<table>
<thead>
<tr>
<th>CRL Name and Groups</th>
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<tbody>
<tr>
<td><strong>BgVV-CRL</strong></td>
<td>CRL Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmmedizin Diedersdorfer Weg 1 D-12277 Berlin GERMANY Tel. +49 1888 412 2302 Fax: +49 1888 412 2955 <a href="http://www.bgvv.de">http://www.bgvv.de</a> Director: Dr. Petra Gowik</td>
</tr>
<tr>
<td><strong>RIVM-CRL</strong></td>
<td>EU CRL for residues RIVM – National Institute of Public Health and the Environment P.O. Box 1 / NL-3720 BA Bilthoven / The Netherlands Phone: +31-30-2742717 / 2742613 ; Fax: +31-30-2744403 <a href="http://www.rivm.nl">www.rivm.nl</a> Director Prof. Dr. Rainer Stephany</td>
</tr>
<tr>
<td><strong>ISS-CRL</strong></td>
<td>CRL at the Istituto Superiore di Sanità Viale Regina Elena 299 00161 Rome, Italy Tel.: + 39 06 4990 2052 Fax + 39 06 4990 2366 <a href="http://www.iss.it">http://www.iss.it</a> Director: Prof. Dr. Sergio Caroli</td>
</tr>
<tr>
<td><strong>AFSSA-LMV-CRL</strong></td>
<td>Laboratoire d’études et de recherches sur les médicaments vétérinaires et les désinfectants AFSSA-Site de Fougères BP 90203 F-35302 Fougères Tel : 33 (0)2 99 94 78 90 Fax : 33 (0)2 99 94 78 77 <a href="http://www.fougeres.afssa.fr">http://www.fougeres.afssa.fr</a> Director: Dr. Pascal Sanders</td>
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</table>
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
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 I (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 I (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:**


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. Norchlorotestosterone acetate was included in the animal experiment and studies were undertaken to elucidate the metabolism. Studies at the CRL tentatively identified 17α-Norchlortestosterone and 4-chloro-17α-hydroxy-19-norandrostane-5α-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-17ß-hydroxy-19-norandrostane-5α-3one was synthesized by pressurized hydrogenation of 17ß-Norchlortestosterone. 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 ultrafiltration, 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 TWINNING 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.
Annually, the CRL conducts an animal experiment in order to obtain 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), 17ß-Oestradiol and Ethynylestradiol (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 reconfirmed. 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 17a-ethyl-5ß-androstane, 3a(17ß)-diol. 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 17a-ethyl-5ß-androstane,3a(17ß)-diol. 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 17a-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/AOAC 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
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)


Blokland MH, Zomer G, Sterk SS, Herbold HA, Wubs KL, Ginkel LA van, Stephany RW. Identification of a novel pharmacological active agent in an illegal growth promoting


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

**Period: 2004.01.01 – 2004.12.31**

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WORK PROGRAMME OF THE COMMUNITY REFERENCE LABORATORY AT THE FRENCH FOOD SAFETY AGENCY (AFSSA)

Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants

Programme for the contract period: January 2004 - December 2004

FINAL REPORT

P. SANDERS
Head of C.R.L.
1 Legal functions and duties


2 Objectives for the period January 2004 – December 2004

2.1 General tasks

2.2 Development and validation of analytical methods

   Annex V, chapter 2, section 1 (a, c, d)

2.3 Production of incurred sample material

   Annex V, chapter 2, section 1 (a, b, g)

2.4 Quality Assurance and quality control including organization and implementation of proficiency tests

   Annex V, chapter 2, section 1 (a, b, c, g)

2.5 Technical and Scientific Support to NRLs and Third Countries

   Annex V, chapter 2, section 1 (d, f, h, l)

3.1 General tasks

- Research and identification of unknown compounds *Annex V, chapter 2, section 1 (a, j)*
- Analysis of official samples *Annex V, chapter 2, section 1 (h)*
- Meeting 4 CRLs *Annex V, chapter 2, section 1 (j)*
- Visit of NRLs *Annex V, chapter 2, section 1 (b)*
- Co-operation with international Organisation *Annex V, chapter 2, section 1 (j)*
- Maintenance QS *Annex V, chapter 2, section 1 (a-l) and Decision 98/179/EEC, No 1.2*
- Techn. and scientific support *Annex V, chapter 2, section 1 (j)*
- Compilation of annual report and cost estimate *Annex V, chapter 2, section 1 (h)*

3.2 Development and validation of analytical methods and animal studies *Annex V, chapter 2, section 1 (a, c, d)*

- Confirmatory method Antimicrobials (extension)

The method developed and validated has the capacity to confirm 44 substances in meat and milk. Compounds (Lincomycin, Spectinomycin, Trimethoprim and Florfenicol) not studied at this moment have to be added in the list of substances to be confirmed. Limited studies have been performed but the extension was not validated.

- Malachite green (Fish) – Screening and Confirmation

Malachite green is a forbidden substance used in fish production. Presence of malachite green and leuco-malachite is regularly detected in fish produced in the European Union or outside the European Union. The screening can be based on a LC/DAD method and confirmation on a LC/MSMS method. Several NRLs need to improve their control strategy. The work will make it possible to improve existing methods used in the laboratory for screening and confirmation, give updated information and provide screening and confirmatory methods validated according to the new MRPL to the NRL. The development of a new confirmatory method has been planned and performed. Unexpected technical problems have been meet during the spring period due to an instability of reproducibility of our auto-sampler system coupled with a new LC/SMSM purchased in spring. The method was developed and validated on two LC/MSMS systems to give a choice of different options to the NRLs. The method was described by B Delepine during the training session organized in October 2004.

- Nifursol (Poultry-Turkey) – Screening and Confirmation

Nifursol, a compound of the nitrofuran family, a former feed additive has been banned since 2003. A study of the applicability of the nitrofuran metabolite LC-MSMS method for screening and confirmation of nifursol bound metabolites has been investigated. The major nifursol protein-bound metabolite, DNSAH, was identified after an *in vitro* metabolism study. The work will continue on this subject in 2005 with a final
development of a specific LC/MSMS method for screening and confirming nifursol metabolite in turkey muscle and a validation of the method according to the decision 657/2002/EC (an ongoing collaboration with the BVL CRL-Berlin will permit the testing of turkey naturally incurred tissue samples).

- Biospecific interaction analysis and detection of residues

A new monoclonal antibody known to be a multi-sulfonamide antibody was kindly supplied to us by an Israeli team. We have worked on the production of a sensor chip able to interact with this antibody. The selectivity of this antibody makes this task more difficult than usual. Then the second task was the study of interactions between this antibody and a wide selection of sulfonamides. The antibody cross-reacts with at least 8 different sulfonamides. Finally a protocol has been validated for the screening of 8 sulfonamides, in milk first. All of these sulfonamides are detected at or below the MRL of 100 µg/kg. Then complementary studies will be performed to adapt this protocol to the analysis of muscle.

- Comparative study of ELISA Kits (antibacterial substances)

A study was performed on the performance of ELISA kits for the detection of chloramphenicol in shrimps with the Eurodiagnostica kit. The results were really satisfactory to detect chloramphenicol at or below the MRPL (0.3 µg/kg).

New ELISA kits for the screening of nitrofuran metabolites AOZ and AMOZ proposed by industry (r-Biopharm) have to be validated independently to provide information to the NRLs about their performance. The validation of the AOZ ELISA kit has been performed in shrimp and in chicken and turkey muscle. The results were really satisfactory. The kit was able to detect AOZ in all of these matrices at or below the MRPL (1 µg/kg). The validation of the AMOZ ELISA kit has already been finished in shrimp. The results were also very satisfactory. The validation of the AMOZ screening in chicken and turkey muscle is on going and results are promising.

- Microbiological methods

* Some work has been done to adapt the STAR protocol to the detection of antibiotic residues in kidney and liver. However the results were not satisfactory. Many false positive results were observed with kidney and liver, mainly on the plate Bst dedicated to the detection of beta-lactams and sulfonamides.

* A comparative study was implemented on field samples (145) during several months between a commercial microbiological test named Premi Test (DSM), the French official method (Four Plate Test) and the STAR protocol developed at the CRL. Positive results after at least one of the 3 methods were confirmed by LC/MS/MS. The aim of the study was to provide NRLs with information about the performance of this rapid test compared with plate tests. The results of the Premi test were really satisfactory in term of reproducibility. Moreover 3 times more positive samples were detected with the Premi Test than with the 4PT. The Premi Test is more sensitive than the 4PT for the detection of beta-lactams and sulfonamides. However the 4PT
and the STAR protocol have shown better sensitivity for tetracyclines than the Premi Test. Moreover this study made it possible to compare the 4PT with the STAR protocol results. The agreement between the 2 methods was of 70% (102/145 samples). Concerning the remaining 30%, 38 samples were detected negative with the 4PT instead of positive with the STAR protocol. Among them, 30 samples confirmed by LC/MS-MS were declared compliant, but 8 samples were confirmed non-compliant. The presence of sulfonamides was confirmed in 3 samples, some macrolides in 2 samples, beta-lactams in 1 sample and a mix of beta-lactams and sulfonamides in 2 samples. Half of them contained antibiotic residues at concentrations higher than the respective MRLs. Finally 5 samples were detected positive by the 4PT and negative by the STAR protocol. None of them was confirmed as non-compliant by LC/MS-MS analysis. Therefore this study was in favour of the Premi Test as a commercial rapid test and in favour of the STAR protocol compared to the 4PT as a plate test.

3.3 Production of incurred sample material  

*Annex V, chapter 2, section 1 (a, b, g)*

Incurred sample material of different species and substances has been produced in order to support the development and validation of test methods and for use in proficiency testing and distribution studies.

- Treatment and slaughter of animals
- Determination of residue concentrations
- Packaging and shipment of samples

Two proficiency tests had been planned in 2004 to analyse the capacity of NRL to detect and confirm the presence of antimicrobials in muscle and milk without previous information of the chemical family. The objective is to test simultaneously the capacity of the two steps of antimicrobial residue control (screening and confirmation).

- **Antimicrobials - Milk (laboratory incurred)**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MRL (µg/kg)</th>
<th>Spiking concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxytetracycline</td>
<td>100</td>
<td>240</td>
</tr>
<tr>
<td>tetracycline</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>neomycin</td>
<td>1500</td>
<td>2400</td>
</tr>
<tr>
<td>penicillin G</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>cloxacillin</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>tylosin</td>
<td>50</td>
<td>120</td>
</tr>
<tr>
<td>sulfamethazine</td>
<td>100</td>
<td>300</td>
</tr>
</tbody>
</table>

All the spiked materials contained antibiotics at concentrations above their respective MRL. Three blank samples were also prepared. In February 2004 participants received 10 milk samples (3 blank and 7 spiked) to be analysed by a screening and a confirmation method. Samples have been analysed for their homogeneity and stability.

- **Antimicrobials - Muscle**

Due to the absence of two scientists in 2004 and to the planning of a new building for our animal facilities, the project was postponed to 2005. It was replaced by a proficiency test on nitrofuran residues in chicken and aquaculture fish products.
Nitrofuran metabolites - Chicken and Aquaculture Fish Products

A MRPL of 1.0 µg/kg for nitrofuran metabolite in tissues was published in 2003. Four nitrofuran contaminated materials, either naturally or artificially incurred, have been prepared in September and October 2004 along with 3 blank materials of the same biological matrices:

<table>
<thead>
<tr>
<th>Material</th>
<th>MRPL (µg/kg)</th>
<th>Estimated Incurred concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total/Bound SEM (art)</td>
<td>1.0</td>
<td>2.4 / 0.2</td>
</tr>
<tr>
<td>Total/Bound AOZ (nat)</td>
<td>1.0</td>
<td>2.2 / 2.0</td>
</tr>
<tr>
<td>Total/Bound AMOZ (nat)</td>
<td>1.0</td>
<td>0.5 / 0.3</td>
</tr>
<tr>
<td>Total/Bound AMOZ (nat)</td>
<td>1.0</td>
<td>1.4 / 1.2</td>
</tr>
</tbody>
</table>

In November 2004 all participants received 8 samples (3 blank and 5 incurred) to be analysed by a screening and a confirmatory method. Materials have also been analysed for their homogeneity and stability.

Chloramphenicol - Porcine Muscle

A MRPL of 0.3 µg/L of chloramphenicol in muscle was published in 2003. A proficiency test has been organized to verify the capacity to screen and confirm the presence of chloramphenicol at concentration close to this value.

In February 2004, 8 samples were prepared at the laboratory, 2 blank materials and 4 incurred materials containing:

<table>
<thead>
<tr>
<th>Material</th>
<th>CAP concentrations (µg/kg) (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAP 1-4</td>
</tr>
<tr>
<td></td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>CAP 2</td>
</tr>
<tr>
<td></td>
<td>0.65 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>CAP 3</td>
</tr>
<tr>
<td></td>
<td>0.22 ± 0.01</td>
</tr>
</tbody>
</table>

Material CAP 1-4 has been sent in blind duplicate.

In March participants received samples to be analyzed by a screening and a confirmatory method.

3.4 Quality Assurance and quality control  

Annex V, chapter 2, section 1 (a, b, c, g)

- Maintenance of the QA/QC system to widen ISO 17025 accreditation

Developed work in QA/QC for the mass spectrometry sector in order to comply with the ISO 17025 standard. Procedure and quality records have been established between the users. The objective of the working group is to limit the introduction of errors into analytical data, and also permit the accreditation of screening and confirmation methods by LC/MS/MS.

- Proficiency test: Antimicrobials Milk - Screening and Confirmation

24 participants (13 from EU and 11 from candidate countries) received 10 milk materials (3 blank, 7 incurred) to be analysed in February 2004. The final report of the study was been sent on 1st July 2004.

21 participants performed the analyses with screening methods for the detection of antibiotic residues. Among them, 15 laboratories performed post-screening methods to direct the confirmation towards specific antibiotics or families. Moreover 17
participants implemented confirmatory methods to identify and quantify some families of antibiotics.

<table>
<thead>
<tr>
<th>antibiotic</th>
<th>MRL (µg/kg)</th>
<th>Spiking concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxytetracycline</td>
<td>100</td>
<td>240</td>
</tr>
<tr>
<td>tetracycline</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>neomycin</td>
<td>1500</td>
<td>2400</td>
</tr>
<tr>
<td>penicillin G</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>cloxacillin</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>tylosin</td>
<td>50</td>
<td>120</td>
</tr>
<tr>
<td>sulfamethazine</td>
<td>100</td>
<td>300</td>
</tr>
</tbody>
</table>

All the spiked materials contained antibiotics at concentrations above their respective MRL.

The most often detected antibiotics were: penicillin G, neomycin, tylosin and sulfamethazine at 1.25, 1.6, 2.4 and 3 MRLs respectively. Then about 70 % of the laboratories succeeded in detecting oxytetracycline, tetracycline and cloxacillin which were spiked at 2.4, 1.8 and 2 MRLs. Whichever the screening test (s) performed, the least detected molecules were tetracycline and oxytetracycline.

Most of the participants were able to confirm the presence of oxytetracycline (15 labs), tetracycline (14) and sulfamethazine (17). Ten participants were able to confirm the presence of penicillins (penicillin G and cloxacillin) and very few were able to confirm tylosin (4) and neomycin (2) even if macrolide or aminoglycoside residues were sometimes detected by post-screening. These results are an indicator of the difficulty encountered by the NRLs to develop some simple and reliable methods for the confirmation of these two families. Moreover when laboratories were able to identify the true molecules in the milk samples, it is important to notice that the accuracy and repeatability z-scores were very satisfactory.

This kind of interlaboratory comparison, gathering screening, post-screening and confirmatory methods was organised for the first time this year. The first point to underline is that the evaluation of the laboratories' performance was obviously depending on the choice of the antibiotics, made by the Community Reference Laboratory (CRL). This choice has been done among the major families of antibiotics used in all the countries of the European Union. Moreover the concentrations were chosen in relation to the respective MRLs and the sensitivities of the usual screening tests. The compilation, the data collection and analysis were obviously heavier and more time-consuming than usual.

However some major advantages could be put forward:
- First, the 3 possible steps of the analytical strategy were evaluated in one unique interlaboratory study: screening, post-screening and confirmation,
- Secondly the ability of screening and post-screening methods was studied (detection and direction towards one antibiotic or one family of antibiotics),
- Thirdly regarding the confirmatory methods, this unique interlaboratory comparison could be assimilated to 5 different usual interlaboratory studies because 5 different confirmatory methods could be evaluated,
- Fourthly the analytical strategy of each participant could be evaluated.
• Proficiency test: Antimicrobials Muscle – Screening and Confirmation

Postponed and replaced by a proficiency test with nitrofuran contaminated samples, planned for the 4th trimester of 2005 with the agreement of DG-Sanco.

• Proficiency test: Nitrofuran metabolites – Screening and Confirmation

24 participants (16 from EU and 8 from Third Countries) received 8 samples to be analysed at the end of November 2004. Results are foreseen for January 2005. A report will be released for Springtime 2005.

• Proficiency test: Chloramphenicol – Screening and Confirmation

25 participants (14 from EU and 11 from candidate countries) received 6 muscle materials (2 blank, 4 incurred) to be analyzed in March 2004. 24 laboratories sent back their results. The final report of the study was sent at the end of July 2004.

**Estimated concentrations of the 3 incurred pig muscle materials.**

<table>
<thead>
<tr>
<th>Material</th>
<th>CAP 1-4</th>
<th>CAP 2</th>
<th>CAP 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP concentrations (µg/kg) (n=2)</td>
<td>0.43 ± 0.07</td>
<td>0.65 ± 0.08</td>
<td>0.22 ± 0.01</td>
</tr>
</tbody>
</table>

Material CAP 1-4 was sent in blind duplicate.

Chloramphenicol was detected with screening tests in porcine muscle at concentrations between 0.35 and 1.20 µg/kg. Then about 95% of the laboratories (18/19) succeeded in detecting CAP at the lowest concentration (0.35 µg/kg). ELISA kits as well as GC/MS methods were able to detect CAP at a concentration near to the MRPL (0.3 µg/kg). Most of the participants were able to confirm the presence of CAP (21 labs) at the lowest concentration 0.35 µg/kg.

• Proficiency test: Malachite Green – Screening and Confirmation

Planning was established during the training session planned in October 2004. An announcement was published in December 2004 for a sending of samples in April 2005 with a final reporting during summer 2005.

3.5 Technical and Scientific Support to NRLs and Third Countries Annex V, chapter 2, section 1 (d, f, h, l)

• Analytical support to NRLs and other official laboratories (ongoing task)

**Visitors - Trainees**

Mr. Maodo Malick DIOP – DVM, Dakar, Senegal, January-June 2004

Mr. Mike CLEAR – NZ-Food Standard Agency – Wellington, New Zealand, May 17-18, 2004
Reference confirmatory analyses performed for the NRLs, for the Foreign Inspection Services and for several Third Countries

<table>
<thead>
<tr>
<th>Country - Administration</th>
<th>Nb Requests</th>
<th>Nb Samples</th>
<th>Compounds and Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greece Inspection Services (BIPs of Thessaloniki &amp; Piraeus)</td>
<td>18</td>
<td>21</td>
<td>Nitrofurans by LCMSMS</td>
</tr>
</tbody>
</table>

- Promotion of exchange of information via the web-site

After the official opening of the CRL web site on December 2002, this is now the 2nd complete year for the CRL website activity. About 1500 visitors and 4000 requests were recorded for access to the worldwide-opened area of the CRL web site since its opening and up to October 2004.

The restricted area contains about 80 documents published to the attention of the DG-SANCO and of the NRLs experts from the 25 EU-Member States.

About 150 scientists entered the restricted area. They belong essentially to the 25 MS-NRLs and the 3 CC-NRLs but some are also coming from administrations such as DG-SANCO-Brussels, AOV-FVO-Dublin and IAEA-Vienna and finally some are from Third Country assimilated-NRLs. They all received on their demand a password for entry into one of the specifically authorized restricted areas on the website.
The last survey from January 2004 to December 2004 indicated about 300 different connections into the restricted area. The entries were sorted as follows: 66% MS-NRL scientists, 24% new MS-NRL scientists, 4% CC-NRL scientists, 4% from DGSANCO & FVO and 3% scientists from Third Country official laboratories.

- Provision of standard substances to the NRLs in the Member States and to official laboratories in third countries (ongoing task)

- Provision of training to scientists from the EU Member States and from third countries (ongoing task)

Training course for MS-NRLs experts entitled "Malachite Green Analysis in Fish"
Held at AFSSA-LERMVD, October 21-22, 2004, Fougères, France
Number of participants: 23
Report sent December 9, 2004 (via CRL website)

Training course for a Ukrainian NRL expert "Analysis of Vet Drug Residues in Food"
Held at AFSSA-LERMVD, August 30-September 3, 2004, Fougères, France
Number of participants: 1

Training course for Taiwanese experts "Analysis of Banned Vet Drugs in Food"
Held at AFSSA-LERMVD, November 2-5, 2004, Fougères, France
Number of participants: 3

Training course for a Cyprus expert "Microbiological screening of antimicrobials in Food"
Held at AFSSA-LERMVD, November 22-24, 2004, Fougères, France
Number of participants: 1

- Diffusion of scientific information

  o Participation to the kick-off meeting on Semicarbazide, January 19, 2004 - Brussels, Belgium

  o Participation to the Meeting regarding Scientific and Technical co-operation between Residues-CRLs and JRC-IRMM, March 15, 2004 - Geel, Belgium

  o Participation to EuroResidue V, May 10-12, 2004 - Noordwijkerhout, The Netherlands

  o Participation to the 2nd International Conference on antimicrobial agents in veterinary medicine, June 13-17, 2004, Ottawa - Canada

  o Participation to the meeting "Amendment of Decision 2002/657/EC", on July 15, 2004, Brussels, Belgium

  o Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL, August 24-26, 2004, Bangkok, Thailand

  o AOAC 118th Annual Meeting, September 18-23, 2004, St-Louis, Missouri, USA
Publications:


Gaudin V., Maris P., Fuselier R., Ribouchon JL., Cadieu N., Rault A. Validation of a microbiological method: the STAR protocol, a five-plate test, for the screening of antibiotic residues in milk. *Food and Agricultural Immunology*, 2003, **15** (3-4), 143-157

Posters & Oral Communications:

- Poster: « Validation of a multi-quinolone, multi-matrix, multi-species method for the determination of quinolone residues by HPLC with fluorescence detection » - Verdon E., Couédor P., Sanders P. - Vth International Conference on Residues of Veterinary Drugs in Food (10 au 12/05/04, Noordwijkerhout, Pays-Bas) [online CRL website]

- Poster: « Results of a Proficiency Study for the Determination of Nitrofuran Metabolites in Shrimps» - Hurtaud-Pessel D., Verdon E., Blot J., Sanders P. - Vth International Conference on Residues of Veterinary Drugs in Food (10 au 12/05/04, Noordwijkerhout, Pays-Bas) [online CRL website]

- Poster: « European Proficiency Testing of National Reference Laboratories for the Confirmation of Sulfonamide residues in Muscle and Milk » - Juhel-Gaugain M., Fourmond MP., Delépine B., Laurentie M., Roudaut B., Sanders P. - Vth International Conference on Residues of Veterinary Drugs in Food (10 au 12/05/04, Noordwijkerhout, Pays-Bas) [online CRL website]

- Results of a European Proficiency Test for the Detection of streptomycine/dihydrostreptomycine, gentamicin and neomycin by ELISA and Biacore methods - V.Gaudin, N.Cadieu & P.Sanders - th International Conference on Residues of Veterinary Drugs in Food (10 au 12/05/04, Noordwijkerhout, Pays-Bas) [online CRL website]

- Day-to-Day Evaluation of the performance of a LCMSMS method through a statistical calculation of the limit of decision and capacity of detection according to ISO Standard 11843 – Application to routine analysis of Nitrofuran Metabolites in Food - Verdon E., Hurtaud-Pessel D., Sanders P. - 118th Meeting of the AOAC International (19 au 24/09/04, Saint-Louis, Missouri, USA) [online CRL website]

- Results of a Proficiency Testing Study for the Determination of Nitrofuran Metabolites in Shrimps - Verdon E., Hurtaud-Pessel D., Sanders P. - 118th Meeting of the AOAC International (19 au 24/09/04, Saint-Louis, Missouri, USA) [online CRL website]
**PTS Reports:**

- « Proficiency Testing Study for the control of antibiotic residues in Milk - screening and confirmation », R. Fuselier, V. Gaudin, M. Juhel-Gaugain & P. Sanders, June 2004. ([online CRL website](#))

**Other Reports:**

- List of analytical methods routinely used in the MS-NRLs and CC-NRLs for screening and confirming antimicrobial residues in food from animal origin (2004 updating - [online CRL website](#))
- List of commercially available antibiotic standards and their possible suppliers (2004 updating - [online CRL website](#))
- Conclusions of the 15.07.04 meeting on the interpretation of the implementation of Decision 657/2002/EC ([online CRL website](#))
- Recommendation on SEM analysis in food from animal products ([online CRL website](#))
- CRL Work Programme 2005 ([online CRL website](#))
- Malachite green Training Session documents ([online CRL website](#))
Technical Report on the Activities of the Community Reference Laboratory
for Residues of β-Agonists, Coccidiostats, Anthelmintics and NSAIDs
for the Period 1 January 2004 to 31 December 2004

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)
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Fax: + 49-1888-412 2300
E-mail: crlvetdrug@bvl.bund.de
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General

The CRL Berlin for residues of beta-agonists, anticoccidials including nitroimidazoles, anthelmintics and non-steroidal anti-inflammatory drugs (NSAIDs) appertains to the division “Food, Feed and Commodities” of the BVL. The Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (Federal Office of Consumer Protection and Food Safety), BVL, where the CRL is located now, was officially established in November 2002. Since May 2002, it had been operating in its preliminary form as Bundesanstalt. The capacities of the CRL, which is also NRL for all substance groups according to Council Directive 96/23/EC, are still occupied considerably by the continuous demands regarding organisation and management inherent to an enlargement of responsibilities and personnel. Moreover, the routine administrative procedures in the newly founded office are not yet fully established, which also entails time losses.

The analytical activities of the CRL Berlin are pursued by two specialised sub-units, one being responsible for GC and GC-MS, one for HPLC and LC-MS. They are supplemented by a third sub-unit in charge of the preparation of incurred test materials to be used as in-house reference samples and for proficiency testing.


A General tasks

1 Identification of new and unknown compounds illegally used for growth promoting purposes

   Annex V, chapter 2, section 1 (a, j)

The efforts undertaken in cooperation with WITEGA Laboratorien Berlin Adlershof GmbH to isolate a new beta-agonist from feeds were continued in 2004. A publication by Nielen et al. (Rapid Commun. Mass Spectrom. 2003; 17: 1-9), who, by means of HR-MS, suggest a structure for a new beta-agonist which was found in feeds, was the basis for this project. First it was tried to synthesise the substance in the dimension of grams. These experiments failed. The further efforts concentrated on trying to isolate the new beta-agonist from feeds in quantities sufficient for $^1$H- and $^{13}$C-NMR-measurements. However, due to the limited
quantities of material of origin available and the very low concentration of the investigated substance, these experiments were not successful, either.

As new beta-agonist, brom-chlorbuterol was included in the multi-method for beta-agonists. Brom-chlorbuterol is available as standard substance from the CRL Berlin.

2 Analysis of official samples

During the period covered by this report the CRL Berlin carried out a total number of 10 confirmatory analyses for nicarbazin in eggs for AGES, Austria, as well as one confirmatory analysis for ronidazole in rabbit muscle for the SGL, Cyprus.

3 EC-4 CRLs residue management (Meeting of four CRLs)

Annex V, chapter 2, section 1 (k)

Did not take place during the period covered by this report.

4 Visit to NRLs

In December 2004 a two-day supporting visit to the NRL in Vienna/Mödling, Austria, took place. The NRL was contacted due to the reorganisation of the institute. It was very interested in a cooperation, especially regarding confirmatory analyses of suspect samples, and in information on method validation. The lab was visited and found not entirely well-equipped. A report was issued and sent to the Commission.

5 Co-operation with international organisations

Annex V, chapter 2, section 1 (j), 2 (g)

Since March 2004 one representative of the CRL/NRL is head of the German delegation at CCMAS. The CRL was asked for and issued statements on reports of the CCMAS working group, especially on questions concerning the establishment of criteria for single laboratory validation and recovery correction. A statement of the German delegation on the application of recovery corrections was sent to the EU codex secretariat via the German ministry for consumer protection (BMVEL). Also for the CCRVDF statements on the criteria approach for the evaluation of methods were issued. With respect to traceability and comparability questions the CRL is still in contact with the Physikalisch-Technische Bundesanstalt (PTB) in
Braunschweig, Germany (the National Metrology Institut of Germany). A contract for an official cooperation is in preparation.

6 Maintenance QS

*Annex V, chapter 2, section 1 (a-l)*

The maintenance of the quality management system and the adaptation to the requirements of ISO 17025 are an ongoing task.

A working group, established in 2003 for the development of a concept which allows to use part of both, the examination data necessary for the optimisation and the establishment of a method for validation purposes, held three meetings in 2004. A software, InterVal II, was produced (financed partly by the BVL) and is now operational as beta-version. It is not yet commercially available. The CRL carried out several experiments on this topic.

7 Technical and scientific support to the Commission

*Annex V, chapter 2, section 1 (j)*

The CRL provided support to the corresponding Commission services regarding questions concerning Commission Decision 2002/657/EC:

- proficiency testing
- validation
- Codex Alimentarius (CCMAS, CCRVDF)
- reflection paper
- guidelines for the implementation of CD 2002/657/EC
- MRPL

The suggestions for MRPLs for Beta-Agonists and nitroimidazoles were prepared by a survey among the NRLs which was refined twice. The suggestions were sent to the Commission in January 2004, a newly revised version in December 2004.

TAIEX was supported by providing information on validation to the new MS and the CCs in form of a workshop held at the BVL's facilities and in form of presentations at the TAIEX workshop in Bilthoven, NL.
DG JRC was supported with regard to proficiency testing in form of a presentation held at the IRMM workshop at Budapest.

8 Reports, cost estimates

The CRL management drew up cost estimates and work plans for the next contract period 1 January to 31 December 2005. Additionally, the report on the reference period January 2003 to December 2003 and the interim report for 2004 were prepared. The reports of both reference periods 2002 and 2003 were adapted according to the new regulations and were provided to the CRL Bilthoven and the CRL Rome for the establishment of the joint report.

B Development, optimisation and validation of analytical methods

Annex V, chapter 2, section 1 (a, c, d)

1 Anthelmintics

1.1 Validation of the method for avermectines in milk by HPLC-FLD

As it was agreed with the Commission at short term to carry out a proficiency test for avermectines in milk in 2005, the validation of the existing method was included into the examination programme.

The establishment of a multi-residue method for screening and confirmatory purposes for all relevant avermectines was performed on the basis of the matrix-comprehensive in-house validation procedure according to Commission Decision 2002/657/EC. The target matrix was milk of different species (cattle, sheep, goat), different fat contents, different storage and transport conditions and analysed by different operators.

marker residues: moxidectin, abamectin, emamectin, doramectin, ivermectin
internal standard: nemadectin
matrix: milk
**Principle of the method**

Sample preparation
- Liquid-liquid extraction using acetonitrile
- SPE on C18-cartridges
- Derivatisation with trifluoro acetic acid anhydride and N-methyl nitroimidazole
- Injection of derivatisation mixture

**HPLC**
- Stationary phase: Prontosil C18, 150 x 2.5 µ
- Mobile phase A: Water
- Mobile Phase B: Acetonitrile/water 98:2
- Gradient: - 0 - 2.8 min 90 % B
- 3.8 min 100 % B
- 35 min 100 % B
- 36 min 90 % A
- 45 min 90 % A
- Col. temp.: 40 °C
- Flow: 200 µl / min
- Inj. vol.: 10 µl
- Fluorescence-detector: Excitation: 365 nm
  Emission: 455 nm

**Validation parameters for selected substances:**

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<tr>
<th>Compound</th>
<th>MRL [µg/kg]</th>
<th>CC&lt;sub&gt;alpha&lt;/sub&gt;</th>
<th>CC&lt;sub&gt;beta&lt;/sub&gt;</th>
<th>Within-laboratory reproducibility</th>
<th>Repeatability</th>
<th>Recovery</th>
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<tr>
<td>Emamectin</td>
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</table>
1.2 Development of a method for the determination of avermectines in liver and muscle

The method for the determination of avermectines in milk was taken over for the determination of abamectin, doramectin, ivermectin and moxidectin in liver and muscle. The matrix-comprehensive in-house validation procedure according to 2002/657/EC by means of InterVal was begun. However, due to the additional time that became necessary for the determination of eprinomectin and the validation of the milk method, it could not be finalised.

1.3 Development of a method for eprinomectin in milk, liver and muscle

The derivatisation of avermectines with trifluoroacetic acid, as it is performed for abamectin, doramectin, ivermectin, moxidectin and emamectin, cannot be applied for eprinomectin. Therefore a separate method was developed for eprinomectin. Instead of trifluoroacetic acid anhydride/ N-methyl nitroimidazole, the derivatisation is realised with a mixture of acetic anhydride/ N-methyl nitroimidazole/DMF = 6:2:9. As this reagent is not suitable for the derivatisation of the other avermectines, it is not possible to establish a comprehensive method to cover all substances of this group with fluorescene detection.

The present method is generally suitable for the determination of eprinomectin in milk, liver and muscle, but is still to be validated.

2 NSAIDs

2.1 Validation of a method for acidic NSAIDs in muscle, liver and kidney by LC-MSMS

In contrast to the work plan for 2003 indicating that a method was to be developed for NSAIDs in muscle or liver, we succeeded in analysing all three matrices (muscle, liver and kidney) with the method developed at the CRL. This means, however, that for the validation study a lot more samples had to be investigated. For this reason it was not completely possible to finalise the validation study. The analysis of the samples has been finished. The evaluation of the data is in process.
The validation experiment was performed by means of InterVal.

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<th>muscle, liver, kidney</th>
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<td>duration of sample</td>
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<td>pH-value</td>
<td>pH 2 - pH &lt;2</td>
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</table>

**Number of factors** 7 + 1 leading factor

**Number of leading factor**

**Levels:**

- 3

**Number of samples** 24

**Number concentration levels** 5

**Total number of analyses** 120

**Samples per day** 2
### Analyte(s)

- Carprofen (CPF)
- Diclofenac (DC)
- Flunixin (FLU)
- Ibuprofen (IP)
- Ketoprofen (KTP)
- Mefenamic acid (MFAS)
- Meloxicam (MLX)
- Niflumic acid (NFA)
- Naproxen (NP)
- Oxyphenbutazone (OPB)
- Phenylbutazone (PBZ)
- Salicylic acid (SA)
- Tolfenamic acid (TLF)
- Vedaprofen (VDP)
- Flunixin hydroxide (FLU-OH)

**Internal standards:** PBZ-d10, MLX-d3, FLU-d3 and TLF-d4

### Principle of the method

- Enzymatic hydrolysis with glucuronidase
- Liquid-liquid extraction with acetonitrile and Na-acetate buffer
- Defattening procedure with hexane
- Solid-phase extraction on C18-material
- Elution with hexane/diethylether and acetonitrile/methanol
- Detection by means of LC-MS/MS

### Method parameters

**HPLC column:**

- **Length:** 150 mm
- **ID:** 2.0 mm
- **Particle size:** 5 µm
- **Column material:** C18, Inertsil ODS2
- **Gradient elution**
MS/MS operating conditions:
- Source: ESI, negative mode
- Scan type: MRM
- Resolution: Q1 and Q3 unit
- Gases: Nitrogen (collision gas)
- Temperature: 400 ºC

This method has been validated in the following concentration ranges:

- 1 to 32 µg/kg for FLU-OH, DC, NFA, KTP, IP, NP; OPB and PBZ
- 2.5 to 80 µg/kg for MLX
- 4 to 128 µg/kg for VDP
- 10 to 320 µg/kg for FLU
- 20 to 640 µg/kg for TLF
- and 40 to 1280 µg/kg for CPF

The higher concentration ranges for MLX, VDP, FLU, TLF and CPF have been adapted to the respective MRL-values existing for muscle, liver and kidney.

3 Animal studies

More studies than originally planned were carried out, as during the course of the reference period a higher need for incurred material for method development and validation purposes arose. The material for beta-agonist proficiency test BETA_06/04 had to be produced.

4.1 Treatment of cattle with beta-agonists

A cow was treated with the beta-agonists brombuterol and ractopamine for one week per substance to gain incurred urine. After urine collection the animal was slaughtered to gain further incurred tissue material (plasma, eye, liver, bile, kidney, muscle). The animal as well as the feeding stuff were financed out of the budget of the Federal Institute for Risk Assessment (BfR).
4.2 Treatment of cattle with an NSAID

A cow was treated with the non-steroidal anti-inflammatory drug phenylbutazone for one week to gain incurred urine. After urine collection the animal was slaughtered to gain further incurred tissue material (plasma, eye, liver, bile, kidney, muscle). The animal as well as the feeding stuff were financed out of the BfR’s budget.

4.3 Treatment of cattle with avermectines and NSAIDs

A lactating cow was treated with the avermectines ivermectin and doramectin and with the non-steroidal anti-inflammatory drugs diclofenac and meloxicam for one week to gain incurred milk. After milk collection the animal was slaughtered to gain further incurred tissue material (plasma, serum, eye, liver, bile, kidney, muscle, fat). The animal as well as the feeding stuff were financed out of the BfR’s budget.

4.4 Treatment of cattle with an avermectine and an NSAID

A cow was treated with the avermectine ivermectin and with the non-steroidal anti-inflammatory drug diclofenac to gain incurred sample material (plasma, serum, eye, liver, bile, kidney, muscle, fat). The animal as well as the feeding stuff were financed out of the BfR’s budget.

4.5 Treatment of turkeys with an anticoccidial

Fifteen turkeys financed out of the BVL’s budget were treated with the anticoccidial salinomycin for two weeks to gain incurred sample material (plasma, serum, eye, liver, muscle, fat). The feeding stuff was in part also financed out of the BVL’s budget.

4.5 Treatment of turkeys with nitroimidazoles

An animal study investigating the nitroimidazoles dimetridazole, metronidazole and ronidazole was performed with 8 turkeys financed out of the BVL’s budget. After two weeks of treatment the animals were slaughtered to gain incurred sample material (plasma, serum, eye, liver, muscle and fat). The feeding stuff was financed out of the BfR’s budget.

4.6 Treatment of laying hens with anticoccidials

This animal study, which started in September 2004, will be continued until March 2005 to produce enough material for an interlaboratory study. Twenty laying hens bought out of the BVL’s budget were treated with the anticoccidials lasalocid, salinomycin and halofuginone. The eggs were collected. The feeding stuff was in part also financed out of the BVL’s budget.
4.7. Results of the animal study on nitroimidazoles

In several animal studies turkeys were treated with different nitroimidazoles (dimetridazole, metronidazole, ronidazole, ipronidazole). After slaughtering, different matrices (breast muscle, leg muscle, liver, plasma, retina) were analysed for their analyte content, for the percentage of hydroxy-metabolites, for homogeneity, stability and bound and conjugated residues. It was investigated how a more targeted sampling and sample handling could be reached in order to control the residues of nitroimidazoles more efficiently.

The studies were finalised in 2004. The results of these studies were put together in two publications (cf. Annex, point 2, no. 4 and no. 7), where target matrices, target analytes as well as a recommended sample treatment procedure were defined.

The main finding was that the repeatability of the analysis of muscle samples, i.e. of samples taken from the same piece of fresh muscle, was unsatisfying due to the inhomogeneity of the matrix. Stability studies showed that nitroimidazoles were not stable in muscle and liver. A rapid degradation of the analytes occurred already during sampling and continued during storage in a non-frozen state. Therefore care must be taken to ensure an immediate and efficient cooling as well as to deep-freeze muscle and liver samples directly after sampling. But even if all precautionary measures are taken the situation is still unsatisfying because of the rapid depletion of the nitroimidazole residues in muscle and liver as shown in the depletion studies with dimetridazole. In plasma a longer detection of the residues is possible, as the residues are more stable and there is no problem with inhomogeneity.

The same is true for retina. In this matrix the highest concentrations of nitroimidazoles were measured. An even longer persistence of the residues than the 5 days of withdrawal tested can be expected from the experiments.

From measurements of parent drugs and the corresponding main hydroxy-metabolites in various incurred materials it can be concluded that HMMNI should be chosen as target analyte to prove a treatment with DMZ. The metabolite of ipronidazole, IPZ-OH, is recommended to detect an illegal medication with IPZ. To check for a treatment with ronidazole or metronidazole, the measurement of the parent drug is to be preferred. Nevertheless, the ratio of parent drug to metabolite was found to vary in relation to the duration of the withdrawal period in the case of a treatment with DMZ. Since respective data on the behaviour of the other nitroimidazoles are not available to date, it is recommended to monitor both, the parent drug and the respective metabolite, whenever possible in order to get more reliable results.
In order to specifically check the influence of the sampling conditions on the amount and distribution of the nitroimidazoles a follow-up animal study was performed. Turkeys were medicated with ronidazole and ipronidazole, the animals were slaughtered and four different ways of treating the muscle samples prior to freezing at –24°C were tested and compared. The four procedures were:

1. Cutting the muscle into sub-samples, vacuum sealing within 45 min;
2. Immediately cooling down the muscle to 0°C (ice water), cutting into sub-samples and vacuum sealing;
3. Cutting the muscle into sub-samples, vacuum sealing and storing at +4°C for 24 h;
4. Cutting the muscle into sub-samples, vacuum sealing and storing at room temperature for 4 h.

Again the results reaffirmed that the immediate freezing of the muscle samples is essential for an effective residue control, since nitroimidazoles and the corresponding hydroxy-metabolites degrade rapidly unless the samples are frozen. Each thermal stress caused a significant loss of analytes, even at +4°C. The nitroimidazoles and metabolites were again inhomogeneously distributed in the muscle samples. The tested sampling procedures only had a minor influence on the homogeneity of the muscle samples as compared to their significant influence on residue levels. Due to the inhomogeneity of the analytes in muscle and liver it is recommended to thoroughly homogenise sufficient quantities of these matrices in order to obtain representative sample material, e.g. by lyophilisation.

C Production of incurred sample material

Sample material incurred with beta-agonists, NSAIDs, avermectines, anticoccidials and nitroimidazoles was produced (Tab. 1). It was used for proficiency testing and quality assurance purposes as well as for the validation of methods.
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<tr>
<th>Substance group</th>
<th>Substances</th>
<th>Matrices</th>
<th>Species</th>
<th>Number of animals</th>
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<td>serum</td>
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<td>muscle</td>
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<td>turkey</td>
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<td>fat</td>
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<td></td>
<td>blank</td>
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<td>chicken</td>
<td>20</td>
</tr>
</tbody>
</table>

Tab. 1: Production of incurred sample material
D QA and QC including organisation and implementation of a proficiency test

1 Maintenance of equipment, documentation, audits, management

*Annex V, chapter 2, section 2 (a, b, c, d, e, f)*

The CRL Berlin continued to adapt its QM system to the requirements of ISO 17025. In October 2004 an audit by the German accreditation office AKS Hannover took place for re-accreditation purposes. According to the report the re-accreditation was successful. The certificate has not yet been issued.

2 Proficiency test: characterisation of the material, packaging, evaluation, report

*Annex V, chapter 2, section 1 (g)*

Official residue control laboratories have to prove their competence by regularly participating in proficiency tests organised or recognised by NRLs or CRLs. These proficiency tests are intended to provide an opportunity to check routine test methods and thus contribute to an objective evaluation of the performance of the participating laboratories.

2.1 Beta-agonists BETA_06/04

The interlaboratory study on beta-agonists in liver and urine was prepared and organised during this reference period. The selected substances were brombuterol, ractopamine, zilpaterol and mabuterol in urine (fresh material), and clenbuterol, brombuterol, ractopamine, zilpaterol, cimaterol and clen cyclohexerol in liver (lyophilisate). The animals were treated as described under B, point 4.1, the urine and liver were collected, the material was lyophilised (liver) and homogenised (liver and urine). The investigations on homogeneity and stability were performed according to international standards. The samples were shipped to the participants on 12 June 2004. The deadline for the submission of the participants' results was 31 August 2004, but had to be extended until mid-September, as many participants did not submit their results in time. Therefore the evaluation could only start in September. The new Member States and the Candidate Countries were contacted and informed, as well, but only 10 of them participated (Czech Republic, Estonia, Hungary, Latvia, Poland, Slovakia and Cyprus [new MS]; Bulgaria, Serbia and Montenegro and Turkey [Candidate Countries]). Only two NRLs of the new Member States and none of the Candidate Countries successfully passed the proficiency test. In general many laboratories failed, mainly because of the high
false-negative rates they got due to the non-inclusion of certain analytes in their multi-methods.
The report on results is still being prepared. Due to the high number of analyte/matrix combinations to be assessed the report could not yet be finalised. Nevertheless, the participants were informed on their results in form of short preliminary reports.

3 Participation in proficiency tests

*Annex V, chapter 2, section 2 (a, b, g)*

The CRL participated in the proficiency tests on beta-agonists in liver and urine and achieved satisfying results. Since the CRL is simultaneously NRL for all substance groups, it also participated in the following proficiency tests demonstrating the competence of the entire lab:

- Proficiency test Fougères AFSSA, nitrofurane, November 2004 (no results yet)
- Proficiency test Fougères AFSSA, CAP in porcine muscle, March 2004 (successful)
- Comparative test, IRMM, Geel, fluoroquinolones in kidney, January 2004 (successful)
- Proficiency test Bilthoven, trenbolone, methylboldenone in urine, December 2004 (no results yet)
- Proficiency test Berlin CAP_11/04 (no results yet)

E Organisation and performance Workshops

1 Workshop on beta-agonists and anticoccidials

The CRL Berlin organised two Workshops entitled "Beta-Agonists and Coccidiostat” in May 2004: on 03-04 May 04 for the representatives of the old Member States, and on 06-07 May 04 for the representatives of the new Member States; in total the Workshops were attended by 38 scientists from the NRLs of the Member States. The Workshops were intended to contribute to the development and harmonisation of the approach to the residue control of beta-agonists and anticoccidials in the Member States.
1.1 **Objective**

In general, workshops are aimed at enhancing the harmonisation of the analytical performances of the National Reference Laboratories on a European scale as well as at promoting the dissemination of important information.

The importance of a harmonised approach in residue analysis was emphasised again. For this reason the significance of multi-residue methods was pointed out to the participants. Moreover, the suggestions for MRPLs for beta-agonists were presented and put up for discussion.

In addition to methodical aspects the participants heard presentations on findings in the field of residue behaviour. Moreover they were informed on the main fields of application of coccidiostats, which were compiled from literature and assessed according to efficiency and profitability aspects. The CRL also suggested sampling strategies it considers sensible as a consequence of the above information. Furthermore, an overview on the anticoccidial and beta-agonist residues found in the past was given based on the available literature.

1.2 **Course of the Workshops**

The programme of the Workshop, the main topics of the presentations and the list of participants as well as some useful supplementary information were compiled in the Workshop manuals, which were handed out to all participants at the beginning of the Workshops.

The main topics covered were:

1. Overview of the activities of the CRL Berlin in 2003 and envisaged activities for 2004
2. Interpretation of "new" analytical terms
3. Compilation of data on world-wide casualties and new findings concerning beta-agonists
4. Presentation of a multi-residue method for beta-agonists by LC-MSMS as developed at the CRL Berlin
5. Demonstration of the beta-agonist method (only on 06 May 2004 for the new MS)
6. Summary of the results of proficiency test COCC_06/03 on anticoccidials and discussion
7. Presentation of a multi-residue method for anticoccidials by LC-MSMS as developed at the CRL Berlin

8. Overview of different modes of application of anticoccidials and of preferences in different countries regarding substances for the application in animal production

9. Report on known sources of anticoccidial contamination (presentation by UK, only on 04 May 2004 for the old MS)

10. Survey on anticoccidials among the participants regarding the analysis performed in the routine laboratories, the matrices used and the positive results found in the last few years

11. Summary of the results gained in nitroimidazole animal studies and ensuing recommendations on sampling as well as on target matrices and analytes

12. Recent results on nifursol including the results of our animal studies on turkeys

1.3 Evaluation

Most participants found the Workshop informative and interesting. The enormous importance of the exchange of information and the possibility to discuss problems with colleagues was emphasised again. The survey carried out at the end of the Workshop showed that, as last year, the new MS were more interested in practical exercises than the old MS.

The implementation of multi-residue methods was regarded as very difficult by some NRLs, as for this purpose extensive validations would have to be carried out, an effort which these NRLs did not consider feasible. The NRLs therefore suggested to validate only a few individual substances to represent the whole substance group. The CRL Berlin regards this proceeding as unacceptable and cannot support it under any circumstances.

The topic of anticoccidials - and not only of nitroimidazoles - will have to be treated intensively in future, as well. A survey among all NRLs, which is currently being undertaken, is to give an overview about which coccidiostat residues have mainly been found in which matrices, and which concentrations these positive findings have had.

2 Workshop on method validation financed by TAIEX

The CRL Berlin organised a “Training Workshop on an In-house Validation Procedure by means of InterVal” from 01 to 03 November 2004 at the BVL Dahlem. The Workshop was to
contribute to the harmonisation of validation procedures in the New Member States and was attended by 14 representatives of the New Member States and the Candidate Countries (incl. Turkey). TAIEX provided the financing of the Workshop.

Validation is a subject of utmost importance in the frame of the control of pharmacologically active substances in food-producing animals and their products. This does not only concern the field of quality assurance but it is also an important element which allows to assess the proficiency of methods and the comparability of measurement results. Moreover, for all persons involved, thoroughly validated methods mean a greater confidence in the reliability of the measurement results.

2.1 Objective

The harmonisation of the methods’ proficiencies as well as their comparability are important objectives of the CRL. These aims are also expressed in Commission Decision 2002/657/EC by laying down, amongst others, precise requirements for the performance of validation studies. However, the application of different statistical concepts and as little as a difference in the number of samples used for the validation already leads to different validation results which no longer permit the comparability of the proficiencies.

2.2 Course of the Workshop

At first, basic problems were discussed, such as the interpretation of the Decision. Especially parameters which are difficult to understand, e.g. $CC_{\alpha}$, $CC_{\beta}$ and MRPL, were explained. In addition, the latest developments on Commission level were presented (Guidelines for the implementation of Decision 2002/657/EC).

The participants were given the opportunity to ask questions and discuss the topics extensively.

Subsequently, the programme InterVal was introduced by actually using the software. The features and possibilities of the software were presented shortly. Then the participants had the chance to test the software themselves by means of two exercises for which they could use their own data. Staff members of quodata and the Reference Laboratory supported the participants intensively.

At the end, the evaluation and report modules of the software were introduced and discussed.
2.3 Evaluation

The majority of the participants regarded the software as very helpful and as a way of alternative validation worth considering. According to the questionnaire that was handed out in the end, the Workshop was regarded as a success. How far this particular validation approach will be implemented in the individual laboratories is not yet foreseeable.

F Technical and Scientific Support to NRLs and Third Countries

Annex V, chapter 2, section 1 (a,b, d, f, h, l)

1 Analytical Support and Training

Annex V, chapter 2, section 1 (h)

1.1 Training courses

The CRL Berlin continued to provide training courses to scientists from EU Member States and Third Countries:

- 2 colleagues (Poland) 01 – 05 March 04: evaluation, assessment of measurement results, interpretation of CD 2002/657/EC
- 1 colleague (Slovenia) 30 Aug – 03 Sept 04: NSAIDs in liver, muscle, kidney and milk, nitroimidazoles in muscle and plasma, anthelmintics in milk
- 1 colleague (Slovakia) 03 – 05 Nov 04: nitroimidazoles in muscle (fresh and lyophilised), plasma and serum (confirmatory methods); validation concepts
- 1 colleague (Turkey) 15 – 18 Nov 04: beta-agonists in liver and urine, evaluation, confirmation, validation
- 2 colleagues (Germany) 22/23 Nov 04: validation, mycotoxines in milk, tetracyclines in muscle, kidney
- 4 colleagues (Russia) 29 Nov – 03 Dec 12.04: beta-agonists in urine and liver, validation according to 2002/657/EC, proficiency testing, German Residue Control Plan

1.2 Visits of different scientists introducing them to the European reference laboratory system and/or showing them through the laboratory

<table>
<thead>
<tr>
<th>Date</th>
<th>Participants</th>
<th>Institute and Country</th>
<th>Purpose of visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Group Size</td>
<td>Organisation/Delegation</td>
<td>Event Description</td>
</tr>
<tr>
<td>-----------</td>
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<td>----------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>27 Jan</td>
<td>1 person</td>
<td>Bundesamt für Materialprüfung (BAM), Berlin</td>
<td>possible collaboration regarding homogenisation of tissue samples</td>
</tr>
<tr>
<td>19 March</td>
<td>9 persons</td>
<td>government delegation from China</td>
<td>tour of the laboratory</td>
</tr>
<tr>
<td>02 April</td>
<td>8 persons</td>
<td>Delegation of the Dutch consumer protection authorities and delegation of the Federal</td>
<td>tour of the laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ministry of Consumer Protection, Food and Agriculture (BMVEL)</td>
<td></td>
</tr>
<tr>
<td>27 April</td>
<td>1 person</td>
<td>Food Safety Agency (FSA) UK</td>
<td>presentation of the activities of the European and National Laboratory for Residues, Berlin</td>
</tr>
<tr>
<td>08 Sept</td>
<td>1 person</td>
<td>Director of the National Service for Health and Quality of Food (SENASA), Argentina</td>
<td>tour of the laboratory, presentation of the European reference laboratory system</td>
</tr>
<tr>
<td>08 Sept</td>
<td>2 persons</td>
<td>Head of the Spanish Food Safety Agency (AESA)</td>
<td>presentation of the activities of the European and National Laboratory for Residues, Berlin</td>
</tr>
<tr>
<td>09 Sept</td>
<td>4 persons</td>
<td>Delegation from Thailand from the National Food Institute; BAM, Berlin</td>
<td>tour of the laboratory, presentation of the European reference laboratory system, quality assurance management system</td>
</tr>
<tr>
<td>27 Sept</td>
<td>1 person</td>
<td>Physikalisch-Technische Bundesanstalt (PTB), Braunschweig</td>
<td>traceability in chemical analysis</td>
</tr>
<tr>
<td>20 Oct</td>
<td>1 person</td>
<td>CFIA, Canada</td>
<td>tour of the laboratory, presentation of proficiency testing systems</td>
</tr>
</tbody>
</table>
1.3 **One-week training course in Thailand on quality assurance/quality management**

A one-week training course from 13 to 20 November 2004 on accreditation requirements according to ISO 17025 for laboratories was carried out in the Thai laboratory responsible for the control of poultry products (Veterinary Public Health Laboratory, Bureau of Quality Control of Livestock Products, Thailand, Bangkok). The scientists of the laboratory were familiarised with the CRL system in the EC and the basics of the quality management system according to ISO 17025. The current laboratory situation was evaluated.

2 **Reference Material**

2.1 **Standard Substances**

*Annex V, chapter 2, section 1 (a, f)*

In accordance with the responsibilities laid down in Council Directive 96/23/EC of 29 April 1996, the CRL/NRL Berlin provided more than 1114 (820 substances thereof in its responsibility as CRL) units of reference standards to the NRLs in the EU Member States, to Third Countries as well as to official residue control laboratories in Germany during this reference period. Standard substances of a total value of 95,900 € for the CRL/NRL (49,031.05 € thereof for CRL tasks) were procured. They had to be financed completely out of the BVL’s budget.

2.2 **Shipment of in-house reference material**

*Annex V, chapter 2, section 1 (a, b)*

Incurred in-house reference material was provided to:

- RFL, Oldenburg, Germany:
  
  1 x 5 g lyophilised muscle, pig, blank
  1 x 3 g lyophilised muscle, pig, DMZ
  1 x 3 g lyophilised muscle, pig, MNZ
- NRL, The Netherlands:
  4 x 30 ml plasma, pig, blank
  4 x 30 ml plasma, pig, zilpaterol
  2 x 9 ml plasma, cattle, blank
  2 x 10 ml plasma, cattle, zilpaterol

- NRL, Denmark:
  4 x 20 g muscle, turkey, nifursol

- NRL, Slovakia:
  1 x 14 g lyophilised muscle, pig, blank
  1 x 14 g lyophilised muscle, pig, DMZ
  1 x 14 g lyophilised muscle, pig, MNZ
### 1  Breakdown of personnel and financial capacities

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<th>sub-item</th>
<th>share of staff time</th>
<th>share % of total staff time</th>
<th>staff costs</th>
<th>staff costs % of total budget</th>
<th>consumables costs</th>
<th>consumables % of total budget</th>
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<td>3,793 €</td>
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<td>5. Co-operation with internat. organisat.</td>
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<td>7. Tech. and scientific support</td>
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<td>8. Reports, cost estimate</td>
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<td><strong>19</strong></td>
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<td>2. Optimisation of a multi-residue method for avermectines in liver</td>
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<td><strong>33.3</strong></td>
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<td>sub-item</td>
<td>share of staff time [d]</td>
<td>share % of total staff time</td>
<td>staff costs</td>
<td>staff costs % of total budget</td>
<td>consumables costs*</td>
<td>consumables % of total budget</td>
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<tr>
<td>Production of incurred sample material</td>
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<td>4.4</td>
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<tr>
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<td><strong>Σ</strong></td>
<td>60</td>
<td><strong>4.4</strong></td>
<td><strong>15,789 €</strong></td>
<td><strong>3.7</strong></td>
<td>0 €</td>
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</tr>
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<td>1. Maintenance of equipment, documentation, audits, management; re-accreditation in 2004 !</td>
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<td>30,344 €</td>
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<td>Technical and scientific support to NRLs and Third Countries</td>
<td>1. Analytical support and training</td>
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<td>6.7</td>
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<td>2. Provision of standard substances incl. procuring, storage, administration, documentation, shipment etc.</td>
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<td><strong>Total sum</strong></td>
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<td><strong>1359.77</strong></td>
<td><strong>100.0</strong></td>
<td><strong>378,802 €</strong></td>
<td><strong>89.6</strong></td>
<td>0 €</td>
<td>0.0</td>
</tr>
</tbody>
</table>
2 Publications, Reports and Contributions


17. Presentations


25. J. Polzer: “Measurement Services and Requirements of National Food Safety Laboratories - the German System”, Presentation at the CCQM Focus Group Meeting on Reference Measurement


Laborvergleichsstudie zum Nachweis von Organophosphor-Verbindungen in tierischer Matrix”.


Preliminary Results of an Animal Study” Presentation at the CRL-Workshop Berlin, 06-07 May
2004.
3 Staff of the CRL Berlin

Management
P. Gowik Director of the CRL Berlin (not financed by the EC)
C. Stachel Deputy Director,
Head of the division for animal studies, reference material
(not financed by the EC)

Analytical Services
W. Radeck Senior Scientist, Head of HPLC division
J. Polzer Senior Scientist, Head of GC-Division
Quality Assurance Officer
M. Stoyke Scientist, HPLC division

S. Zschieck Technician (88.3%, 4months/100%, 8months)
B. Matthes Technician (62,34%)
S. Maidhof Technician (58,9%)

Administration, Documentation, Translation
M. Jüsgen Translator
Organigramme of the Unit the CRL is connected to

---

Head CRL/NRL
Dr. P. Gowik

Deputy Head CRL/NRL
Dr. C. Stachel

QO CRL
Dr. J. Polzer
Deputy QO CRL Dr. Stoyke

Assistance CRL
Ms. M. Jüs

Assistance NRL
Ms. K. Hahn

---

Residue Analysis, Animal Studies, Pharmacokinetics

---

Animal Studies, Reference Material
Dr. F. Hamann
Dr. C. Stachel

---

CRL/NRL Tasks

<table>
<thead>
<tr>
<th>Dr. W. Radeck</th>
<th>Dr. J. Polzer, QO, Head GC</th>
<th>Dr. M. Stoyke, Deputy QO, Prof. Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms. S. Maidhof, Ms. B. Matthes, Ms. S. Rahn</td>
<td>Ms. A. Hiller, Ms. A. Neumärker</td>
<td>Ms. C. Bieber</td>
</tr>
<tr>
<td>Beta-Agonists, Benzimidazoles, Metamizol, Anticoccidials</td>
<td>CAP, Nitroimidazoles</td>
<td>NSAIDs, Anthelmintics, Rest</td>
</tr>
</tbody>
</table>

---

NRL Tasks

<table>
<thead>
<tr>
<th>Dr. D. Bohm</th>
<th>Dr. K. Schmidt</th>
<th>Dr. R. Hackenberg</th>
<th>Dr. C. Bock</th>
<th>Dr. A. Möller</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms. S. Mönch</td>
<td>Ms. D. Holzkamp</td>
<td>Ms. S. Zschlieck</td>
<td>Mr. A. Petruck</td>
<td>Ms. M. Schramm, Ms. C. Siering</td>
</tr>
</tbody>
</table>

---

Cleaning of Glassware
Ms. Fröse, Ms. Buschenhagen-Vieluf
Annual Report
on the Activities
of the ISS-CRL (Rome)

1 July – 31 December 2004

(point A1. in the 2004 Work Plan)
Preface ............................................................................................................... 3
Organisational chart of the ISS-CRL ................................................................. 4
Activities performed .......................................................................................... 5
Annexes ........................................................................................................... 24
Preface

The ISS-CRL has performed the activities detailed in the relevant Work Programme for the period July 1 - December 31, 2004, as approved by DG SANCO.

In doing so, the ISS-CRL has discharged its duties as prescribed by the mandate assigned by the Council Directive 96/23 of April 29, 1996 (Annex 5).

All the objectives set forth in the above Work Programme have been achieved and results are summarized in Section 3 below.
Organisational chart of the ISS-CRL

ISS-CRL
S. Caroli
Director

Section of Chemical Elements
- S. Caroli, head
- A. Colabucci, scientist
- M. D'Amato, scientist
- C. Frazzoli, scientist
- D. Pino, scientist

Section of Organics (I)
(Carbamates, Organophosphorus Compounds, Organochlorine Compounds, Pyrethroids)
- R. Dommarco, head
- F. Aureli, scientist
- A. Sorbo, scientist

Section of Organics (II)
(Polychlorobiphenyls, Polychlorodibenzo-\textit{p}-dioxins, Polychlorodibenzofurans)
- A. di Domenico, head
- S. De Luca, scientist
- A. M. Ingelido, scientist
Activities performed

A. General Tasks.


Harmonisation of the approaches developed by each of the four CRLs for residues and coordination of their activities toward the NRLs was pursued throughout the duration of the contract in conformity with the exchange of views had during the CRLs meeting of 11 December 2003. This was achieved basically by frequent contacts through e-mail and personal talks on the occasion of meetings and conferences.

Detailed technical and financial reports were prepared as regards the results obtained in the context of the previous Work Plan for the period 1 January - 31 December 2003 and of the Workshop on the Second Proficiency Test on PCBs, PCDDs and PCDFs.

All other administrative duties, including contacts with the European Commission, were discharged as necessary.


The usual systematic semiannual revision and updating of the Handbooks of Analytical Methods in use at NRLs for chemical elements, carbamates, pyrethroids, organophosphorus compounds, organochlorine compounds, polychlorobiphenyls, polychlorodibenzo-p-dioxins and polychlorodibenzofurans has been regularly accomplished and circulated (both in the electronic and hard version) among the NRLs for further comments and remarks. The updated versions are available upon request.
A3. Publication of technical reports and scientific papers.

The Report on the Second Proficiency Test for Organophosphorus Compounds has been revised by taking into account all comments and remarks. The report on the First Proficiency Test on PCBs, PCDDs and PCDFs has also been revised and incorporates now all comments and remarks. Both reports have been sent to DG SANCO and the relevant NRLs. The reports on the Eighth Proficiency Test for Chemical Elements and on the First Proficiency Test on the Determination of Trace Elements in Real Matrices have been already submitted to DG SANCO. Scientific papers on specific activities of the ISS-CRL have been published or are in the peer reviewing process as set forth in Annex 1.

A number of scientific events were also attended and/or organised to further spread the information on the activities of the CRL-NRL network, as detailed in Annex 2.


Contacts have been developed with the Organisation for Economic Cooperation and Development (OECD), the Institute for Reference Materials and Measurements, Joint Research Centre, European Commission (EC-JRC-IRMM) and the European Agency for the Evaluation of Medicinal Products (EMEA) to exchange information on activities of mutual interest, in particular, recent developments in the application of the Good Laboratory Practice Principles and of the ISO/IEC 17025 Standard.

Contacts with the OECD.

i) XVIII OECD Working Group on GLPs, Paris, May 3-4, 2004;

Contacts with the EC-JRC-IRMM.


ii) Meeting of the four CRLs for residues to illustrate the current activities of the four CRLs, promoted by and held at the EC-JRC-IRMM, Geel, March 15, 2004.

Contacts with EMEA. The ISS-CRL regularly receives, archives and makes available to the interested scientific community all the analytical methods in accordance with Article 12 of Council Regulation (EEC) No. 2377/90, as amended by Council Regulation (EC) No. 1308/99. Such methods are supplied by EMEA.
B. Development and validation of analytical methods.

B.1. Development of the analytical method for the determination of organophosphorus pesticides in pork lard.

The already validated method “Analytical method for the determination of organophosphorus pesticides in pork lard” (VM-C-1) has been improved. In particular, the measuring range has been increased including a fifth point at a concentration level lower than the relevant Maximum Residue Limits (MRLs). The linearity has been tested by triplicate analyses of matrix-matched standards. The samples have been injected and the peak area vs. the concentration of active ingredients has been plotted. The internal standard method has been used.

An excellent linear correlation between the injected amount and the detector response has been observed for all active ingredients within the range of 0.03 µg/ml to 1.6 µg/ml for matrix-matched standards.

The data obtained using the linear regression are summarized in Table 1.

Table 1. Linearity data for matrix-matched standards.

<table>
<thead>
<tr>
<th>Active ingredients</th>
<th>Slope</th>
<th>y-axis intercept</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>0.803</td>
<td>0.030</td>
<td>0.998</td>
</tr>
<tr>
<td>Chlorpyrifos methyl</td>
<td>1.022</td>
<td>-0.004</td>
<td>0.999</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>1.020</td>
<td>-0.021</td>
<td>0.998</td>
</tr>
</tbody>
</table>

The recovery rates have been reconsidered using three different spiking levels (about 0.5, 1 and 1.5 MRL) for each active ingredient. As far as each single level is concerned, six different samples have been tested. The preparation of three mixtures containing diazinon, chlorpyrifos methyl and chlorpyrifos at different levels of concentration has been also performed from proper stock solutions. The concentration level in each mixture is reported in Table 2.
Table 2. Concentration levels of the analytes in the analytical solutions.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Concentration level (µg/ml)</th>
<th>Mixture 1</th>
<th>Mixture 2</th>
<th>Mixture 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td></td>
<td>3.25</td>
<td>5.42</td>
<td>7.59</td>
</tr>
<tr>
<td>Chlorpyrifos methyl</td>
<td></td>
<td>3.22</td>
<td>5.37</td>
<td>7.52</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td></td>
<td>3.26</td>
<td>5.43</td>
<td>7.61</td>
</tr>
</tbody>
</table>

A suitable amount of blank pork lard has been fortified in order to ascertain the recovery rates for each active ingredient. Eighteen aliquots of blank material have been selected and divided into three sets of 6 aliquots. Each set has been fortified at about 0.5, 1 and 1.5 times the relevant MRL, respectively.

The average recoveries range from 93.8 % to 96.8 % for diazinon, from 90.2 % to 102.2 % for chlorpyrifos methyl and from 97.5 % to 99.7 % for chlorpyrifos. In particular, the average recoveries are reported in Table 3, where each fortification level is also specified.

Table 3. Fortification levels and recoveries.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Fortification level at about 0.5 times the MRL (µg/ml)</th>
<th>Recovery (%) ± S.D.</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>0.0325</td>
<td>96.8 ± 3.54</td>
<td>3.66</td>
</tr>
<tr>
<td>Chlorpyrifos methyl</td>
<td>0.0322</td>
<td>102.2 ± 8.42</td>
<td>8.24</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.0326</td>
<td>97.5 ± 6.66</td>
<td>6.83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Fortification level at about the MRL (µg/ml)</th>
<th>Recovery (%) ± S.D.</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>0.0542</td>
<td>95.0 ± 6.36</td>
<td>6.69</td>
</tr>
<tr>
<td>Chlorpyrifos methyl</td>
<td>0.0537</td>
<td>98.8 ± 3.97</td>
<td>4.02</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.0543</td>
<td>99.7 ± 5.39</td>
<td>5.41</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Fortification level at about 1.5 times the MRL (µg/ml)</th>
<th>Recovery (%) ± S.D.</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>0.0759</td>
<td>93.8 ± 2.40</td>
<td>2.56</td>
</tr>
<tr>
<td>Chlorpyrifos methyl</td>
<td>0.0752</td>
<td>90.2 ± 3.76</td>
<td>4.17</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.0761</td>
<td>98.5 ± 3.27</td>
<td>3.32</td>
</tr>
</tbody>
</table>
The above mentioned method has been extended to other organophosphorus compounds selected out of those listed in the European Directives (Part A). The relevant clean-up phase has been also tested in order to optimise the recovery rates. The concentration levels have encompassed the relevant MRLs. Further refinement of this extended method is now in progress.

B.2. Development and validation of analytical methods for the determination of PCBs, PCDDs and PCDFs.

Analytical methods have been improved so as to measure in addition to non-dioxin-like PCBs and PCDDs/PCDFs also the twelve dioxin-like PCBs (the four non-ortho and the eight mono-ortho substituted PCB congeners).

As regards the determination of non-“dioxin”-like and mono-ortho “dioxin”-like PCBs in food and feed (NDL method), according to the strategy described in the US EPA Method No. 1613 (1994), fully $^{13}$C-labeled PCB congeners — isotopically-labeled internal standards (ISs) — are added to the homogeneous specimen matrix (oil and fats, milk, eggs, dairy products, meat, fish, fishery products, vegetable material, pellet-like feed), or a portion thereof, prior to extraction or a critical analytical pre-treatment (e.g., freeze-drying).

The lipid component is then quantitatively separated (extracted) from the matrix under assay with an organic solvent (or solvent mixture), an operation that removes the highly lipophilic (generally, log[$K_{OW}$] > 5.5) PCBs as well.

The extract obtained by Soxhlet or “accelerated solvent extraction” (ASE) is subject to a multi-step clean-up comprising a treatment with concentrated sulphuric acid meant to destroy the degradable organic load co-extracted with the analytes. The acid treatment is followed by chromatographic filtration steps to concentrate the analytes in a purified form.

After clean-up, the extract is quantified against as many external standards (ESs) as are the congeners to be determined by High Resolution Gas Chromatography coupled with Low Resolution Mass Spectrometry operated in the single ion monitoring mode [HRGC-LRMS(SIM)]. HRGC coupled with High
Resolution Mass Spectrometry [HRGC-HRMS(SIM)] is generally used as a confirmatory technique.

This method deals with the determination of (in brackets, IUPAC ID numbers): (I) ndl PCB congeners belonging to the homologues T$_3$CB, T$_4$CB, P$_5$CB, H$_6$CB, H$_7$CB and O$_8$CB and, in particular, the six indicator ndl congeners 2,4,4’-T$_3$CB [28], 2,2’,5,5’-T$_4$CB [52], 2,2’,4,5,5’-P$_5$CB [101], 2,2’,3,4,4’,5’-H$_6$CB [138], 2,2’,4,4’,5,5’-H$_6$CB [153] and 2,2’,3,4,4’,5,5’-H$_7$CB [180]; (II) the eight mono-ortho chlorosubstituted dl PCB congeners 2,3,3’,4,4’-P$_5$CB [105], 2,3,4,4’,5-P$_5$CB [114], 2,3’,4,4’,5-P$_5$CB [118], 2’,3,4,4’,5-P$_5$CB [123], 2,3,3’,4,4’,5-H$_6$CB [156], 2,3,3’,4,4’,5-H$_6$CB [157], 2,3’,4,4’,5,5’-H$_6$CB [167] and 2,3,3’,4,4’,5,5’-H$_7$CB [189]. The dl congener 2,3’,4,4’,5-P$_5$CB [118] may alternatively be determined together with Group I analytes. This method also applies to the determination of the perchlorinated D$_{10}$CB [209] with Group I analytes. This congener is eventually used as a homogeneity indicator of matrices that were previously spiked with it. With reference to the existing Italian regulations, the method dealt with has a required application range for cumulative PCBs within approximately 10 and 1000 ng/g (lipid base).

As to the reference PCBs, as many ESs are used as many native congeners are determined. With the possible exception of D$_{10}$CB [209], this also applies to the ISs of the aforesaid 14 congeners and, more in general, of all the congeners that are quantified.

Table 4 reports the performance characteristics of the method.
Table 4. Performance parameters determined for the NDL method.

<table>
<thead>
<tr>
<th>Instrumental characteristic</th>
<th>Reference value/range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variability of relative retention time(s)</td>
<td>&lt; ±0.1 %</td>
</tr>
<tr>
<td>Congener peak resolution (GC valley overlapping)</td>
<td>&lt;10 %</td>
</tr>
<tr>
<td>Variability of relative response factor(s)</td>
<td>&lt; ±15 %</td>
</tr>
<tr>
<td>Recovery rate of internal standards (13C-labeled)</td>
<td>60–120 %</td>
</tr>
<tr>
<td>Variability of critical isotopic mass ratio</td>
<td>&lt; ±20 %</td>
</tr>
<tr>
<td>Deviance of parallel quantitations by two isotopic masses</td>
<td>&lt; ±20 %</td>
</tr>
<tr>
<td>Assessment of background interferences</td>
<td>—</td>
</tr>
<tr>
<td>Deviance of CRM-based intra-laboratory accuracy</td>
<td>&lt; ±20 %</td>
</tr>
<tr>
<td>Variability of single congeners or cumulative values</td>
<td>&lt; ±20 %</td>
</tr>
<tr>
<td>Linearity response</td>
<td>LOQs ≤ 10 pg, over two orders of magnitude</td>
</tr>
<tr>
<td>Variability of over-time laboratory response</td>
<td>&lt; ±20 %</td>
</tr>
</tbody>
</table>

As regards the determination of polychlorodibenzodioxins, polychlorodibenzo furans and coplanar “dioxin”-like polychlorobiphenyls in food and feed (DL method), according to the US EPA Method No. 1613 (1994), fully 13C-labeled PCDD, PCDF and dl PCB congeners — isotopically-labeled internal standards (ISs) — are added to the homogeneous specimen matrix (oil and fats, milk, eggs, dairy products, meat, fish, fishery products, vegetable material, pellet-like feed), or a portion thereof, prior to extraction or a critical analytical pre-treatment (e.g., freeze-drying).

The lipid component is then quantitatively separated (extracted) from the matrix under assay with an organic solvent (or solvent mixture), an operation that removes the highly lipophilic (generally, log[Kow] > 6) PCDDs, PCDFs and dl PCBs as well.

The extract obtained by Soxhlet or “accelerated solvent extraction” (ASE) is subject to a multi-step clean-up comprising a treatment with concentrated
sulphuric acid meant to destroy the degradable organic load co-extracted with the analytes. The acid treatment is followed by chromatographic steps to concentrate the analytes in a purified form. This solution undergoes quantification.

After clean-up, the extract is quantitated against 21 external standards (ESs) (as many as the congeners to be determined) by HRGC-HRMS(SIM).

This method deals with the determination of (in brackets, IUPAC ID numbers): (I) PCDD and PCDF congeners belonging to the homologues T₄CDD, P₅CDD, H₆CDD, H₇CDD, O₈CDD, T₄CDF, P₅CDF, H₆CDF, H₇CDF and O₈CDF and in particular the 17 congeners 2,3,7,8-T₄CDD, 1,2,3,7,8-P₅CDD, 1,2,3,4,7,8,-H₆CDD, 1,2,3,6,7,8-H₆CDD, 1,2,3,7,8,9-H₆CDD, 1,2,3,4,6,7,8-H₇CDD, O₈CDD, 2,3,7,8-T₄CDF, 1,2,3,7,8-P₅CDF, 2,3,4,7,8-P₅CDF, 1,2,3,4,7,8,-H₆CDF, 1,2,3,6,7,8-H₆CDF, 1,2,3,7,8,9-H₆CDF, 2,3,4,6,7,8-H₆CDF, 1,2,3,4,6,7,8-H₇CDF, O₈CDF; (II) the four coplanar dl PCB congeners 3,3′,4,4′-T₄CB [77], 3,4,4′,5-T₄CB [81], 3,3′,4,4′,5-P₅CB [126] and 3,3′,4,4′,5,5′-H₆CB [169]. It technically also applies to the determination of the perchlorinated D₁₀CB [209], eventually used as a homogeneity indicator of matrices that were previously spiked with it. With reference to the existing regulation(s), the method dealt with has a required application range within approximately 0.1 and 10 pgWHO-TE/g, lipid base.

As to the reference PCDDs, PCDFs and dl PCBs as many ESs are used as many native congeners are determined. With the possible exception of D₁₀CB [209], this also applies to the ISs of the aforesaid 21 congeners.

Table 5 reported the performance characteristics of the method.
### Table 5. Performance parameters determined for method DL

<table>
<thead>
<tr>
<th>Instrumental characteristic</th>
<th>Reference value/range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variability of relative retention time(s)</td>
<td>&lt; ±0.1 %</td>
</tr>
<tr>
<td>Congener peak resolution (GC valley overlapping)</td>
<td>&lt; 10 %</td>
</tr>
<tr>
<td>Variability of relative response factor(s)</td>
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<td>60–120 % ((dl) PCBs, 40–120 %)</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>Assessment of background interferences</td>
<td>—</td>
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<tr>
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</tr>
<tr>
<td>Linearity response</td>
<td>LOQs ≤ 0.5 pg, over two orders of magnitude</td>
</tr>
<tr>
<td>Variability of over-time laboratory response</td>
<td>&lt; ±20 %</td>
</tr>
</tbody>
</table>

**B.3. Development and validation of a new analytical method for the determination of Pd, Pt and Rh in milk.**

Twenty different samples of milk were collected in different areas in Italy, packed and frozen at – 20 °C and stored at this temperature until pretreatment.

After freeze-drying samples were chemically digested by microwave (MW) assisted acid treatment in a high performance MW oven.

After cooling at room temperature, the content of each vessel was quantitatively transferred into polyethylene tubes and gravimetrically diluted with deionised water. Sample solutions were then stored at 4 °C in the dark until
analysis. Blank samples were prepared along with the samples, following exactly the same procedure, but omitting the test material.

The matrix effect (non-spectral interferences), was minimised by optimising the digestion efficiency and the dilution factor.

All sample manipulations took place in a Class 100- clean room to minimise the risk of analyte loss or contamination throughout the whole analytical process. The determination of traces of PGEs in milk was performed resorting to Sector Field Inductively Coupled Plasma-Mass Spectrometry (SF-ICP-MS). The relative concentration of analyte and mass interferences, as from the preliminary study on a matrix-matched sample, requires the reduction of mass interferences by optimising the instrumental settings and the physical resolution of the mass overlaps, when the required resolution is compatible with the isotope counts.

For the quantification the analytical isotopes $^{105}$Pd, $^{195}$Pt and $^{103}$Rh were selected. This choice was based on the natural abundances of the analytical isotopes and the ascertainment of the relative abundances of the potential atomic and molecular interfering ions along with the physical resolution required to separate analyte masses from interfering masses.

Tables 6 report the figures of merit for PGEs determination by Guard Electrode (GE) Pneumatic Nebulization (PN) SF-ICP-MS in a milk certified reference material (BCR 151). The detection power offered by the technique in the investigated matrices was checked by analysing 10 independent procedural blanks and calculating the net signal after spiking the matrix. LoDs based on the
3σ criterion applied to the matrix solution turned out to be adequate for the quantification of PGEs in milk samples.

The same procedure was applied to calculate the LoQs and, therefore, the reporting limits (equal to or higher than the LoQs). The whole process reproducibility was assessed by calculating the RSD (%) of measurements performed on 5 independent aliquots in the same analytical run. The instrumental imprecision was evaluated and monitored over the entire working range by performing replicate analyses of standards. As regards the test for accuracy, unfortunately no reference materials certified for PGEs in the said matrices are commercially available. Consequently, reliability of measurements was checked through recovery tests by including in the analytical runs a set of 5 independent samples which were fortified with the expected mass of the analytes prior to the digestion process.

To establish the comparability of results, the uncertainty associated to the analytical methods developed was estimated according to the EURACHEM/CITAC Guide.

Control charts were set up to check the performance of the method by daily plotting results from the measurement of the pooled sample.
Table 6. Method’s parameters for PGEs determination by GE-PN SF-ICP-MS in milk samples.

<table>
<thead>
<tr>
<th></th>
<th>Pd (HR)</th>
<th>Pt (LR)</th>
<th>Rh (MR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoDs (ng kg(^{-1}))(^a)</td>
<td>59.5</td>
<td>0.8</td>
<td>7.4</td>
</tr>
<tr>
<td>Reporting limit (ng kg(^{-1}))(^b)</td>
<td>198.2</td>
<td>2.8</td>
<td>24.7</td>
</tr>
<tr>
<td>Whole process reproducibility (%)(^c)</td>
<td>6.9</td>
<td>4.1</td>
<td>4.6</td>
</tr>
<tr>
<td>Recovery (%)(^d)</td>
<td>125</td>
<td>97</td>
<td>154</td>
</tr>
<tr>
<td>Uncertainty (%)(^e)</td>
<td>14</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^a\)Calculated on the basis of the 3\(\sigma\) criterion in the matrix.
\(^b\)Calculated on the basis of the 10\(\sigma\) criterion in the matrix.
\(^c\)Five independent aliquots were analyzed.
\(^d\)Calculated on five independent fortified samples.
\(^e\)Calculated according to the EURACHEM/CITAC Guide.
C. Quality assurance and quality control programmes, including the organization of proficiency tests.

C.1. Support to the NRLs.

As for the implementation of quality assurance schemes by the NRLs, the most recent developments in the application of quality systems based on accreditation (ISO/IEC 17025 criteria) and conformity compliance (OECD Principles of Good Laboratory Practice) were put at the disposal of the NRLs, thus further promoting the adoption of quality criteria, as applicable. In particular, information on current possibilities of better harmonisation between the two quality systems (as discussed in the meeting of the OECD Group on Good Laboratory Practice in Paris, May 3-4, 2004) was made available to the NRLs.

C.2. Organization and conduct of proficiency tests.

C.2.1.

The ISS-CRL organized and carried out the second Proficiency Test (PT-B2) on the determination of the six WHO indicator non-dioxin-like polychlorobiphenyls (ndl-PCBs) (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180), 12 WHO dioxin-like polychlorobiphenyls (dl-PCBs) and 17 2,3,7,8-chlorosubstituted polychlorodibenzo-p-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs), all of them naturally present in crude fish oil at trace level.

In order to assess the performance of each National Reference Laboratory (NRL) for residues as regards the determination of the aforesaid analytes, flasks and bottles containing fish oil for the determination of ndl-PCBs (flasks) and dl-PCBs and PCDDs+PCDFs (bottles) were prepared and shipped
to each NRL. Together with fish oil samples, ndl-PCB, dl-PCB and PCDD+PCDF standards were also delivered as common analytical checks.

The NRLs were requested to analyze the samples using their own methods. Their results were evaluated by the ISS-CRL using the z-score approach and were discussed during the workshop held in Rome on 12 November, 2004.

All NRLs (with the exception of Portugal L-06 for the analysis of ndl-PCBs and Belgium L-17 for the analysis of PCDDs+PCDFs) participated in the exercise. The majority of them performed satisfactorily for all analytes.

A summary of Z-scores are reported in Tables 7, 8 and 9.

Table 7. Z-Score outcomes for ndl-PCBs using the overall mean estimate of each congener as the target value and RSDs as estimate of results variability (except where noticed).

| x-Scores of ndl-PCBs | \( z \) | ≤ 2 | \( 2 < |z| < 3 \) | \( z \) | ≥ 3 |
|---------------------|---------|--------|-----------------|---------|
| \( T_3CB-28 \) | All NRLs except DEU L-11b, DNK L-15 and FRA L-12 | DEU L-11b, DNK L-15 and FRA L-12 | All NRLs except DEU L-11b, DNK L-15 and FRA L-12 |
| \( T_4CB-52 \) | All NRLs except FRA L-12 | FRA L-12 |
| \( P_5CB-101 \) | All NRLs except FRA L-12 | FRA L-12 |
| \( H_6CB-138 \) | All NRLs except FRA L-12, GBR L-01 and NIR L-08 | FRA L-12, GBR L-01 and NIR L-08 |
| \( H_6CB-153 \) | All NRLs except FRA L-12 | FRA L-12 |
| \( H_7CB-180 \) | All NRLs except FRA L-12 | FRA L-12 |

(a) 0.2 used as estimate of results variability.
(b) FRA L-12 result qualified as an outlier.
Table 8. z-Score outcomes for dl-PCBs using the overall mean estimate of each congener as the target value and RSDs as estimate of results variability (except where noticed).

### z-Scores of non-ortho-PCBs

| Congener | \(|z| \leq 2\) | \(2 < |z| < 3\) | \(|z| \geq 3\) |
|----------|----------------|-----------------|----------------|
| T4CB-77  | All NRLs except ESP L-05 and PRT L-07 | ESP L-05 | PRT L-07<sup>a</sup> |
| T4CB-81  | All NRLs except PRT L-07 and SWE L-03 | PRT L-07 and SWE L-03<sup>b</sup> |
| P5CB-126 | All NRLs | |
| H6CB-169 | All NRLs | |

### z-Scores of mono-ortho-PCBs

| Congener | \(|z| \leq 2\) | \(2 < |z| < 3\) | \(|z| \geq 3\) |
|----------|----------------|-----------------|----------------|
| P5CB-105 | All NRLs | | |
| P5CB-114 | All NRLs except DEU L-11b and ITA L-09 | DEU L-11b and ITA L-09<sup>d</sup> | |
| P5CB-118 | All NRLs | | |
| P5CB-123 | | | |
| H6CB-156 | All NRLs except DNK L-15 | DNK L-15<sup>f</sup> | |
| H6CB-157 | All NRLs | | |
| H6CB-167 | All NRLs | | |
| H7CB-189 | All NRLs except GBR L-01 | GBR L-01 | |

<sup>a</sup> PRT L-07 result qualified as an outlier.

<sup>b</sup> PRT L-07 and SWE L-03 results qualified as outliers.

<sup>c</sup> 0.2 used as estimate of results variability

<sup>d</sup> ITA L-09 result qualified as an outlier.

<sup>e</sup> Statistics not performed

<sup>f</sup> DNK L-15 result qualified as an outlier.
Table 9. z-Score outcomes for PCDDs+PCDFs using the overall mean estimate of each congener as the target value and RSDs as estimate of results variability.

<table>
<thead>
<tr>
<th>z-Scores of PCDDs</th>
<th>2,3,7,8-T4CDD</th>
<th>2,3,7,8-P3CDD</th>
<th>1,2,3,4,7,8-H6CDD</th>
<th>1,2,3,6,7,8-H6CDD</th>
<th>1,2,3,7,8,9-H6CDD</th>
<th>O6CDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>z ≤ 2</td>
<td>All NRLs except AUT L-19</td>
<td>All NRLs</td>
<td>All NRLs except ITA L-09</td>
<td>All NRLs except GBR L-01</td>
<td>All NRLs except PRT L-07</td>
<td>All NRLs</td>
</tr>
<tr>
<td>2 &lt; z &lt; 3</td>
<td>AUT L-19</td>
<td>All NRLs</td>
<td>ITA L-09</td>
<td>GBR L-01 (^a)</td>
<td>PRT L-07</td>
<td>All NRLs</td>
</tr>
<tr>
<td>z ≥ 3</td>
<td>All NRLs except AUT L-19</td>
<td>All NRLs</td>
<td>All NRLs except ITA L-09</td>
<td>All NRLs except GBR L-01</td>
<td>All NRLs except PRT L-07</td>
<td>All NRLs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>z-Scores of PCDFs</th>
<th>2,3,7,8-T4CDF</th>
<th>1,2,3,7,8-P5CDF</th>
<th>2,3,4,7,8-P5CDF</th>
<th>1,2,3,6,7,8-H6CDF</th>
<th>1,2,3,7,8,9-H6CDF</th>
<th>2,3,4,6,7,8-H6CDF</th>
<th>1,2,3,4,6,7,8-H7CDF</th>
<th>1,2,3,4,6,7,8-H7CDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>z ≤ 2</td>
<td>All NRLs</td>
<td>All NRLs</td>
<td>All NRLs except DEU L-11b</td>
<td>All NRLs except GBR L-01</td>
<td>All NRLs except PRT L-07 and GRC L-10</td>
<td>All NRLs except GRC L-10</td>
<td>All NRLs except GCR L-10</td>
<td>All NRLs except GRC L-10</td>
</tr>
<tr>
<td>2 &lt; z &lt; 3</td>
<td>DEU L-11b</td>
<td>All NRLs</td>
<td>All NRLs except DEU L-11b</td>
<td>GBR L-01</td>
<td>PRT L-07</td>
<td>GRC L-10</td>
<td>GRC L-10</td>
<td>GRC L-10</td>
</tr>
<tr>
<td>z ≥ 3</td>
<td>All NRLs</td>
<td>All NRLs</td>
<td>All NRLs except DEU L-11b</td>
<td>All NRLs</td>
<td>All NRLs except PRT L-07 and GRC L-10</td>
<td>All NRLs except GRC L-10</td>
<td>All NRLs except GCR L-10</td>
<td>All NRLs except GRC L-10</td>
</tr>
</tbody>
</table>

\(^a\) GBR L-01 result qualified as an outlier.
D. Assistance to the Commission, EU Member States, Accession Countries and Third Countries through technical and scientific support.

**D1. Training of technical personnel.**

This task was carried out as necessary. In particular, the ISS-CRL has participated in the planning, organisation and conduct of the *Balkan Conference on: National/Reference Measurement Infrastructure for Environmental and Food Chemical Measurements* (Plovdiv, Bulgaria, 2 – 4 September 2004), where information on the CRLs – NRLs network was conveyed to some new EU Member States as well as to other countries of future accession. Training stages are being agreed upon in collaboration with EC-JRC-IRMM. Furthermore, in the frame of the bilateral governmental cooperation programme between Italy and Hungary, Dr. V. Mihucz from the University of Budapest spent one month (August 2004) at the ISS-CRL to be trained in the use of SF-ICP-MS for the analysis of Pt in food matrices.

**D2. Organisation of Workshops.**

A Workshop on the Second Proficiency Test on PCBs, PCDDs and PCDFs was held in Rome on November 12, 2004, to debate the performance of the NRLs (see point C2.1 above). The Workshop allowed the discussion on the results obtained by the NRLs for PCBs, PCDDs and PCDFs in the frame the relevant PT and the mutual understanding of the NRLs as regards the analytical approaches used, the identification of procedural errors and pitfalls and the identification of corrective actions as necessary.
D3. Minimum Required Performance Levels.

The task was carried out in several occasions as regards specific aspects of the Decision 2002/657/EC.


During the period of validity of this contract no controversies arose among Member States or among Member States and non-EU countries that required the formal intervention of the ISS-CRL.

D5. Technical assistance.

The ISS-CRL responded to all technical requests posed by the NRLs as regards applicability of analytical methods and their further development.

D6. Assistance to Third Countries.

The Serbian Centre KIBID requested the analysis of a sample of milk to quantify the content of Zn. The sample was received on 5 November 2004 and the results of the analysis were submitted to KIBID on 23 November 2004. The analysis was performed by SF-ICP-MS and confirmed by Dynamic Reaction Cell Inductively Coupled Plasma Mass Spectrometry (DRC-ICP-MS).
Annexes
Scientific reports and publications


2. A. Sorbo, R. Dommarco, S. Caroli. Handbook of Analytical Methods for Carbamates as Adopted by National Reference Laboratories


8. S. De Luca, A. M. Ingelido, A. di Domenico, S. Caroli, Report on the results of the Second Proficiency Test on the determination of Polychlorobiphenyls (PCBs), Polychlorodibenzo-p-dioxins (PCDDs) and Polychlorodibenzofurans (PCDFs).
Annex 2

Participation in conferences, workshops and courses.

1. 2004 Winter Conference on Plasma Spectrochemistry, Fort Lauderdale, Florida (USA), January 2-21, 2004 (Session chair; one presentation and two posters).


4. XIII Convegno Nazionale Gruppo Italiano per la Quality Assurance nella Ricerca, Pomezia (Italy), March 1-2, 2004 (one presentation).


6. Training Course on Good Laboratories Practice, Pomezia (Italy), April 1-2, 2004 (one presentation).


9. International Symposium on Metal Ions in Biology, Budapest (Ungheria), May 18-24, 2004 (membro del Comitato Scientifico; due relazioni ad invito).

10. XIII National Conference GIQAR, Sorrento (Napoli), May 27-28, 2004 (Two presentations).


13. Training Course “Suole e acque interne”, Viterbo, September 13-17, 2004 (one presentation).

14. 2nd International IUPAC Symposium on “Trace Elements in Food”, Brussels (Belgium), October 7-8, 2004 (Member of the Scientific Committee, one presentation).


17. Workshop on the Second Proficiency Test on the Determination of PCBs, PCDDs and PCDFs in Matrices of Animal Origin, Rome (Italy), November 12, 2004 (organisation and conduct of the workshop).
