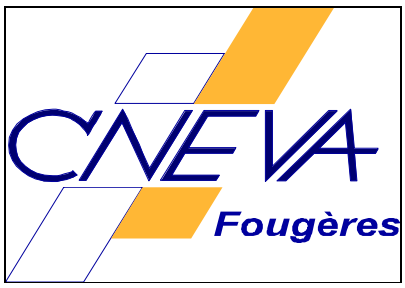
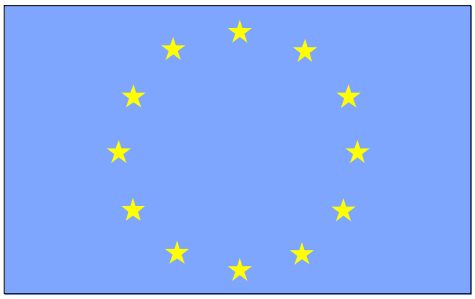
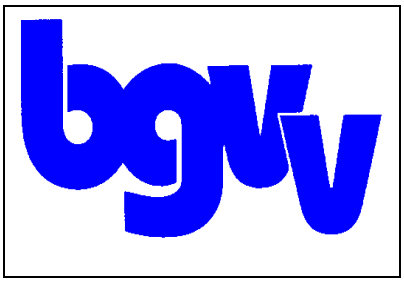
JOINT ANNUAL REPORT OF THE EUROPEAN UNION COMMUNITY REFERENCE LABORATORIES FOR RESIDUES

EUROPEAN COMMISSION CONTRACT PERIOD: AUGUST 1996 - JULY 1998







I. Contents

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I. Introduction

The mandate and conditions of operation of the four European Union Community Reference Laboratories (CRLs) for residues were established through the Council Directive 86/469/EC of 16 September, 1986, later replaced by Directive 96/23/EC of 29 April, 1996. In particular, the latter legal provision prescribes the performance of a number of tasks to the benefit of the European residue control system as well as of the National Reference Laboratories (NRLs) for residues in the Member States, Third Countries and the European Commission (EC). In this context emphasis is laid upon the fact that the activities carried out by the four CRLs over the period 1 August, 1996 – 31 July, 1998, witnessed further progress in the integrated approach adopted to implement their duties. This fact positively impacted on the NRLs perception of the role played by the CRLs as an interactive system. The sections which follow give a detailed account of the results achieved in the period under consideration for each of the four CRLs for residues.

This second Joint Annual Report of the four CRLs for residues has been prepared following the guidelines agreed upon during a meeting of the four Directors of the CRLs held in Brussels, 29 May, 1998. It is a further attempt to improve not only the quality of the scientific activities of the CRLs in conjunction with their respective NRLs, but also to improve the quality of communication and understanding within the various EC services involved, Member States, Third Country Administrators and, last but not least, among the four CRLs for residues themselves.

This Report has been edited by the ISS-CRL in consultation with the other three CRLs and the EC. It is available upon request either directly from the EC in Brussels, Belgium, or from any of the four CRLs for residues.

Residues Within the Mandate of the CRLs as Prescribed by the Council Directive 96/23/EC of 29 April, 1996

RIVM-CRL

- Stilbenes, stilbene derivatives and their salts and esters
- Antithyroid agents
- Steroids
- Resorcylic acid Lactones, including Zeranol
- Sedatives
- Mycotoxins

CNEVA-LMV-CRL

- Antibacterial substances, including sulphonamides and quinolones
- Dyes
- Carbadox residues and Olaquindox residues
- Chloramphenicol

BgVV-CRL

- Beta-agonists
- Anthelmintics
- Anticoccidials, including nitroimidazoles
- Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

ISS-CRL

- Carbamates and pyrethroids
- Organochlorine compounds, including PCBs
- Organophosphorus compounds
- Chemical elements

II. Activities of the Community Reference Laboratory at the Rijkinstituut voor Volksgezondheid en Milieu (RIVM-CRL)

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1. General

Within the RIVM the CRL is part of the Laboratory for Residue Analysis (Laboratorium voor Analytisch Residu Onderzoek, ARO). The infrastructure of ARO and the CRL are such that there are two designated areas for the CRL, *i.e.*, the laboratory and the documentation centre, respectively. This infrastructure reflects two of the main CRL activities, namely, analytical services and documentation services. The third activity, Quality Assurance (QA) and Quality Control (QC) assistance is integrated with the QA/QC facility of ARO. However, special facilities are available to the CRL with respect to studies to be performed in compliance with the Good Laboratory Practice (GLP) principles.

As a consequence of Council Directive 96/23/EC of 29 April, 1996, RIVM now also has extended responsibilities with respect to residues of sedatives and mycotoxins. Further, the area of hormonal growth promoters was extended to all steroids, now also including corticosteroids.

The Bilthoven CRL for residues has a staff of 10 persons *i.e.,* 8.4 Full - Time Equivalent (FTE), namely 4.8 for analytical tasks and 3.6 for documentation. For management, QA/QC, scientific assistance and infrastructural support an additional 2 FTE ARO staff are available to the CRL.

2. Method Development

In a number of areas significant improvements have been made in the development and validation of analytical procedures. A method for the determination of the group of Resorcylic Acid Lactones (RALs) based on Immuno - Affinity Chromatography (IAC) was developed and is currently under validation. A detailed report and Standard Operating Procedures (SOPs) are under preparation. The detection and identification of RALs, among which group of compounds are the estrogenic anabolic compound Zeranol and the f2-toxine Zearalenone, are of importance since recent studies have demonstrated that metabolites of Zearalenone, possibly including Zeranol and Taleranol (a Zeranol metabolite), can be present in biological samples taken for regulatory inspections. In such cases the use of Zeranol as growth promoter is difficult to prove. The method already was used on request of several Member States. Together with one of the NRLs a proposal for additional EU funding was submitted to EC FAIR Programme (DG VI) and already granted, to further investigate this issue.

Preliminary studies on the detection and identification of the anabolic steroid Stanozolol were undertaken for which several approaches based on Gas Chromatography - Mass Spectrometry (GC-MS) were tested.

Based on the results of these studies, a method for the detection and identification of Stanozolol in biological matrices was implemented. These studies were undertaken in close collaboration with the second Dutch NRL (RIKILT-DLO, Wageningen, NL) and several other laboratories in The Netherlands and Belgium. Further, improved methods for the detection and identification of residues of Trenbolone in samples of urine, liver and muscle were developed and validated using IAC and Liquid Chromatography - Mass Spectrometry (LC-MS). LC-MS equipment was implemented in December 1997 at the Bilthoven CRL. With this technique a method for the quantification of Stanozolol and metabolites in urine was developed and validated. This procedure is planned to be demonstrated to, and discussed with, representatives of the NRLs during the annual workshop (September 1998).

A method for the determination of gestagens with Supercritical Fluid Extraction (SFE) is currently being developed and validated. The preliminary results were presented at Wintergreen, Virginia (USA), 18-22 May, 1997, at the 19th International Symposium on Capillary Chromatography and Electrophoresis in a poster titled Comparison of a Liquid Extraction and a Supercritical Fluid Extraction for the Analysis of Gestagens in Samples of Bovine Fat.

A multi - residue method for the screening of samples of urine for anabolic compounds was validated under conditions of method development, establishing a full set of performance characteristics and under practical conditions for which purpose the method was implemented

in one NRL. A detailed report on the validation of the method was prepared and is now available, together with the relevant SOP. Further work was done to modify the method for the detection of anabolic compounds in samples of faeces. The method is being tested with routine samples from the Dutch Control Programme.

A new procedure developed by De Brabander (RUG, Ghent, Belgium) for the detection and identification of thyrostatic compounds was implemented in the Bilthoven CRL. This procedure is based on the new technology of multiple MS (MS^n). A validation study was performed and a contribution to a paper by De Brabander was made. The method is used on a regular basis to assist NRLs. Methods of analysis for natural hormones were extended and validated. A deuterated analogue to be used as the internal standard for the analysis of Progesterone is being synthesized.

The extraction method for steroids from (fatty) tissue samples by using SFE was validated and applied to a range of different samples. SFE was applied to matrices such as fat, meat and skin. Different steroids could be extracted and analyzed using one and the same method. The method was presented during the Bruges Conference in June 1998.

Different Liquid Chromatogaphy (LC) methods were developed for the analysis of corticosteroids from biological matrices. For the analysis of samples of animal feedstuffs a High Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD) and a High Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS) method are available.

For the analysis of corticosteroids in hydrolyzed urine samples, a SFE LC-MS method was developed and validated. For samples of liver a method of analysis based on IAC-LC-MS was devised. These methods were also presented during the Bruges Conference in June 1998. The methods, along together with those for Stanozolol, will be demonstrated during the annual Bilthoven CRL Workshop.

A number of methods were developed and validated which are to be applied in various EC-sponsored WTO studies for natural hormones, Trenbolone, Zeranol and metabolites, Melengestrol as well as a number of hormones not registered in the USA in samples of meat and liver. All methods are based on either GC-MS or LC-MS and were optimized for low limits of detection in combination with reliable identification criteria.

3. Other Research

Research

Research into the natural occurrence of α-Nortestosterone in different species of pregnant animals, such as sheep, goats and mares, was continued. Samples of urine from such animals were analyzed for this analyte. Results of this study were presented in a meeting in Bruges, June 1998. Research on the occurrence of “new” and/or “unknown” compounds is ongoing. In materials of different sources a number of anabolic compounds, not yet included in residue control programmes, was identified, including Stanozolol, Norgestrel, Ethylnortestosterone and the corticosteroid Clobetasol. The use of the $^{13}C/^{12}C$ isotope ratio analysis was

explored for the detection of natural hormones. Contacts were established for cooperation with sport - doping laboratories already using this novel technique.

Within the working party Uncertainty of Chemical Measurements of Eurachem the subgroup Uncertainty of Qualitative Measurements, *casu quo* Reliability of Identification, has begun its activities. The Bilthoven CRL bears the responsability of convening this subgroup. A representative of the Bilthoven CRL is a member of a working group revising the Commission Decision 93/256/EEC on criteria for analytical methods to be used for residue control.

Ring Tests

A ring test on the determination of thyreostatic compounds was organized in preparation of a workshop. All participants received a set of four samples. Results were discussed during the workshop and are published in detail in the proceedings of this event. The workload for laboratories, both NRLs and Routine Field Laboratories (RFLs), in relation to ring tests is high. A declining willingness and/or ability is apparent for an increasing number of laboratories to participate in ring tests that do not precisely fit into their actual work programme. This is a matter of increasing concern as most laboratories do not structurally plan additional capacity and time for mandatory ring tests, even not so after accreditation.

The Bilthoven CRL organized certification studies within the EC DG XII Standards, Measurements and Testing (SMT) programme on the development of Certified Reference Materials (CRMs). During this period studies on Zeranol and Trenbolone in samples of liver were organized. The Bilthoven CRL document no. 389002 064 on the ring test for Estradiol in serum was finalized. This report describes the results of a cooperative study organized in 1995. The objective of this study was to evaluate the analytical possibilities within the responsible control laboratories, *i.e.*, the RFLs and NRLs with respect to the control of residues of 17 β-Estradiol. Two types of methods were used, *i.e.*, (Radio) Immunochemical Analysis [(R)IA] methods and methods based on GC-MS. The evaluation was based on z-scores, a quantitative value describing the difference between an individual mean from the overall average value as a function of the standard deviation of the population of results.

For GC-MS all laboratories found mass concentrations with a z-score between -2 and +2 ($|z| \leq 2$), this indicating a good agreement between laboratories. For (R)IA most laboratories found mass concentrations with a z-score between -2 and +2, also indicating good agreement. A limited number of laboratories reported doubtful results ($2 < |z| < 3$). In general, it was concluded that immunochemical and GC-MS procedures give on average similar quantitative results for the different samples. The variability between laboratories using GC-MS, expressed as Relative Standard Deviation (RSD), ranged from 37 % (40 ng l^{-1}) to 27 % (400 ng l^{-1}). For immunochemical procedures the corresponding values were slightly higher (60 % and 28 %, respectively).

However, a significant difference was observed between immunochemical methods with a preliminary extraction procedure and those without. Inclusion of an extraction procedure resulted in lower values for the content of Estradiol.

In preparation of the annual workshop of September 1998, a small ring test on Stanozolol and corticosteroids was organized. Since most Member States have not get incorporated these compounds within their residue control programme, only a limited number of laboratories plan to participate.

4. Quality Assurance

The quality system within the Bilthoven CRL [incorporating the EN 45001 criteria plus International Standardisation Organization (ISO)/IEC Guide 25 accreditation and compliance with GLP principles] was maintained during the contract period. The further introduction and maintenance of QA and QC in NRLs was actively supported and the RIVM-CRL served as a help desk for NRLs in this matter.

The results of the second QA Inventory were first presented at the workshop held in Bilthoven, 7-9 April, 1997. A response of 100 % was eventually achieved. The main conclusion of the Inventory was that almost all EU NRLs at present have a described quality programme based on an international standard. However, only 33 % of the EU NRLs were officially accredited, certified or acknowledged to be in compliance with GLP principles. In comparison with the results of the first Inventory (1994) it is

evident that the EU NRLs have made significant progress in the implementation of quality systems. It is, however, also apparent that still a substantial number of EU NRLs is lacking essential QA facilities. These results have been reported in the CRL document no. 389002 062 and were among others also presented at the Bruges Conference in June 1998.

After completion of its first period of four years, ARO (and the Bilthoven CRL which is a part of it) was fully reaudited by a team of the Dutch Council for Accreditation (formerly STERLAB) in September 1996. This resulted in a full reaccreditation covering research and development. The full accreditation for research and development as such is particularly important because it is for the first time within the EU that such an accreditation has been achieved. So far, in fact, only routine work had been accredited. In 1997 the accreditation has been endorsed. In January 1998 the Veterinary Public Health Inspectorate (VPHI), section GLP, has also inspected the RIVM-CRL. The GLP compliance was endorsed and the Bilthoven CRL operates now in compliance with the Organization for Economic Cooperation and Development (OECD) GLP Principles which are also recognized by the USA Food and Drug Administration (FDA).

As a consequence of the inclusion of mycotoxins in the mandate of the Bilthoven CRL, preliminary steps were taken to prepare an inquiry about QA related to mycotoxin activity in the NRLs.

On 13 February, 1997, one of the Spanish NRLs (Instituto de Salud Carlos III) was audited. A visit report was prepared (RIVM-CRL document no. 389002 056). In June 1997 a laboratory under contract of the Argentinean government was audited at Buenos Aires in cooperation with auditors from Canada and USA. For QA-involving audit missions to USA and Canada headed by the EC reference is made to Section 7.

5. Technical and Scientific Support to NRLs and Third Countries

Reference Materials

The work done in the preparation of the Bank of Reference Samples of Blank Urine from Livestock was presented at the Biological and Environmental Reference Materials Symposium (BERM-7) in Antwerp (B), 21-25 April, 1997. A detailed report on this collection of samples was prepared and published in the Proceedings of BERM-7 (Special Issue of the *Fresenius' Journal of Analytical Chemistry*).

The additional preparation of nine batches of ampouled deuterated and non - deuterated anabolic compounds for the Bank of Reference Standards started in May 1997 and has now been completed. The CRL is coordinating the production, QC and distribution of the materials and is extending the number of compounds on a regular basis. During this contract period a total of 765 ampoules was supplied to NRLs and RFLs in the EU and Third Countries. Of these, 154 ampoules were distributed to non-EU countries. A total of 145, non - ampouled standards and 9 sets of

Blank Bovine Urine, 3 sets of Blank Ovine Urine and 6 sets of Blank Porcine Urine were distributed.

Advisory and Documentation Services

In the period of interest the advisory service dealt with 123 requests for advice, 79 of them from laboratories from EU Member States. In total 178 documents were distributed on request including SOPs of analytical methods. The catalogue of analytical methods CB/METHODS was further improved and extended. The Database was completed with records for the standard and reference substances. On numerous occasions scientists were made aware of the existence and possibilities for use of the catalogue of methods. Results of the CB/METHODS were made available on request. An overview of papers, report abstracts, posters and documents produced by the RIVM-CRL (partially in cooperation with other institutes) is given in Annex II.

A document is in preparation, in which the problems related to mycotoxins in animal products are mapped. This document is aimed at providing information about which groups of toxins can be found in animal products, providing an overview of available and suitable methods of analysis and providing recommendations to NRLs about investigations to be incorporated in National Plans. The document is planned to be ready by early 1999.

The electronic Database with addresses of the staff of NRLs, RFLs and other relevant laboratories is maintained on a regular basis, partly in relation with the method catalogue CB/METHODS. Pilot batches of a first CD ROM edition of CRL-INFO have been prepared containing among others a read - only version of the Database CB/METHODS. A cooperation has been started with the University of Ghent (B) and the University of Liège (B) to combine CB/METHODS with a Database developed for a similar purpose and which is accessible *via* Internet. Copyright problems still have to be solved.

In the framework of the support to Third Countries an overview of the EU regulatory CRL-NRL residue control system was presented on invitation of the organizers at the 8th International Technical Consultation on Veterinary Drug Registration (ITCVDR) at Prague (CZ) in September 1996, on invitation of the Association of Official Analytical Chemists (AOAC) International Section Latin America during the Second AOAC Latin America Meeting at Buenos Aires (Argentina) in June 1997, on invitation of the 12th World Association of Veterinary and Food Hygienists at The Hague (NL) in August 1997 and on invitation of the AOAC International during the 110th Annual Meeting at San Diego, CA (USA), in September 1997.

Arbitration at the Member State Level

Analytical assistance was given to France (RALs in urine), Sweden (natural hormones in blood serum), Germany [for Nortestosterone (4

cases), Methyltestosterone (1 case) and natural hormones (1 case) in blood serum] and Portugal (confirmation of thyreostats in thyroid gland samples). Analytical support for quantification and confirmation of compounds was given to a variety of third countries including Cyprus, Argentina, Brasil and the USA.

6. Technical and Scientific Assistance to the European Commission

Support has been given to the EC Legal Service at Geneva (CH) in February 1997 during the WTO hearings for Arbitration between the EU and USA/Canada as regards hormones in meat. In this WTO case in appeal in 1998 again support was given by the CRL to DG XXIV to develop study plans for residue analyses and to consult in other case - related studies. Under guidance of EC DG XXIV the Bilthoven CRL and the Berlin CRL participated in two exploratory missions to USA (November 1997 and July 1998) and to one such mission to Canada (May 1998). The report of the second USA mission has been published on the Internet (EC DG XXIV Homepage). All the *ad hoc* DG XXIV activities called for a substantial additional effort especially by the senior staff of the RIVM-CRL. Specific documentary support was given to the DG VI with regard to the veterinary hormonal active drugs Busereline and Fertiriline.

As regards reference methods and method validation, support and advice were given to the EC DG VI in November 1996 and January, March and May 1997, respectively, as prescribed by the EU Directive 96/23/EC. A draft guideline for the establishment of reference methods was developed as a joint task of the four CRLs. The draft was finalized at the workshop

organized by the Berlin CRL in December 1997. At that workshop also the formal assignment of coordination for the revision of the method qualifying criteria was returned to the EC because of lack of support by the 30 responsible participating EC delegate. Management and financial items have also been discussed with EC DG VI in Brussels (B) in coordination meetings held on 24 October, 1996, 27 November, 1996, 14 January, 1997, 12 March, 1997, 27 May, 1997, 4 September, 1997, and 29 May, 1998.

Arbitration at the European Union Level

An arbitration case between two Member States about Progesterone analyses was handled by the RIVM-CRL.

7. Workshops and Scientific Events

On 7-9 April, 1997 a workshop was organized (Thyreostats in Farm Animals - Regulatory Residue Analysis within the European Union). The workshop programme was organized in close cooperation with the University of Ghent (B). For the purpose of and prior to this workshop the NRLs participated in a small ring test. The results of the analyses and the performance of the laboratories were discussed during the workshop. Fourteen participants coming from NRLs from all EU Member States participated in this workshop. Three observers from Third Countries and one observer from a non-NRL laboratory also participated. Hands - on work was done on the analysis with different methods of the same samples as those sent to the NRLs. Discussion on methods, validation analysis and performance were held in small groups and evaluated during a plenary

session. Conclusions and recommendations (see Annex I) were drawn up at the end of the workshop whose proceedings are in preparation. A report was set up on the evaluation of this and other previously held workshops.

The senior staff of the RIVM-CRL contributed to the organization of the 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis held in June 1998 at Bruges (B) as Members of the Scientific Committee. The staff of the whole Bilthoven CRL contributed a variety of papers and posters to this Symposium, a major scientific event within their mandate.

III. Activities of the Community Reference Laboratory at the Centre National d'Etudes Vétérinaires et Alimentaires - Laboratoire des Médicaments Vétérinaires (CNEVA-LMV-CRL)

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1. General

In order to meet the obligations prescribed by the Directive 96/23/EC a staff of six scientists and four technicians are directly involved in the activities of the Fougères CRL. With the important extension of responsibilities (new compounds such as Chloramphenicol (CAP), Sulphonamides, Carbadox, Olaquindox and dyes in addition to the usual antibacterial substances and all the new species and target tissues), priorities had to be established further to much debate and exchange of views. The overall programme includes method development, new research activity, ring tests and training courses. To comply with this increase of activities a technician had to be engaged in January 1997 and a scientist in May 1997.

2. Method Development

To take into account these responsibilities and in order to harmonize SOPs in the EU and to organize training courses, the team was strongly

involved in the development of numerous analytical procedures using different method.

Development in the Use of HPLC

The validation of a multiresidue method for determining tetracyclines in muscle tissue including Doxycycline was achieved. Quantitative analysis of amphoteric and neutral penicillin antibiotics in muscle tissue was carried out by HPLC with precolumn derivatization. Quantitative analysis of nine quinolone substances was performed in chicken tissues with fluorescence detection. Quantitative analysis of four macrolides (Tilmicosin, Tylosin, Spiramycin and Neospiramycin) in muscle tissue was attained. In addition to this, two projects are running for the quantification of aminoglycosides and cephalosporine families.

Development in the Use of LC-MS

Particular efforts have been devoted to this technical aspect following the increasing amount of laboratories well equipped with this kind of apparatus. Confirmation of the macrolides Spiramycin, Tilmicosin, Tylosin, Erythromycin and Josamycin in bovine muscle was attained. Confirmation of six quinolones (Ciprofloxacin, Enrofloxacin, Danofloxacin, Sarafloxacin, Marbofloxacin and Difloxacin) was performed. Confirmation of four nitrofurans (Nitrofurazone, Nitrofurantoine, Furalidone and Furaltadone) was achieved.

3. Other Research

Two main research activities in the field of screening and post - screening of antibacterial substances in meat and milk have been undertaken, *i.e.*: *i*) the study of the sensitivity of 100 lactic bacteria against 30 antibiotics using the impedance - metric approach. These results bring out three interesting strains with internal code *Enterococcus* 94 and *Lactobacillus* 07 and 08. This good outcome will pave the way to the improvement of the routine methodologies used by the EU countries; *ii*) starting from June 1997 a new research project has been undertaken for the detection of CAP and Sulfamethazine (SMZ) in milk and meat using real - time biospecific interaction analysis based on surface plasma and resonance measurement. The analysis lasts about 10 min per sample. Repeatability and reproducibility were tested. The detection limit for CAP and SMZ is less than 1 ng ml^{-1} in milk and no significative cross - reactivity with other antibiotics was observed. Furthermore, an investigation on Tetracycline and Benzylpenicillin will be developed.

4. Quality Assurance

<u>Organization of a Proficiency Test</u>

In accordance with one of the conclusions of the meeting of June 1996 (Screening and Post - screening Tests to Detect Antibacterial Residues in Meat) a ring test was planned in May 1997 with the partecipation of 18 laboratories. The main goal was to test the most routinely used plates (*Bacillus subtilis* at pH 6 and pH 7.2) from the Four

Plate Test (FPT) with two volumes of agar medium. Following the results and the relevant debate, all the NRLs decided to work out in 1999 a common test which would be in compliance with the majority of Maximum Residues Levels (MRLs) for antibiotics in meat.

Reference Materials

In the framework of different projects of the SMT Programme, the Fougères CRL organized collaborative tests for the certification of four CRMs, namely, Oxytetracycline residues in milk (produced by the Fougères CRL) and Chlortetracycline in muscle, liver and kidney (produced by the Belfast NRL). Moreover, steps are being taken with the Institute for Reference Materials and Measurement (IRMM) of the Joint Research Centre (JRC) at Geel to plan the production of new CRMs and exchanges of views took place with the NRLs to know their needs in this specific field.

Accreditation

In 1997 the Fougères CRL obtained the renewal of its accreditation by the French Official Body COFRAC.

5. Technical and Scientific Support to NRLs and Third Countries

Updating of the List of Validated Methods (HPLC and MS) Routinely Used by the NRLs

Once more after sending a questionnaire the list of methods was updated and discussed during the meeting held on 26-27 May, 1998, on the possibility of extending the list to other techniques [screening Thin Layer Chromatography (TLC), Enzyme Linked Immuno - Specific Assay (ELISA), microbiology]. All the participants agreed upon the importance of performing this task.

Training Courses

To meet the demands of numerous NRLs and with the purpose of harmonizing analytical procedures as well as of organizing a ring test in the near future, two training courses were held, *i.e.*, the quantitative determination of seven penicillin residues in muscle by HPLC with precolumn derivatization (September and October 1997), attended by 17 participants, and determination of Tetracycline residues in pig muscle using electrospray LC-MS, attended by 15 participants.

Technical Assistance to NRLs and Third Countries

At the request of Portugal, Ireland, France and Austria 30 samples were analyzed to confirm results or to identify substances. Numerous documents, analytical procedures and sometimes chemical products were

sent to NRLs and third countries. During this period scientists and technicians from Belgium, Algeria, Tunisia, Niger, Morocco and South - Korea were hosted at the Fougères CRL.

6. Technical and Scientific Assistance to the European Commission

The Fougères CRL has been involved in various coordination or expert meetings organized by the EC in Brussels. Comments were made on the document Research on the Presence of Antibiotic Residues in Meat Originating from the Fifteen EU Member States (13 November, 1996).

7. Workshops and Scientific Events

Meeting of the NRLs, Fougères (26-27 May, 1998)

Twentythree representatives of the NRLs of 15 EU countries discussed about the priorities to be set for the four next years (1998-2002). These consist of a network of laboratories in order to prepare a project to be submitted to the fifth Framework Programme, starting by the end of 1998, for the organization of two ring tests before finalizing a new European test for antibacterial residues screening in meat, for the development of HPLC, LC-MS and LC-MS-MS analytical procedures concerning aminoglycosides, cephalosporins and quinolones and for the organization of practical training courses on methods recently developed by the Fougères CRL or other NRLs.

Third International Symposium on Hormone and Veterinary Drug Residues Analysis, Bruges (2-5 June, 1998)

The Fougères CRL substantially contributed to the success of this event with the participation of its six scientists and the presentation of eight posters and one oral contribution.

IV. Activities of the Community Reference Laboratory at the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV-CRL)

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1. General

According to Council Directive 96/23/EC of 29 April, 1996, the Berlin CRL is responsible for residues of β-agonists, anticoccidials including nitroimidazoles, anthelmintics and Non - steroidal Anti - inflammatory Drugs (NSAIDs). Within the BgVV-CRL the laboratory belongs to the Division of Chemistry and Technology of Foods and Consumer Goods. The analytical activities of the Berlin CRL are pursued by three specialized sub - units (see also Annex 3), which are responsible for Gas Chromatography (GC), GC-MS, HPLC, LC-MS and immuno - assay methods, respectively.

2. Method Development

During the 1996-1998 reference period the Berlin CRL began to establish analytical methods as required by the groups of substances presently assigned to it in accordance with the Council Directive 96/23/EC, namely NSAIDs and nitroimidazoles.

Development of a GC-MS Method for the Determination of Nitroimidazoles

The activities of the GC-MS unit were focused on the establishment of adequate methods for the confirmation and quantification of some nitroimidazoles, *e.g.*, Metronidazole (MNZ), Dimetridazole (DMZ) and Ronidazole (RNZ), as well as their main metabolites in muscle of swine and poultry. The method consists of the following steps: spiking of samples with internal standard, *i.e.*, d_3-Ronidazole (RNZ-d_3) or Ternidazole (TNZ); extraction at pH 4.2 using a NaCl-KH_2PO_4 buffer; solid phase extraction; derivatization; measurement by GC-MS. The SIM measurements are carried out in the Electron Ionization (EI) mode by monitoring fragment ions of the silylated analytes. These are listed in Table 1. The next step in this investigation area will be the optimization of the method, to be followed by a comprehensive validation by means of a recently developed in - house validation concept.

Table 1. Fragment ions of silylated analytes

Analyte	Ion 1		Ion 2		Ion 3		Ion 4	
	M/e (relative abundance, %)		M/e (relative abundance, %)		M/e (relative abundance, %)		M/e (relative abundance, %)	
RNZ	214	(100)	168	(50)	167	(40)	229*	(5)
HMMNI	214	(100)	168	(50)	167	(40)	229*	(5)
MNZ	167	(100)	182	(80)	228	(50)	243*	(15)
RNZ-d_3	217	(100)	171	(50)	169	(30)	232*	(5)
TNZ	181	(100)	211	(70)	121	(30)	242	(25)

* molecular ion

Validation of a GC-MS Method

The method for the determination of CAP in muscle (GC-MS-NCI) was validated applying the in - house validation concept developed by Jülicher *et al.* (1998). It takes into account matrix - and time - induced deviations and is based on a statistical variance component model. The calibration runs were performed at four different concentration levels (0.3, 0.6, 0.9 and 1.2 µg kg^{-1}) of CAP in muscle. The operational conditions and matrices were chosen randomly. According to the criteria laid down in the Commission Decision 93/256/EEC, four diagnostic ions were monitored. The relative abundances did not exceed those of the corresponding standard substances by more than ± 20 %. The relative retention time of the detected compounds was equal to that of the corresponding standard substance with a tolerance of ± 0.5 % and a recovery of 100.4 %. The calculated repeatability of the method was ± 0.0762 µg kg^{-1}. The in - house reproducibility at different concentration levels was: ± 0.0762 µg kg^{-1} at 0.30 µg kg^{-1}, ± 0.0877 µg kg^{-1} at 0.75 µg kg^{-1} and ± 0.103 µg kg^{-1} at 1.20 µg kg^{-1}, with CC_α = 0.42 µg kg^{-1}, α = 1 % and CC_β = 0.50 µg kg^{-1}, β = 1 % (see also Section 3, Other Research). The applicability and ruggedness of the method were proved by the performance of validation experiments.

Validation of an HPLC Method

A multi - residue HPLC method with Photo - Diode Array (PDA) detection was developed for the determination and confirmation of selected

NSAIDs, namely: Salicylic Acid (SA), Oxyphenbutazone (OPB), Flunixin (FLU), Carprofen (CPF), Niflumic Acid (NFA), Diclofenac (DC), Phenylbutazone (PBZ), Mefenamic Acid (MFAS) and Vedaprofen (VDP). The method was validated in plasma in a concentration range of 0.05-64 ng ml^{-1}.

The experimental approach was based on a fractional factorial scheme. Potential outliers were not eliminated. The validation was performed with underlying α- and β-error probabilities of 1 % each for the calculation of the power curve and the critical concentrations CC_α and CC_β (see also Section 3, Other Research). Table 2 summarizes the results obtained. As expected, the in - house repeatability was found to be independent of matrix influences since it did not decrease with increasing content levels. The method is suitable for the screening and confirmation of the aforementioned NSAIDs (with the exception of SA) in calf, bovine, ovine, porcine and equine plasma. It was also demonstrated that the method is applicable to differently treated plasma samples in terms of sampling, storing and thawing processes, to different matrix volumes and to samples of calves and bovines fed on different feeding conditions (intensive or extensive) and that it is independent of both operational and personnel conditions. The critical concentrations CC_α and CC_β calculated for the different analytes are sufficiently low to guarantee an efficient control.

Table 2. Figures of merit for the determination of NSAIDs.

	In-house repeatability[a)] (%)	In-house repeatability[a)] (%)	CC_α (µg ml^{-1})	CC_β (µg ml^{-1})
SA	30; 30; 30	63; 41; 41	0.184	3.60
OPB	24; 22; 20	31; 27; 24	0.088	0.260
FLU	9; 9; 9	11; 10; 10	0.042	0.057
CPF	10; 10; 10	11; 12; 12	0.063	0.086
DC	7; 7; 7	10; 9; 9	0.061	0.081
NFA	8; 8; 8	10; 10; 10	0.060	0.078
PBZ	18; 18; 18	24; 23; 23	0.086	0.184
MFAS	8; 8; 8	14; 10; 9	0.076	0.111
VDP	8; 7; 7	9; 9; 8	0.064	0.084

a) The in-house repeatability and reproducibility are given for three different concentration levels, *i.e.*, 0.1, 1.0 and 32 µg ml^{-1}.

Development of a Method for the Determination of Acid Non - Steroidal Anti - Inflammatory Drugs in Liver Using HPLC

Endogenous interferences on the analytes are considerably stronger in the extracts of liver samples than in those of other matrices. It was therefore necessary to develop a special gradient allowing a satisfactory separation of SA, PBZ, VDP, CPF, FLU, NFA, MFAS, Tolfenamic Acid (TLF), Suxibuzone (SXB) and Flurbiprofen (FBP). In the framework of such performance tests, the variances of peak areas, peak heights and retention times were examined by ten - fold injections at six concentration levels for each analyte. These concentration levels were covered by the previously determined working range of the instrument. Furthermore, the peak shape (symmetry, half - band width, tailing) was assessed and

long - term stability tests of the instrument performance and the standard solutions were carried out.

Development of the Sample Pretreatment Method for Acid Non - Steroidal Anti - Inflammatory Drugs in Liver

Literature scans showed that so far there is no multi - method available for the pretreatment of acid NSAIDs. The development of a new method included liquid - liquid and solid phase extractions, both allowing for good recovery rates from standard solutions. Satisfactory recovery rates from matrix samples have not yet been achieved, since the substances under test are subject to very strong metabolization and are very unstable in aqueous solutions.

Development of a Method for the Determination of 4-Methylamino-Antipyrine, Ramifenazone and Antipyrine in Liver Using HPLC

A gradient method was developed to separate the target analytes mentioned above. The variances of the peak areas, peak heights and retention times were determined at five different concentration levels for each analyte. The symmetry, tailing and half - band width of the peaks were examined and the capacity, resolution and selectivity factors were determined. Considerable retention time fluctuations were observed in the long - term stability tests of the instrument, which led to further modifications of the chromatographic conditions.

Development of the Sample Pretreatment Method for 4-Methylamino-Antipyrine, Ramifenazone, Antipyrine and their Metabolites in Liver

After investigating the solution and distribution behaviour of the analytes from standard solutions, the optimized pretreatment steps were applied to the matrix samples (liver, calf). Preliminary results showed recovery rates of 60 to 80 %. Further examinations concerning the reproducibility data, sensitivity ranges and an adaptation of the method to other compartments or species are still to be performed.

Development and Optimization of an ELISA Multi - method for the Extraction of Anilinic, Phenolic and Resorcinolic β-agonists from Different Bovine Matrices

The simultaneous extraction of different types of β-agonists with an organic solvent under alkaline conditions is rather ineffective, especially with respect to resorcinolic compounds like Terbutaline. Acidic pH values provide an almost uniform state of charge for the different types of β-agonists. Under these conditions anilinic, phenolic and resorcinolic β-agonists are positively charged allowing for the simultaneous extraction with a polar solvent. Buffer solutions of different pH values were spiked with Clenbuterol, Salbutamol and Terbutaline. Subsequently they were cleaned (*tert*-butyl methyl ether) and extracted with different organic solvents. All fractions were separately collected, dried, redissolved in ELISA buffer and measured with the Biognost® "Bronchodilator" ELISA. The optimized method was applied to bovine plasma and urine. Results

showed that a simultaneous extraction of anilinic, phenolic and resorcinolic β-agonists is possible using *iso*-Butanol at a pH value ranging from 4.8 to 6.

Determination of the Cross - reactivities of Some β-agonist Compounds in Nine Different Commercially Available ELISA Test Kits for β-agonist Detection

In order to further expand the findings of the comparative ELISA test kit study published in 1996, four further compounds were tested for their cross - reactivities. The substances were diluted with the respective test buffer at different concentrations. Cross - reactivities were determined comparing the B/B_0 - 50 % - concentration of the calibration standard with the B/B_0 - 50 % - concentration of the test standard. The results listed in Table 3 show that all β-agonist test kits available on the European market are able to detect Brombuterol, which has recently been misused as β-agonist in certain Member States. The Biognost® "Clenbuterol" kit provided the best results with respect to Clenisopenterol, Clenproperol and Clencyclohexerol.

Table 3. Cross - reactivities (in %) of β-agonists and β-antagonists in different ELISA test kits

Test kit	Brom-buterol	Cleniso-penterol	Clen-properol	Clency-clohexerol	Remarks
Randox Clenbut	123	0.06	6.7	0	own clen-standard
Randox Beta-Ago	80	0.04	8.9	0	own clen-standard
CER Clenbuterol	119	0.04	5.8	0	own clen-standard
CER Beta-Ago	82	0.01	5.9	0	own clen-standard
Eurodiagnostica	194	0.01	6.5	0	own clen-standard
	104	0.02	13.1	0	salb-standard kit
Eurokit (Genego)	72	0	9.4	0	own clen-standard
	80	0	10.5	0	clen-standard kit
r-Biopharm	117	0	31.2	0	own clen-standard
	101	0	26.8	0	clen-standard kit
Biognost Clenbut	49	94.8	76.7	91.2	own clen-standard
Biognost Broncho	96	11	7	0	own clen-standard

Experiments on the Suitability of Bovine Hair for β-agonist Analysis

A method was optimized to extract Clenbuterol from the matrix. The highest recoveries of Clenbuterol were found after a 60 min extraction with a Dithiotreitol (DTT) solution. After nine successive washing steps, the washing solution of samples contaminated with 4 µg g^{-1} of Clenbuterol of hair still produced positive results. The data of further washing experiments of the hair samples showed that even more than ten consecutive washing steps of incurred hair samples were still not enough to obtain a completely Clenbuterol - free washing solution. These findings confirmed the unsuitability of hair analysis for providing sound evidence of Clenbuterol misuse in animal fattening since it is impossible to clearly differentiate between incurred samples and samples contaminated only externally. Nevertheless, hair analysis may be recommended for screening purposes in the examination of live animals.

European Union - wide Survey on the Situation of the Anticoccidials and Nitroimidazole Analysis in the Official Residue Control Laboratories

A questionnaire was sent out to 36 laboratories in Germany and 28 laboratories in the EU Member States, including the NRLs and the CRLs. 77 % of the addressed laboratories responded (26 laboratories from Germany and 23 laboratories from other Member States). As many as 61 % of the participants deemed Anticoccidial analysis to be of low importance for their laboratories, while only two participants had a different opinion. The majority (80 %) covered a spectrum of up to five

compounds in their residue control programmes (only 20 % of all relevant compounds). 82 % of the laboratories expressed a need for methodological improvement. More than 90 % of the laboratories declared their interest in participating in interlaboratory studies or workshops. The general opinion was that an improvement of this unsatisfactory situation in Nitroimidazole and Anticoccidial analysis would be difficult to achieve.

Development of an ELISA Test for the Determination of 5-Nitroimidazoles

The developed ELISA tests are suitable for the screening for residues of MNZ, DMZ, RNZ, Ipronidazole and their metabolites in slaughtered animals. The residues of the 5-Nitroimidazoles and their metabolites are extracted with an acidic medium from samples of slaughtered poultry (muscle, liver) and pig (muscle). Subsequently, the samples are hydrolyzed with Protease, defatted with *n*-heptane and extracted again with *tert*-butyl methyl ether and ethyl acetate. The obtained extract is subjected to the ELISA measurement. The antibodies of the developed test system react selectively with 5-Nitroimidazoles. All hydroxy - metabolites of the 5-Nitroimidazoles can be detected simultaneously as well. No cross - reactivities with 2- or 4-Nitroimidazoles, Nitrofuranes or Benzimidazoles were observed. The metabolites account for 40-50 % of the overall residues, except for DMZ, where metabolites account for a much higher percentage. A final validation of the tests is still to be performed. Such tests are planned to be commercialized.

3. Other Research

Development of a Statistically Based In - house Validation Concept

Previous validation concepts either neglect matrix - and time - induced effects or consider them only to a limited extent. Therefore, research during the last reference period concentrated on the development of an in - house validation concept based on an uncertainty model which takes into account matrix - and time - induced deviations. This concept determines the in - house repeatability and reproducibility standard deviation on the basis of the matrix, time and random measurement errors, which can be calculated individually with the help of a mathematical model and the underlying experiment design. In a first step the experiment plan is drawn up on the basis of a random sampling scheme. The concept was subsequently extended to include a fractional factorial experiment design, which, apart from the calculation of the in - house repeatability and reproducibility, also allows for the assessment of the influence of the selected factors on the distribution of the measurement results. Thus, the extended in - house validation concept also allows fundamental uncertainty components to be determined, namely, matrix mismatch, run - bias and random measurement error.

The laboratory bias, which obviously cannot be determined by this concept, can be estimated afterwards with the help of the RSD of the Laboratory in interlaboratory studies performed in the context of proficiency tests. The still missing method bias can only be determined by means of CRMs. With regard to the evaluation of a method’s efficiency the

validation concept offers a number of advantages, *i.e.*: different matrices can be covered by a single validation procedure provided that the required sampling plans select factors and factor levels in a meaningful way; time - induced deviations are covered as well; the validation procedure offers the possibility of determining a critical concentration CC_α, a method - specific limit from which it can be assumed, with an underlying error probability α for false positive decisions, that the analyte is present; furthermore, the "power curve" allows the probability of an analyte being detected within the validated concentration range to be calculated; finally, a critical concentration CC_β is determined at which the probability of not being able to detect the true concentration (false negative rate) equals β. Hence, CC_β describes the true "detection capability" of the method.

The newly developed concept was applied to two validation experiments performed at the Berlin CRL, *i.e.*, the determination of CAP in muscle by GC-MS/NCI and the multi - residue method for the determination of NSAIDs in plasma by means of HPLC with PDA detection. The practicability of the in - house validation procedure was further corroborated by these two applications.

4. Quality Assurance

Proficiency Testing

Once an appropriate internal QC system has been implemented, approved laboratories are authorized to perform official residue controls in

accordance with Council Directive 96/23/EC. Such laboratories must be integrated in an internationally recognized external QA and accreditation system. Within this context laboratories must prove their competence by regularly and successfully participating in appropriate proficiency tests which are organized and/or recognized by NRLs or CRLs (Commission Decision 98/179/EC). The Beta - Agonist Interlaboratory Studies 5/96 and 11/97 organized by the Berlin CRL is to be considered as a contribution to the required external QC of the participating laboratories. They are in particular intended to offer the laboratories an opportunity to objectively check their routine methods for the detection of β-agonists. The studies were designed in compliance with the International Harmonized Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories (ISO/REMCO N 280) jointly elaborated by ISO, International Union for Pure and Applied Chemistry (IUPAC) and AOAC. The participants were basically free to use an analytical method of their choice and to decide on adequate number of replicates. It was, however, recommended to analyze the samples for their possible β-agonist content with the help of the laboratory's routinely applied screening and confirmatory methods. The detected residues of β-agonists had to be quantified. Furthermore, participants were asked to give an outline of their sample preparation and the analytical methods applied. For positive results the way of identification of the detected substances and the criteria to be taken into account had to be explained. Out of the 48 laboratories which declared their participation in the Beta - Agonist Interlaboratory Study 5/96, 4 laboratories submitted no results and 10 submitted only screening results. As a consequence, only 34 laboratories could be integrated in the z-score

evaluation. In 1997 a total of 44 laboratories declared their willingness to participate in the Beta - Agonist Interlaboratory Study 11/97. Six laboratories submitted no results and 9 laboratories submitted only screening results. The outcome of the Interlaboratory Study 5/96 revealed that 23 % of the participants were unable to confirm Clenbuterol, especially in liver, when present at concentrations below 1 µg kg^{-1}. More than 80 % of the laboratories failed to detect Salbutamol in the plasma sample which contained both Salbutamol and Clenbuterol at the 1 µg kg^{-1} level. Compared with the results of the Interlaboratory Study 5/96, those of the Interlaboratory Study 11/97 showed an apparent improvement of the participants' ability to quantify Salbutamol. However, in spite of the extremely high concentration of the analyte, 7 % of the participants were still unable to detect Salbutamol in urine, whereas 13 % of the participants were unable to detect it in liver. More than 30 % of the laboratories could not detect Cimaterol in the urine sample containing also Salbutamol, although the former was present at a concentration above 20 µg l^{-1}.

Since not all participants of the Interlaboratory Study 5/96 analyzed both matrices and also because of the high number of false negative results in this interlaboratory study, only 4 laboratories reached a high score, *i.e.*, a total of 5 satisfactory results. In the Interlaboratory Study 11/97, on the other hand, there were only 2 laboratories which achieved the maximum number of 3 satisfactory z-score results.

Satisfactory results had been achieved for Clenbuterol at high concentration levels in liver and plasma for both z-score evaluation and

false positive/negative rates (Interlaboratory Study 5/96). As regards the analysis of Cimaterol in urine, an unacceptably high number of laboratories (30 %, Interlaboratory Study 11/97) produced false negative results. Those laboratories which were able to analyze Cimaterol in urine obtained more or less satisfactory quantitative results in terms of z-scores. A weak point of the European residue control laboratories seems to be the analysis of Salbutamol. This becomes obvious not only from the rate of false negative results in plasma (80 %, Interlaboratory Study 5/96), but also from the high number of questionable and unsatisfactory results in liver and urine (Interlaboratory Study 11/97). The results of both Beta -Agonist Interlaboratory Studies confirmed that proficiency tests are an indispensable tool for external QC. The evaluation of the two studies revealed on the one hand a good performance of some participating laboratories, on the other hand a high number of false negative results produced by European residue control laboratories. This underlines the need for further cooperation supported by interlaboratory studies to be carried out at regular intervals.

Collaborative Study

During the 1996-1998 reference period the Berlin CRL successfully participated in the collaborative study Sulphonamides in Meat organized by the Chemische Landesuntersuchungsanstalt Freiburg (Germany) on behalf of the Working Group acting on the basis of Article 35 of the Lebensmittel- und Bedarfsgegenständegesetz (LMBG).

Standards, Measurements and Testing Programme

The Berlin CRL participated in the EU DG XII SMT programme Certification of Clenbuterol in Bovine Eye (certification campaign held in spring 1998) organized by the Bilthoven CRL. The results of the measurements, including the required quality data, were submitted.

5. Technical and Scientific Support to NRLs and Third Countries

Analytical Support

During the 1996-1998 reference period the Berlin CRL carried out confirmation analyses of CAP and β-agonists in samples submitted by the NRLs of Austria, Portugal and Cyprus. Technical advice was given to a number of NRLs with regard to analytical questions concerning the confirmation analyses of CAP and β-agonists.

Training

The Berlin CRL provided training support to several scientists from EU Member States and from third countries.

Standard Substances

The provision and distribution of standard substances to the NRLs in charge of the substance groups assigned to the Berlin CRL has been extended during the reference period. The number of reference standards

provided by the Berlin CRL increased to a total of 312 substances, some of which had especially been synthesized upon request of the CRL. During this period a total of 408 reference standards was shipped to laboratories in Germany, other EU countries and third countries.

6. Technical and Scientific Assistance to the European Commission

Working Groups of the European Commission and the European Council

In several working groups the members of the staff of the Berlin CRL contributed to the redrafting of the EU legislation on residue control and provided assistance in reviewing the criteria for residue detection methods in the framework of the revision of Commission Decision 93/256/EEC. The Director of the Berlin CRL actively participated in the working group Risk Assessment of Hormonal Growth Promoters. He was also one of the experts involved in several missions of the Food and Veterinary Office, Dublin, as regards the inspection of residue control systems in third countries, namely, Evaluation on Residue Control in Meat (USA, 3-14 November, 1997), Evaluation on Residue Control in Meat (Canada, 5-14 May, 1998) and Evaluation on Residue Control in Meat, Milk, Dairy Products, Aquaculture Fish and Game Meat (USA, 20 July - 1 August, 1998).

7. Workshops and Scientific Events

CRL Workshop on the Establishment of Reference Methods, Berlin (10-12 December, 1997)

The workshop was attended by 27 scientists from 23 NRLs of the Member States. At present the confirmation of all positive findings for banned substances by reference methods required by Article 15 of Directive 96/23/EC cannot be fulfilled. This is above all due to the lack of methods meeting the criteria formulated in the provisions of Commission Decisions 93/256/EEC and 93/257/EEC as well as to the inadequacy of some of these criteria.

With the purpose of protecting consumers from unacceptable health hazards as well as preventing market distortions it is of vital importance to ensure analytical quality, to guarantee a comparable, reliable control of established threshold levels and to discourage the use of banned substances. Validated analytical methods and/or reference methods are a prerequisite to achieve these objectives. Therefore, the workshop aimed at discussing a harmonized approach for developing, evaluating and applying reference methods in order to contribute to the implementation of the new residue control Directive. Participants expressed their concern that at present the impact of Article 15 of Council Directive 96/23/EC in connection with the Commission Decisions 93/256/EEC and 93/257/EEC severely endanger the implementation of the residue control system in the European Union. Participants stated that the validation of analytical methods and the establishment of reference methods in accordance with the Commission

Decisions 93/257/EEC and 93/256/EEC can be hardly achieved. The revision of these two decisions is therefore urgently needed. Methods for the control of banned substances must also be validated copying with minimum validation limits to assess the performance level of such methods. The assessment of the reliability and comparability of methods mainly depends on their error probabilities (false positive and false negative results). The establishment of maximum tolerable error probabilities for the different levels of analytical methods (screening, confirmatory, reference) should be a political decision. The empirical determination of these error probabilities forms an integral part of the entire validation process. An agreed framework for the establishment of reference methods with regard to the provisions of Article 15 of Council Directive 96/23/EC as drafted by the four CRLs is necessary.

All participants agreed that the accepted framework has to be integrated in a new Commission Decision laying down detailed rules for routine and reference methods. This decision must also include the above mentioned minimum validation limits and the definition of tolerable α and β errors for the different levels of analytical methods.

<u>3rd International Symposium on Hormone and Veterinary Drug Residue Analysis, Bruges (2-5 June, 1998)</u>

Collaborating in the Scientific Committee of the 3rd International Symposium on Hormone and Veterinary Drug Residue Analysis, held in Bruges (Belgium), 2-5 June, 1998, and acting as a session chairman, the

Director of the Berlin CRL supported the preparation of this event. Some scientific results of the CRL research activities were also presented.

V. Activities of the Community Reference Laboratory at the Istituto Superiore di Sanità (ISS)

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1. General

The ISS is the main Italian institute of technical - scientific research, control and advice in public health. Founded in 1943, it has become since 1978 the technical and scientific body of the Italian National Health Service. It is under the authority of the Minister of Health and it has its own structures, particular rules and scientific autonomy. Its role within the Italian health system is to promote public health through scientific research, surveys, controls and analytical tests in the different fields of health sciences, namely, drugs, environment, food safety, health services planning and evaluation and infectious and non - infectious pathology. The Institute cooperates with the major international organizations.

The CRL for Residues at the Istituto Superiore di Sanità (ISS-CRL) is responsible for five broad categories of chemicals, namely trace elements, organochlorine and polychlorobiphenyl compounds, carbamates and pyrethroids and organophosphate compounds. The ISS-CRL consists of twelve people, four of which are on the regular staff of ISS and eight are hired.

2. Method Development

In continuation of the steps already taken in the previous period and as prescribed by the mandate assigned to the ISS-CRL, particular attention was devoted to improvement of analytical approaches already in use and the development of novel ones. In this context, extraction and separation of pesticide residues from fatty matrices on the basis of non - instrumental techniques were further developed. In the new approach, the classical separatory funnel *n*-hexane/acetonitrile partition step for the said separation was replaced by a solid matrix partition. In addition, the use of a small cartridge of silica-C18 on line with the partition step allowed for the removal of most of the fatty material and the recovery of organochlorinated pesticide residues. The method offered some advantages when compared to classical approaches, *i.e.*, reduced solvent volumes, absence of emulsions and use of disposable items. The performance of the method was compared to that of instrumental techniques, such as Size - Exclusion Chromatography (SEC). Using the same approach of extraction of residues from materials dispersed over a large - surface matrix, an innovative selective extraction of organophosphate and pyrethroid pesticide residues from milk was also undertaken.

As regards milk, also the obtainment of baseline figures for a number of essential and potentially toxic elements was considered compulsory for the assessment of the nutritional integrity of this fluid as well as of its conformity with general health requirements. Recommendations were recently issued on desirable concentration ranges of essential nutrients and acceptable non - toxic levels of inorganic polluting agents. In addition to

this, it became clearly evident over the last few years that there is a keen need to ascertain the binding form, *i.e.*, the particular species of a chemical element in a given matrix. Only when this information is available, in fact, one can formulate assumptions about its mobility, resorption, bioavalaibility, accumulation and toxicity. For the purpose of speciation studies, therefore, it was decided to combine Inductively Coupled Plasma Atomic Emission (ICP-AES) or Mass Spectrometry (ICP-MS) and HPLC and to apply the coupled techniques to detect the chemical forms under which some elements of primary importance, such as Al, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Pt, Sr and Zn are present in milk. This allowed for the assessment of the overall intake of the elements of interest as well as of their bioavailability as a function of diet. In the ultimate, this was deemed to facilitate the identification of possible states of undue exposure and the interpretation of the metabolic fate of toxic trace elements.

On the basis of these data, it was possible to reach some interesting general indications, *i.e.*: *i)* elements such as Ba, Bi, Cd, Li, Mn, Mo, Pb, Tl and Zn appeared to be equally distributed among components with high (*e.g.*, Bovine Serum Albumine, BSA) as well as with low (*e.g.*, lactose, mineral salts, citrates.) molecular weight components. A second group of elements, containing Ca, Cu, Sr and especially Mg were bound preferably to low molecular weight components; *ii)* the second consideration centered on the diversification in the element contents of the various chromatographic fractions. The results obtained support the view that the major portion of the total amount of each of the elements tested is contained in the first (proteins having a molecular weight around

20,000 Da) and fifth chromatographic fractions (substances with a relatively low molecular weight, *i.e.*, mineral salts). The present findings can contribute to clarify some problems as regards the metabolic fate of important essential and potentially toxic trace elements in milk.

3. Other Research

An investigation was undertaken to ascertain the average levels of a number of key elements in several types of honey with special regard to the influence of the various processing steps. Honey is a natural sticky material elaborated out of the nectar of flowers and of plant secretions in the honey sac of bees. The nectar is collected, transformed, combined with proper substances by bees and stored in honeycombs where ripening occurs. The chemical composition of nectar may depend on the botanical species, although the percentage amount of constituents is generally given by *ca.* 81 % carbohydrates, *ca.* 18 % water and *ca*. 1 % proteins, aminoacids, vitamins, organic acids and minerals all together. Four types of samples were considered useful for the purpose of this study, namely freshly collected, sealed, extracted and ripened honey. The so - called freshly collected honey is the nectar picked up by the bees, concentrated and enriched with enzymes from glandular secretions and stored in the hexagonal cells of the honeycomb. At this step the water content is around 70-80 %. The ventilation produced by the beating of bee wings makes humidity decrease down to 17-18 %. The cells are then capped with a thin layer of wax (honey is now named sealed). The frames are subsequently withdrawn from the beehives, the wax removed and the honey inside collected after slow centrifugation and filtration (extracted honey). In the

last phase the honey is stored in a steel container and kept a few days until complete maturation (ripened honey). During this period of time, the scattered exogenous particolate gets to the surface of honey and is afterward removed.

Samples of sunflower honey at different stages of production were collected in an uncontaminated area near Siena (Italy). This area is generally considered to be relatively unpolluted as it is far from large industrial plants and major railways and highways. Different types of honey were analyzed to determine their content in As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Pt, Sn, V and Zn. On the one hand, this work aimed as ascertaining and quantifying inorganic contaminants in honey which are potentially hazardous to the environment and human health; on the other hand, the lack of CRMs for trace elements in a host matrix of honey prompted the investigation also in view of the possible launch of a certification project for the production of a new CRM based on this matrix . In order to obtain a more homogeneous and fluid material suited to the analysis, samples of *ca*. 10 g of honey were put into carefully decontaminated glass containers, precisely weighed and added with 5 g of high purity deionized water. This mixture was then heated up to 50 °C and sonicated. Subsamples of *ca*. 1.8 g of this viscous solution were transferred into the microwave teflon vessels, weighed and digested. In order to check the possible release of analytes from these containers, blanks were prepared following the same procedure adopted for the samples.

Plasma - based techniques were employed in the determination of trace elements in honey samples, namely ICP-AES, Quadrupole (Q) ICP-MS and High Resolution (HR) ICP-MS (depending on the actual concentration of each element). In particular, As, Cd, Cr, Ni, Pb, Pt, Sn and V were determined by Q-ICP-MS and HR-ICP-MS, Cu by the three methods, Fe by ICP-AES and HR-ICP-MS and Mn and Zn by ICP-AES only. The accuracy of measurements was tested by including in the analytical runs the Bureau Communautaire de Référence (BCR) CRM 191 (Brown Bread), *i.e.*, the only CRM available with a matrix of carbohydrates. The ranges ascertained were as follows (in ng/g): As < 0.50-0.70; Cd < 0.50-0.74; Cr, 1.03-3.93; Cu, 127-216; Fe, 191-651; Mn, 223-580; Ni, 17-49; Pb, 3.20-186; Pt < 0.50; Sn < 4-27; V, 1.22-1.94; and Zn, 565-1144.

On the basis of these findings, there is no doubt that a major difficulty in the obtainment of reliable data was the heterogeneity of the raw material, as also highlighted by the significant differences in concentrations noted when testing more subsamples from the same raw mass. Sample dissolution in water certainly mitigated such heterogeneity and facilitated manipulation, but without overcoming completely the problem. Furthermore, as regards the possible preparation of a honey - based multielemental CRM, this can be successfully undertaken once the difficulties caused by matrix heterogeneity are solved.

It is worth mentioning in this context that contamination of honey from trace elements can occur in many different ways, not always easily

recognizable. A major cause for this fact is due to the acidity of honey (pH 3.5-4). Under such conditions, metal release can occur during the centrifugation and ripening steps which are generally performed in stainless steel containers, as is the case of Pb. Similar considerations can be made in the case of Sn, the presence of which can be traced back to the release of the metal from the lid of the glass pot where honey is stored. The preliminary findings of this investigation were presented as an invited key - note lecture at the Deauville Conference 6th Symposium on Analytical Sciences (Valencia, 24-26 June, 1998) and are now being published as a full paper in *Talanta.*

4. Quality Assurance

A partial repetition of Phase 1.2a of the ongoing QA Programme for Trace Element Determinations was carried out in consequence of the relatively unsatisfactory performance of the former run. Participants were asked to analyze As, Cd, Hg and Pb in known and unknown solutions enriched with salts to simulate an acidic digestion of real meat sample. Results of this trial were evaluated and discussed during the third Workshop on Trace Elements held in Rome, 13-15 November, 1997.

A further step (Phase 2.1a) in the conduct of proficiency tests for trace elements was also undertaken. The purpose of this phase, of greater difficulty when compared to the previous ones, was the assessment of the capabilities of each laboratory in the analysis of As, Cd, Hg and Pb in real meat samples. The entire process of meat pretreatment (digestion, homogeneization, dilution and bottling) was under the sole responsibility of

the Rome CRL in order to eliminate, at this stage of the proficiency testing, all possible differences in the final product which could arise from specific procedures in use at each NRL. The stock solution thus obtained was subdivided into four aliquots, three of which were spiked with known amounts of As, Cd and Hg. Finally, after appropriate coding, one bottle from this set was randomly selected and shipped together with a bottle of the unspiked solution to each participant. Results and their statistical evaluation were discussed during the above mentioned Workshop. Different kinds of sample pretreatment were compared in order to assess the most effective matrix destruction procedure. Pilot analyses were carried out by plasma - based spectrochemical techniques to quantify the concentrations expected for the elements under test in the original meat mass. Results showed that several laboratories experienced some difficulties in the analysis of pre - digested samples. The same participants supplied unsatisfactory results even in the case of solutions enriched with salts to simulate an acidic digestion of real meat samples, where the pre - established concentration values were much higher than those of the really digested samples. Thus, it was decided to repeat Phase 1.2a in order to reassess the laboratory performances. At the same time it was decided to start Phase 2.2a concerning the quantification of the As, Cd, Cu, Hg and Pb levels in freeze - dried bovine muscle. At this stage, the collaboration of the IRMM was requested for the production of this material. It was also decided that these two parts of the exercise should be conducted at the same time.

Another proficiency test exercise for organochlorine pesticides was also organized. Solutions of 15 chemicals in soya oil were prepared at concentrations in the vicinity of 0.05 mg kg^{-1}. The participating laboratories received two glass ampoules containing the spiked soya oil and the blank soya oil, respectively, and they were asked to carry out two replicate determinations of the concentrations of the 15 compounds in the spiked oil. An ampoule containing a concentrated standard solution of the 15 compounds in *iso*-octane was also supplied to be used for quantification. A workshop was organized and held in 17-19 October, 1996, to discuss and evaluate the results. A report was produced, containing the data as supplied by the NRLs together with some statistical evaluation. After the discussion had during the workshop the laboratories reconsidered their own results and introduced some amendments arising from merely trivial errors, such as typing, calculation and the like. A new report was then produced to include the definitive results of the second proficiency test on organochlorine pesticide residues in oil solution. This was circulated among the participants.

The samples for the third exercise on organochlorine compounds were prepared and shipped to the participating laboratories. Unknown organochlorine pesticide residues have to be determined in two spiked soya oils and two *iso*-octane solutions, both at two different levels of concentration.

5. Technical and Scientific Support to NRLs and Third Countries

The most recent literature references were scanned in order to update the Data Bank of Analytical Methods for Chemical Elements. The greatest care was devoted to the elaboration, for each method, of a short text highlighting the novelty brought about by the study and to the harmonization of key words for quick and unambiguous retrieval of subgroups of papers, technical reports and other published material. The same approach was followed to expand the section of the Data Bank devoted to Organochlorine Compounds in Fatty Matrices. The information contained therein was made available to all interested NRLs. The computer software designed to host the Data Bank was further optimized in order to make it even more flexible and interactive. In this context, all the fields to be filled in by data were further expanded in order to contain detailed information of value to the users. Moreover, it was possible to move across the various fields of the Data Bank more easily than in the case of the previous version.

The NRLs supplied the methods they used for the determination of organochlorine residues in fatty material. Methods were collected as given and arranged in a Handbook entitled Collection of Methods for the Determination of Organochlorine Compounds in Foods of Animal Origin. This was distributed to each participating laboratory prior to the second Workshop on trace organics held in Rome, 17-19 October, 1996. Subsequently, the participating laboratories were invited to produce a new version of their own methods. It was also agreed to present future contributions on a floppy disk using a Winword format. A revised edition

of the Handbook of the methods will be produced and circulated by the end of the year. The Handbook of Methods was made available, upon request, to the Ministry of Agriculture of the Kingdom of Morocco.

In the case of trace elements, the current version of the Handbook on the Analytical Methods for Trace Elements in Use at NRLs was expanded and updated. In particular, a new section with additional information on CRMs currently employed by NRLs was included. Subsequently, the revised version of the Handbook will be supplied to the NRLs for further comments and remarks.

6. Technical and Scientific Assistance to the European Commission

In the light of the mandate assigned by the Council Directive 96/23/EC, the Rome CRL actively cooperated with the EC in projects for the certification of trace elements in candidate CRMs as undertaken in the frame of the SMT Programme. These were as follows: CRMs 609, 610, 611 and 612 (Trace Elements in Groundwater); CRM 185R (Trace Elements in Bovine Liver) and CRM 278R (Trace Elements in Mussel Tissue). The first project was completed in June 1997, while the other two were concluded in December 1997.

Coordination meetings of the four CRLs for Residue Analysis took place in Brussels on 24 October, 1996; 27 November, 1996; 14 January, 1997; 12 March, 1997; 27 May, 1997; 4 September, 1997 and 29 May, 1998.

The ISS-CRL also participated in the meetings of the OECD Panel for GLP held in Paris, 4-5 March, 1997 and 27-28 January, 1998.

7. Workshop and Scientific Events

1) Training Course on Applied Toxicology, Milan, 8 October, 1996 (one oral contribution on analytical techniques for pesticide determination).
2) Second Workshop on Quality Assurance of Organochlorine Compounds Determination, Rome, 17-19 October, 1996 (organization and conduct of the meeting to assess the performance of the NRLs in the frame of the second exercise on proficiency testing for the determination of organochlorine compounds).
3) Principles of Good Laboratory Practice and Performance of Inspections, Rome, 30-31 October, 1996 (organization and conduct of a course aimed at training GLP inspectors).
4) Food Quality Assurance in Chemical Laboratories, Rome, 5-6 November, 1996 (one oral contribution on analytical QC for pesticides).
5) Accreditation of Laboratories: from Theory to Practice, Bari, 21 February, 1997 (one oral contribution on analytical QC for trace elements).
6) Fourth National Conference Associazione Italiana per lo Studio degli Elementi in Traccia negli Organismi Viventi (AISETOV) Biological Role of Trace Elements, Rome, 20-22 March, 1997 (one oral contribution on analytical QA for trace elements).

7) Seventh International Symposium on Biological and Environmental Reference Materials, Antwerpen, Belgium, 21-25 April, 1997 (one oral contribution and one poster on CRMs).
8) VIII Hungarian - Italian Symposium on Spectrochemistry, Analytical Techniques in Environmental Chemistry, Debrecen, Hungary, 29 June - 4 July, 1997 (organization of the conference with presentation of two contributions on the role of the Rome CRL in the analysis of residues in matrices of animal origin as well as on the importance of CRMs in environmental chemistry).
9) XIII National Congress on Analytical Chemistry, San Martino al Cimino (Viterbo), 7-11 September, 1997 (one oral contribution on analytical QA for trace elements).
10) Principles of Good Laboratory Practice and Performance of Inspections, Rome, 5-6 November, 1997 (organization and conduct of a course aimed at training GLP inspectors).
11) Third Workshop on Quality Assurance of Trace Elements Determination, Rome, 13-15 November, 1997 (organization and conduct of the meeting to assess the performance of the NRLs in the frame of the third exercise on proficiency testing for the determination of trace elements).
12) 1998 Winter Conference on Plasma Spectrochemistry, Scottsdale (USA), 5-10 January, 1998 (collaboration to the organization of the conference and one oral contribution on the importance of CRMs in environmental chemistry).
13) First International Conference on Trace Element Speciation in Biomedical, Nutritional and Environmental Sciences, Munich

(Germany), 4-7 May, 1998 (collaboration to the organization of the conference and one oral contribution on the speciation of elements in milk).

14) Quality Performance in Chemical Laboratories, Marghera (Venezia), 1 June, 1998 (one oral contibution on analytical QA for trace elements).
15) National Congress of Quality Assurance, Grottaferrata (Roma), 18-19 June, 1998 (one oral contribution on analytical QA for trace elements).
16) Deauville Conference 98 - 6th Symposium on Analytical Sciences, Valencia (Spain), 22-24 June, 1998 (collaboration to the organization of the conference and one oral contribution on the analysis of honey).

VI. Annexes

Annex 1 - Publications, Reports and Other Informative Material

Publications, Reports and Other Informative Material of the RIVM-CRL

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66) H. F. Brabander, K. de Wasch, L. A. van Ginkel, S. S. Sterk, Ph. Delahaut, X. Taillieu, M. Dubois, C. J. M. Arts, M. J. van Baak, L. G. Gramsberg, R. Schilt, E. O. van Bennekom, D. Courtheyn, J. Vercammen, R. F. Witkamp, Multi - laboratory Study of the Analysis and Kinetics of Stanozolol and Metabolites in Treated Calves, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, June 1998, Poster B11.

67) P. Gaspar, G. van Vyncht, G. N. Kramer, L. A. van Ginkel, C. Dirscherl, G. Maghuin - Rogister, Preparation of Lyophilized Bovine Liver Samples Containing Trenbolone Residues, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, June 1998, Poster A18.

68) H. A. Herbold, S. S. Sterk, L. A. van Ginkel, R. W. Stephany, Multi - residue Screening for Anabolic Compounds in Faeces Using Direct Injection/Coupled - Column HPLC and GC/MS, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, June 1998, Poster B22.

69) R. C. Schothorst, R. W. Stephany, Results of the Second Inventory on QA/QC and GLP for EU-National Reference Laboratory, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, June 1998, Poster A44.

70) A. A. M. Stolker, P. L. W. J. Schwillens, M. H. Blokland, S. S. Sterk, L. A. van Ginkel, Comparison of Different LC - Methods for Determination of Corticosteroids in Biological Matrices, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, June 1998, Poster B44.

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72) S. S. Sterk, E. F. van Tricht, L. A. van Ginkel, R. W. Stephany, Production and Stability Testing of Incurred Reference Materials for Trenbolone in Bovine Liver, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, June 1998, Poster A4798.

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74) R. W. Stephany, S. S. Sterk, R. C. Schothorst, M. A. Jonker, L. A. van Ginkel, European Union Reference Laboratories – A Quality Challenge, *12th WAVFH Congress*, The Hague, August 1997.

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1) V. Gaudin, P. Maris, Detection of Chloramphenicol and Sulfamethazine Residues in Milk and Meat Using Real - time Biospecific Interaction Analysis (BIA), *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, 2-5 June, 1998.

2) R. Fuselier, P. Maris, An Impedance - metric Method Used as an Alternative Screening Test for Veterinary Drug Residues in Milk, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis*, Bruges, 2-5 June, 1998.

3) R. Fuselier, P. Maris, N. Cadieu, European Interlaboratory Test 1997, Microbiological Screening for Antibiotic Residues in Pork Muscle, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis*, Bruges, 2-5 June, 1998.

4) B. Delépine, D. Pessel, P. Sanders, Simultaneous Determination of Six Quinolones in Pork Muscle by Liquid Chromatography (APCI) Mass Spectrometry, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis*, Bruges, 2-5 June, 1998.

5) D. Pessel, B. Delépine, Determination of Amoxycillin and Ampicillin Residues in Bovine Muscle by Liquid Chromatography - Mass Spectrometry, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis*, Bruges, 2-5 June, 1998.

6) M. Juhel - Gaugain, P. Sanders, M. Laurentie, B. Anger, P. Maris, Results of a European Interlaboratory Study for the Determination of Oxytetracycline in Pork Muscle, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis,* Bruges, 2-5 June, 1998.

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15) P. Maris, E. Verdon, D. Hurtaud, List of the HPLC and LC-MS Methods for Antibiotic Residues Analysis Used in NRLs, June 1997.

Publications, Reports and Other Informative Material of the BgVV-CRL

1) B. Jülicher, P. Gowik, S. Uhlig, Assessment of Detection Methods in Trace Analysis by Means of a Statistically Based In - house Validation Concept, *Analyst*, 123 (1998), 173-179.
2) P. Gowik, B. Jülicher, S. Uhlig, A Multi - residue Method for Non - steroidal Anti - inflammatory Drugs in Plasma with High - Performance Liquid Chromatography - Photodiode - Array Detection: Method Description and Comprehensive In - house Validation, *J. Chromatogr. B*, 716 (1998), 221-232.
3) P. Gowik, B. Jülicher, Entwicklung eines in - house Validierungskonzeptes für die Spurenanalytik, *Nachr. Chem. Tech. Lab*., 46 (1998), 841-844.
4) Ch. Wolf, D. Behrendt, B. Conradi, B. Jülicher, Results of Two European Interlaboratory Studies for the Determination of β-Agonists in Various Matrices, *Analyst*, 123 (1998), 2719-2723.
5) M. Friederichs, I. Schmädicke, B. Jülicher, Rückstände in Lebensmitteln tierischer Herkunft - Stoffe mit pharmakologischer Wirkung, In: *Ernährungsbericht* 1996, Deutsche Gesellschaft für Ernährung e.V., Frankfurt, (1996), 109-117.
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7) Ch. Wolf, D. Behrendt, B. Jülicher, CAP Interlaboratory Study 3/95 - *Report on Results*, 1996.

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9) D. Behrendt, B. Jülicher, *Internal Report on the Visit at the National Residue Laboratories in Ireland (the State Laboratory and the Central Meat Control Laboratory, Abbotstown)*, 1997.

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14) V. Wesseling, S. Hahnau, B. Jülicher, K. Rubach, Multi - residue Screening of 5-Nitroimidazoles and their Major Metabolites in Incurred Swine Samples using ELISA Technique, *Third International Symposium on Hormone and Veterinary Drug Residue Analysis*, Bruges, 2-5 June, 1998.

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1) A. Di Muccio, P. Pelosi, I. Camoni, D. Attard Barbini, R. Dommarco, T. Generali, A. Ausili, Selective Solid - matrix Dispersion Extraction of Organophosphate Pesticide Residues from Milk, *J. Chromatogr. A*, 754 (1996), 497-506.

2) A. Di Muccio, T. Generali, D. Attard Barbini, P. Pelosi, A. Ausili, F. Vergori, S. Girolimetti, Single - step Separation of Organochlorinated Pesticide Residues from Fatty Materials by Combined Use of Solid - matrix Partition and C18 Cartridges, *J. Chromatogr. A*, 765 (1997), 61-68.

3) A. Di Muccio, P. Pelosi, D. Attard Barbini, T. Generali, A. Ausili, F. Vergori, Selective Extraction of Pyrethroid Pesticide Residues from Milk by Solid - matrix Dispersion, *J. Chromatogr. A*, 765 (1997), 51-60.

4) T. Generali, P. Pelosi, A. Di Muccio, A Collection of Methods for the Determination of Organochlorine Compounds in Foods of Animal Origin as Supplied by EU-National Reference Laboratories, Rev. 2, *Internal Report*, July 1996.

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10) S. Caroli, O. Senofonte, S. Caimi, P. Pucci, J. Pauwels, J. N. Kramer, A Pilot Study for the Preparation of a New Reference Material Based on Antarctic Krill, *Fresenius' J. Anal. Chem.*, 360 (1998), 410-414.

11) S. Caroli, G. Forte, A. L. Iamiceli, Quantification of Trace Elements in the Marine Macroalga *Fucus sp*., *Microchem. J.*, 1998 (accepted).

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13) S. Caroli, O. Senofonte, S. Caimi, P. Pucci, J. Pauwels, P. Robouch, J. N. Kramer, Certified Reference Materials for Research in Antarctica: the Case of Marine Sediment, *Microchem. J.*, 59 (1998), 136-143.

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Annex 2 – Audits

Audits of the RIVM-CRL

1) CRL audited by STERLAB (45001 EN accreditation), 9 October, 1996.
2) CRL audited by STERLAB (45001 EN accreditation), 15 October, 1997.
3) CRL audited by VHI, GLP/OECD, 5-16 January, 1998.
4) Evaluation on Residue Control Meat, USA, 3-14 November, 1997.
5) Evaluation on Residue Control Meat, Canada, 5-14 May, 1998.
6) Evaluation on Residue Control Meat, Milk, Dairy Products, Aquaculture Fish and Game Meat, USA, 20 July-1 August.

Audits of the CNEVA-LVM-CRL

1) Institut Scientifique de la Santé Publique Louis Pasteur, Brussels, Belgium, 28 May, 1997.
2) Staatliches Veterinäruntersuchungsamt Hannover, Hannover, Germany, 18 June, 1997.

Audits of the BgVV- CRL

1) The State Laboratory, Abbotstown, Castlenock, Dublin, Ireland, 9-10 April, 1997
2) The Central Meat Control Laboratory, Abbotstown, Castlenock, Dublin, Ireland, 10-11 April, 1997.
3) Evaluation on Residue Control Meat, USA, 3-14 November, 1997.
4) Evaluation on Residue Control Meat, Canada, 5-14 May, 1998.
5) Evaluation on Residue Control Meat, Milk, Dairy Products, Aquaculture Fish and Game Meat, USA, 20 July-1 August.

Audits of the ISS-CRL

1) SIAPA, Divisione delle Industrie Chimiche, Galliera , Bologna, Italy 2-3 December, 1997.

Annex 3 – Organigrams and Staff

Organigram and Staff of the RIVM-CRL

R. W. Stephany, Director of the RIVM-CRL, Head of the Laboratory for Residue Analysis.

L. A. van Ginkel, Deputy Director, Deputy Head of Laboratory for Residue Analysis, Research Leader, Head of Section of Veterinary Drugs and Medicine.

H. P. van Egmond, Research leader, Head of Section of Natural Toxins and Nitro Substances,.

S. S. Sterk, Scientist, Deputy Research Leader, Responsible for the Analytical Services.

H. A. Herbold, Senior Scientist.

M. Blokland, Scientist (since 12 May, 1997).

A. Kieft, Scientist (until 1 January, 1998).

B. Le, Scientist (since 1 January, 1998).

P. L. W. J. Schwillens, Scientist.

A. Spaan, Scientist.

A. A. M. Stolker, Scientist, Responsible for Scientific Assistance.

H. J. van Rossum, Scientist.

F. van Tricht, Scientist (until 1 January, 1997).

K. L. Wubs, Scientist.

P. W. Zoontjes, Scientist.

C. van de Kamp, Senior Documentalist, Literature Scientist.

M. Jonker, Literature Scientist, Responsible for Documentation Services.

R. Jansen, Information and Automation Manager (until 1 April, 1996).

E. Sahertian, Information and Automation Manager (since 1 May 1995).

G. Wilbers, Documentalist, Responsible for Maintenance and Updating Databases.

A. Hulsbosch - Aalpol, Documentation Supporter (since 1 February, 1998).

J. ten Have, Documentation Supporter, Archiver (since 1 February, 1998).

R. C. Schothorst, Responsible for QA/QC Infrastructure, QA/QC Officer.

A. van der Berg, QA/QC Officer.

A. M. Kartassamita, QA/QC Secretary.

E. van Tamelen, Management Assistant (since 1 June, 1997).

S. van Laer - van Zalinge, Management Assistant (until 1 April, 1997).

M. van Tuyl, Amanuensis, Logistic Supporter.

I. Aitton – Huijsmans, Financial Contract Supporter (until 15 May, 1998).

Organigram and Staff of the CNEVA-LVM-CRL

P. Maris, Director of the CNEVA-LVM-CRL.

J. P. Abejan, QA/QC Officer.

V. Juban, QA/QC Officer.

B. Anger, Member of the Unit for Analysis of Antibacterial Substances by HPLC.

N. Cadieu, Member of the Unit for Microbiological Analysis.

P. Couedor, Member of the Unit for Analysis of Antibacterial Substances by HPLC.

R. Fuselier, Member of the Unit for Microbiological Analysis.

V. Gaudin, Member of the Unit for Microbiological Analysis.

D. Hurtaud - Pessel, Member of the Unit for Analysis of Antibacterial Substances by MS.

M. Juhel, Member of the Unit for Analysis of Antibacterial Substances by HPLC.

A. Rault, Member of the Unit for Microbiological Analysis.

E. Verdon, Member of the Unit for Analysis of Antibacterial Substances by HPLC.

J. C. Yorke, Member of the Unit for Analysis of Antibacterial Substances by HPLC.

C. Marcault, Secretary.

C. Gervis, Secretary.

Organigram and Staff of the BgVV-CRL

B. Jülicher, Director of the BgVV-CRL.

D. Behrendt, Deputy Director, QA/QC Officer.

P. Gowik, Senior Scientist, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by HPLC and LC-MS.

A. Preiß - Weigert, Senior Scientist, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by GC and GC-MS.

L. Benesch - Girke, Scientist, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by HPLC and LC-MS.

S. Hahnau, Scientist, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by Immunoassay.

V. Wesseling, Scientist, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by Immunoassay.

Ch. Wolf, Chemical Engineer, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by GC and GC-MS.

A. Hiller, Technician, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by Immunoassay.

B. Conradi, Translator, Administrator.

S. Maidhof, Technician, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by HPLC and LC-MS.

B. Matthes, Technician, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by Immunoassay.

A. Neumärker, Technician, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by GC and GC-MS.

S. Rahn, Technician, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by GC and GC-MS.

M. Schramm, Technician, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by HPLC and LC-MS.

H. Thurau, Technician, Member of the Unit for Residue Analysis and Pharmacokinetic Studies by HPLC and LC-MS.

Organigram and Staff of ISS

S. Caroli, Director of the ISS-CRL, Director of the Analytical Chemistry Section of the Applied Toxicology Laboratory.

A. Di Muccio, Scientist Responsible for Organic Compounds, Director of the Section of Pesticide Residues of Applied Toxicology Laboratory (until February 1998).

R. Dommarco, Scientist Responsible for Organic Compounds, Director of the Section of Pesticide Residues of Applied Toxicology Laboratory (since February 1998).

M. Alessandrelli, Scientist, Member of the Unit of Chemical Elements (since May 1998).

G. Amendola, Scientist, Member of the Unit of Chemical Elements (until July 1998).

R. Cresti, Scientist, Member of the Unit of Chemical Elements (since May 1998).

S. D'Ilio, Scientist, Member of the Unit of Chemical Elements.

G. Forte, Scientist, Member of the Unit of Chemical Elements.

A. L. Iamiceli, Scientist, Member of the Unit of Chemical Elements (until April 1998).

R. Miniero, Scientist, Member of the Unit of Chemical Elements (until April 1998).

P. Pelosi, Scientist, Member of the Unit of Organic Substances (until April 1997).

M. Spagnoli, Scientist, Member of the Unit of Chemical Elements (since May 1998).

M. Delle Femmine, Technician.

C. Ferreri, Technician (since May 1998).

B. Galoppi, Technician (until April 1998).

Annex 4 – Workshops

RIVM-CRL

Title: Thyreostats in Farm Animals. Regulatory Residue Analysis within the EU Member States
Date: 7-9 April, 1997
City: Bilthoven
Participants: 16 Representatives of the NRLs of the EU Member State, 3 observers, 4 co-organizers

Proceedings: published

CNEVA-LMV-CRL

Title : Quantitative Determination of 7 Penicillin Residues in Muscle Tissue by HPLC
Date : September - October 1997
City : Fougères
Participants: 17 Analysts from NRLs of the EU Member States

Title : Determination of Tetracycline Residues in Pig Muscle Using LC-MS (Electrospray)
Date : March 1998
City : Fougères
Participants: 15 Analysts from NRLs of the EU Member States

Title : Meeting of the NRLs, Anti - microbial Residues in Food

Date : May 1998

City : Fougères

Participants: 23 Representatives of NRLs of the EU Member States

BgVV-CRL

Title: Establishment of Reference Methods

Date: 10-12 December, 1997

City: Berlin

Participants: 27 Analysts from the NRLs of the EU Member States

ISS-CRL

Title: Second Workshop on QC of Organochlorine Compound Determinations

Date: October 17-19, 1996

City: Rome

Participants: 14 Analysts of the NRLs of the EU Member States

Title: Third Workshop on QC of Trace Elements Determinations

Date: 15-17 November, 1997

City: Rome

Participants: 14 Analysts of the NRLs of the EU Member States

Annex 5

STAATSTOEZICHT OP DE VOLKSGEZONDHEID

VETERINAIRE HOOFDINSPECTIE

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF

GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 88/320/EEC the conformity with the OECD Principles of GLP was assessed on 15-16 January 1998 at

National Institute of Public Health and the Environment (RIVM)
Laboratory for Residue Analysis (ARO)
European Community Reference Laboratory
Antonie van Leeuwenhoeklaan 9, Postbus 1
3720 BA BILTHOVEN, The Netherlands

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following area of expertise: Analytical Chemistry.

Rijswijk, 16 March 1998

Th. Helder, DVM
Ministry of Health, Welfare and Sport
State Supervisory Public Health Service
Veterinary Public Health Inspectorate
GLP Monitoring Unit

VHI 4

Annex 6 - Glossary and Abbreviations

ANP	Annual National Plan
AISETOV	Associazione Italiana per lo Studio degli Elementi in Traccia negli Organismi Viventi
AOAC	Association of Official Analytical Chemists
ARO	Analytisch Residu Onderzoek
BERM	Biological and Enviromental Reference Materials
BCR	Bureau Communautaire de Référence
BgVV	Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin
BSA	Bovine Serum Albumine
CAP	Chloramphenicol
CPF	Caroprofen
CMCL	Central Meat Control Laboratory
CNEVA-LMV	Centre National d'Etudes Vétérinaires et Alimentaires, Laboratoire des Médicaments Vétérinaires
CRL	Community Reference Laboratory
CRM	Certified Reference Material
DAD	Diode Array Detector
DDT	Dithiotrertol
DC	Diclofenac
DMZ	Dimetridazole
EC	European Commission
EI	Electron Ionization
EEC	European Economic Communities
ELISA	Enzyme Linked Immuno - Specific Assay
EU	European Union
FAO	Food and Agriculture Organization
FBP	Fluribiprofen
FDA	Food and Drug Administration
FLU	Flunixin
FPT	Four Plate Test
FTE	Full - Time Equivalent
GC	Gas Chromatography
GC-MS	Gas Chromatography - Mass Spectrometry
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography

HR-ICP-MS	High Resolution - Inductively Coupled Plasma - Mass Spectrometry
IAC	Immuno - Affinity Chromatography
ICP-AES	Inductively Coupled Plasma - Atomic Emission Spectrometry
IRMM	Institute for Reference Materials and Measurements
ISO	International Standardisation Organization
ISS	Istituto Superiore di Sanità
ITCVDR	International Technical Consultation on Veterinary Drug Registration
IUPAC	International Union for Pure and Applied Chemistry
JRC	Joint Research Centre
LMBG	Lebensmittel- und Bedarfsgegenständegesetz
LC	Liquid Chromatography
LC-MS	Liquid Chromatography - Mass Spectrometry
MFAS	Mefenamic Acid
MRL	Maximum Residue Limit
MS	Mass Spectrometry
MNZ	Metronidazole
NFA	Niflumic Acid
NRL	National Reference Laboratory
NSAID	Non - Steroidal Anti - Infiammatory Drugs
OECD	Organization for Economic Cooperation and Development
OPB	Oxyphenbutazone
PBZ	Phenylbutazone
PDA	Photo - Diode Array
ppb	parts per billion
Q-ICP-MS	Quadrupole - Inductively Coupled Plasma - Mass Spectrometry
QA	Quality Assurance
QC	Quality Control
RAL	Resorcylic Acid Lactone
RFL	Routine Field Laboratory
RIA	Radio Immuno - chemical Analysis
RIVM	Rijkinstituut voor Volksgezondheid en Milieu
RNZ	Ronidazole
RSD	Relative Standard Deviation
SA	Salicyilic Acid

SEC	Size - Exclusion Chromatography
SFE	Supercritical Fluid Extraction
SMT	Standards, Measurements and Testing
SMZ	Sulfametrazine
SOP	Standard Operating Procedure
SXB	Suxibuzone
TLF	Tolfenamic Acid
VDP	Vedaprofen
VPHI	Veterinary Public Health Inspectorate