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The four EU Community Reference Laboratories (CRL) for Residues.

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I. Introduction

The powers and conditions of operation of the four European Union Community Reference Laboratories (CRLs) for residues were established through the Council Decisions 89/187/EEC and 91/664/EEC. These legal provisions, full text of which is attached to this report as Annexes 5 and 6, respectively, prescribe the performance of a number of tasks to the benefit of the European residue control system and in particular of the National Reference Laboratories (NRLs) for residues in the Member States, Third Countries as well as of the European Commission. In this connection emphasis is laid upon the fact that the activities carried out by the four CRLs over the period August 1, 1995 - July 31, 1996, witnessed further progress in the integrated approach adopted to implement their duties. This positively impacted on the NRLs perception of the role played by the CRLs as a unitary 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 first Joint Annual Report of the four residue CRLs has been drafted following the guidelines as agreed upon during a special coordinative meeting of the four Directors of the CRLs in Rome, 6 September 1996.

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 involved EC Services, the EC Services and the Member State and Third Country Administrators and, last but not least, between the four residue CRLs themselves.

Each CRL processed its part of the draft report electronically in Microsoft WORD (TM), which parts subsequently have been joint and edited by the RIVM-CRL in consultation with the Commission.

This report is available on request either directly or via the CRLs from the European Commission at Brussels, Belgium.
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1 General

At RIVM all scientific and professional activities are planned and performed according to a strictly project-based (multi)annual programme. All staff capacity involved is recorded and monitored in a central time registration system.

The CRL project is nearly fulltime performed within the Laboratory for Residue Analysis (ARO), which is a part of the RIVM Division for Analytical Chemical Laboratories.

ARO primarily is a research and development facility and is staffed with about 40 people. It has a long lasting history of experience in the field of residue and trace analysis for, amongst others, growth promoters, veterinary and human drugs and natural toxins. Whenever possible, ARO cooperates in multi-disciplinary and/or (inter)national multi-laboratory programmes like projects within the EU (SMT/BCR, AIR, HCM, Eurachem) or global projects of organizations like IUPAC, AOAC International, WHO/FAO and IOC.

As required by the implemented QA systems and to avoid any possible conflict of interest with the NRL functions of ARO, the CRL laboratory part is completely separated from the NRL part.

2 Method Development

A method for analyzing gestagens in kidney fat was developed and validated. Chlormadinone, megestrol, melengestrol and medroxyprogesterone and their respective acetates are analyzed now in a multi-residue procedure with a solid phase and HPLC clean-up. Final determination is performed on a GC-MS. The analytical procedure is described in a SOP and a RIVM report.

A multi-residue method for 17 anabolic compounds was developed and validated. The method was presented and demonstrated during a workshop. The method exists of an automated solid phase clean-up coupled to an HPLC system. Subsequent screening is performed on a GC-MS. The detection limit for the screening of these substances is approximately 1 ppb.

A publication on this topic was presented during the EuroResidue III Conference. A SOP and a RIVM report are in preparation.

The development and implementation of a method of analysis for thyreostats was postponed to the second half of 1996 due to other priorities.

The research in the analysis of trenbolone in samples of urine is ongoing. Trenbolone is one of the compounds most difficult to analyze for efficiently. This was also concluded by the participants of the workshop.
Methods of analysis for the natural hormones were extended and validated. The report of the ring test for estradiol in serum was finalized and send for comments to all participants.

Finally, the CRL anticipated to the new substance groups tranquillizers (sedativa) and corticosteroids. The development and validation of a method for synthetic corticosteroids in animal feed was started.

3 Other Research

The preparation of a “Bank of Blank Reference Urines” was completed. Now sets of reference samples are available for Bovine (N=20), Ovine (N=20) and Porcine (N=10) lyophilized urine. The sets were analyzed with the multi-residue method of analysis. Datasheets for all sets and a report on the preparation are available. The sets can amongst others be used for method validation and for proficiency testing.

A feasability study into the possibility of producing other sets of blank reference samples, e.g. serum or tissues, is still ongoing.

Reference materials for trenbolone in urine were prepared. Incurred material was used. The homogeneity of the materials was tested. For this homogeneity test the method of analysis for trenbolone had to be modified and validated. A study on the stability of the reference materials is still ongoing. Data for a half year storage are available.

The SMT project “Bank of Reference Standards” was finished. This “Bank” contains isotope enriched (mainly deuterated) standards and a number of non-deuterated veterinary drugs and metabolites. The CRL is coordinating the QA and the shipment of the materials. The number of compounds is under extension now with relevant anabolic steroids.

Research is being continued as regards the natural occurrence of alpha-nortestosterone in different species of pregnant animals like sheep, goat and mares.

Research is being continued as regards the natural occurrence of the zeranol-related compounds (RALs) like the metabolite beta-zearalanol and the metabolite of the f2-toxin: alpha-zearalenol.

The metabolism of boldenone and the possible natural occurrence of this compound was studied. A publication was presented during the EuroResidue III Conference.

Research into the occurrence of “new” and/or “unknown” compounds is continuously ongoing to update what is “going on” in the “black market scene”. Especially here communication with and input from the related NRLs proved to be very useful.
4 Quality Assurance

The two operational quality systems within the CRL (EN45001 and GLP) were maintained during the contract period. The further introduction and maintenance of QA/QC in NRLs was actively and continuously supported, mostly at the request of NRLs.

A second Quality Assurance inventory, a follow-up of the first one held in 1994, was organized among nearly 40 NRLs. This inventory has to investigate the progress made during the past 2 years. The results can be used during discussions within DG VI and between DG III and DG VI about the appropriate system for both food and residue control. Also ongoing discussion with DG XII (SMT/BCR) and with Third Countries (eg USA, Australia, Bulgaria and Argentina) can benefit from this inventory. The report on the inventory is in preparation.

On behalf of the RIVM Board of Directors the CRL itself was audited for its scientific competence by an independent team of international scientists in March 25-27, 1996. The overall conclusion of the audit team was, amongst others, that the CRL-NRL system provides an adequate system to stimulate and co-ordinate residue control programmes within the EU, both scientifically and economically (value for money). The scientific competence of the CRL was judged as "on top of the state of art". It was recommended that the EC in cooperation with the CRLs develop and implement a transparent system of auditing the scientific competence of NRLs. A full report of the scientific audit is available on request from the CRL.

The CRL was audited at Bilthoven by the Commission for professional, technical and financial compliance regarding the second contract period at January 18-19, 1996.

Audits of NRLs were performed in Portugal (2 laboratories). The NRLs in Finland, Sweden and Austria were visited for a first orientation visit. Audit and visit reports were prepared and are available.

The CRL trained two visiting QA-officers of Spain and Argentina.

A ring test on estradiol in serum was organized during the winter. Laboratories using immunoochemical procedures or procedures based on gas chromatography-mass spectrometry participated in this study of which the draft report was send to the participants for comments. The agreement between laboratories and between methods was rather good.

Within the framework of the SMT-programme a ring test "zeranol in muscle, kidney and liver was organized. Evaluation of the results is scheduled for December 1996.

The organization of a ring test for trenbolone and nortestosterone is planned for the winter of 1997.

A multi-residue ring test was organized prior to the annual workshop for NRLs.
5 Technical and Scientific Support to NRLs and Third Countries

Analytical assistance was given to Austria and Portugal for the confirmation of results they found in screening. This concerned natural hormones, zeranol and thyreostats.

Analytical support was also given to Cyprus, a Third Country, for quantification and confirmation of testosterone, trenbolone and zeranol in samples of urine and meat.

An arbitration case between two Third Countries about DES analyses was handled by the CRL on request of the EC DG VI.

Still ongoing is an arbitration case between two Member States about natural levels of steroid hormones.

Over 60 requests were received for advice, reference standards or training. In total 55 batches of reference substances were distributed of which 46 to EU Member States.

The electronic catalogue of analytical methods “CB\METHODS” was further improved and extended, amongst others, with regulatory data. On numerous occasions scientists were made aware of the existence and possibilities for use of this catalogue. At the EuroResidue III Conference 150 copies of a “read only” demo version of this database on diskette were supplied to the participants parallel to the presentation of a poster on the topic.

6 Workshops and Scientific Events

From 20-23 May 1996 the annual workshop was organized with the title: “Evaluation and validation of multi, residue procedures for anabolic compounds”. Prior to the workshop the NRLs for anabolic compounds participated in a small ring test. The results of the analysis and the performance of the laboratories were evaluated during the workshop. 17 Participants from NRLs representing all EU Member States participated in this workshop.

Hands-on work was done on the analysis of the same samples as were sent to the NRLs with the method developed by the CRL. Discussions on methods, validation analysis and performance were held in small groups and evaluated later in the whole group of participants.

Conclusions and recommendations were drawn up at the end of the workshop and proceedings will be prepared.

A report was prepared on the evaluation of this and the previous workshop.

EuroResidue III Conference on Veterinary Drug Residues.

The CRL played a leading role in the organization of this very successful, meanwhile major and global scientific event in Veldhoven, The Netherlands, on May 6-8, 1996. Nearly all CRL staff contributed and/or participated to the Conference. Due to the very regrettable decease of the Chairman of the Organizing Committee (Dr. Nel Haagsma, Veterinary Faculty, University of Utrecht, NL), the CRL also had to support the technical organization.
7 Technical and Scientific Assistance to the European Commission

Demo diskettes and updates of the database CB\METHODS containing methods of analyses for veterinary drugs, pesticides, heavy metals and anabolic compounds were supplied to the Commission.

Support has been given for the evaluation of the Annual National Plan, e.g. by clarifying the meaning of analytical limits used.

Contribution (two papers) to the “EC Hormone Conference” at Brussels, B, November-December 1995 were made.

A paper and an additional file with documentation on the progestative steroid Melengestrol acetate (used legally as growth promoting feed additive in the USA) has been supplied to the Commission in December 1995.

Papers were supplied on violative Estradiol levels in calf urine, on the natural occurrence of the steroid Boldenone in calves and on a new multi-residue procedure.

Advice and support were given to the Commission for the revision of the Directive 86/469/EEC and related directives. The new Council Directives 96/22/EC and 96/23/EC were adopted in April 1996.

Co-ordination of the CRL-NRL expert group for revision of the criteria was performed. For this revision the working paper “Reliability of Residue Analysis; for What Purpose and What Price” was drafted. For the hot item of “Reliability” or its complement “Uncertainty” in qualitative analyzes (as applicable to most residues in the CRLs mandate) other organizations with related interest, like Eurachem, IOC and USA-FDA, were consulted and/or stimulated to support the revision process. Due to a considerable amount of “pioneer work” to be done and due to the very severe consequences of the revised criteria in the context of the new Council Directives, the revision process proceeded only slowly.

The CRL participated in various coordinative or expert meetings with the Commission in Brussels.

The CRL participated in or was consulted with regard to two related EC DG VI AIR-projects.
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1 General

Amongst the official functions of the CRL the main expectations of the NRLs concerning the work to be done by CRL-Fougères and decided two years ago at the meeting in May 1994 are:

To give advice and to develop analytical methods
To harmonize the analytical methods
To organize ring tests
To organize training sessions
To coordinate research activities

Taking into account these needs, the program defined in June 1995 for the third year allowed the reinforcement of the relations with the NRLs.

2. Method Development

In our field we have a broad diversity of analytes and the development of new analytical procedures using high performance liquid chromatography (HPLC), mass spectrometry (MS) and microbiological assays is very important. This effort has to be continued because in the near future our responsibilities will be extended to others substances (sulphonamides, carbadox, olaquindox and dyes) and other animals or animal products (poultry, aquaculture, milk, eggs, honey and meat of rabbit and wild game) following the new Council Directive 96/23/EC.

Development using HPLC
- Penicillins: a multi-residue method for 7 analytes in bovine muscle was validated (amoxicillin, ampicillin, penicillin G, penicillin V, oxacillin, cloxacillin, dicloxacillin) - E. Verdon and P. Couëdor.
- Tetracyclines: validation of a protocol in order to prepare the ring test - M. Juhel-Gaugain and B. Anger.
- Macrolides: we have just started this development at the end of this third contract - M. Juhel-Gaugain.

*Development using mass spectrometry*

Our research ended with the development of two methods concerning ampicillin and tilmicosin in bovine muscle - D. Hurtaud-Pessel.

### 3. Other Research

Besides the short term research and development we developed research at the mean term:

*Screening of antibacterial substances using agar diffusion test*

With two objectives in mind, to improve the quality of this kind of test in order to realize monitoring plan and to prepare the meeting of June 11 and 12, the topics are the screening and post-screening methods. The utilization of other culture media and bacterial strains allowed the improvement of this kind of test close to the maximum residue limits (MRLs) in order to reduce the "false-negative" results - R. Fuselier and J.L. Ribouchon.

### 4. Quality Assurance

*Organization of two ring tests*

According to the conclusions of the workshop held in Fougères on June 13-14, 1995 two ring tests were carried out on May and June 1996.
High Performance Liquid Chromatography Proficiency Test on oxytetracycline in porcine muscle:
15 Laboratories agreed to participate and 8 coded frozen samples were dispatched. The results obtained were globally satisfactory: most of the laboratories using their own method were able to measure oxytetracyclin in accordance with the required accuracy and repeatability - M. Juhel-Gaugain and B. Anger.

Mass Spectrometry Proficiency Test on tetracyclines:
7 Laboratories agreed to participate and 6 coded frozen samples were sent. Finally, very few of them gave results. A report will be sent in September 1996 - D. Hurtaud-Pessel.

Analysis certificate for antibiotics standard.
During the last meeting of the NRLs held in Fougères we agreed upon the task to solve problems concerning the use of antibiotic standards, in particularly by the definition of the analytical specifications of these products. The NRLs received a draft from our laboratory in July 1996 for further discussion. An agreement upon a model of certificate of analysis could be found in October. - E. Verdon

Reference materials
In the framework of a project of the Standards, Measurements and Testing Programme (SMT) we organized two circular tests "Oxytetracycline residue in milk" and "Neomycin residue in milk" and the participants came to Fougères in January 1996 to discuss and comment the results. In the same framework the stability study is being carried out for a third reference material "Ampicillin residues in milk".
In other respects, the CRL sent to the NRLs a questionnaire to know their needs in this field: the reference materials the most frequently quoted are: oxytetracycline, tetracycline and penicillin G in muscle.
Our laboratory also participated in the "Chloramphenicol Interlaboratory Study 3/95" purpose of which was to demonstrate the reliability of the analytical results.

Audits
In order to better know the organization of the NRLs and to evaluate their analytical capabilities, three of them were visited: Denmark, Austria and Greece. The reports are available.
5. Technical and Scientific Support to NRLs and Third Countries

Update of the list of the validated methods (HPLC. and LC-MS) used by NRLs

One of the conclusions of the workshop held in June 1995 was to set up a list of methods used as confirmatory analytical methods or in development through the 15 countries in the EU. To complete, to modify or to add any new information (e.g. new methods or new level of validation), the CRL sent the first list in May 1996 in order to provide the updated list in September 1996.

Training courses

With the purpose to organize training courses for the next two years, a questionnaire was sent to the NRLs. From the six answers obtained, the most important topic turns to the analyse of tetracyclines or penicillins with HPLC, combined or not with mass spectrometry.

Technical assistance to NRLs and Third Countries

On the request of Portugal, we analysed for identification and quantification oxytetracycline residues in two samples of bovine muscle.

In other respects numerous documents, analytical procedures and standards were sent to the NRLs and to third countries (Israel, Slovakia, Thailand, Canada, Morocco, Botswana, Hungary, India, etc.).
6. Workshops and Scientific Events

On June 11-12, 1996, the CRL of Fougères organized a meeting on "Screening and post-screening tests to detect antibacterial residues in meat". 18 Representatives from the NRLs participated and discussed the following topics: the last ring test organized in 1995, determination of the criteria of a common test, comparison of different post-screening tests using microbiological assays and definition of the requirements of the ELISA techniques to detect antibiotic residues. A report will be sent in September 1996.

Among the main conclusions it was decided to choose two bacterial strains for a first common European test and a ring test is planned for the winter of 1997.

In the framework of the Euroresidue III Conference (May 1996), five scientists of our laboratory presented their research work through one oral and four posters contributions (Annex 1).

7. Technical and Scientific Assistance to the European Commission

The CRL participated in coordinative and expert meetings with the Commission in Brussels.
IV. Activities of the Community Reference Laboratory at the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV)

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1. General

The CRL for residues of ß-agonists, chloramphenicol and sulfonamides is allocated within the BgVV in the unit “Residue Control Reference Laboratory“ of the division “Animal Drug Registration, Residue Control and Feed Additives“. The analytical activities of the CRL Berlin are pursued by three specialized sub-units (see Annex 3) :

- GC and GC-MS,
- HPLC and LC-MS,
- Immuno-Assays.

2. Method Development

According to the plans of the Commission to integrate new substance groups within the residue control plans and to rearrange the responsibilities of the CRLs for certain groups of substances - as now layed down in Council Directive 96/23/EC from 29 th April 1996 - the CRL made efforts to prepare the practical application of this Directive. For this reason it started to establish analytical methods necessary for the substance groups then newly assigned to its responsibilities, namely non steroidal anti-inflammatory drugs (NSAIDs), anthelmintics and anticoccidials including nitroimidazoles.

**GC-MS**

Some further ß-agonists (clenproperol, brombuterol, ritodrine) were analytically investigated and included in the general screening programme.

The existing analytical methods for chloramphenicol were adapted to the matrices plasma and urine.

The establishment of an adequate confirmation method for nitroimidazoles by GC-MS is in preparation.
**HPLC, LC-MS**

A screening method for the analysis of 10 non-steroidal anti-inflammatory drugs (NSAIDs) commonly used in veterinary practice in plasma by HPLC-DAD-detection including a sample preparation method for plasma was established.

Furthermore efforts are focused on the development of a new confirmation method of NSAIDs by LC-MS/APCI-technique. The results so far obtained reveal, that not all of the investigated substances may be ionized under negative ion electrospray conditions. However, a number of important NSAIDs like phenylbutazone, flunixin, oxyphenbutazone and diclofenac are particularly suited for the detection and identification under these conditions. For these substances one can observe in the SIM mode a sensitivity higher than that achieved by UV- or PDA-detection.

The first results of this work were presented during the EuroResidue III - Conference in May 1996 in Veldhoven/NL.

A method for the chromatographic separation and simultaneous screening of avermectines (abamectine, ivermectine, moxidectine and doramectine) by HPLC-DAD-detection was introduced in the HPLC/LC-MS laboratory.

**ELISA**

ELISA screening methods for the detection of clenbuterol in muscle, liver and kidney including an appropriate extraction procedure were established and validated. The minimization of matrix effects gave rise to a limit of detection of 0,03 ng/g (liver, muscle) and 0,13 ng/g (kidney), respectively. The method had been successfully used for the analysis of a large number of samples resulting from the pharmacokinetic study of clenbuterol in veal calves. Moreover, a screening method for the detection of β-agonists of the aniline-, phenol- and resorcinol type in poultry liver was established and validated. Depending on the different polarities of the investigated substances the recovery is between 50 and 95 %.

The development of an ELISA-test for the determination of nitrofuranes in animal tissues and in body fluids yields promising interim findings - so far sensitive antisera were produced.

**3. Other research**

*Residue kinetic study of clenbuterol in veal calves*
Besides the general objective of method development and research, during the period under review, a certain part of the analytical capacity was taken by the high number of samples coming from the pharmacokinetic study of clenbuterol in veal calves.

The research project concerning residue kinetics of clenbuterol in veal calves has continued by its second part testing dosages which are said to be used in illegal veal calf fattening.

For this purpose 31 veal calves were treated with different dosages of clenbuterol and slaughtered on 8 slaughtering days. Samples were taken muscle, liver, bile, kidney, fat, hair, eye, plasma, urine and faeces. The analysis of these samples carried out by ELISA and by GC-MS has not yet been finished.

The results so far achieved prove that after the application of a fattening dose the clenbuterol concentration in liver fell below 0.5 µg/kg as early as after approximately 14 days after the end of the treatment. The preliminary results of muscle (proposed MRL 0.1 µg/kg) show an even earlier falling below this level. Actually the third (final) part of the study is in preparation.

4. Quality Assurance

Proficiency testing

44 European official residue control laboratories from 12 EU Member States participated in the “Chloramphenicol Interlaboratory Study 3/95”. It was the aim of the study to determine chloramphenicol (CAP) in incurred plasma and urine samples providing the participating laboratories with an objective means of assessing and demonstrating the reliability of the analytical results they produce.

The study was designed and evaluated according to the "International Harmonised Protocol for the Proficiency Testing of (chemical) Analytical Laboratories" jointly elaborated by ISO, IUPAC and AOAC.

Immunochemical methods like ELISA and RIA were predominantly employed for screening analyses. For the confirmation of CAP results the participants mainly used GC-MS with negative chemical ionisation apart from GC-MS-EI and GC-ECD.

Out of the usable screening results only 3 urine samples proved to be false positive but 22 plasma samples had false negative results (i.e. 16 % of urine and 21 % of plasma samples). Concerning the confirmatory results out of 175 examined positive urine and 148 positive plasma samples 28 urine and 30 plasma samples could not be confirmed (i.e. 16 % of urine and 20 % of plasma
samples). Even taking into account the rather low concentration of some samples there still remained 12 false negative samples (i.e. 7 %) with CAP concentrations above 1 µg/l.

Good results obviously may also be achieved if less sensible analytical systems are duly optimized. A sensible and specific analytical system does not automatically guarantee good results. In general the results reveal that considerable differences exist between the European residue control laboratories analytical performance. It may be assumed that the comparability of analytical results is not yet assured in the European residue control system. In spite of the high number of satisfactory results achieved in this study the number of false negative results underlines the need for further co-operation accompanied by interlaboratory studies to be carried out in regular intervals.

An interlaboratory study for the detection of β-agonist residues in bovine plasma and liver was organized in May 1996 for the NRLs of the Member States. The evaluation of this study will be done during the next period under review.

*Audits*

The visits of the NRLs in Finland and Sweden allowed an evaluation of their analytical capacities and performance.

The NRL of Sweden revealed a high level of professional and analytical performance. The established quality assurance system is convincing. The laboratory represents a very good standard as a European residue control laboratory.

The NRL of Finland with a highly qualified and motivated staff is well on the way to establish an efficient reference laboratory for residues on a high level in a European scale.

*Sample treatment*

As a contribution to the establishment of an adequate sampling strategy the impact of sampling and of different conditions of storage, transport and sample treatment was investigated using clenbuterol as an example.

No significant differences concerning the distribution of clenbuterol in the liver lobes could be found. Clenbuterol concentrations found in the medulla of the kidney were higher than that in the cortex. Differences in residue concentrations between the left and the right kidney can be excluded. The very low coefficients of variation of the averaged results during a 5 months storage period of both liver and muscle show that an analysis can be repeated after several months
without losses of the analyte provided that the sample has been stored non-homogenised at low temperature.

Standards Measurements and Testing (SMT) Programme
Within the framework of the SMT-Programme of the European Commission the CRL participated in different projects. A certification study of reference material for clenbuterol and salbutamol in urine carried out under the collaboration of 26 laboratories was organized. The CRL participated in a collaborative study “Clenbuterol and salbutamol in feeding stuffs” organized in the framework of another SMT project.

5. Technical and Scientific Support to NRLs and Third Countries

Analytical Support
During the period under review the CRL carried out confirmation analyses of chloramphenicol and of β-agonists in samples sent by the NRLs of Austria and Portugal. Technical advice was given to a number of NRLs regarding analytical questions concerning the analysis of chloramphenicol and β-agonists

Training
During the period under review 6 trainees coming from Europe, Africa, South America and Asia have been introduced in special analytical operations.

Standard Substances
Provision and distribution of standard substances for the detection of β-agonists and chloramphenicol to the NRLs has been extended during the reported year.
6. Workshops and Scientific Events

CRL-Workshop in Berlin

From 3rd to 5th July 1996 the CRL in Berlin organized a workshop “β-agonists: method validation and surveillance” which brought together 20 analysts of the NRLs of the EU Member States. The workshop was intended to contribute to the harmonisation of the validation procedures, which is a fundamental requirement and a prerequisite for the comparability of analytical results in the European Union and to draw the attention to new emerging aspects of β-receptor research which seem to be of interest for residue control.

Two invited speakers presented a key lecture to the main topics of the workshop: Prof. M. Thompson from the Birbeck College, University of London, on “Uncertainty in analytical measurement” and Prof. D. Strossberg, Institut Cochin, Université de Paris, “Function and regulation of the different β-receptors” - the latest findings of β-receptor research.

The CRL Berlin presented a draft concept of a statistically safe validation procedure allowing to verify the performance of analytical methods.

This concept as well as the individual approaches of the participants to method validation outlined by 8 participants as an example were discussed within sub-groups and in a plenary session.

The workshop participants agreed on the necessity to establish a model to harmonize the validation of analytical methods. This model should be applicable to the different analytical techniques and should provide the laboratories with sufficient information to assess the methods performance level by means of objectifyable parameters.

EuroResidue III in Veldhoven

Collaborating in the scientific committee of the EuroResidue III Conference in Veldhoven the CRL Berlin supported the preparation of this event and made efforts to introduce the CRL-system as well as to present the scientific results of its research work by 4 oral and 2 poster contributions.
7. Technical and Scientific Assistance to the European Commission

Working groups of the European Commission and the Council

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

Viale Regina Elena 299, Rome, Italy,

telephone **6-49902052, telefax **6-49902366, telex 610071 ISTISAN, E-mail crl@istsan.interbusiness.it or caroli@istsan.interbusiness.it

1 General

The Community Reference Laboratory for Residues at the Istituto Superiore di Sanità (CRL-ISS) is responsible for two broad categories of chemicals, namely trace elements (in particular arsenic, cadmium, lead and mercury) and organochlorine and polychlorobiphenyl compounds. The CRL-ISS consists of twelve people, four of which are on the regular staff of ISS and eight are hired.

2. Method development

In fulfilment of the mandate of the CRL-ISS, several investigations were undertaken to develop and improve present methods of analysis for the assigned residues. In particular, an experimental study devoted to the assessment of the capabilities of Anodic Stripping Voltammetry was undertaken in order to evaluate its applicability to environmental analytical studies as an alternative to atomic spectrometric and mass spectrometric techniques. The content of cadmium and lead in some environmental certified reference materials was quantified by using the Hanging Mercury Drop Electrode. The experimental results show that the instrumental detection power is inadequate to determine the concentration values of the above elements in real world samples. The use of buffers (such as sodium acetate, citric acid, ammonium citrate and sodium chloride) added to the specimens under study does not seem to improve the performance of this technique for the levels of analytes expected in the matrices of interest. The same results were also obtained for samples where hydrochloric acid or ammonia were added to modify the pH values of the solutions.

Further investigations were carried out as regards non-instrumental techniques for the separation of organochlorine pesticide residues from fatty matrices. In the new approach followed the classical separatory funnel hexane:acetonitrile partition step for the said separation has been adapted so that it can now be performed on disposable cartridge filled with a wide-pore diatomaceous material. The solid-matrix partition step developed offers some advantages
compared to classical schemes. Among these mention should be made of the simple and fast operation, the reduction of solvent consumption and the use of disposable items. A manuscript on this subject has been submitted to *J. Chromatogr*.

### 3. Other research

An investigation was started to ascertain the conditions under which the concentration of mercury can be considered to remain substantially unaltered. In particular, pure aqueous solutions as well as solutions enriched with various chemicals to simulate the digestion process were taken into account. This study was performed on the basis of the findings of the first interlaboratorial trial, namely, the difficulties encountered by participants in analysing mercury solutions in aqueous nitric acid which were unstable in the medium term. To solve or alleviate this problem the effects of various amounts of nitric acid and potassium dichromate (alone or in combination) were elucidated by measuring the variations with time in the actual concentration of mercury. Determinations were made both by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Mass Spectrometry (ICP-MS).

Results show that solutions added with 5 % (w/w) nitric acid and 0,01 % (w/w) potassium dichromate do not undergo any significant alteration in the nominal content of the analyte for at least one month. Similar conclusions are attained in the case of solutions enriched in calcium carbonate, sodium chloride, sodium nitrate, potassium orthophosphate, potassium nitrate and magnesium nitrate to mimic acid digests of meat samples. These findings confirm that the use of oxidising agents together with strong mineral acids can be recommended in order to guarantee preservation of mercury at trace and ultratrace levels. In addition, the presence of major inorganic components of biological samples after their acid digestion does not interfere negatively with the stability of mercury solutions when the analyte is present at such low concentrations. Experimental results and conclusions are illustrated in a manuscript entitled “Stability of Mercury Dilute Aqueous Solutions: An Open Issue” by S. Caroli, G. Forte, A. L. Iamiceli and A. Lusi, published in *Microchem. J*.
4. Quality Assurance

A new phase of the quality control exercise has been organised and conducted. Fourteen National Reference Laboratories and five additional public laboratories (included in the exercise upon their request) took part in the collaborative test. This trial included the repetition of the analysis of acid aqueous solutions containing arsenic, cadmium, lead and mercury (Phase 1.1a) and the analysis of digestion-simulating samples (Phase 1.2a) with the same elements at various concentrations. Participants received sixteen specimens for each phase. These contained trace elements at known and unknown levels. Statistical treatment of data and evaluation of the laboratory capabilities were discussed during the Second Workshop held in Rome on May 9-11, 1996. On that occasion a general improvement of the capabilities of NRLs in analysing trace elements was highlighted. If the two sets of results are compared (Phase 1.1a and 1.2a) it clearly emerges that the general outcome of Phase 1.2a was significantly better than the former phase. As regards the determination of mercury, this is possibly due to the better stabilising effect of the addition of potassium dichromate. Main sources of error found during the trial were evaluated, such as the use of blanks containing appreciable amounts of the analytes, old calibrants, calibrants prepared without the previous additions of salts and / or potassium dichromate as stabilisers, errors in calculating the dilution factor, etc.

The preliminary organisational steps were undertaken as regards the second ring test on organochlorine compounds involving sixteen National Reference Laboratories to implement the decisions taken during the previous meeting held in Rome, May 4-6, 1995. For the exercise, solutions of fifteen organochlorinated pesticides in “blank” Soya oil was prepared, each compound being at concentration in the order of magnitude of 50 µg/kg. Each participating laboratory received three ampoules containing the “blank” Soya oil, the spiked oil and a concentrated standard solution of the fifteen organochlorine compounds.
Each laboratory was asked to analyse two subsamples of the spiked oil. Results have been received and are being processed and assessed for presentation and discussion at the next meeting of the National Reference Laboratories.

5. Technical and scientific support to NRLs and Third Countries

The most recent and innovative literature references were scanned in order to update the data bank of analytical methods for inorganic residues. The greatest care was devoted to the elaboration, for each publication selected, of a short free text highlighting the novelty brought about by the study and to the harmonisation of key words for quick and unambiguous retrieval of subgroups of papers, technical reports and the like. The information contained therein is made available upon request to all interested National Reference Laboratories. An ad hoc designed computer software to host the data bank was developed in conformity with the programmes prepared by the CRL at the RIVM.

Moreover, it was possible to set up the complete version of the handbook collecting the methods in use at the National Reference Laboratories for the determination of trace elements (in particular arsenic, cadmium, lead and mercury) and a revised version of the handbook for the determination of organochlorine compounds. These manuals, supplied to all National Reference Laboratories, form the core of an information system to be constantly expanded and updated for enhancing harmonisation of analytical approaches among the National Reference Laboratories as well as for providing each of them with a unique reference source.

6. Workshop and scientific events

Participation in the following conferences:

1. First Mediterranean Basin Conference on Analytical Chemistry, Córdoba, November 5-10, 1995 (organisation of a session and presentation of a plenary lecture on the activities of the CRL at ISS);
2. VII Italian-Hungarian Symposium on Spectrochemistry - Innovative Methodologies for Health and Environmental Protection, Rome, November 27- December 1, 1995 organisation of the conference with the preparation and presentation of two contributions on the role of the CRL at the ISS and on the stability of mercury dilute aqueous solutions, respectively;

3. EuroResidue III, Veldhoven, The Netherlands, May 6-8, 1996 (collaboration to the presentation of a key-note lecture);

4. Second Workshop on Quality Control of Trace Element Determinations, Rome, May 9-11, 1995 (organisation and conduct of the meeting to assess the performance of the NRLs in the framework of the second exercise on Analytical Quality Control of trace elements (see Section 4 above);

5. XIX National Conference “Ricerca e Tecnologia”, Riccione, June 9-14, 1996 (presentation of a lecture on analytical quality control for trace elements);

6. First European Pesticide Residue Workshop, Alkmaar, The Netherlands, June 10-12, 1996 (organisation of the event with the preparation and presentation of a contributions on non-instrumental separation of organochlorine pesticide residue from fatty materials).

7. **Technical and Scientific Assistance to the European Commission**

Participation in the Co-ordination Meetings of the four CRL Directors (Brussels, September 12, 1995; February 13, 1996; June 18 1996).
VI Annexes

Annex 1 Publications & reports

Publications & reports of the RIVM (number with postscript “n”)

RIVM list of productions of CRL-ARO for the 3rd CRL contract year as extracted from the ARO Management Information System ARO-MIS CBARODOC. Documents are unambiguously identified by their ARODOC-nr.

   Extraction of anabolic steroids from bovine urine using coupled SPE-SFE-SPE technique
   **Abstract** Combined 3rd European symposium on analytical SFC and SFE & the 6th international symposium on SFC and SFE, Uppsala (SE), 6-8 September (1995).
   ARODOC-nr 13804

2n. Stephany, R.W. and L.A. van Ginkel
   European Union Community Reference Laboratories for regulatory residue analysis of veterinary drugs: A strategic system on search for reliability targets
   **Abstract** Eurachem meeting, Prague (CZ) May 8 - 10 (1996) [room document pp 4].
   ARODOC-nr 13927

3n. Stephany, R.W. and L.A. van Ginkel
   Analytical strategies to survey the veterinary (mis)use of hormonal anabolic agents in food animals
   ARODOC-nr 13928

4n. Stephany, R.W. and L.A. van Ginkel
   Regulatory monitoring in the European Union for residues of veterinary drugs
   **Abstract** KNCV "chemie & recht" najaars-symposium, Ede (NL) 9 November 1995.
   ARODOC-nr 13929 (Proceedings in print).
5n. Kamp, C.G. van de
Read only Demo Version of CB\METHODS

**Diskette** 1.44 Mbyte read only, May 1996. ARODOC-nr 16207

6n. Berg, A. van den (Editor)
Kwaliteitshandboek ARO uitgave 960116 [ QA Manual ARO 1996 ]

**Handboek** RIVM-ARO (1996) pp 73 *(manual)*. ARODOC-nr 16283

CB\METHODS A CRL catalogue for residue analyses

**Poster** EuroResidue III Veldhoven, (NL), 6-8 May (1996). ARODOC-nr 16209

Extraction of anabolic agents from biological matrices by using a combined sfe/spe technique

ARODOC-nr 13806

Extraction of zearalenone, zeranol and their metabolites from fortified bovine urine using a coupled SPE/SFE technique

**Poster & handout** 7th International symposium on SFC and SFE, Indianapolis, IN (US) March-April 1996. ARODOC-nr 16540

10n. Ginkel, L.A. van and R.W. Stephany
Residue analysis of anabolic compounds; the implementation of control strategies

European Reference Laboratories for residue analysis a quality challenge
**Ware(n)-chemicus** 25 (1995) 124 (**abstract of poster**). ARODOC-nr 14780

12n. Ginkel, L.A. van and S.S. Sterk
Evaluation and validation of multi residue procedures for anabolic compounds
**Workbook** EU workshop at RIVM May 20-23 1996, Bilthoven (NL) pp 49. ARODOC-nr 14796

13n. Maxwell, R.J., O.W. Parks, A.R. Lightfield and A.A.M. Stolker
Recovery of trace level veterinary pharmaceutical residues from biological matrices
**Proceedings** 7th International symposium SFC-SFE, Indianapolis, IN (US) (1996) l-16 pp 2. ARODOC-nr 13808

Extraction of zearalenone, zeranol and their metabolites from fortified bovine urine using a coupled SPE/SFE technique
**Proceedings** 7th International symposium SFC-SFE, Indianapolis, IN (US) (1996) c-15 pp 2. ARODOC-nr 13809

15n. Stephany, R.W. and R.C. Schothorst
EU-inventory on QA-systems. Part 1: format items and applicability

16n. Schothorst, R.C. and R.W. Stephany
EU-inventory on QA-systems. Part 2: results, conclusions and recommendations

17n. Stephany, R.W.
European Community Reference Laboratories and quality assurance: History and backgrounds


18n. Stephany, R.W. and R.C. Schothorst
RIVM ARO-CRL experiences with GLP and EN 45001
389002 024 CRL document chapter 29 (1995) 250 - 258. ARODOC-nr 15001

19n. Stephany, R.W., [G. Pottie and R. Wennig]
Quality assurance of Benelux regulatory analysis for residues of illegal veterinary anabolic agents
389002 024 CRL document chapter 30 (1995) 259 - 263. ARODOC-nr 15541

20n. Sterk, S.S.
Presentation of CRL for hormonal and veterinary anabolics at the National Institute of Public Health and Environmental Protection in Bilthoven, The Netherlands
389002 024 CRL document chapter 4 (1995) 33 - 34. ARODOC-nr 13914

21n. Stephany, R.W. and L.A. van Ginkel
Yield or Recovery : A world of difference
ARODOC-nr 13918

22n. Stephany, R.W. and L.A. van Ginkel
European Union Community Reference Laboratories for regulatory residue analysis of veterinary drugs : A strategic tool

26n. Ginkel, L.A. van and R.W. Stephany
How to detect residues ? Analytical strategies for residue control
Proceedings Scientific Conference on growth promotion in meat production,
27n. Ginkel, L.A. van and C. Dirschel
The EC Standards, Measurements and Testing programme - Results for veterinary drugs
ARODOC-nr 16396

Boldenone is a naturally occurring anabolic steroid in cattle
ARODOC-nr 16397

A multi residue procedure for the detection of anabolic compounds of bovine urine using coupled column HPLC and GC-MS
ARODOC-nr 16398

30n. Stephany, R.W., J. Boisseau, B. Jülicher and S. Caroli
The four European Union CRLs for residues: An overview of output and targets
ARODOC-nr 16400

Quality assurance and quality control for National Reference Laboratories for detecting residues in biological samples

32n. Verduyn, R
Report of syntheses of reference standards. (in Dutch)
389002 032 CRL-ARO report August 1995. ARODOC-nr 14782
33n. Sipoli-Marques, M.A.
A new concept in analytical SFE: a coupled (SPE)-SFE-SPE technique and its application to the recovery of anabolic mycotoxin zearalenone, zeranol and their metabolites from fortified bovine urine
389002 031 CRL-ARO report August 1995. ARODOC-nr 13798

389002 030 CRL-ARO report September 1995 pp 5. ARODOC-nr 13921

Quantification and confirmation of 17 - beta-estradiol in samples from Germany
389002 026 CRL-ARO report (research study plan 4.1995.09). ARODOC-nr 14789

37n. Stephany R.W.
Reliability of residue analyses for what purpose and what price ?
389002 033 CRL-ARO report October 1995 pp 15. ARODOC-nr 15867

Report certification study zeranol in bovine muscle liver and urine
389002 054. CRL-ARO report [(part) study plan 4.1995.06 (1995)]
ARODOC-nr 15161

39n. Stephany, R.W. and R.C. Schothorst
2nd Inventory on QA/QC and GLP for EU-NRLs
389002 037 CRL-ARO report February 1996 pp 9. ARODOC-nr 15045

40n. Kamp, C.G. van de
CB\METHODS a residu analyses methods electronic catalogue based on CARDBOX-plus MS-DOS software
389002 053 CRL-ARO report May 1996 (manual). ARODOC-nr 16208
41n. Ginkel L.A. van and R.W. Stephany
2nd Annual progress report on EC contract MAT 1 - CT93 - 0014 "Hormones and veterinary drugs in farm animals (phase 1)". Period: December 1994 - November 1995
573004 002 EC SMT/MAT-ARO report March 1996 pp 9. ARODOC-nr 15185

42n. Vermeulen, R.K., Ginkel L.A. van and R.W. Stephany
Progress report contract AIR3-CT95-1511 “Development of cost effective systems for the multi-residue analysis in food producing animals”
573008 001 EC DG6-AIR report Nov 1995 pp 7. ARODOC-nr 15108

43n. Ginkel L.A. van and R.W. Stephany
1st Annual progress report on EC contract MAT 1 - CT94 - 0011 "Hormones and veterinary drugs in farm animals (phase 2)". Period: December 1994 - November 1995
574005 002 EC SMT/MAT-ARO report March 1996 pp 28. ARODOC-nr 15184

Publications & reports of the CNEVA-LMV (number with postscript “f”)

1f. Delepine B., Hurtaud-Pessel D., Sanders P.
Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry.

2f. Verdon E., Couëdor P.
Determination of ampicillin residues in milk by ion-pair RP-HPLC after precolumn derivatization.
J Pharm. and Biomed Analysis, (accepted)

3f. Gaugain M., Abjean J.P.
High performance thin-layer chromatographic method for the fluorescence detection of three nitroimidazole residues in pork and poultry tissue.
J Chromatogr A, (accepted)
4f. Verdon E., Couëdor P.
Determination of ampicillin an amphoteric penicillin antibiotic at the residue level in bovine muscle by HPLC with pre-column derivatization

5f. Juhel-Gaugain M.
Screening of quinolone residues in pork muscle by planar chromatography

6f. Hurtaud-Pessel D., Delepine B.
Determination of ampicillin residues in bovine muscle by liquid chromatography / electrospray / mass spectrometry

7f. Delepine B., Hurtaud-Pessel D., Sanders P.
Identification of tilmicosin in bovine muscle at maximum residues limit level by LC/MS, using a particle-beam interface.

8f. Roudaut B., Verdon E., Maris P.
Determination of ampicillin residues in bovine milk, comparison of liquid chromatography and microbiological assay

9f. Stephany, R.W., J. Boisseau, B. Jülicher and S. Caroli
The four European Union CRLs for residues: An overview of output and targets
Publications & reports of the BGVV (numbers with postscript “d”) 

1d. Hahnau, S. and B. Jülicher
Evaluation of commercially available ELISA test kits for the detection of clenbuterol and other β 2-agonists
Food Additives and Contaminants 13 (1996) 259-274

The influence of the choice and the pretreatment of suitable tissue samples on the results of residue analyses using clenbuterol as an example

3d. Gowik, P. and B. Jülicher
Behaviour of some selected NSAID's under electrospray LC-MS conditions

4d. Wolf, Ch., Behrendt, D. and B. Jülicher
Results of a european interlaboratory study for the determination of chloramphenicol in bovine urine and plasma

Detection of clenbuterol in cattle hair by IAC/cELISA

6d. Wolf, Ch., Behrendt, D., Neumärker A. and B. Jülicher
Chloramphenicol detection in plasma and urine

7d. Wesseling, V., Hahnau, S., Jülicher, B. and K. Rubach
Generation of polyclonal antibodies for the detection of nitroimidazol derivatives
8d. Stephany, R.W., Boisseau, J., Jülicher, B. and S. Caroli
The four European Union CRLs for residues: An overview of output and targets

*Proceedings* EuroResidue III, Veldhoven, (NL), May 6-8 (1996) 149 - 155

9d. Wolf, Ch., Behrendt, D. and B. Jülicher
CAP Interlaboratory Study 3/95 - *Report* on Results

**Publications & reports of the ISS** (numbers with postscript “i”)

1i. Caroli, S. Forte, G. Iamiceli, A.L. and A. Lusi
Stability of Mercury Dilute Aqueous Solutions: An Open Issue
Microchem J, (accepted)

J Chromatogr. (accepted)

3i. Generali, T. Pelosi, P. and A. Di Muccio
ISS internal *report*

4i. Dommarco, R. Santilio, A. Generali, T. Pelosi, P. and A. Di Muccio
ISS internal REPORT

Handbook of Analytical Methods for Trace Elements in Use at NRLs
Data Bank of Literature References for Trace Elements in Food Matrices (1975-1995)
ISS internal report

7i. Stephany, R.W., J. Boisseau, B. Jülicher and S. Caroli
The four European Union CRLs for residues: An overview of output and targets.

8i. Caroli, S.
Harmonization of Procedures for the Analysis of Residues in Meat. Activities of the European Commission Reference Laboratory at the Istituto Superiore di Sanità

9i. Caroli, S. Forte, G. Iamiceli, A.L. and A. Lusi,
Stability of Mercury Dilute Aqueous Solutions: An Open Issue

Abstract First European Pesticide Residue Workshop, Alkmaar, (NL), June 10-12, 1996.

ANNEX 2 Audits
Audits of the RIVM
1. September 15, 1995
   Bundesanstalt für Tierseuchenbekämpfung, Mödling (Austria)

   Professional, technical and financial audit of CRL by EC, Bilthoven (The Netherlands)

3. February 14-16, 1996
   Laboratoria Nacional de Veterinaria, Lisbon and Oporto (Portugal)

   National Food Administration, Uppsala (Sweden)

5. March 19-20, 1996
   National Veterinary and Food Research Institute (EELA), Helsinki (Finland)

   International Scientific Audit of Division 3 Analytical Chemical Laboratories of RIVM
   Bilthoven (The Netherlands)

Audits of the CNEVA
1. May 22, 1996
   Residue Analysis Laboratory, Institute of Food Hygiene, Athens (Greece)

2. May 23, 1996
   Bundesanstalt für Tierseuchenbekämpfung, Mödling (Austria)

   Food Control Laboratory, Danish Veterinary Service, Ringsted (Denmark)

Audits of the BGVV
1. September 4-5, 1995
   National Veterinary and Food Research Institute (EELA), Helsinki (Finland)
2. September 6-7, 1995
   Livsmedelverket, National Food Administration (NFA), Uppsala (Sweden)

**Audits of the ISS**

Not applicable in the period considered
Dr. Rainer W. Stephany (Ph.D., Chemistry)  
Director of the CRL, Head of Laboratory for Residue Analysis.  
Role in the CRL: Executive and project management. Responsible for acquisition, implementation and maintenance of EC/RIVM CRL contract. Responsible for arbitration matters. First and general CRL contact point.
- Dr. Leendert A. van Ginkel (Ph.D., Chemistry)
  Deputy director of the CRL, Research leader, Head of section of Veterinary Drugs and
  Medicine, Deputy Head of Laboratory for Residue Analysis.
  Role in the CRL: Exploration of new research topics in the field of anabolics, responsible
  for the research done at CRL.

- Drs. Saskia S. Sterk (M.Sc., Pharmacy, Pharmacist)
  Scientific researcher CRL, deputy research leader, responsible for documentation and advisory
  service of the CRL, responsible for daily research supervision CRL.
  Role in the CRL: Exploration of new research topics in the field of anabolics, daily
  supervision of research, supervision of documentation and advisory service.

- Mr. Henk A. Herbold (Analytical senior Technician) Senior researcher CRL.
  Role in the CRL: Development of multi-residue methods of analysis in the field of
  anabolics and natural hormones. Validation of the methods.

- Mr. André Spaan (Analytical Technician) Researcher CRL.
  Role in the CRL: Development of multi-residue methods of analysis in the field of
  anabolics and natural hormones. Validation of the methods. Preparation of reference and
  ring test materials.

- Miss Frederike van Tricht (Analytical Technician) Contractor, researcher CRL.
  Role in the CRL: Development of methods of analysis in the field of anabolics and
  Storage and logistics of reference materials.

- Miss. Anneke Kieft (Analytical Technician) Contractor, researcher CRL.
  Role in the CRL: Development of methods of analysis in the field of anabolics. Validation
  of the methods. Preparation of reference materials.

- Mr. Cor van de Kamp (Documentalist) Senior documentalist and literature researcher.
  Role in the CRL: Responsible for CB/METHODS database and helpdesk. Evaluation of
  literature and developing of files with information about the substances in the CRL field.
  Responsible for computerized literature search and Internet.

- Miss Gonny Wilbers (Documentalist)
  Junior Documentalist and responsible for maintenance and updating databases.
  Role in the CRL: Archiving and input in databases, maintenance of databases, screening
  information of suppliers and products. Surveillance of logistics of reference materials
  (Amongst others Acknowledgements of receipt)

- Drs. Marco Jonker (M.Sc. Chemistry) Scientific Documentalist, literature researcher.
  Role in the CRL: Evaluation of literature and developing of files with information about the
  substances in the CRL field. Drafting monographs.

- Dr. Ronald C. Schothorst (Ph.D., Chemistry) QA/QC-officer.
Role in CRL: Responsible for implementation and maintenance of quality systems of the CRL.
Auditor of studies under GLP regime

- Mrs. Saskia van Laer-van Zalinge (Management assistant)
  Role in the CRL: Support of the management and secretarial duties.

- Mrs. Irene Aitton-Huijsmans (Financial and contract support)
  Role in CRL: Support of the management on financial and contract matters. Liaison officer with the EC in these matter.

- Mr. Henk van Blitterswijk (Information and automation manager)
  Role in the CRL: Implementation and maintenance of automation systems network and hard and software. Responsible for data back-up regime. Advisor in information matters
Organigram & staff of CNEVA-LMV

Head : Dr J. BOISSEAU

Deputy : Dr P. MARIS

Scientists : D. Hurtaud-Pessel, M. Juhel-Gaugain, J.C. Yorke, E. Verdon, R. Fuselier (*)

Technicians : B. Anger, P. Couëdor, J.L. Ribouchon, A. Rault (*)

Responsible for Quality Assurance : V. Juban

Assistant : C. Gervis, C. Marcault

(*) for the scientific areas involved, see pg. 9 and 10.
Organigram & staff of BGVV

CRL for Residues at the BgVV

Residue Control, Reference Laboratory (ZERF)

Head: Dr B. Jülicher    Assistant: C. Wendland
Deputy and QM Officer: Dr D. Behrendt

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<tr>
<th>CENTRAL UNIT FOR THE COORDINATION OF RESIDUE CONTROL IN GERMANY</th>
<th>RESIDUE ANALYSIS / PHARMACOKINETIC STUDIES</th>
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<td>I. Schmädicke</td>
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<td>Dr M. Friederichs</td>
<td>Ch. Wolf</td>
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<td>A. Neumärker</td>
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<td>S. Rahn</td>
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### Organigram & staff of ISS

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<tr>
<th>Name</th>
<th>Position within the ISS</th>
<th>Position within the CRL</th>
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<tbody>
<tr>
<td>Dr. S. Caroli</td>
<td>Director of the Section of Analytical Chemistry, Department of Applied Toxicology</td>
<td>Director, Head of the section for trace elements</td>
</tr>
<tr>
<td>Dr. A. Di Muccio</td>
<td>Director of the Section of Pesticide Residues, Department of Applied Toxicology</td>
<td>Head of the section for organochlorine compounds</td>
</tr>
<tr>
<td>Dr. F. Zanasi</td>
<td>Director of the section of Additives, Contaminants and Preserved Foods, Department of Food Chemistry</td>
<td>Head of the section for inorganic residues in preserved foods</td>
</tr>
<tr>
<td>Dr. M. Baldini</td>
<td>Section of Additives, Contaminants and Preserved Foods, Department of Food Chemistry</td>
<td>Senior Scientist</td>
</tr>
<tr>
<td>Dr. A. Lusi</td>
<td>Section of Analytical Chemistry, Department of Applied Toxicology</td>
<td>Hired Scientist</td>
</tr>
<tr>
<td>Mr. M. Delle Femmine</td>
<td>Section of Analytical Chemistry, Department of Applied Toxicology</td>
<td>Hired Technician</td>
</tr>
<tr>
<td>Dr. G. Forte</td>
<td>Section of Analytical Chemistry, Department of Applied Toxicology</td>
<td>Hired Scientist</td>
</tr>
<tr>
<td>Dr. A.L. Iamiceli</td>
<td>Section of Analytical Chemistry, Department of Applied Toxicology</td>
<td>Hired Scientist</td>
</tr>
<tr>
<td>Dr. R. Miniero</td>
<td>Section of Additives, Contaminants and Preserved Foods, Department of Food Chemistry</td>
<td>Hired Scientist</td>
</tr>
<tr>
<td>Mrs B. Galoppi</td>
<td>Section of Additives, Contaminants and Preserved Foods, Department of Food Chemistry</td>
<td>Hired Technician</td>
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<tr>
<td>Dr. P. Pelosi</td>
<td>Section of Pesticide Residues, Department of Applied Toxicology</td>
<td>Hired Scientist</td>
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<tr>
<td>Dr. T. Generali</td>
<td>Section of Pesticide Residues, Department of Applied Toxicology</td>
<td>Hired Scientist</td>
</tr>
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</table>
Annex 4 Workshops

RIVM

Title: Evaluation and validation of multi-residue procedures for anabolic compounds
Date: May 20-23, 1996
City: Bilthoven
Participants: 17 Analysts of the NRLs of the EU Member States

CNEVA-LMV

Title: Screening and post-screening tests to detect antibacterial residues in meat
Date: June 11-12, 1996
City: Fougères
Participants: 18 Analysts from NRLs of the EU Member States

BGVV

Title: β-Agonists: Method Validation and Surveillance
Date: July 3-5, 1996
City: Berlin
Participants: 20 Analysts of the NRLs of the EU Member States

ISS

Title: Second Workshop on Quality Control of Trace Elements Determinations
Date: May 9-11, 1996
City: Rome
Participants: 14 Analysts of the NRLs of the EU Member States

COUNCIL DECISION
of 6 March 1989
determining the powers and conditions of operation of the Community reference laboratories provided for by Directive 86/469/EEC concerning the examination of animals and fresh meat for the presence of residues

(89/187/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 86/469/EEC of 16 September 1986 concerning the examination of animals and fresh meat for the presence of residues (1), and in particular Article 8 (2) thereof,

Having regard to the proposal from the Commission,

Whereas Article 8 (2) of the aforementioned Directive provides that the Council shall designate Community reference laboratories which shall be responsible for coordinating inspections for residues and shall determine the powers and conditions for the activities of such laboratories;

Whereas it is appropriate to define such powers and conditions at this stage while informing the laboratories, which will be designated later, of the functions they will be required to fulfil and of the minimum requirements which they will have to satisfy,

HAS ADOPTED THIS DECISION:

Article 1

The functions of Community reference laboratories shall be:

(a) to coordinate the application, within the various national reference laboratories, of good laboratory practice, in accordance with Directives 87/18/EEC (2) and 88/320/EEC (3);

(b) to provide national reference laboratories with details of analytical methods and the comparative tests to be conducted, and to inform them of the results of such tests;

(c) to provide national reference laboratories, at their request, with technical advice on the analysis of the substances for which they have been designated the Community reference laboratory;

(d) to distribute blank samples and samples containing known amounts of analyte to be analysed blind in comparative tests to be carried out by national reference laboratories;

(e) to organize comparative tests between the various reference laboratories, the frequency of which shall be determined under the contracts to be concluded between the Commission and such laboratories and each time a new reference method is introduced under Community rules;

(f) to promote and coordinate research into new analytical methods and to inform national reference laboratories of advances in analytical methods and equipment;

(g) to identify residues and determine their concentration in cases where the results of an analysis give rise to a disagreement between Member States;

(h) to conduct initial and further training courses for the benefit of analysts from national laboratories;

(i) to provide the Commission services, including the Community Reference Bureau, with technical and scientific assistance;

(j) to compile a report on each year's work and transmit it to the Commission;

(k) to liaise, in the field of analytical methods and equipment, with the national reference laboratories designated by third countries in the plans to be submitted in accordance with Article 7 (2) of Directive 86/469/EEC.

Article 2

In order to perform the functions specified in Article 1, Community reference laboratories must satisfy the following minimum requirements:

(a) have suitably qualified staff who are adequately trained in analytical methods used for the residues for which they have been designated the Community reference laboratory;

(b) possess the equipment and substances needed to carry out the analyses for which they are responsible;

(c) have an adequate administrative infrastructure;

(d) have sufficient data-processing capacity to produce statistics based on their findings and to enable rapid communication of those statistics and other information to national reference laboratories and the Commission;

(e) ensure that their staff respect the confidential nature of certain issues, results or communications;

(f) have sufficient knowledge of international standards and practices;
(g) have available an up-to-date list of reference substances held by the Community Reference Bureau and an up-to-date list of manufacturers and vendors of such substances.

Article 3

This Decision is addressed to the Member States.

Done at Brussels, 6 March 1989.

For the Council
The President

C. ROMERO HERRERA

31. 12. 91  Official Journal of the European Communities  No L 368/17

II

(Acts whose publication is not obligatory)

COUNCIL

COUNCIL DECISION

of 11 December 1991

designating the Community reference laboratories for testing certain substances for residues

(91/664/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 86/469/EEC of 16 September 1986 concerning the examination of animals and fresh meat for the presence of residues (1), and in particular Article 8 (2) thereof,

Having regard to the proposal from the Commission,

Whereas coordination between the national reference laboratories responsible in the Member States for the examination of animals and their meat for the presence of residues must be assigned to highly specialized laboratories possessing the installations and equipment necessary for this type of analysis;

Whereas the Council, in its Decision 89/187/EEC (2), determined the powers and conditions of operation of the Community reference laboratories provided for in Directive 86/469/EEC;

Whereas, in accordance with Article 8 (2) of Directive 86/469/EEC, it is important to designate at this point the Community reference laboratories for testing certain substances for residues,

Whereas reference laboratories are eligible for Community aid according to the conditions referred to in Article 28 of Council Decision 90/424/EEC of 26 June 1990 on expenditure in the veterinary field (3),

HAS ADOPTED THIS DECISION:

Article 1

The following laboratories are hereby designated Community reference laboratories:

(a) for the residues included in Annex I, groups A. I and A. II, of Directive 86/469/EEC:

Rijksinstituut voor de Volksgezondheid en Milieuhygiëne
Antonie van Leeuwenhoeklaan 9
NL-3720 Bilthoven;

(b) for the residues included in Annex I, group A. III. (a), of Directive 86/469/EEC, with the exception of sulphonamides:

Laboratoire des Médicaments vétérinaires
(CNEVA-LMV)
La Haute Marché, Javené
F-35133 Fougeres;

(1) OJ No L 275, 26. 9. 1986, p. 36.
(2) OJ No L 64, 10. 3. 1989, p. 37.
Annex 6 EU Council Decision 91/664/EEC (Continued)

Article 2

This Decision is addressed to the Member States.


For the Council
The President
P.BUKMAN

(c) for the residues included in Annex I, group A.III. (b), of Directive 86/469/EEC, and the residues of beta-agonists and sulphonamides:

Bundesgesundheitsamt
Thielallee 88—92
D-1000 Berlin 33;

(d) Directive 86/469/EEC:

Istituto Superiore di Sanità
via Regina Elena 299
I-00161 Roma.
### Annex 7 Glossary & Abbreviations

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<tr>
<th>Acronym</th>
<th>Abbreviation</th>
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<tr>
<td>AIR</td>
<td>Agro-Industrial Research</td>
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<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemistry International</td>
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<tr>
<td>BgVV</td>
<td>Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin</td>
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<tr>
<td>CAP</td>
<td>Chloramphenicol</td>
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<tr>
<td>CNEVA-LMV</td>
<td>Centre National d’Etudes Veterinaires et Alimentaires - Laboratoire des Medicaments Vétérinaires</td>
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<tr>
<td>CRL</td>
<td>Community Reference Laboratory</td>
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<tr>
<td>DG</td>
<td>Directorate-General</td>
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<td>EC</td>
<td>European Community</td>
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<tr>
<td>ECD</td>
<td>Electron Capture Detector</td>
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<tr>
<td>EEC</td>
<td>European Economic Community</td>
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<tr>
<td>EI</td>
<td>Electron Impact</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immuno Sorbent Assay</td>
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<td>EU</td>
<td>European Union</td>
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<td>FDA</td>
<td>Food and Drug Agency</td>
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<td>GC-MS</td>
<td>Gas chromatography-Mass spectrometry</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<td>HPTLC</td>
<td>High Performance Thin Layer Chromatography</td>
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<tr>
<td>HCM</td>
<td>Human Capital and Mobility Programme</td>
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<tr>
<td>IOC</td>
<td>International Olympic Committee</td>
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<td>ISO</td>
<td>International Organisation for Standardization</td>
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<tr>
<td>ISS</td>
<td>Istituto Superiore di Sanità</td>
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<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
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<tr>
<td>LC-MS</td>
<td>Liquid chromatography-Mass spectrometry</td>
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<tr>
<td>MRL</td>
<td>Maximum Residue Limit</td>
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<tr>
<td>NRL</td>
<td>National Reference Laboratory</td>
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<tr>
<td>ppb</td>
<td>parts per billion (microgram per kilogram)</td>
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<tr>
<td>QA</td>
<td>Quality Assurance</td>
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<tr>
<td>QC</td>
<td>Quality Control</td>
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<tr>
<td>RAL</td>
<td>Resorcylic Acid Lactones</td>
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<td>RIA</td>
<td>Radio Immuno Assay</td>
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<td>RIVM</td>
<td>National Institute of Public Health and the Environment</td>
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<tr>
<td>SMT</td>
<td>Standards, Measurements and Testing Programme</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
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<tr>
<td>µg/kg</td>
<td>microgram per kilogram</td>
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