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Community Reference Laboratory For Feed Additives

Annual Report

Authorisation Activities 2009

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Feed Additives

The mission of the JRC-IRMM is to promote a common and reliable European measurement system in support of EU policies.

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FOREWORD

With this report the team of the Community Reference Laboratory for Feed Additives would like to present the 2009 activities and achievements.

Like in the previous years, the evaluation of analytical methods submitted by the applicants makes up the primary activity of the CRL team. In 2009 we evaluated in close cooperation with the National Reference Laboratories analytical methods related to 24 dossiers. Again, we had to deal with feed additives from quite different fields such as vitamins, enzymes, trace elements, coccidiostats or probiotics.

One of the major modifications compared to former years was the new requirement for the applicants to conduct a verification study of those analytical methods that has been exclusively single laboratory validated. After it became mandatory in 2008, we have been discussing with many applicants the implementation of the verification concept, explaining the corresponding guidelines that we have placed on our web site. The first experiences gained with the evaluation of dossiers in which the new concept has been applied clearly demonstrate the usefulness of the verification study to strengthen the conclusions and recommendation in our reports.

Another very important achievement gained in 2009 was the amendment of Commission Regulation EC (No) 378/2005 which specifies the duties and tasks of the CRL. The amendment (Commission Regulation (EC) No 885/2009) has been drafted with strong involvement of the CRL team and introduced various modifications such as a flexible fee system, the grouping of applications and specific conditions for dossiers on flavourings. These modifications are crucial for the submission of all the applications that we expect in 2010 and which are linked to the re-evaluation of feed additives that have been authorised under the previous legislation. Thus the new rules will play a major role in order to cope with the huge number of dossiers that we have to evaluate in the upcoming years.

Christoph von Holst
Operating manager
CRL – FA

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Main Activities of the CRL-FA Authorisation in 2009 *(by P. Robouch)*

In addition to the scientific evaluations of analytical methods the CRL-FA organised in 2009 a workshop, maintained a sample bank of feed additives and a database on methods of analysis, and drafted the CRL-FA Guidance for applicants.

Evaluation of Dossiers

In 2009, a total of 24 dossiers were evaluated and the corresponding reports were submitted to EFSA on time.

The dossier distribution - related to the different active substances - covers a variety of categories and functional groups, as presented hereafter:

Category	Functional group	Count
coccidiostats		2
nutritional additives	amino acids	1
	trace elements	1
	vitamins	1
technological additives	acidity regulators	1
zootechnical additives	enzymes	10
	microorganisms	5
	other	3
	Total	24

Table 1: Overview of dossiers evaluated in 2009

FAD number	Product / Additive Name	Active Substance	Rapporteu	Date of Repor
FAD-2007-0036	Roxazyme G2G_G2L	Endo-1-4-beta-xylanase; Endo-1,3(4)-beta-gluconase; Endo-1,4-beta-glucanase	CRL-FA	03/04/2009
FAD-2007-0050	Biokey Zink	hydrate)	*CReAA	03/12/2009
FAD-2007-0051	Rovimix Hy D	25-hydroxycholecalciferol	CRL-FA	29/01/2009
FAD-2008-0008	Ronozyme NP (CT)/(L)/(M)/-(C	6-phytase	*DGCCRF	06/03/2009
FAD-2008-0010	Finase L & P	3-phytase	*PL	18/03/2009
FAD-2008-0011	AveMix XG10	Endo-1-4-beta-xylanase; Endo-1,3(4)-beta-gluconase;	CRL-FA	26/01/2009
FAD-2008-0013	Ronozyme WX	Endo-1-4-beta-xylanase	*AGES	12/08/2009
FAD-2008-0021	Cylactin LBC - Cernivet LBC	Enterococcus faecium NCIMB 10415	CRL-FA	26/01/2009
FAD-2008-0022	Avemix 02 CS & L	Endo-1-4-beta-xylanase; Endo-1,3(4)-beta-gluconase; Pectinase	CRL-FA	03/04/2009
FAD-2008-0023	Natugrain Wheat TS & L	Endo-1-4-beta-xylanase	CRL-FA	06/01/2009
FAD-2008-0026	Ronozyme ProAct	Serine Protease	CRL-FA	06/01/2009
FAD-2008-0037	Maxiban G160	Narasin Nicarbazin	CRL-FA	07/08/2009
FAD-2008-0039	Bacillus subtilis PB6	Bacillus subtilis ATCC PTA-6737	*TLL	15/04/2009
FAD-2008-0040	Finase EC	6-phytase	*PL	16/07/2009
FAD-2008-0043	Natuphos	3-phytase	*AGES	24/06/2009
FAD-2008-0044	Formi LHS	Potassium diformate	CRL-FA	31/07/2009
FAD-2008-0045	Soda ash	Sodium carbonate	CRL-FA	05/10/2009
FAD-2008-0049	Aviplus	Citric acid Sorbic acid Thymol	CRL-FA	20/08/2009
FAD-2008-0052	Cycostat 66G	Robenidine hydrochloride	CRL-FA	21/08/2009
FAD-2008-0056	Animavit	Bacillus subtilis KBL001 CBS117162	CRL-FA	28/09/2009
FAD-2008-0060	Lactiferm	11181	CRL-FA	22/09/2009
FAD-2009-0001	L-isoleucine	L-isoleucine	CRL-FA	17/07/2009
FAD-2009-0005	Protural	Sodium benzoate	*SVA	22/10/2009
FAD-2009-0013	Calsporin	Bacillus subtilis C-3102	CRL-FA	27/11/2009

(*) NRLs

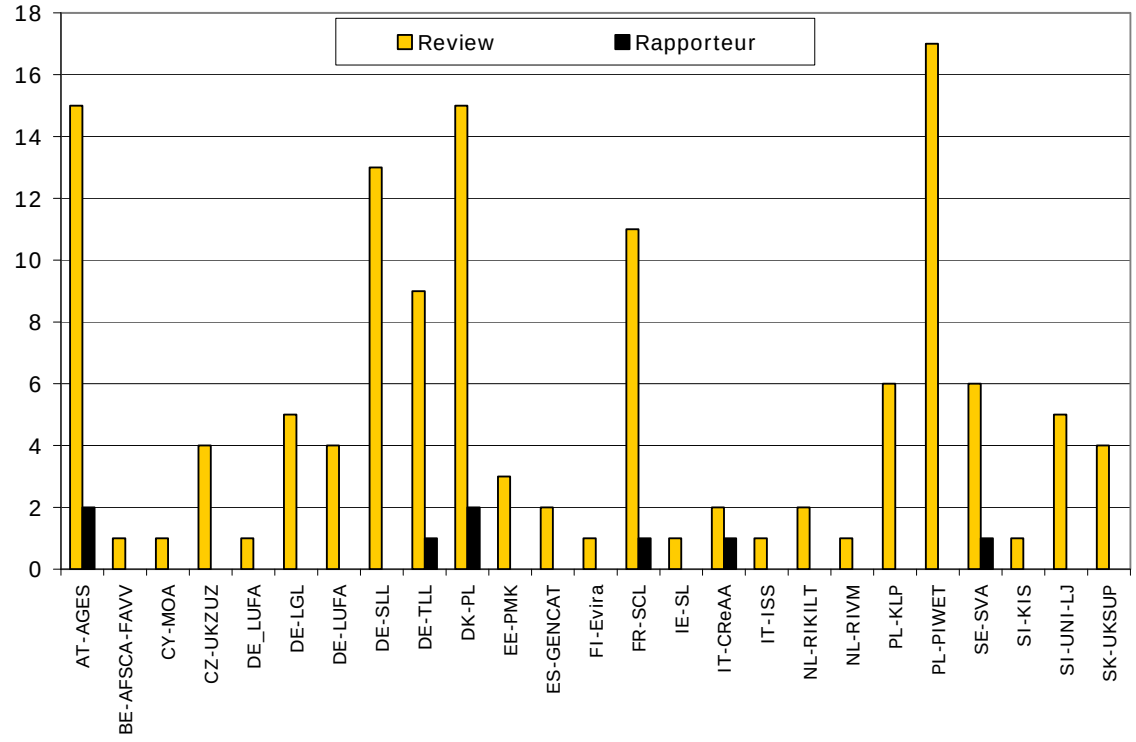
As a result of the training seminars organised in 2008 and 2009 for NRL Rapporteurs, the number of dossiers outsourced to NRLs has significantly increased from two in 2008 to eight in 2009. The following Rapporteur Laboratories are acknowledged:

- Österreichische Agentur für Gesundheit und Ernährungssicherheit, Wien, Austria (AT-AGES);
- Thüringer Landesanstalt für Landwirtschaft, Jena, Germany (DE-TLL);
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby, Danemark (DK-PL);
- Service Commun des Laboratoires, Rennes, France (FR-SCL);
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali, Torino, Italy (IT-CReAA);
- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala, Sweden (SE-SVA)

(sorted by country).

The enthusiastic and professional involvement of NRLs to the CRL network is demonstrated by the critical and constructive comments in the frame of the thorough peer review of Initial FAD reports. The CRL wishes to acknowledge the contribution of 25 NRLs (out of 35) listed in Figure 1. Five NRL laboratories commented more than ten reports: PL-PIWET (17); AT-AGES (14), DK-PL (14); DE-SLL (13) and FR-SCL (11).

Figure 1: Number of comments by NRLs during the review process 2009



(List of NRLs, Page 10-11)

CRL-FA Workshop 2009 - Executive Summary *(by P. Robouch)*

The 9th Workshop of the CRL-FA network was organised at the IRMM on the 11-12 June 2009. A total of forty-three participants attended the event, including representatives from twenty seven National Reference Laboratories.

The 2008 activities of the CRL-FA were reviewed and the important apects of the dossier evaluations were discussed. Participants were updated about (i) the CRL guidance documents for Applicants and Laboratories; (ii) the revised CRL database of official methods; (iii) the implementation of the verification concept and (iv) the CRL e-Room. Furthermore, the CEN activities in the field of feed and the EFSA request for advanced analytical methods were presented. Two other presentations on the determination of flavouring compounds and vitamins (A & E) completed the program of the event.

It was noticed that most expert Working Groups were idle. The chairperson or any active members are encouraged to re-activate the group, if deemed relevant.

The CRL organised since 2008 several training sessions for rapporteurs. Before organising other sessions, the CRL asked for expression of interests from the NRLs. The CRL committed to outsource more evaluations of dossiers to the NRL network.

As for the next Workshop, participants were requested to come with suggestions: - NRLs could present their activities and major achievements during a poster session; - technical or experimental questions/problems could be raised for discussion among participants.

The workshop was concluded with the award ceremony. Four rapporteurs were awarded by Giuseppe Simone, on behalf of the CRL-FA, as "CRL-FA Certified Rapporteurs", having successfully attended the training workshop and prepared a FAD report compliant with the latest CRL requirements. The successful awardees are:

- Annette Plöger from Danish Plant Directorate,
- Irmengard Strnad from AGES, Austria,
- Otto Jahn from TLL, Germany, and
- Roger Ziebal from SCL, France

Many other experienced Rapporteurs are expected to be certified in the next future.

Regulation (EC) No 885/2009 and the new "Administrative guidance to applicants" *(by G. Simone)*

A new "Administrative guidance to applicants" was published on the CRL-FA website on 1 December 2009 in order to take into account and to further explain the provisions laid down by Regulation (EC) No 885/2009 amending Regulation (EC) No 378/2005. The document aim is to help applicants with the administrative procedure for the payment of the flexible fee and for the submission of the samples to the CRL-FA.

Indeed, new legal provisions were introduced by Commission Regulation (EC) No 885/2009 in order to simplify, whenever possible, the existing provisions related to the reference samples and to the CRL-FA Evaluation Reports, to clarify some issues such as those related to flavourings and other non-holder specific products, to differentiate the fee on the basis of the type of application.

Key aspects of Regulation (EC) No 885/2009): simplification and new fee system:

(New) samples are not required when the application concerns a:

- New use (Article 4(1) of Regulation (EC) No 1831/2003)
- Modification (Article 13(3) of Regulation (EC) No 1831/2003)

…if some conditions are satisfied

A (new) evaluation report is not required when the application concerns a:

- New use (Article 4(1) of Regulation (EC) No 1831/2003)
- Modification (Article 13(3) of Regulation (EC) No 1831/2003)

…if some conditions are satisfied

New system of fees based on two components:

- One component is intended to support the CRL administrative costs and the costs related to the handling of the reference samples (2000 €)
- One component is intended to support the costs of the Rapporteur Laboratory for the scientific evaluation and preparation of the report (4000 €)

The "Administrative guidance" document describes in detail the implementation of these provisions, providing to the applicants useful flowcharts and forms to be submitted to the CRL-FA.

CRL Training Seminars *(by P. Robouch)*

The CRL continued the training seminars in 2008 to increase the number of experienced Rapporteurs drafting the CRL Evaluation Reports on analytical methods submitted in connection with the application for authorisation as feed additives according to Regulation (EC) 1831/2003. The seminars followed the structure developed in 2008.

Menu of the training seminar

* *Starter*: "Typical" difficulties encountered during a "first" dossier evaluation are presented. Some of them relate to the Commission Administrative jargon and procedures, not trivial to all National Administrations. Others concern the challenge of translating the contract before acceptance by the National Institution or the challenge in finding the right path through the many Commission decisions and regulations.

* *Main Course*: The evaluation process is then thoroughly explained. This includes an overview of the legislative requirements relevant to the Feed Additive Authorisation. It stresses also the main topics to be considered and presenting the common pitfalls to be avoided. Finally an "ideal" report is presented. Many issues need to be considered and well understood (i.e. cascade approach; active agent vs. feed additive; provisional requirements; etc.).

* *Dessert*: After these academic presentations it is time for some hands-on practice. Two Feed Additives (FAD) applications are to be evaluated. For the first case participants perform the evaluation of a dossier (later disclosed as already "authorised") with the support of the Training moderator. The conclusions elaborated by the participants are compared to the CRL's evaluation report and discussed. The second case relates to a "new/ongoing" dossier. Participants need to (a) evaluate whether the applicant provided all the relevant information, (b) draw the recommendations to be put forward and (c) draft the initial report.

Two Training Seminars were organised:

- Jan 22, 2009 on *Probiotics*; and
- May 6-8, 2009 on *Coccidiostats.*

Twelve potential Rapporteurs from the National Reference Laboratories listed bellow attended the events.

- AGES, Wien (AT)
- LGL, Oberschleißheim (DE)
- LUFA, Leipzig (DE)
- TLL, Jena (DE)
- Evira, Helsinki (FI)
- State Laboratory (IE)
- PIWET, Pulawy (PL)
- SVA, Uppsala (SE)
- VF Uni-LJ, Ljubljana (SLO)

Breaking News .....

Consequences of the Treaty of Lisbon

The Treaty of Lisbon (OJ C 306, 17/12/2007, link), amending the Treaty on European Union and the Treaty establishing the European Community signed by the twenty seven member states under the general provisions that '*The Union shall be founded on the present Treaty and on the Treaty on the Functioning of the European Union (hereinafter referred to as "the Treaties"). Those two Treaties shall have the same legal value.* ***The Union shall replace and succeed the European Community.'.***

As of January 2010, the Community Reference Laboratory for Feed Additives therefore becomes the European Union Laboratory for Feed Additives

EURL
European Union Reference Laboratory
Feed Additives

As explained in "Your Guide to the Lisbon Treaty" (link, cf. "Some technical terms")

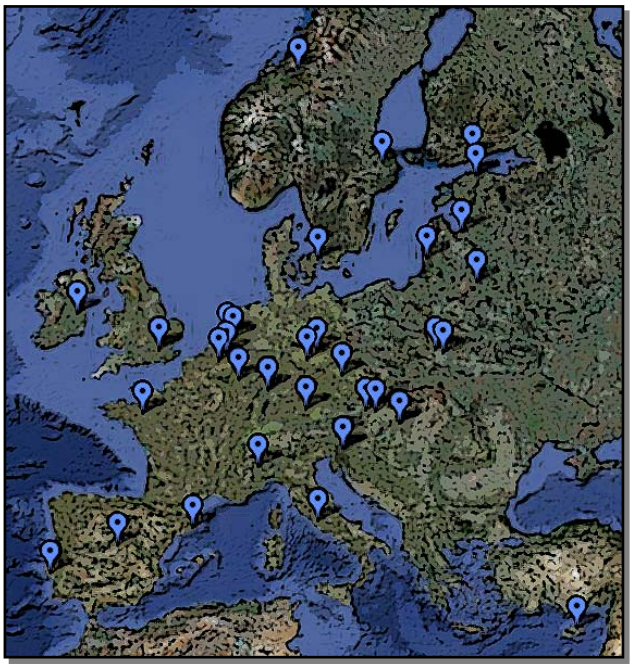
> *The Lisbon Treaty amends the Treaty on European Union and the Treaty establishing the European Community. It is the latest in a series of treaties updating and consolidating the EU's legal base.*
> *The EU will be given a single legal personality under the Lisbon Treaty.*
> *Currently, the European Community and the European Union have different statutes and do not operate the same decision-making rules. The Lisbon Treaty will end this dual system and the European Union will have its own legal personality.*
> *This change will improve the EU's ability to act, especially in external aff airs. The Lisbon Treaty will allow the EU to act more effectively, coherently and credibly in its relations with the rest of the world.*

Acknowledgment

We sincerely thank our colleagues within the Institute for their strong support and interest in the CRL-FA activities, both with regards to secretarial support, review of reports and development of tailor made systems. We would like to thank Sulhattin Yasar for his contribution to the evaluation of dossiers and drafting of reports.

We are also very grateful to all experts from the NRLs for contributing to the evaluation of the dossiers and to the discussions in the workshops and working groups which was indispensable for the successful operation of the evaluation procedure. The List of NRLs follows.

The CRL-FA Network Map



The list of NRLs of the CRL-FA network

Country	National Reference Laboratory
	- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren. BE - Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol. BE - Centre wallon de Recherches agronomiques (CRA-W), Gembloux, BE
	- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha. CZ
	- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby. DK
	- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim. DE - Landwirtschaftliches Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer. DE - Sächsische Landesanstalt für Landwirtschaft. Fachbereich 8 – Landwirtschaftliches Untersuchungswesen, Leipzig. DE - Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen, Jena. DE
	- Põllumajandusuuringute Keskus (PMK). Jääkide ja saasteainete labor, Saku, Harjumaa. EE - Põllumajandusuuringute Keskus (PMK), Taimse materjali labor, Saku, Harjumaa. EE
	- Laboratorio Arbitral Agroalimentario, Ministerio de Agricultura, Pesca y Alimentación, Madrid. ES - Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Cabrils. ES
	- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes. FR
	- The State Laboratory, Kildare. IE

Country	National Reference Laboratory
	- Istituto Superiore di Sanità. Dipartimento di Sanità alimentare ed animale, Roma. IT - Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino. IT
	- Feedingstuffs Analytical Laboratory, Department of Agriculture, Nicosia. CY
	- Valsts veterinārmedicīnas diagnostikas centrs (VVMDC), Riga. LV
	- Nacionalinis maisto ir veterinarijos rizikos vertinimo institutas, Vilnius. LT
	- Laboratoire de Contrôle et d'essais – ASTA, Ettelbruck. LU
	- Mezőgazdasági Szakigazgatási Hivatal Központ, Élelmiszer- és Takarmány-biztonsági Igazgatóság, Takarmányvizsgáló Nemzeti Referencia Laboratórium, Budapest. HU
	- RIKILT- Instituut voor Voedselveiligheid, Wageningen. NL - Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven. NL
	- LabNett AS, Agricultural Chemistry Laboratory, Stjørdal. NO
	- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien. AT
	- Instytut Zootechniki w Krakowie. Krajowe Laboratorium Pasz, Lublin. PL - Państwowy Instytut Weterynaryjny, Pulawy. PL
	- Instituto Nacional dos Recursos Biológicos, I.P./Laboratório Nacional de Investigação Veterinária (INRB,IP/LNIV), Lisboa. PT
	- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana - Kmetijski inštitut Slovenije, Ljubljana. SL
	- Skúšobné laboratórium - Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava. SK
	- Elintarviketurvallisuusvirasto/Livsmedelssäkerhetsverket (Evira), Helsinki/Helsingfors. FI
	- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala, SE
	- The Laboratory of the Government Chemist, Teddington

Annex I

CRL-FA Evaluation Reports
Executive Summaries

FAD number	Product/Additive Name
FAD-2007-0036	Roxazyme G2G_G2L
FAD-2007-0050	Biokey Zink
FAD-2007-0051	Rovimix Hy D
FAD-2008-0008	Ronozyme NP (CT)/(L)/(M)/-(CT)
FAD-2008-0010	Finase L & P
FAD-2008-0011	AveMix XG10
FAD-2008-0013	Ronozyme WX
FAD-2008-0021	Cylactin LBC - Cernivet LBC
FAD-2008-0022	Avemix 02 CS & L
FAD-2008-0023	Natugrain Wheat TS & L
FAD-2008-0026	Ronozyme ProAct
FAD-2008-0037	Maxiban G160
FAD-2008-0039	Bacillus subtilis PB6
FAD-2008-0040	Finase EC
FAD-2008-0043	Natuphos
FAD-2008-0044	Formi LHS
FAD-2008-0045	Soda ash
FAD-2008-0049	Aviplus
FAD-2008-0052	Cycostat 66G
FAD-2008-0056	Animavit
FAD-2008-0060	Lactiferm
FAD-2009-0001	L-isoleucine
FAD-2009-0005	Protural
FAD-2009-0013	Calsporin

Full reports available on the CRL-FA website
http://irmm.jrc.ec.europa.eu/html/CRLs/crl_feed_additives/authorisation/evaluation_reports/index.htm

FAD-2007-0036	
Product Name	Roxazyme G2 G& L
Active substance	Endo-1,4-beta-xylanase (EC 3.2.1.8) Endo-1,3(4)-beta-glucanase (EC 3.2.1.6) Endo-1,4-beta-glucanase (EC 3.2.1.4)
Rapporteur	CRL-FA

The current application authorisation is sought for Roxazyme G2 G and L under the category 'zootechnical additives', group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Roxazyme G2 G and L as a digestibility enhancer for chicken and turkey for fattening, laying hens, ducks and piglets (weaned). The product is intended to be marketed as solid (Roxazyme G2 G) and liquid (Roxazyme G2 L) formulations.

The active agents of Roxazyme G2 G and L are (1) endo-1,4-β-xylanase (2) endo-1,3(4)-β-glucanase and (3) endo-1,4-β-glucanase produced by Trichoderma reesei. The enzymatic activities are expressed in U-unit. According to the applicant one U-unit is the amount of the enzyme (endo-1,4- β-xylanase or endo-1,3(4)- β-glucanase or endo-1,4-β-glucanase) which releases 1 µmol of reducing sugar (glucose or xylose equivalent) per minute at 40°C, pH = 5.0 from the wheat arabinoxylan or barley β-glucan or carboxymethylcellulose, respectively.

The solid and liquid formulations (Roxazyme G2 G and L) have an endo-1,4- β-xylanase activity of 2700 U/g of product, an endo-1,3(4)- β -glucanase activity of 700 U/g of product and an endo-1,4- β-glucanase activity of 800 U/g of product. Both formulations are intended to be mixed into premixtures and/or feedingstuffs to provide in feedingstuffs a minimum endo-1,4- β -xylanase activity of 135 U/kg, endo-1,3(4)- β -glucanase activity of 35 U/kg and endo-1,4- β -glucanase activity of 40 U/kg.

For the determination of the activity of endo-1,4- β -xylanase, endo-1,3(4)- β -glucanase and endo-1,4- β-glucanase in the feed additives the applicant proposes a colorimetric method based on the formation of reducing sugar moieties released by the enzymes reacting with dinitrosalysilic acid (DNS). The method was ring-trial validated by four external laboratories for the feed additives.

- For the determination of endo-1,4- β–xylanase activity the reported performance characteristics were: (1) a relative standard deviation for repeatability (RSDr) ranging from 4.0 to 5.0% and (2) a relative standard deviation (RSD) for between-laboratory reproducibility ranging from 5.0 to 10.0%.

- For the determination of endo-1,3(4)- β–glucanase activity the reported performance characteristics were: (1) a RSDr ranging from 3.0 to 7.0% and (2) a RSD of reproducibility ranging from 4.0 to 9.0%.

- For the determination of endo-1,4- β–glucanase activity the reported performance characteristics were: (1) a RSDr ranging from 4.0 to 8.0% and (2) a RSD of reproducibility ranging from 2.0 to 9.0% for endo-1,4-betaglucanase.

Based on these acceptable performance characteristics, the applicant method is found suitable for official controls of the activities of the above mentioned three active substances in the feed additives.

For the determination of the activity of endo-1,4- β -xylanase, endo-1,3(4)- β -glucanase and endo-1,4- β -glucanase in the premixtures and feedingstuffs the applicant proposed three in-house validated colorimetric methods, based on the same analytical principle, but using different substrates compared to the assay on the feed additive: the measurement of the rate of release of water soluble dyed fragments by the enzymes from the dye cross-linked substrates.

- For the determination of endo-1,4- β -xylanase in the premixtures and feedingstuffs the reported performance characteristics were: (1) a RSDr ranging from 10 to 15% and (2) a relative standard deviation for intermediate precision (RSDR) ranging from 12 to 15%. The applicant determined only for the feedingstuffs a recovery rate ranging from 97 to 105% and the limit of detection (LOD) and limit of quantification (LOQ) of 15-30 and 70 U/kg feedingstuffs, respectively.

- For the determination of endo-1,3(4)- β -glucanase in the premixtures and feedingstuffs the reported performance characteristics were: (1) a RSDr ranging from 3 to 5% and (2) a RSDR ranging

from 1 to 4%. The applicant determined only for the feedingstuffs a recovery rate ranging from 96 to 106% and the LOD and LOQ of 4-7 and 18 U/kg feedingstuffs, respectively.

- For the determination of endo-1,4- β -glucanase in the premixtures and feedingstuffs the reported performance characteristics were: (1) a RSDr ranging from 3 to 16% and (3) a RSDR ranging from 10 to 16%. The applicant determined only for the feedingstuffs a recovery rate ranging from 98 to 104% and the LOD and LOQ of 4-8 and 20 U/kg feedingstuffs, respectively.

The reported LOD and LOQ values were found to be below the minimum recommended activity levels in feedingstuffs of 135, 35 and 40 U/kg for endo-1,4- β xylanase, endo-1,3(4)- β glucanase and endo-1,4- β -glucanase, respectively. The premixture samples were validated at a target activity level of endo-1,4- β xylanase of around 18000 U/kg, endo-1,3(4)- β -glucanase of around 5500 U/kg premixture and endo-1,4- β -glucanase of around 5000 U/kg premixture.

Based on these acceptable performance characteristics the CRL recommends three in-house validated methods for official controls for the determination of endo-1,4- β -xylanase, endo-1,3(4)- β -glucanase and endo-1,4- β -glucanase activities in the feedingstuffs and premixtures in the frame of Authorisation. Further testing or validation is not considered necessary.

FAD-2007-0050	
Product Name	Biokey Zn
Active substance	Zinc (as chelate of amino acids hydrate)
Rapporteur	C.Re.A.A.

In the current application authorisation is sought for Zinc chelate of amino acids hydrate (Biokey Zn) under the category 'nutritional additives', functional group 3(b), 'compounds of trace elements', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically authorisation is sought for Biokey Zn for all species, according to Article 4(1) of regulation (EC) No 1831/2003.

The product is intended to be marketed in a powder form containing 14 to 17 % Zinc chelate of amino acids hydrate as active agent. It is intended to be added to complete feed to supplement Zn for all species within legal limits for total zinc ranging from 150 to 250 mg/kg of feedingstuffs for all species.

For the determination of Zinc in the feed additive, premixtures and feedingstuffs, the applicant proposed the ISO 11885:1998 standard method and the Lebensmittel-und Bedarfsgegenständegesetz (LMGB) method #35. These methods apply to water and food matrices while the applicant did not provide any experimental evidence to demonstrate the applicability of these methods to the matrices of relevance.

However, several official methods are available for the determination of total zinc in premixtures and feedingstuffs. The Community Method (Commission Regulation (EC) No 152/2009) for the determination of trace elements such as zinc in feed is based on the sample digestion by hydrochloric acid, followed by atomic absorption spectrometry (AAS) determination. Only one performance characteristic is provided: the limit of quantification (LOQ) of 20 mg/kg of feedingstuffs. The same experimental principles are applied in the ISO 6869 standard method, which was ring-trial validated on various matrices with total zinc concentrations ranging from 30 to 15000 mg/kg. The reported performance characteristics are:

- a relative standard deviation of repeatability (RSDr) ranging from 1.7 to 7.6 %;
- a relative standard deviation of reproducibility (RSDR) ranging from 3 to 15 %, and
- LOQ = 5 mg/kg of feedingstuffs.

Based on these acceptable performance characteristics the CRL recommends for official controls: - the Commission Regulation (EC) No 152/2009 and the ISO 6869 for the determination of total zinc in the feedingstuffs and - the ISO 6869 standard method for the determination of total zinc in premixtures and as well as in feed additives. Further testing or validation is not considered necessary.

FAD-2007-0051	
Product Name	Rovimix Hy D
Active substance	25-hydroxycholecalciferol
Rapporteur	CRL-FA

25-hydroxycholecalciferol is a product already authorised as feed additive by Regulation (EC) No 1443/2005, under the category 'Nutritional Additive', functional group 'Vitamins, provitamins and chemically well defined substances, having a similar effect' according to the classification system of Annex I of Regulation (EC) No 1831/2003 for chickens for fattening, laying hens and turkeys.

The current application is for the re-evaluation according to Article 10(2) of 25-hydroxycholecalciferol and for its new use for poultry and pigs according to Article 4(1) of Regulation (EC) No 1831/2003

This authorization is sought to use 25-hydroxycholecalciferol for poultry and pigs and the proposed inclusion level of active substance ranges from 0.05 to 0.100 mg/kg complete feedingstuffs, depending on the target animal species. If the 25-hydroxycholecalciferol is combined with vitamin D3 the proposed inclusion level of the active substance ranges from 0.05 to 0.125 mg/kg complete feedingstuffs.

The active substance shall have a minimum purity of 25-hydroxycholecalciferol of 94% measured by a Reverse Phase High Performance Liquid Chromatography (RF-HPLC) with Diode Array Detection (DAD) or Ultraviolet (UV) detection at 270 nm.

For the determination of the active substance (25-hydroxycholecalciferol) in the feed additive the applicant proposed a Normal Phase High Performance Liquid Chromatography (NP-HPLC) method equipped with UV detection at 260 nm.

The following acceptable performance characteristics obtained using a Rovimix formulation were reported: - a relative intermediate precision standard deviation (RSDR) of 2.6 % and – a recovery rate close to 100 %. The method is therefore considered suitable for official control.

For the determination of 25-hydroxycholecalciferol in premixtures the applicant proposed a NP-HPLC method with DAD or UV detection at 265 nm. A 25-hydroxyergocalciferol internal standard is used for determination of 25-hydroxycholecalciferol in premixtures with a content of 25-hydroxycholecalciferol lower than 100 mg/kg of premixtures. The method was validated with respect to selectivity, linearity, range of application, recovery, accuracy and intermediate precision. The following acceptable performance characteristics were reported : - a limit of quantification (LOQ) of 2 mg/kg premixtures; - a recovery rate close to 100 % determined at different concentration levels; - a repeatability relative standard deviations (RSDr) ranging from 1.0 to 3.25 % and a RSDR ranging from 1.5 to 4.3 %. The method is considered suitable for official control.

For the determination of 25-hydroxycholecalciferol in feedingstuffs the applicant proposes a HPLC method connected with to a mass spectrometer (MS) using a 26,27-d6-25-hydroxycholecalciferol internal standard.

The method has been single-laboratory validated with respect to selectivity, linearity, range of application, recovery, accuracy and intermediate precision and showing an acceptable performance profile. The following acceptable performance characteristics were reported: - a LOQ of 0.005 mg/kg feedingstuffs; - a recovery rate ranging from 100 to 110 %; - a RSDr ranging from 8.5 to 13.2 %; and - RSDR ranging from 8.8 to 17.5 %. The method is considered suitable for official control of the content of 25-hydroxycholecalciferol in feedingstuffs.

For the analytical determination of vitamin D3, (included as additional requirement in Annex III), the applicant suggests the CEN method (EN 12821:2000). This method was originally validated for food and the applicant provided additional data demonstrating that the method is suitable for the analysis of vitamin D3 in feedingstuffs. The following performance characteristics were reported: a limit of detection (LOD) and a LOQ of 0.008 and 0.02 mg/kg feedingstuffs, respectively. From the information provided by the applicant the CRL calculated a RSDR of 17 % for concentration level of vitamin D3 around 0.020 mg/kg feedingstuffs. The method is considered suitable for official control of vitamin D3 content in feedingstuffs at the concentration range covered in the validation. Further testing or validation is not considered necessary.

FAD-2008-0008	
Product Name	Ronozyme NP
Active substance	6-phytase (EC 3.1.3.26)
Rapporteur	CRL-FA

In the current application authorisation is sought for Ronozyme NP (CT, L, M) under the category "zootechnical additives", functional groups 4(a) and 4(c), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought to use this product as a digestibility enhancer for poultry, piglets (weaned) and pigs for fattening, and as a substance which favourably affects the environment.

The active agent of Ronozyme NP (CT, L, M) is 6-phytase (EC 3.1.3.26), produced by Aspergillus oryzae (DSM 17594). The activity of 6-phytase is expressed in FYT (phytase) units. According to the applicant, one FYT unit is the quantity of enzyme which liberates 1 micromole of inorganic phosphate per minute from sodium phytate at pH = 5.5 and 37oC. The product is intended to be placed on the market as a coated thermo tolerant granulate formulation (Ronozyme NP CT) containing 10000 FYT/g of product, as a granulate formulation (Ronozyme NP M) containing 50000 FYT/g of product and as a liquid formulation (Ronozyme NP L) containing 20000 FYT/g of product. The product is intended to be incorporated into premixtures and/or complete feedingstuffs to obtain a minimum enzyme activity level of 600 FYT/kg of feedingstuffs for laying hens, 1000 FYT/kg of feedingstuffs for piglets (weaned) and pigs for fattening and 1500 FYT/kg of feedingstuffs for poultry excluding laying hens.

For the determination of the activity of 6-phytase in the feed additive and premixtures, the applicant submitted an in-house validated colorimetric method, based on the release by the 6-phytase of inorganic phosphate during the hydrolysis of sodium phytate at pH = 5.5 and 37oC. The released phosphate forms with molybdate and vanadate ions a coloured complex that is measured at 405 or 415 nm and quantified against a phosphate curve. The content of endogenous phosphate - present in the samples and not related to the phytase activity - is measured in a separate analysis and subtracted from the response of the enzymatic activity measurement.

For the determination of the enzyme activity of 6-phytase in the feed additive, the applicant submitted two protocols, which differ in terms of the equipment used - robot versus conventional instruments. Since both methods show comparable performance characteristics, the CRL recommends for official control the use of the method requiring conventional instruments, easily available in official feed laboratories.

The method for the determination of the enzyme activity in premixtures is similar to the corresponding method for the analysis of feedingstuffs. The method was validated on two different premixtures at the activity range of 80000 to 1700000 FTY/kg of premixture. The following performance characteristics were reported: (1) a relative standard deviation for repeatability (RSDr) ranging from 1.2 to 5.1%, (2) a relative standard deviation for intermediate precision (RSDR) ranging from 2.4 to 4.2% and (3) a recovery rate ranging from 95 to 99%. Based on these acceptable performance characteristics the method is considered to be suitable for official control at the target activity ranges.

For the determination of the 6-phytase activity in feedingstuffs, the applicant submitted the harmonised method developed on behalf of the European Association of Feed Additive Manufacturers (FEFANA). This method is currently under evaluation to become a CEN (European Committee for Standardisation) and ISO (International Organisation Standardization) standard. This method is similar to the one for the determination of the phytase activity in the feed additive. The method was ring trial validated covering a phytase activity from 500 to 1500 FYT/kg of feedingstuffs on various feed samples including different phytase products such as Ronozyme P. The performance characteristics obtained were: (1) a RSDr of 10%, (2) a relative standard deviation for between-laboratory reproducibility of 12% and (3) a limit of detection (LOD) and limit of quantification (LOQ) of 20 and 60 FTY/kg of feedingstuffs, respectively. Both limits are well below of the minimum enzyme activity level of 600 FYT/kg proposed by the applicant. These precision data have been calculated from pooled results of all enzyme products including a feed additive that contained the specific enzyme of the present application. Based on the acceptable method performance characteristics the CRL recommends this method for official controls to determine the activity of 6-phytase in feedingstuffs at the target activity levels. Further testing or validation is not considered necessary.

FAD-2008-0010	
Product Name	Finase L & P
Active substance	3-phytase (EC 3.1.3.8)
Rapporteur	Danish Plant Directorate

In the current application authorisation is sought for FINASE L & P under the category zootechnical additives, group 4(a) and 4(c), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use FINASE L & P as a digestibility enhancer for laying hens, turkeys for fattening, sows, ducks for fattening, pheasants and other game birds and as a substance which favourably affects the environment. The additive is intended to be marketed in two forms, as a powder (FINASE P) and as liquid (FINASE L) formulations.

The active agent of FINASE L & P is 3-phytase (EC 3.1.3.8), produced by a microorganism Trichoderma reesei. The enzyme activity is expressed in PPU units. According to the applicant, one PPU unit is the quantity of enzyme which liberates one µmole of inorganic phosphate from sodium phytate per minute at pH = 5.0 and 37oC. According to the applicant, Finase L & P have a guaranteed minimum activity of 5000 PPU/g of product. Finase L & P are intended to be incorporated into premixtures and/or complete feedingstuffs to obtain an enzyme activity level ranging from 250 to 1000 PPU/kg of feedingstuffs for laying hens, turkeys for fattening, sows, ducks for fattening, pheasants and other game birds.

For the determination of the 3-phytase activity in the feed additive, the applicant proposes a spectrophotometric method. The method is based on the release by the 3-phytase of inorganic phosphate during the hydrolysis of sodium phytate. The released phosphate forms a colour with phosphomolybdate complex that is measured at 820 nm and quantified against a phosphate standard curve. The method is in-house validated and the following performance characteristics are reported: (1) a relative standard deviation for repeatability (RSDr) ranging from 3.0 to 6.1 %, (2) a relative standard deviation for intermediate precision (RSDR) ranging from 4.9 to 10.8 % and (3) a recovery rate of 100%. Therefore, the method is considered suitable for the intended purposes.

For the determination of the 3-phytase activity in premixtures, the applicant proposes a method, based on the same method principle as for the additive. The assay requires an extraction of the enzyme in a buffer containing EDTA, albumin and Tween followed by centrifugation. The method is in-house validated and the following performance characteristics are reported: (1) a RSRr ranging from 2.3 to 3.5%, (2) a RSDR ranging from 3.9 to 6.0 % and (3) a recovery rate ranging from 99 to 114%. The results of a ruggedness test indicated that the addition of 20 mM copper reduces the enzyme activity by 17 to 25 %. Therefore the applicant submitted method is not considered suitable for the intended purposes due to the interference of copper – a substance which is often present in premixtures at high levels. An alternative approach, considered valid by the CRL for the determination of the phytase activity in premixtures, is based on the dilution of the premixture sample into blank feed matrix and applying the corresponding method for the determination of the phytase activity in feedingstuffs. However, this method is not applied in the present dossier and the corresponding validation data is missing; hence the suitability of such method for official controls could not be evaluated.

For the determination of the 3-phytase activity in feedingstuffs, the applicant proposes a method, based on the same principles as for the feed additive. The method is in-house validated and the following performance characteristics are reported: (1) a RSDr ranging from 3.9 to 8.9%, (2) a RSDR ranging from 5.1 to 7.4 % and (3) a limit of detection (LOD) and limit of quantification (LOQ) of 23 and 36 PPU/ kg of feedingstuffs, respectively. The recovery rate ranges from 82 to 94% within the target activity range of 250 and 500 PPU/kg of feedingstuffs.

A harmonised method is available for the determination of the phytase in feedingstuffs, and is currently evaluated to become a standard of the European Committee for Standardisation (CEN). However, this harmonised method requires a pH = 5.5 which is different from the one used by the applicant (pH = 5.0) at which the enzymatic activity unit of the applicant product is defined. Hence, the CRL could not evaluate the harmonised method for official control for the determination of 3-phytase activity at the defined condition within the authorisation frame of FINASE L & P.

Based on the acceptable performance characteristics the method submitted by the applicant is considered suitable for official controls to determine the activity of 3-phytase in feedingstuffs at the target activity level.

FAD-2008-0011	
Product Name	AveMix XG 10
Active substance	Endo-1,4-beta-xylanase (EC 3.2.1.8) Endo-1,3(4)-beta-glucanase (EC 3.2.1.6)
Rapporteur	CRL-FA

The current application authorisation is sought for AveMix XG 10 under the category 'zootechnical additives', group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use AveMix XG 10 as a digestibility enhancer for chickens for fattening. The product is intended to be marketed as solid and liquid formulations.

The active agents of AveMix XG 10 are (1) endo-1,4-□-xylanase and (2) endo-1,3(4)- β-glucanase produced by the strain X-252 (MUCL 49755) and the strain A-34 (MUCL 49754)) of Trichoderma reesei, respectively. The enzymatic activities are expressed in xylanase unit (XU) and betaglucanase unit (BGU). According to the applicant one XU-unit is the amount of enzyme which releases 1 µmol of reducing sugar (xylose equivalent) per minute from xylan of oat spelt at 50°C, pH = 4.8, whereas one BGU-unit is the amount of enzyme which releases 1 µmol of reducing sugar (cellobiose equivalent) per minute from betaglucan of barley at 50oC, pH = 5.0.

Both solid and liquid formulations of Avemix XG10 have an endo-1,4-β-xylanase activity of 40 000 XU/g and an endo-1,3(4)–β-glucanase activity of 6 600 BGU/g. The product is intended to be mixed into premixtures and/or feedingstuffs to provide an endo-1,4-β-xylanase activity of 4 000 XU/kg feedingstuffs and an endo-1,3(4)-β–glucanase activity of 660 BGU/kg feedingstuffs.

For the determination of the activity of endo-1,4-β-xylanase in the feed additives and premixtures a colorimetric method based on the formation of reducing sugar reacting with Dinitrosalicylic acid (DNS) is proposed. The method is in-house validated only for the feed additives, and the following performance characteristics were reported: -a recovery rate of 104%, - a relative standard deviation for repeatability (RSDr) of 4%, - a relative standard deviation for intermediate precision (RSDR) of 5%, - a limit of detection (LOD) and a limit of quantification (LOQ) of 722 and 820 XU per gram of the product. No sufficient validation parameters have been provided for the determination of the activity of endo-1,4-□-xylanase in the premixtures. For the determination of the activity of endo-1,4-β-xylanase in the feedingstuffs the applicant provided upon request from the CRL an in-house validated method based on the measurement of the rate of release of water soluble dyed fragments by endo-1,4-ß-xylanase from the dye cross-linked wheat arabinoxylan in a form of "Xylazyme AX tablet". The following performance characteristics were reported: - a recovery of 104%, - a RSDr of 4%, - a RSDR of 4%, - an LOD and LOQ of 939 and 1878 XU/kg feedingstuffs.

For the determination of the activity of endo-1,3(4)-β–glucanase in the feed additives and premixtures a colorimetric method based on the formation of reducing sugar reacted with DNS is proposed. The method is in-house validated only for the feed additives, and the following performance characteristics were reported: -a recovery rate of 107%, - a RSDr of 2%, - a RSDR of 4%, - an LOD and LOQ of 100 and 116 BGU/g product. No sufficient validation parameters have been provided for the determination of the activity of endo-1,3(4)-β-glucanase in the premixtures. For the determination of the activity of endo-1,3(4)-□-glucanase in the feedingstuffs the applicant provided upon request from the CRL an in-house validated method based on the measurement of the rate of release of water soluble dyed fragments by endo-1,3(4)-□-glucanase from the dye cross-linked barley glucan. The following performance characteristics were reported: - a recovery of 109%, - a RSDr of 6%, - a RSDR of 5%, - an LOD and LOQ of 111 and 222 BGU/kg feedingstuffs.

Based on acceptable performance characteristics, the proposed methods are considered suitable for determination of endo-1,4-β-xylanase and endo-1,3(4)-β–glucanase activities in feed additives and feedingstuffs (not in premixtures) for official control purposes in the frame of authorisation. Further testing or validation is not considered necessary.

FAD-2008-0013	
Product Name	Ronozyme WX
Active substance	endo-1,4-β-xylanase (EC 3.2.1.8)
Rapporteur	AGES

The current application authorisation is sought for endo-1,4-β-xylanase (Ronozyme WX) under the category 'zootechnical additives', group 4(a), 'digestibility enhancer' for poultry, piglets (weaned) and pigs for fattening, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for endo-1,4-β-xylanase (Ronozyme WX) according to Article 4(1) and Article 10(2) of Regulation (EC) No 1831/2003.

The active agent of Ronozyme WX is endo-1,4-β-xylanase produced by submerged fermentation of Aspergillus oryzae (DSM 10287). The enzymatic activity of endo-1,4-β-xylanase is expressed in xylanase unit (FXU). One FXU-unit is the amount of endo-1,4–β-xylanase which liberates 7.8 micromoles of reducing sugars (xylose equivalents) per minute from azo-wheat arabinoxylan at pH 6.0 and 50 oC. The product is intended to be marketed in two forms, as liquid formulation (Ronozyme WX L) with an enzymatic activity of 650 FXU/ml and as granulate formulation (Ronozyme WX CT) with an enzymatic activity of 1000 FXU/g. Both products are intended to be incorporated into premixtures and/or complete feedingstuffs to obtain a minimum enzyme activity level of 100 FXU/kg of feedingstuffs for poultry and pigs for fattening, and a minimum enzyme activity level of 200 FXU/kg of feedingstuffs for piglets (weaned).

For the determination of endo-1,4-β-xylanase activity in feed additives, premixtures and feedingstuffs the applicant proposes in-house validated colorimetric methods based on the quantification of water soluble dyed fragments produced by the action of endo-1,4,-□-xylanase on azo-wheat arabinoxylan substrate for feed additives and azurine-crosslinked wheat arabinoxylan substrate for premixtures and feedingstuffs. The intensity of the colour is proportional to the endo-1,4,-β-xylanase activity, which is quantified based on a reference enzyme Ronozyme, available from the applicant upon request. For the quantification of endo-1,4-β-xylanase activity in premixtures and feedingstuffs the analytical method foresees using the standard addition technique.

The results of in-house validation of the analytical method determining endo-1,4-β-xylanase activity in feed additives are: - a relative standard deviation for repeatability (RSDr) ranging from 1.3 to 7.0 %; - a relative standard deviation for intermediate precision (RSDR) ranging from 7.0 to 12.3 %; - a limit of quantification (LOQ) of 0.7 FXU/g of product and - a recovery rate (RR) of 97.8 %. The reported method performance characteristics are acceptable. Therefore, the proposed method is considered suitable for determination of endo-1,4-□-xylanase in feed additives for official control purpose in the frame of authorisation.

For the determination of endo-1,4-β-xylanase activity in premixtures the following performance characteristics of the analytical method based on a standard addition technique, were provided, upon request by the CRL: - RSDr = 14.2 %, - RSDR = 38.5 %, - LOD = 75 FXU/kg of premixture, and - a RR ranging from 85 to 90 %. The performance characteristics were determined for premixtures samples with an enzyme activity range of 15000 to 30000 FXU/kg of premixture. Better method precision values may be obtained by diluting the premixture sample into blank feed matrix and applying the corresponding method for the determination of endo-1,4-β-xylanase activity in feedingstuffs. However, this alternative approach was not applied in the present dossier and the corresponding validation data is missing; hence the suitability of such method for official controls could not be evaluated. Therefore, the method proposed by the applicant is considered suitable for determination of endo-1,4-β-xylanase in premixtures for official control purpose within the range of the activity levels included in the validation which is 15000 to 30000 FXU/kg.

For the determination of endo-1,4-β-xylanase activity in feedingstuffs the following performance characteristics for the method based on a standard addition technique were reported: - RSDr ranging from 6.6 to 16.8 %, - RSDR ranging from 16.5 to 28.1 %, - LOD = 50 FXU/kg of feedingstuffs, and - RR ranging from 96 to 112 %. The performance characteristics were determined for the feed samples with an enzyme activity range of 160 to 2000 FXU/kg of feedingstuffs. The reported method performance characteristics are acceptable. Therefore, the proposed method is considered suitable for determination of endo-1,4–β-xylanase in feedingstuffs for official control purpose in the frame of authorisation. Further testing or validation is not considered necessary.

FAD-2008-0021	
Product Name	Cylactin/Cernivet LBC ME 10 and 20
Active substance	Enterococcus faecium
Rapporteur	CRL-FA

In the current application authorisation is sought for the microbial feed additive Cylactin/Cernivet LBC ME 10 and 20 under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. The active agent in the additive is Enterococcus faecium NCIMB 10415. The additive is available in micro-encapsulated form and contains a minimum concentration of 1 x 1010 colony forming units (c.f.u.) per gram. Specifically, authorisation is sought to use Cylactin/Cernivet LBC ME 10 and 20 for chickens for fattening. The conditions of use are proposed with a recommended dosage of 0.3 to 2.8 x 109 c.f.u./kg feed.

For the quantification of the active agent (Enterococcus faecium NCIMB 10415) of Cylactin/Cernivet LBC ME 10 and 20 in the feed additive, premixtures and feedingstuffs, an appropriate spread plate method was proposed by the applicant. The method was in-house validated and the method precision data were acceptable for the intended purpose.

For official controls regarding the quantitative determination of the colony forming units of the active agent in the feed additive, premixtures and feedingstuffs, a fully ring-trial validated spread plate enumeration method is recommended (J. Appl. Microbiol. 2002, 93, 781-786).

The method's performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.12 – 0.20 log10 and 0.23 – 0.41 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are around 104 colony forming units (c.f.u.) per gram (g) feed additive or premixture and around 107 c.f.u./kg feedingstuff.

The identity of the bacterial strain, Enterococcus faecium NCIMB 10415, was analysed by microscopy, biochemistry and molecular methods such as randomly amplified polymorphic DNA (RAPD) methodology. Pulsed-field gel electrophoresis (PFGE) is recognised as a standard methodology for microbial identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2008-0022	
Product Name	AveMix 02 CS & L
Active substance	Endo-1,4-beta-xylanase (EC 3.2.1.8) Endo-1,3(4)-beta-glucanase (EC 3.2.1.6) Pectinase (EC 3.2.1.15)
Rapporteur	CRL-FA

The current application authorisation is sought for AveMix 02 CS and L under the category 'zootechnical additives', group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use AveMix 02 CS and L as a digestibility enhancer for piglets (weaned). The product is intended to be marketed as solid (AveMix 02 CS) and liquid (AveMix 02 L) formulations.

The active agents of AveMix 02 CS and L are (1) endo-1,4-β-xylanase produced by Trichoderma reesei, (2) endo-1,3(4)-β–glucanase produced by Trichoderma reesei, and (3) pectinase produced by Aspergillus aculeatus. The enzymatic activities are expressed in xylanase unit (XU), betaglucanase unit (BGU) and pectinase unit (PGLU), respectively. According to the applicant: (1) one XU-unit is the amount of enzyme which releases 1 µmol of reducing sugar (xylose equivalent) per minute from xylan of oat spelt at 50°C, pH = 4.8; (2) one BGU-unit is the amount of enzyme which releases 1 µmol of reducing sugar (cellobiose equivalent) per minute from betaglucan of barley at 50oC, pH = 5.0; and (3)

one PGLU unit is the amount of enzyme which releases 1 µmol of reducing (glucose equivalent) sugar per minute from polymethylgalacturonic acid (pectin containing substrate) at 35°C and pH = 4.8.

The solid formulation (AveMix 02 CS) has an endo-1,4-β-xylanase activity of 21400 XU/g, an endo-1,3(4)-β-glucanase activity of 12300 BGU/g and a pectinase activity of 460 PGLU/g. The liquid formulation (AveMix 02 L) has an endo-1,4-β-xylanase activity of 10700 XU/g, an endo-1,3(4)-β-glucanase activity of 6150 BGU/g and a pectinase activity of 230 PGLU/g. Both formulations are intended to be mixed into premixtures and/or feedingstuffs to provide a minimum endo-1,4–β-xylanase activity of 2140 XU/kg, endo-1,3(4)-β–glucanase activity of 1230 BGU/kg and pectinase activity of 46 PGLU/kg in feedingstuffs.

Endo-1,4-β-xylanase: For the determination of the activity of endo-1,4-β-xylanase in the feed additives and premixtures a colorimetric method based on the formation of reducing sugar reacting with Dinitrosalicylic acid (DNS) is proposed. The method is in-house validated only for the feed additives, and the following performance characteristics were reported: -a recovery rate of 104%, - a relative standard deviation for repeatability (RSDr) of 3%, - a relative standard deviation for intermediate precision (RSDR) of 5%. No validation data were provided by the applicant for the determination of the activity of endo-1,4-β-xylanase in the premixtures. For the determination of the activity of endo-1,4-β-xylanase in the feedingstuffs the applicant provided upon request from the CRL an in-house validated method based on the measurement of the rate of release of water soluble dyed fragments by endo-1,4-ß-xylanase from the dye cross-linked wheat arabinoxylan. The following performance characteristics were reported: - a recovery rate of 104%, - a RSDr of 4%, - a RSDR of 4%, - a limit of detection (LOD) and limit of quantification (LOQ) of 939 and 1878 XU/kg feedingstuffs, respectively.

Endo-1,3(4)-β-glucanase: For the determination of the activity of endo-1,3(4)-β-glucanase in the feed additives and premixtures a colorimetric method based on the formation of reducing sugar reacted with DNS is proposed. The method is in-house validated only for the feed additives, and the following performance characteristics were reported: -a recovery rate of 107%, - a RSDr of 4%, - a RSDR of 5%. No validation data were provided by the applicant for the determination of the activity of endo-1,3(4)-β-glucanase in the premixtures. For the determination of the activity of endo-1,3(4)-β-glucanase in the feedingstuffs the applicant provided upon request from the CRL an in-house validated method based on the measurement of the rate of release of water soluble dyed fragments by endo-1,3(4)-β-glucanase from the dye cross-linked barley glucan. The following performance characteristics were reported: - a recovery rate of 109%, - a RSDr of 6%, - a RSDR of 5%, -an LOD and LOQ of 111 and 222 BGU/kg feedingstuffs, respectively.

Pectinase: For the determination of the activity of pectinase in the feed additives and premixtures a colorimetric method based on the formation of reducing sugar reacted with DNS is proposed. The method is in-house validated only for the feed additives, and the following performance characteristics were reported: -a recovery rate of 105%, - a RSDr of 5%, - a RSDR of 14%. No validation data were provided by the applicant for the determination of the activity of pectinase in the premixtures. For the determination of the activity of pectinase in the feedingstuffs a viscosimetric method based on the measurement of reduced viscosity by the enzyme of a pectin substrate, which is then directly related to enzymatic activity. The method is in-house validated and the following performance characteristics were reported: -a recovery rate of 113%, - a RSDr of 9%, - a RSDR of 9%, - an LOD and LOQ of 14 and 28 PGLU/kg feedingstuffs, respectively.

Based on these acceptable performance characteristics, the proposed methods are considered suitable for determination of endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase and pectinase activities in feed additives and feedingstuffs for official control purposes in the frame of authorisation. Since validation parameters of the methods for premixtures are not available, the CRL is unable to comment on the suitability of the proposed methods for this matrix. Further testing or validation is not considered necessary.

FAD-2008-0023	
Product Name	Natugrain Wheat TS & L
Active substance	Endo-1,4-β-xylanase (E.C. 3.2.1.8)
Rapporteur	CRL-FA

In the current application authorisation is sought for Natugrain Wheat TS and Natugrain Wheat TS L, in accordance with article 4(1) and 10(2) of Regulation (EC) No 1831/2003. Authorisation is sought to use Natugrain Wheat TS and Natugrain Wheat TS L as a digestibility enhancer for chicken for fattening and ducks under the category 'zootechnical additives' and the functional group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003.

The active agent of Natugrain Wheat TS and Natugrain Wheat TS L is thermostable endo-1,4-β-xylanase, produced by a strain of Aspergillus niger-CBS 109.713. The additive is intended to be marketed as powder (Natugrain Wheat TS) and as liquid formulation (Natugrain Wheat TS L). Both formulations contain an endo-1,4-β-xylanase activity of 5600 TXU/g product. They are intended to be mixed into premixtures and/or feedingstuffs to obtain a recommended endo-1,4-β–xylanase activity level ranging from 280 to 800 TXU per kg feedingstuffs. Enzymatic activity of endo-1,4-β-xylanase is expressed in thermostable xylanase units (TXU). One TXU is defined as the amount of enzyme that liberates 5 µmol of reducing sugars (xylose equivalents) from wheat arabinoxylan per minute at pH = 3.3 and 55oC.

For the determination of the activity of endo-1,4-β-xylanase in the feed additive, premixtures and feedingstuffs, the applicant proposes an in-house validated viscosimetric method. Endo-1,4-β-xylanase catalyses the hydrolysis of glycosidic bonds in the substrate wheat arabinoxylan to yield xylose and reduces consequently the viscosity of sample solution. The decrease in viscosity of sample solution, expressed in terms of a drop time, is a measure for the endo-1,4-β-xylanase activity and is determined using a falling ball viscosimeter at pH = 3.3 and 55oC. The quantification is performed using an endo-1,4–β–xylanase standard curve based on reference enzyme with known activity provided by the applicant. The method performance characteristics, determined for the feed additive, premixtures and feedingstuffs matrices are: a relative standard deviation for repeatability (RSDr) ranging from 2.4 to 5.7%; - a relative intermediate precision (RSDR) ranging from 3.4 to 11.8%; - a recovery rate ranging from 82 to 115%; - a limit of detection (LOD) of 11 TXU/kg feedingstuffs and - a limit of quantification (LOQ) of 36 TXU/kg feedingstuffs.

Based on acceptable performance characteristics, the applicant method is considered to be suitable for official control purposes in the frame of authorisation. Further testing or validation is not considered necessary.

FAD-2008-0026	
Product Name	Ronozyme ProAct CT & L
Active substance	Serine Protease (EC 3.4.21.)
Rapporteur	CRL-FA

The current application authorisation is sought for Ronozyme ProAct CT and L, in accordance with article 4(1) of Regulation (EC) No 1831/2003. Authorisation is sought to use Ronozyme ProAct CT and L as a digestibility enhancer for chicken for fattening under the category ‘zootechnical additives’, group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. The product is intended to be marketed as solid (Ronozyme ProAct CT) and as liquid (Ronozyme ProAct L) formulations.

The active agent of Ronozyme ProAct CT and L is serine protease produced by submerged batch-fed pure culture fermentation of a genetically modified strain of a Bacillus licheniformis denoted “Rh-3”. The enzymatic activity is expressed in protease unit (PROT) where 1 PROT is the amount of serine protease that liberates 1 µmol para-nitroaniline (pNA) from 1mM Suc-Ala-Ala-Pro-Phe-pNA

(C30H36N6O9) substrate per minute at pH = 9.0 and at 37 oC. The solid and liquid formulations have a target activity of 75 000 PROT/g. The solid formulation (Ronozyme ProAct CT) is intended to be mixed into premixtures and/or feedingstuffs to provide an enzyme activity of 15 000 PROT per kg of feedingstuffs, whereas the liquid formulation (Ronozyme ProAct L) is directly sprayed onto the compound feed to obtain an enzyme activity of 15 000 PROT per kg of feedingstuffs.

For the determinations of serine protease activity in the feed additive, in the premixtures and in the feedingstuffs, the applicant proposes two in-house validated colorimetric methods based on the same principle, where the amount of yellow complex (para-nitroaniline, pNA) released by serine protease enzyme from the substrate "Suc-Ala-Ala-Pro-Phe-pNA" at pH = 9.0 and at 37 oC. The enzyme activity of the unknown sample is quantified against certified Ronozyme ProAct ™ serine protease standard with known enzyme activity.

For determination of the serine protease activity in the feed additives a relative standard deviation for repeatability (RSDr) of 1.1% and a relative standard deviation for within-laboratory reproducibility (RSDR) of 3.8% were reported. Based on the obtained method performance characteristics the method is considered suitable for the intended purpose.

For determination of the serine protease in the premixtures, the following performance characteristics were reported: a percentage recovery rate of 102%, a RSDr of 6.8 % and a RSDR of 6.4%. The validation experiments were conducted on premixture samples covering an activity range of 400 to about 36.000 PROT/g. The method is considered suitable for the intended purpose within the activity range covered by the validation study.

For determination of the serine protease activity in the feedingstuffs the following performance characteristics were reported: a limit of quantification (LOQ) of 1000 Prot/kg, a percentage recovery rate of 101%, a RSDr of 8.9 % and a RSDR of 11.7%. The performance characteristics were determined in feedingstuffs samples containing enzyme activity levels close to the target levels of this application. Based on acceptable performance characteristics, the proposed method is considered suitable for official control purposes for determination of serine protease activity in feedingstuffs for chicken for fattening within the frame of authorisation. No further testing or method validation is considered necessary.

FAD-2008-0037	
Product Name	Maxiban G160
Active substance	Narasin Nicarbazin
Rapporteur	CRL-FA

Maxiban G160 is a product already authorised as feed additive by Regulation (EC) No 2430/1999, under the category 'coccidiostats and histomonostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active agents of Maxiban G160 are narasin and nicarbazin. The authorised inclusion level in complete feed is 40 to 50 mg of narasin + 40 to 50 mg of nicarbazin per kilogram.

In the current application the re-authorisation is sought for Maxiban G160 according to Article 10 (2) of Regulation (EC) No 1831/2003. Specifically, re-authorisation is sought to use Maxiban G160 for the control of coccidiosis in chickens for fattening.

The Maxiban G160 is a free-flowing mixture of tan-to-yellow particles and grey-brown particles, which contains granular narasin, granular nicarbazin, vegetable diluent, anti-dusting oil and microtracer.

The narasin concentration in the feed additive is expressed in terms of narasin 'g activity' which is calculated from the measured concentration of narasin components A, D and I.

The CRL recommends the standardized method EN 14183 for the determination of narasin in the feed additive (Maxiban G160), premixtures and feedingstuffs for official control purposes in the frame of the Maxiban G160 authorisation. The method is based on high performance liquid chromatography (HPLC) with post-column derivatisation (PCD) and ultraviolet (UV) detection and its performance

characteristics are: - a limit of quantification (LOQ) of 2 mg/kg; - a relative standard deviations for repeatability (RSDr) ranging from 1.3 to 5.0 % and a relative standard deviations for reproducibility (RSDR) ranging from 4.6 to 12.6 % depending on matrix and concentration level.

The CRL recommends the standardized method prEN 15782 suitable for the determination of nicarbazin in the feed additive (Maxiban G160), premixture and feedingstuffs for official control purposes in the frame of the Maxiban G160 authorisation. The method is based on high performance liquid chromatography (HPLC) equipped with ultraviolet/visible (UV/VIS) detection and its performance characteristics are: - LOQ = 20 mg/kg; - RSDr ranging from 2.6 to 10.2 % and - RSDR ranging from 4.8 to 12.3 % depending on matrix and concentration levels.

Regarding residues in tissue the applicant proposed for the marker residue 4,4' dinitrocarbanilide (DNC) a maximum residues limit (MRL) of 750 □g/kg in liver of chicken for fattening. For official control of this level the CRL recommends a method of the Community Reference Laboratory for Residues of Veterinary Drugs (Berlin) based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The method has been validated in accordance with the requirements of Commission Decision (EC) No 657/2002. Further testing or validation is not considered necessary.

FAD-2008-0039	
Product Name	Bacillus subtilis PB6
Active substance	Bacillus subtilis ATCC PTA-6737
Rapporteur	TLL

In the current application authorisation is sought for the microbial feed additive Bacillus subtilis ATCC PTA-6737 (Bacillus subtilis PB6) under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of Bacillus subtilis PB6 as a gut flora stabiliser for chickens for fattening. The feed additive has a minimum of 1x1010 colony-forming units (CFU) per gram of viable spores of Bacillus subtilis ATCC PTA-6737 as active agent in maltodextrin carrier. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration ranging from 1x107 to 5x107 CFU/kg of feedingstuffs.

For the enumeration of Bacillus subtilis ATCC PTA-6737 in the feed additive, premixtures and feedingstuffs, the applicant proposes the draft CEN method - prEN 15784:2008 E – an internationally recognised spread plate method. This method was ring-trial validated using the premixtures and feedingstuffs samples containing Bacillus subtilis spores. The performance characteristics of the draft CEN method reported after logarithmic transformation of measured values (CFU) are:

- For the premixtures: (1) a standard deviation for repeatability (sr) of 0.09 log10 and (2) a standard deviation for between-laboratory reproducibility (sR) of 0.32 log10.

- For the feedingstuffs: (1) a sr of 0.07 log10 and (2) a sR of 0.35 log10.

The applicant used the above mentioned spread plate method to analyse the various matrices containing Bacillus subtilis ATCC PTA-6737 spores and reported the following results: (a) 1x109 to 1.5x1011 CFU/g of feed additive; (b) 1x107 to 1.5x109 CFU/kg for premixtures and (c) 1x107 to 1.5x108 CFU/kg for feedingstuffs. The results obtained for feed additive and premixtures are considered acceptable; this method is therefore recommended for official controls for the feed additives and premixtures in the frame of the authorisation.

As regards feedingstuffs, the CRL notes that the limit of quantification reported by the applicant upon request (LOQ = 1x107 CFU/kg feedingstuffs) is identical to the minimum dose proposed and is below the LOQ reported in the draft CEN method (2x107 CFU/kg). On the basis of the available information, the draft CEN method is recommended for official control of the feedingstuffs containing Bacillus subtilis PB6 at the dosages above the LOQ reported by CEN. Below 2x107 CFU/kg the CRL is not able to conclude on the suitability of this method for official control purposes.

Molecular methods were used by the applicant for identification of the active agent. For official controls pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended. Further testing or validation is not considered necessary.

FAD-2008-0040	
Product Name	Finase EC
Active substance	6-phytase (EC 3.1.3.26)
Rapporteur	PDIR

In the current application authorisation is sought for 6-Phytase (Finase EC) under the category zootechnical additives, functional group 4(a) as a digestibility enhancer for chickens for fattening, chickens for laying, laying hens, turkeys for fattening, turkeys for breeding, piglets, pigs for fattening, sows, ducks, and other minor poultry species (i.e. geese, quail, pigeons, pheasants and game birds) and functional group 4(c) as a substance which favourably affects the environment, according to Annex I of Regulation (EC) No 1831/2003. The additive is intended to be marketed in two forms, as a liquid (Finase EC 10 L) and as a solid (Finase EC 40 P) formulation.

The active agent of Finase EC is 6-phytase (EC 3.1.3.26), produced by a fungus Trichoderma reesei. The enzyme activity is expressed in PPU units. According to the applicant, one PPU-unit is the quantity of enzyme which liberates one µmole of inorganic phosphate from sodium phytate per minute at pH = 5.0 and 37oC. Finase EC L and Finase EC P have a minimum 6-phytase activity of 10000 and 40000 PPU/g, respectively.

Both formulations are intended to be incorporated into premixtures and/or complete feedingstuffs to obtain a minimum recommended enzyme activity level of (a) 125 PPU/kg of feedingstuffs for chickens for fattening, chickens for laying, laying hens, turkeys for fattening, turkeys for breeding, ducks and minor species (i.e. geese, quail, pigeons, pheasants and game birds) and (b) 250 PPU/kg of feedingstuffs for piglets, pigs for fattening and sows.

For the determination of 6-phytase activity in the feed additive the applicant proposes a spectrophotometric method. The method is based on the release of inorganic phosphate from 6-phytase during the hydrolysis of sodium phytate and the quantification is done against a phosphate standard curve. The method was in-house validated by the applicant and later was verified by a second independent laboratory. The reported performance characteristics were: - a relative standard deviation for repeatability (RSDr) ranging from 3.5 to 6.1%, - a relative standard deviation for intermediate precision (RSDR) ranging from 3.5 to 5.3%, - a recovery rate (RR) ranging from 100 to 101%, - a limit of detection (LOD) ranging from 0.1 to 1.0 PPU/g of product and - a limit of quantification (LOQ) of product ranging from 1.0 to 1.8 PPU/g

For the determination of 6-phytase activity in premixtures the applicant proposes a method based on the same method principle as for the feed additive. The method was in-house validated by the applicant and later was verified by a second independent laboratory. The reported performance characteristics were: - a RSDr ranging from 3.5 to 5.5%, - a RSDR of 8.5%, - LOD ranging from 0.3 to 10 PPU/g of premixture, - a LOQ ranging from 0.5 to 50 PPU/g of premixture and a RR of 102%. Due to reported matrix effects for some premixtures, the method is not applicable to premixtures with elevated levels of zinc and copper.

For the determination of 6-phytase activity in feedingstuffs the applicant proposes a method, based on the same principles as for the feed additive. The method was in-house validated by the applicant and later was verified by a second independent laboratory The reported performance characteristics were: - a RSDr ranging from 3.9 to 8.9%, - a RSDR ranging from 5.1 to 7.4%, - a LOD ranging from 23 to 35 PPU/kg of feedingstuffs, - LOQ ranging from 36 to 50 PPU/kg of feedingstuffs and - RR ranging from 64 to 101%. A very low RR of 64% was obtained at an enzymatic activity level of 125 PPU/kg of feedingstuffs.

Based on these acceptable performance characteristics the applicant's in-house validated and verified methods are recommended for official control purposes within the frame of authorisation for the determination of 6-phytase activity in - feed additives; - premixtures with the exception of samples with high amounts of zinc and copper; and - feedingstuffs at the activity level of 250 PPU/kg, but not at the activity level of 125 PPU/kg due to the low recovery rate obtained at this level. Further testing or validation is not considered necessary.

FAD-2008-0043	
Product Name	Natuphos
Active substance	3-phytase (EC 3.1.3.8)
Rapporteur	AGES

The current application authorisation is sought for 3-phytase (Natuphos) under the category 'zootechnical additives', group 4(a), digestibility enhancer for pigs for fattening according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for 3-phytase according to Article 13(3) of Regulation (EC) No 1831/2003.

The active agent of Natuphos is 3-phytase produced by submerged fermentation of Aspergillus niger (CBS 101.672). One FTU-unit is the amount of 3-phytase which liberates one µmol of inorganic phosphate per minute from sodium phytate at pH = 5.5 and 37 °C. The product is intended to be marketed in three forms, as powder (Natuphos 5000), as granulate (Natuphos 5000 G and 10000 G) and as liquid formulation (Natuphos 5000 L and 10000 L), containing, either 5000 or 10000 FTU/g or ml of product. These products are intended to be incorporated into premixtures and/or complete feedingstuffs to obtain an enzyme activity level of minimum 100 FTU/kg in complete feedingstuffs for pigs for fattening.

For the determination of 3-phytase activity in feed additives, premixtures and feedingstuffs the applicant proposes a colorimetric method measuring inorganic phosphate released by 3-phytase from phytate substrate at pH = 5.5 and 37 °C. The released inorganic phosphate forms a yellow complex with an acidic molybdate/vanadate reagent, which is measured at 415 nm and quantified against a standard curve of phosphate for feed additives; and is based on a reference enzyme Natuphos, available from the applicant, for premixtures and feedingstuffs.

The applicant submitted the validation data regarding the analytical method determining 3-phytase activity in feed additives, premixtures and feedingstuffs, which were obtained from collaborative studies organised by the Association of German Agricultural Analytical and Research Institutes (VDLUFA).

For the determination of 3-phytase activity in feed additives the following performance characteristics were reported: - a relative standard deviation for repeatability (RSDr) of 2.5 %, - a relative standard deviation for reproducibility (RSDR) of 4.9 % and - recovery rate (RR) ranging from 98 to 102 %. Based on these acceptable performance characteristics the proposed method is considered suitable for determination of 3-phytase activity in feed additives for pigs for fattening for official control purposes in the frame of authorisation.

For the determination of 3-phytase activity in premixtures the method performance characteristics obtained from feedingstuffs are applicable to the premixture samples which are diluted with ground corn meal and therefore behave as a matrix of feedingstuffs.

For the determination of 3-phytase activity in feedingstuffs the performance characteristics of the VDLUFA method, obtained at 600 U/kg enzymatic activity, were: - a limit of detection (LOD) of 45 FTU/kg of feedingstuffs, - a limit of quantification (LOQ) of 90 FTU/kg of feedingstuffs, - a RSDr ranging from 6.4 to 7.0 %, - a RSDR ranging from 11.1 to 12.3 % and RR ranging from 98 to 103 %. Upon request from the CRL the applicant provided additional precision data obtained at lower enzyme activity levels close to the minimum level in feedingstuffs (100 FTU/kg). For enzyme activity levels between 100 and 500 FTU/kg of feedingstuffs the reported relative standard deviation for intermediate precision (RSD) ranges from 11.8 to 15.2 %.

Several other ring trial validated methods for the determination of phytase activity in feedingstuffs exist. These include a colorimetric method which is developed by FEFANA (European Association of Feed Additive Manufacturers) and validated on various phytase products (including Natuphos products). The method is currently under evaluation to become a standard of the European Committee for Standardisation (CEN) and is very similar to the above mentioned VDLUFA method, but the quantification is based on the use of phosphate standard curve. The validation of draft CEN method included a phytase activity level ranging from 700 to 1500 U/kg of feedingstuffs, whereas the validation range of VDLUFA method was extended to cover the low phytase activities ranging from 100 to 500 FTU/kg of feedingstuffs. Therefore, the proposed VDLUFA method is found suitable for

official controls for the determination of phytase activity at minimum proposed level (100 FTU/kg of feedingstuffs) in the frame of present authorisation.

Based on acceptable performance characteristics the applicant proposed VDLUFA method is recommended for official control purpose for the determination of 3-phytase activity in premixtures and feedingstuffs. Further testing or validation is not considered necessary.

FAD-2008-0044	
Product Name	Formi LH
Active substance	Potassium diformate
Rapporteur	CRL-FA

In the current application authorisation is sought for potassium diformate (Formi LHS) under the category "zootechnical additives", group 4(d) - "other zootechnical additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Formi LHS as an additives for sows. The additive is intended to be marketed as a crystalline dry product containing 98% potassium diformate, 1.5% silica and water up to 0.5%.

The active agent of Formi LHS is potassium diformate. The product is intended to be incorporated into premixtures and/or complete feedingstuffs. The minimum and maximum content of potassium diformate in complete feedingstuffs for sows is 8000 and 12000 mg/kg, respectively.

For the determination of potassium diformate in the feed additive, the applicant proposes a method based on the quantification of total formate. The measured formate content allows the calculation of potassium diformate content in the sample. The method is based on oxidation with potassium permanganate followed by iodometric titration. The following acceptable performance characteristics for the determination of total formate content obtained from the in-house validation study were reported: - a recovery rate ranging from 99 to 101 % and a relative standard deviation of repeatability (RSDr) of 0.1 %.

Based on acceptable performance characteristics, the proposed method is recommended for official control purposes for the determination of potassium diformate in feed additives in the frame of authorisation.

For the determination of potassium diformate in premixtures and feedingstuffs, the applicant proposes an ion chromatography method equipped with electrical conductivity detection (IC/ECD). The method is based on the principle that potassium diformate dissociates into formate under the conditions of the analysis. From the measured formate content the potassium diformate content is then calculated. On request of the CRL the applicant provided in-house validation results for the determination of potassium diformate in feedingstuffs only. As no results were reported for premixtures the CRL could not evaluate the suitability of the proposed method for official control purposes.

The following acceptable performance characteristics obtained from the in-house validation study were reported for feedingstuffs: - a limit of detection (LOD) of 100 mg/kg; - a limit of quantification (LOQ) of 500 mg/kg; - a recovery rate close to 100 %; and - RSDr ranging from 3.2 to 3.5 %. The validation experiments were performed with a set of different feed samples covering a formate content ranging from 3600 to 10000 mg/kg. These samples were also analysed by a second independent expert laboratory and all the reported results were in good agreement. Furthermore, the validation report included summary information related to a proficiency test (PT) organised by VDLUFA in 2006. Upon request from the CRL, the organiser of the PT provided the raw data together with the statistical assessment of the trial, showing a relative standard deviation of reproducibility of 16%.

Based on the acceptable performance characteristics mentioned above, the CRL recommends the proposed method for official control purposes for the determination of potassium diformate in feedingstuffs in the frame of authorisation. Further testing or validation is not considered necessary.

FAD-2008-0045	
Product Name	Soda Ash
Active substance	Sodium Carbonate
Rapporteur	CRL-FA

In the current application authorisation is sought for Sodium Carbonate under the category technological additives, functional group 1(j) as an acidity regulator for all animal species, according to Annex I of Regulation (EC) No 1831/2003.

The active agent is sodium carbonate of technical grade with a minimum purity of 99.5%. It is intended to be marketed as a white odourless powder to be incorporated into premixtures and/or complete feedingstuffs to obtain a maximum recommended dosage of sodium carbonate of 400 mg/kg of feedingstuffs for all animal species.

For the determination of sodium carbonate in feed additives the applicant proposes the international standard method ISO 740:1976. The method is based on a titrimetric assay for the determination of total soluble alkalinity of sodium carbonate for industrial use. The CRL recommends this ISO method for official controls for the determination of sodium carbonate in feed additives.

The unambiguous determination of the content of exogenous sodium carbonate added to the premixtures and the feedingstuffs is not achievable by analysis. Nevertheless, several analytical methods are available for the determination of total sodium and total carbonates in premixtures and in feedingstuffs. Among them, the CRL recommends two international standard methods for official controls: - the CEN method EN 15510:2008 for the determination of total sodium and the Community method (Regulation (EC) No 152/2009) for the determination of total carbonates. Further testing or validation is not considered necessary.

FAD-2008-0049	
Product Name	AviPlus
Active substance	Citric acid Sorbic acid Thymol
Rapporteur	CRL-FA

In the current application authorisation is sought for AviPlus according to Article 4 (1) of Regulation (EC) No 1831/2003 under the category "zootechnical additives", group 4(d) "other zootechnical additive", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use AviPlus as additive improving growth and/or feed efficacy of weaned piglets. The additive is intended to be marketed in forms of micro beads, containing 25 % of citric acid, 16.7 % of sorbic acid and 1.7 % of thymol in matrix of saturated vegetable fats.

The active agents of AviPlus are citric acid (E 330), sorbic acid (E 200) and thymol (Flavis Nº 04.006), all approved additives.

The feed additive is intended to be incorporated into premixtures and/or complete feedingstuffs to obtain a recommended concentration ranging from 1000 to 3000 mg feed additive per kg of complete feedingstuffs for piglets. The corresponding concentration ranges in complete feedingstuffs for piglets are: from 250 to 750 mg/kg for citric acid, from 167 to 501 mg/kg for sorbic acid and from 17 mg/kg to 51 mg for thymol.

For the determination of the citric acid (E 330) in the feed additive (AviPlus) and premixtures a reverse phase high performance liquid chromatography method equipped with ultraviolet/diode array detection (RP-HPLC-UV/DAD) is proposed by the applicant. The following acceptable performance characteristics obtained from the in-house validation study were reported: - a limit of determination (LOD) of 5 mg/kg; - a limit of quantification (LOQ) of 10 mg/kg; - a recovery rate of 100 % determined

at different concentration levels; - a repeatability relative standard deviation (RSDr) of 3.3 % for feed additive and 5.2 % for premixtures.

For the determination of citric acid (E 330) in the feedingstuffs the applicant proposes an enzymatic method, based on the CEN standardized method for the determination of citric acid in fruit and vegetable juices (EN 1137). The following acceptable performance characteristics obtained from the in-house validation study were reported: - LOD = 5 mg/kg; - LOQ = 10 mg/kg; - a recovery rate of 100 %; - RSDr = 1.6 %.

Samples of feed additive (AviPlus), premixtures and feedingstuffs were sent to a second independent laboratory for determination of citric acid. The reported results were in agreement with those obtained by the applicant, thus demonstrating the transferability of the applicant's method [7].

Based on these acceptable performance characteristics, the applicant in-house validated and verified methods for the determination of citric acid are recommended for official control purposes in the frame of authorisation.

For the determination of the sorbic acid (E 200) in the feed additive (AviPlus), premixtures and feedingstuffs the RP-HPLC-UV/DAD method is proposed by the applicant. The following acceptable performance characteristics obtained on the in-house validation were reported: LOD = 10 mg/kg; - LOQ = 25 mg/kg; - a recovery rate of 100 % for feed additive (AviPlus) and premixtures, 85 % for feedingstuffs; - RSDr = 2.9 % for feed additive, 4.1 % for premixtures and 4.2 % for feedingstuffs.

Samples of feed additive (AviPlus), premixtures and feedingstuffs were sent to a second independent laboratory for determination of sorbic acid. The reported results were in agreement with those obtained by the applicant, thus demonstrating the transferability of the applicant's method.

Based on these acceptable performance characteristics, the applicant in-house validated and validated method for the determination of sorbic acid is recommended for official control purposes in the frame of authorisation.

For the determination of the thymol in the feed additive (AviPlus), premixtures and feedingstuffs the RP-HPLC-UV/DAD method is proposed by the applicant. The following acceptable performance characteristics obtained on the in-house validation were reported: - LOQ = 2.5 mg/kg; - a recovery rate ranging from 90 to 100 % depending on matrix; - RSDr ranging from 2.9 to 3.4 %, for different matrixes.

Samples of feed additive (AviPlus), premixtures and feedingstuffs were sent to a second independent laboratory for determination of thymol. The reported results were in agreement with those obtained by the applicant, thus demonstrating the transferability of the applicant's method.

Based on these acceptable performance characteristics, the applicant in-house validated and verified method for the determination of thymol is recommended for official control purposes in the frame of authorisation. Further testing or validation is not considered necessary.

FAD-2008-0052	
Product Name	Cycostat 66G
Active substance	Robenidine hydrochloride
Rapporteur	CRL-FA

Cycostat 66G (E 758) is a product already authorised as feed additive for use in rabbits for breeding and chickens, turkeys and rabbits for fattening, under the category 'coccidiostats and histomonostats' according to Annex I of Regulation (EC) No 1831/2003.

In the current application a re-evaluation according to Article 10 (2) of Regulation (EC) No 1831/2003 is sought for Cycostat 66G for rabbits for fattening and rabbits for breeding on the inclusion level ranging from 50 to 66 mg active substance per kilogram of feedingstuffs. The active substance of Cycostat 66G is robenidine hydrochloride. Cycostat 66G contains 6.6 % of the active substance (robenidine hydrochloride), 4.0 % of calcium lignosulfonate (binder) and 89.4 % of calcium sulphate dehydrate (carrier).

The performance characteristics of the Community method based on reverse phase high performance liquid chromatography (RP-HPLC) with ultraviolet (UV) detection are: - a limit of quantification (LOQ) of 5 mg/kg; - a relative standard deviations for repeatability (RSDr) ranging from 3.3 to 4.1 % and a relative standard deviation for intermediate reproducibility (RSDR) ranges from 9.7 to 10.6 % for feedingstuffs for rabbits.

The applicant used the above mentioned Community method to determine robenidine hydrochloride in feedingstuffs and applied it also to feed additive and premixtures. The following method performance characteristics were reported:

For feed additive: - LOQ = 150 mg/kg; - RSDr = 3.4 %; - RSDR = 4.4 % and – a recovery rate of 93 %;

For premixtures: - limit of detection (LOD) of 119 mg/kg; - RSDr = 3.1 %, - RSDR = 7.2 % and - a recovery rate of 99 %;

For feedingstuffs: - LOD = 0.5 mg/kg; - RSDr = 2.6 %; - RSDR = 3.1 % and - a recovery rate ranging from 94 to 98 %.

The reported performance characteristics confirm the applicability of the Community method in the frame of this authorisation, i.e. when robenidin hydrochloride is measured in Cycostat 66G or when robenidin hydrochloride is introduced in premixtures and feedingstuffs via Cycostat 66G. Therefore, the CRL recommends the Community method (Commission Regulation (EC) No 152/2009, Annex IV, Method E) for the determination of robenidine hydrochloride in the feed additive, premixtures and feedingstuffs for official control purposes in the frame of the Cycostat 66G authorisation. Further testing or validation is not considered necessary.

FAD-2008-0056	
Product Name	AnimaVit
Active substance	Bacillus subtilis KBL001 CBS117162
Rapporteur	CRL-FA

In the current application authorisation is sought for the microbial feed additive Bacillus subtilis KBL 001 CBS 117162 under the category 'zootechnical additive', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) 1831/2003. Specifically, authorisation is sought for the use of Bacillus subtilis KBL 001 CBS 117162 for piglets (weaned) and pigs for fattening. The feed additive consists of a minimum of 4x109 colony forming units (CFU) per gram of viable spores of Bacillus subtilis KBL 001 CBS 117162. The feed additive is in the form of granulate and is intended to be mixed into complete feedingstuffs at a final concentration of 2x109 to 1x1010 CFU/kg of feedingstuffs.

For the enumeration of Bacillus subtilis KBL 001 CBS 117162 in the feed additive, premixtures and feedingstuffs, the applicant proposes the draft CEN method - prEN 15784:2008 – an internationally recognised spread plate method. This method was ring-trial validated using the premixtures and feedingstuffs samples containing Bacillus subtilis spores. The performance characteristics of the draft CEN method reported after logarithmic transformation of measured values (CFU) are:

- For the premixtures: (1) a standard deviation for repeatability (sr) of 0.09 log10 CFU/g and (2) a standard deviation for between-laboratory reproducibility (sR) of 0.32 log10 CFU/g.

- For the feedingstuffs: (1) a sr = 0.07 log10 CFU/g and (2) a sR = 0.35 log10 CFU/g and (3) a limit of quantification (LOQ) of 2x107 CFU/kg, well bellow the minimum content proposed by the applicant (2x109 CFU/kg of feedingstuffs)

Molecular methods were used by the applicant for identification of the active agent. For official controls pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended. Further testing or validation is not considered necessary.

FAD-2008-0060	
Product Name	Lactiferm
Active substance	Enterococcus faecium M74
Rapporteur	CRL-FA

In the current application authorisation is sought for the microbial feed additive Enterococcus faecium M74 NCIMB 11181 under the category 'zootechnical additive', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) 1831/2003. Specifically, authorisation is sought for the use of Enterococcus faecium M74 NCIMB 11181 for chickens for fattening. Enterococcus faecium M74 NCIMB 11181 is provided as a powder or in a coated form containing at least 5x1010 CFU/g and also in a water soluble form containing at least 2x1011 CFU/g spores of Enterococcus as active agent. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 2.5x108 to 1.5x1010 CFU/kg of feedingstuffs.

For the enumeration of Enterococcus faecium M74 NCIMB 11181 in the feed additive, premixtures and feedingstuffs, the applicant proposes the CEN method - FprEN 15788 - an internationally recognised spread plate method. This method was ring-trial validated using feedingstuffs samples containing Enterococcus faecium containing enterococci at two different concentrations that cover the target levels of this application. The performance characteristics of the CEN method for the enumeration are, a standard deviations for repeatability (sr) and reproducibility (sR) of around 0.12 – 0.20 log10 and 0.23 – 0.41 log10 (calculated from the base 10 logarithms of the measured CFU/g) in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are around 107 CFU/kg of feedingstuffs.

The applicant used the above mentioned spread plate method to analyse various matrices containing Enterococcus faecium M74 NCIMB 11181 and reported results at concentrations ranging: - from 6.8x1010 to 1.1x1011 (for powder form and coated form) and 2.9x1011 to 3.4x1011 (in water soluble) CFU/g for feed additive; - from 1.8x1012 to 3.9x1012 CFU/kg for premixtures;and - from 1.7x108 to 6.7x1011 (for powder and coated form), and 3.9x1011 to 8.3x1012 (in water soluble), CFU/kg for feedingstuffs.

The CRL recommends the above mentioned draft CEN method for official control of the active agent in feed additive, premixtures and feedingstuffs.

The molecular method, 1-D Protein Gel Electrophoresis (SDS-PAGE) was used by the applicant for identification of the active agent. The CRL recommends for official control pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended. The CEN Technical Committee 327 is currently developing a European Standard for this methodology. Further testing or validation is not considered necessary.

FAD-2009-0001	
Product Name	
Active substance	L-isoleucine
Rapporteur	CRL-FA

In the current application authorisation is sought for L-isoleucine under the category 'nutritional additives', functional group 'amino acids, their salts and analogues', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use L-isoleucine for supplementing feed for all animal species. The product is a crystalline powder with a minimum content of 92 % L-isoleucine. The feed additive is intended to be included into premixtures and feedingstuffs at a final concentration depending on the concentration of L-isoleucine already present in the feed components and on the nutritional requirements of the different animal species.

For the determination of the active substance (L-isoleucine) in the feed additive, premixtures, and feedingstuffs the applicant proposes the official Community and fully ring-trial validated method for determination of amino acids [Commission Regulation (EC) No 152/2009]. The method is applicable

for both the determination of free (synthetic and natural) and the determination of total (peptide-bound and free) amino acids including L-isoleucine, using an amino acid analyser or High Pressure Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. The same method is adopted by ISO and described in the ISO standard 13903:2005 [Animal feedingstuffs – determination of amino acids content], which additionally reports the results from a second intercomparison study performed on different premixtures and feeds [Llames & Fontaine, J. of AOAC Int., Vol. 77, No. 6, 1994]. Performance characteristics for the target analyte (L-isoleucine) include the relative standard deviation for repeatability (RSDr) ranging from 2.00 to 5.38 % and relative standard deviation for reproducibility (RSDR) ranging from 6.84 to 14.62 %, depending on the matrix. The method is suitable for official controls for the determination of free and total L-isoleucine in feedingstuffs. Although performance characteristics for the feed additive itself and, for premixtures are not available, the method can be considered suitable also for official control of active substance in these matrices. It is not suitable to differentiate between the salts or D- and L-forms of amino acids, or between naturally occuring and added L-isoleucine.

Alternatively, validated methods based on the same techniques, such as the method 4.11.6 of the Association of German Agricultural Analytical and Research Institutes (VDLUFA) [Methodenbuch III, 5. Erg. 2004, VDLUFA – Verlag, Darmstadt] and the similar AOAC Method 999.13 [Fontaine and Eudaimon, J. of AOAC Int., Vol. 83, No. 4, 2000] can complement the official Community method for the determination of L-isoleucine in the feed additive and in premixtures and therefore are considered suitable for official control purposes in the frame of the authorisation. Further testing or validation by the CRL is not considered necessary.

FAD-2009-0005	
Product Name	Protural
Active substance	Sodium benzoate
Rapporteur	SVA

In the current application authorisation is sought for Sodium Benzoate (Protural) under the category ‘zootechnical additives’, functional group 4(d), ‘other zootechnical additives’, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically authorisation is sought to use Protural for weaned piglets. It is intended to be marketed in the form of a white, odourless, non-dust forming granule. Its active agent is sodium benzoate with a minimum purity of 99.4%. It is intended to be incorporated into premixtures and/or complete feedingstuffs for weaned piglets to obtain a recommended dosage of sodium benzoate of 4 g/kg in feedingstuffs.

For the determination of sodium benzoate in the feed additive the applicant proposed a potentiometric method for the intended purpose. For official control the CRL recommends the European Pharmacopoeia's titrimetric method [Monograph 01/2008:0123] to determine sodium benzoate in the feed additives.

For the determination of sodium benzoate in the premixtures and feedingstuffs the applicant proposed a method, based on ion chromatograph (IC) coupled to Ultraviolet (UV) detection. The method was in-house validated with acceptable performance characteristics. However, the verification of the IC-UV method was missing as the second laboratory applied a different method based on High Performance Liquid Chromatography (HPLC) coupled to UV detection, instead of the IC UV method. According to the opinion of experts from National Reference Laboratories the application of IC-UV in the frame of official control could be difficult due to the instrumentation required. However, other methods based on HPLC-UV are routinely used by National Reference Laboratories for official control for the determination of benzoic acid in the premixtures and the feedingstuffs. Such a method was developed and single-laboratory validated at the Austrian Agency for Health and Food Safety (AGES) on premixtures and feedingstuffs samples containing benzoic acid at a concentration range of 5 to 10 g/kg. The reported performance characteristics were: - a limit of detection (LOD) of 0.4 g/kg, - a limit of quantification (LOQ) of 1.25 g/kg, - a relative standard deviation for repeatability (RSDr) ranging from 2.3 to 4.9%, - a relative standard deviation for intermediate precision (RSDR) ranging from 4.2 to 6.9% and - a recovery rate (RR) ranging from 96 to 101%.

Based on these acceptable performance characteristics the CRL recommends the HPLC UV method developed and validated by AGES to determine sodium benzoate in the premixtures and feedingstuffs for official controls. Further testing or validation is not considered necessary.

FAD-2009-0013	
Product Name	Calsporin
Active substance	Bacillus subtilis C-3102
Rapporteur	CRL-FA

In the current application authorisation is sought for the microbial feed additive Bacillus subtilis C-3102, DSM 15544 under the category 'zootechnical additive', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) 1831/2003. Specifically, authorisation is sought for the use of Bacillus subtilis C-3102 for piglets (weaned). The feed additive consists of a minimum of 1x1010 colony forming units (CFU) per gram of viable spores of Bacillus subtilis C-3102. The feed additive is a pale granular powder intended to be mixed into complete feedingstuffs at a final concentration of 3x108 CFU/kg.

For the enumeration of Bacillus subtilis C-3102 in the feed additive, premixtures and feedingstuffs, the applicant proposes the CEN method - EN 15784:2009 – an internationally recognised spread plate method. This method was ring-trial validated using the premixtures and feedingstuffs samples containing Bacillus subtilis spores. The performance characteristics of the CEN method - reported after logarithmic transformation of measured values (CFU) - are:

- For the premixtures: - a standard deviation for repeatability (sr) of 0.09 log10 CFU/g and - a standard deviation for between-laboratory reproducibility (sR) of 0.32 log10 CFU/g.

- For the feedingstuffs: - sr = 0.07 log10 CFU/g; - sR = 0.35 log10 CFU/g and - a limit of quantification (LOQ) of 2x107 CFU/kg of feedingstuffs, well bellow the minimum content proposed by the applicant (3x108 CFU/kg).

Molecular methods were used by the applicant for identification of the active agent. The CRL recommends for official control pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification. The CEN Technical Committee 327 is currently occupied with the harmonization of a European Standard for this identification method. Further testing or validation is not considered necessary.

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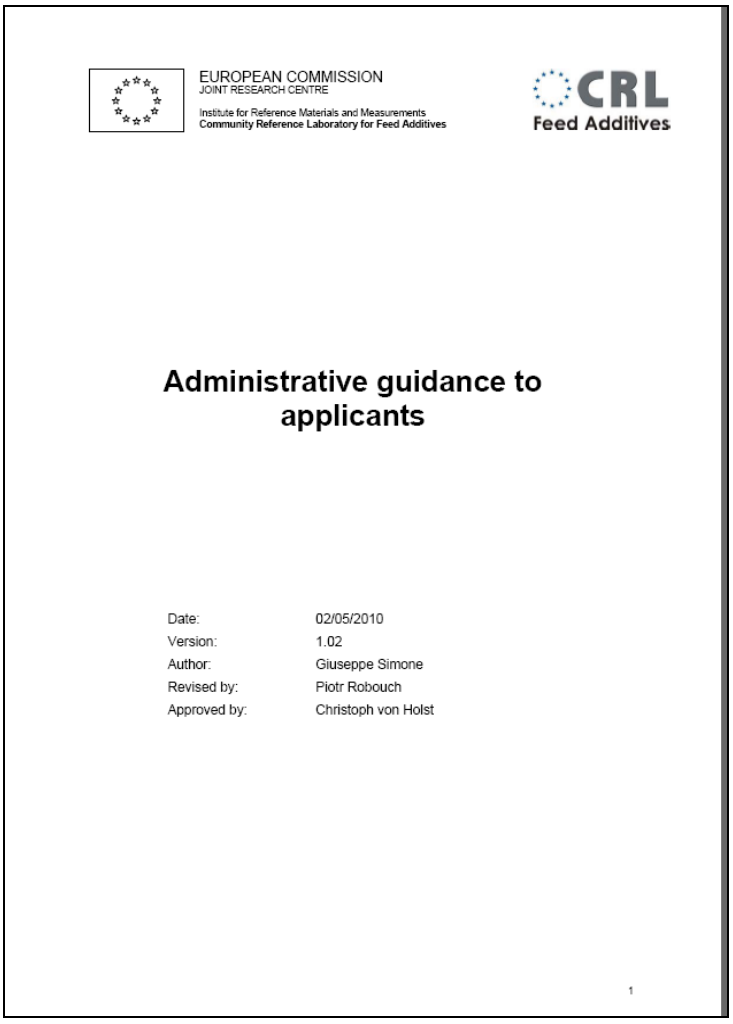
Annex II

CRL-FA Administrative Guidance to Applicants



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

CRL Feed Additives

Administrative guidance to applicants

Date:	02/05/2010
Version:	1.02
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Approved by:	Christoph von Holst

1

Full document available from CRL website
http://irmm.jrc.ec.europa.eu/html/CRLs/crl_feed_additives/authorisation/guidance_for_applicants/Administrative_Guidance_2009_ver1.02_w_annexes.pdf

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Version	Date	Comment	Modified Pages
1.00	01/12/2009	Document created by Giuseppe Simone	

1. INTRODUCTION AND PURPOSE

1.1. Introduction

Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives to use for animal nutrition [1] establishes the Community procedure for authorisation of feed additives.

It also establishes that the Institute of Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC) is the Community Reference Laboratory for Feed Additives (CRL-FA).

The duties and tasks of the Community Reference Laboratory for Feed Additives (CRL-FA) in relation with this procedure are outlined in:

- Annex II of Regulation (EC) No 1831/2003[2];
- Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 of 4 March 2005 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives[3].

The CRL-FA is assisted by a consortium of National Reference Laboratories (NRLs), listed in Annex II of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009[3].

Article 12 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 as last amended by Regulation (EC) No 885/2009 [3] foresees the possibility that the CRL for feed additives establishes guidance for applicants concerning

a) reference samples;

b) the testing of methods of analysis, including in particular criteria about when such testing may be required;

c) the validation of methods of analysis, including in particular criteria about when such validation may be required;

[1] O.J. L 268 of 18.10.2003, p. 29.

[2] as amended by Commission Regulation (EC) No 378/2005 of 4 March 2005, O.J. L 59 of 05.03.2005, p. 8.

[3] O.J. L 59 of 05.03.2005, p. 8, last amended by Commission Regulation (EC) No 885/2009, O.J. L 254 of 26.09.2009, p. 58

d) requirements concerning methods of analysis submitted in accordance with paragraph 2.6 of Annex II to Regulation (EC) No 429/2008[4]

Paragraph 2.6 of Annex II to Commission Regulation (EC) No 429/2008 - which lays down the rules for the implementation of Regulation (EC) No 1831/2003 as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives, stipulates that applicants shall refer to the detailed guidance provided by the CRL (this document).

1.2. Purpose of this Guidance document

This Guidance aims to help applicants in the administrative process for the authorisation of feed additives laid down in Article 2 and Article 3 of Regulation (EC) No 429/2008.

In particular this Guidance lays down the administrative procedure for the payment of the fee foreseen by Article 4 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, as last amended by Regulation (EC) No 885/2009[5] and for the submission of the reference samples to the CRL.

This document will be updated regularly according to new legislation and to the experience that the CRL will develop with the handling samples and applications.

Applicants are therefore recommended to consult the latest version of this document, available on the CRL's Feed Additives website, at the following URL: http://irmm.jrc.ec.europa.eu/crl-feed-additives.

For detailed information related to the administrative procedures for the submission of the applications to the European Commission and for the submission of the corresponding technical dossiers to the European Food Safety Authority (EFSA), applicants are recommended to consult the website of the European Commission – Directorate General Health and Consumers (DG SANCO) [6] and the website of EFSA – FEEDAP[7].

[4] O.J. L133 of 22.05.2008, p. 1

[5] O.J. L 254 of 26.09.2009, p. 58

[6] http://ec.europa.eu/food/food/animalnutrition/feedadditives/index_en.htm

[7] http://www.efsa.europa.eu/EFSA/ScientificPanels/efsa_locale-1178620753812_FEEDAP.htm

2. ADMINISTRATIVE PROCEDURE

The administrative procedure consists of different phases and it involves different actors depending on their respective roles.

2.1. Pre - validation phase

The procedure starts with a pre-validation phase which includes two consecutive steps: **1) Declaration; 2) Application**

2.1.1. Declaration

The applicant shall send to the CRL-FA the **Declaration Form** (**DF**) provided in Annex CRL/I to this Guidance document no later than **six weeks before** the intended date of submission of the corresponding application to the European Commission [8]. The DF shall be completed with the required information depending on the type of application which is intended to be submitted, determining the rate of the applicable fee in accordance with the provisions laid down in Annex IV to Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009[9]. The DF shall be accompanied by a cover letter on headed notepaper carrying the official heading or logo of the applicant.

The information provided in the DF will be checked by the CRL-FA. If no fee is due, the CRL-FA will send the **No Fee Acknowledgement (NFA)** to the applicant. In this case the samples validation procedure described in paragraph 2.2.1 and the CRL evaluation phase described in paragraph 2.3 (see below) are not applicable. If a fee is due, the CRL-FA will send a **Debit Note (DN)** for the appropriate fee rate to the applicant. The applicant shall pay the fee **within two weeks**. Upon reception of the payment, the CRL-FA will send the **Fee Acknowledgement of Receipt (FAR)** to the applicant.

The CRL-FA will also send copy of either NFA or FAR to by to DG SANCO and EFSA.

[8] In case of applications submitted in accordance with Article 10 (2) of Regulation (EC) No 1831/2003 the related Declaration Form shall be submitted to the CRL-FA **no later than 26 September 2010**

[9] O.J. L 59 of 05.03.2005, p. 8, last amended by Commission Regulation (EC) No 885/2009, O.J. L 254 of 26.09.2009, p. 58

2.1.2. Application

1) In accordance with Article 2 of Regulation (EC) No 429/2008 the applicant shall complete the **Application Form** (**AF**) in Annex I to that Regulation and, shall send it – in original and signed, to the European Commission.

   The CRL-FA recommends that corresponding fields in both the DF and the AF are completed providing **exactly the same information**.

   Detailed information about the submission of the AF is available on the website of the European Commission – DG SANCO[10].

2) Article 3 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 stipulates that applicants seeking authorisation for a feed additive shall send **reference samples** to the CRL-FA.

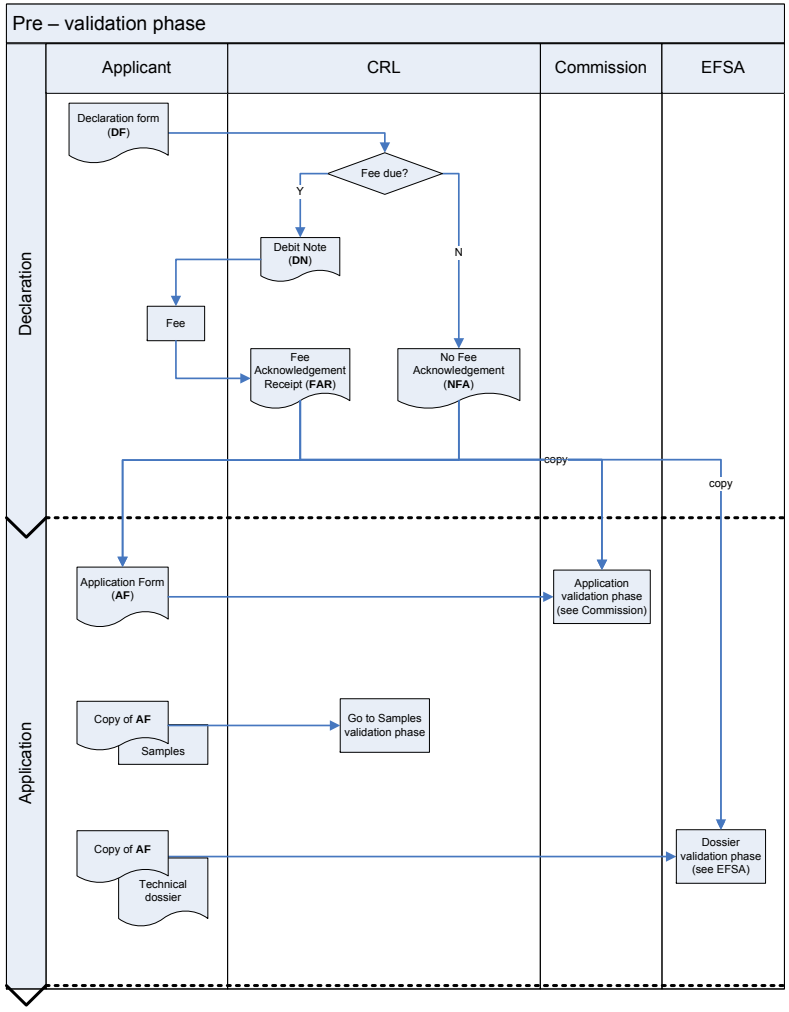
   When sending these samples to the CRL-FA, the applicant shall enclose copy of the AF – as submitted to the European Commission.

   Details concerning on the quantity and packaging of the reference samples, the required accompanying documentation, and the procedure for shipment are described in paragraph 4.2.

3) In accordance with Article 2 of Regulation (EC) No 429/2008 and Article 7 (3) of Regulation (EC) No 1831/2003, the applicant shall send the technical dossier to EFSA. Copy of the AF and other administrative documents shall be enclosed to the technical dossier. Detailed information and requirements related to the submission of the technical dossier are available on the website of the EFSA – FEEDAP[11].

[10] http://ec.europa.eu/food/food/animalnutrition/feedadditives/index_en.htm

[11] http://www.efsa.europa.eu/EFSA/ScientificPanels/efsa_locale-1178620753812_FEEDAP.htm



2.2. Validation phase

The application and the dossier are considered valid, when they fulfil the requirements laid down in Regulation (EC) No 1831/2003 and Regulation (EC) No 429/2008. The description of the validity checks on the application form and on the technical dossier performed by DG SANCO and EFSA respectively is beyond the scope of this guidance

document. Detailed information related to those procedures is available on the DG SANCO[12] and EFSA websites[13].

2.2.1. Samples validation

Article 3 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 stipulates that applicants seeking authorisation for a feed additive, shall send reference samples to the CRL-FA.

Reference samples are considered valid by the CRL-FA when they fulfil the requirements laid down in Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 and further detailed in this guidance document. The checklist used by the CRL-FA for the samples validation procedure is available in Annex CRL/II to this guidance document. The applicant is encouraged to use it as reference checklist before sending the samples to the CRL-FA since in no circumstances the CRL will return to the applicant samples that are considered invalid. These will be immediately disposed according to CRL-FA internal procedures.

If samples are considered not valid, the CRL-FA will send the **Invalid Samples Acknowledgement of Receipt** (**ISAR**) to the applicant, whereby to request a new set of samples and/or related documentation fulfilling the requirements.

When the samples are considered valid, the CRL-FA will send the **Valid Samples Acknowledgement of Receipt (VSAR)** to the applicant. A copy of it is also sent by the CRL-FA to DG SANCO and EFSA.

The applicant is recommended to keep this document for future reference and to retain the **CRL Sample Number** (required in case of future applications related to the same feed additive) and the **Expiry Date** in order to supply replacement samples on time, in accordance with Article 3 (3) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

[12] http://ec.europa.eu/food/food/animalnutrition/feedadditives/index_en.htm

[13] http://www.efsa.europa.eu/EFSA/ScientificPanels/efsa_locale-1178620753812_FEEDAP.htm

Exceptions

As laid down in Article 3 (4) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 reference samples are not required in the following two specific cases:

- when the application is submitted in accordance with Article 4 (1) of Regulation (EC) No 1831/2003 for a new use of an already authorised feed additive;
- when the application is submitted in accordance with Article 13 (3) of Regulation (EC) No 1831/2003 for changing the terms of an existing authorisation.

In both cases, these provisions are only applicable at the condition that:

- reference samples have been previously sent to the CRL

and

- the proposed modification of the terms of the authorisation is not related to the characteristics of the product or its composition.

2.3. CRL-FA Evaluation phase

The CRL-FA evaluation starts when EFSA issues the **Validity Statement** and makes all information supplied by the applicant available to the CRL-FA. EFSA's scientific assessment of the dossier starts in parallel on the same date (see EFSA – FEEDAP website[14] for details on the scientific assessment).

The CRL-FA makes Section 2.6 of the Technical dossier available to the NRLs via eRoom and may appoint one NRL to act as Rapporteur Laboratory (the Rapporteur).

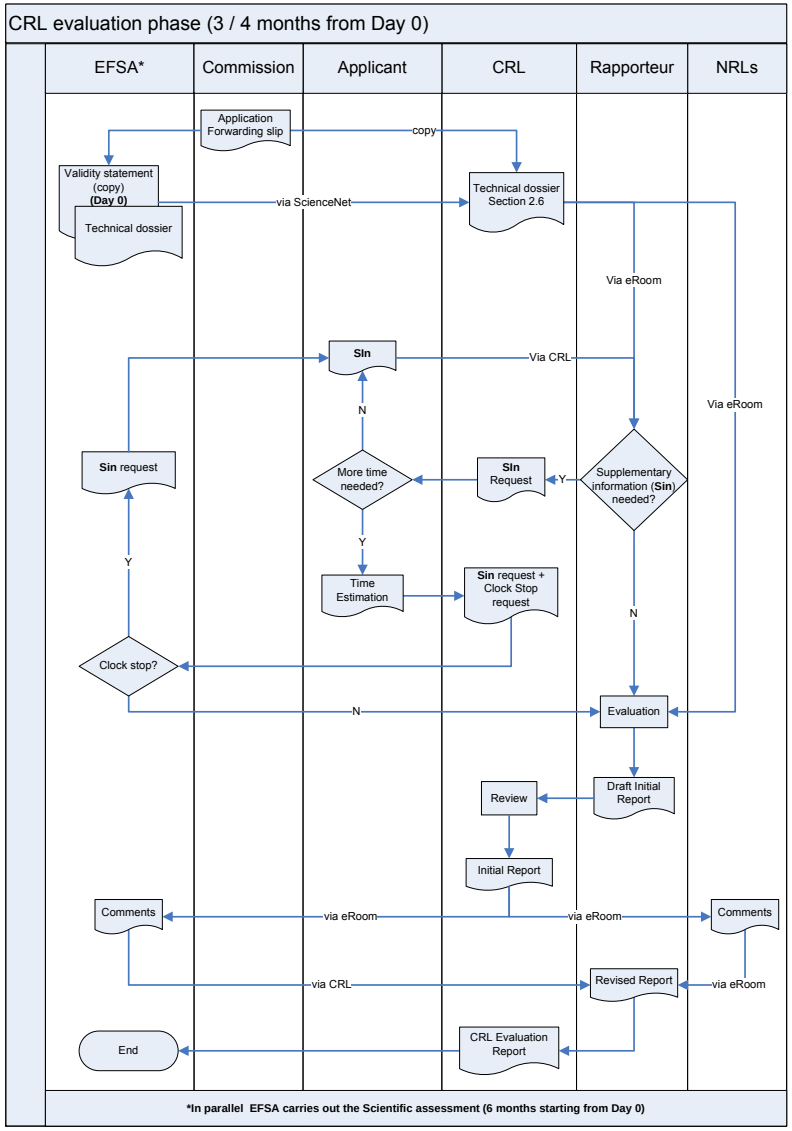
When the information included in the technical dossier do not allow the Rapporteur to conclude on the suitability of the methods of analysis for official control (*cf.* Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009), the Rapporteur informs the CRL-FA. A request to provide **Supplementary Information (SIn)** within a specified deadline is sent to the applicant by the CRL-FA.

Once the applicant has provided the requested information, these are forwarded by the CRL-FA to the Rapporteur in order complete the evaluation.

[14] http://www.efsa.europa.eu/EFSA/ScientificPanels/efsa_locale-1178620753812_FEEDAP.htm

Whenever the applicant anticipates that the **Sin** can not be provided to the CRL-FA within the deadline, the CRL-FA will inform EFSA about the required Sin and will request an extension of the time limit to deliver the CRL Evaluation Report, in accordance with Article 5 (1) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009. Upon agreement of EFSA, the evaluation will be stopped and the Sin request will be forwarded to the applicant. The evaluation will resume when the **SIn** are submitted by the applicant.

The Rapporteur prepares the Initial Report and sends it to the CRL-FA. The Initial Report is reviewed by the CRL-FA and is made available to the NRLs and EFSA for comments. The Rapporteur compiles the comments received and sends the Revised Report to the CRL-FA. Based on the Revised Report the CRL Evaluation Report is then prepared by the CRL-FA and sent to EFSA.



Exceptions

As laid down in Article 5 (4) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, the evaluation of the CRL-FA is not required in the following two specific cases:

— when the application is submitted in accordance with Article 4 (1) of Regulation (EC) No 1831/2003 for a new use of an already authorised feed additive;

— when the application is submitted in accordance with Article 13 (3) of Regulation (EC) No 1831/2003 for changing the terms of an existing authorisation.

In both cases, these provisions are only applicable at the condition that:

— the methods of analysis for the concerned feed additive were already submitted in accordance with paragraph 2.6 of Annex II to Regulation (EC) No 429/2008 for purpose of authorisation and they were evaluated by the CRL-FA;

and

— the proposed conditions for the new use or the proposed modification of the conditions fall within the scope of the methods already evaluated by the CRL-FA.

Nevertheless, if DG SANCO, EFSA, or the CRL-FA considers that a new evaluation is necessary, the applicant will be required to pay the corresponding fee.

2.4. Amendments to the CRL Evaluation Report

EFSA or DG SANCO may request the CRL-FA to amend an Evaluation Report already delivered when, as laid down in Article 5 (3) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, when the conditions for placing the additive on the market resulting from EFSA's opinion are significantly different from those originally proposed by the applicant or when supplementary information relevant to the method of analysis have been provided by the applicant to EFSA. In accordance with Article 5 (4) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 in such cases the CRL may request the Rapporteur to prepare the required amendment.

2.5. Addendum to the CRL Evaluation Report

When the CRL Evaluation Report indicates that the testing and/or the validation of the method of analysis through the consortium of NRLs is considered necessary, the CRL-FA shall inform the applicant and DG SANCO, as provided for in Article 10 (1) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009. At the same time the CRL-FA will provide to the applicant a detailed work plan, including an

estimate of the time schedule and of the fee to be paid. The applicant shall inform the CRL-FA about his agreement within 15 days.

The CRL-FA will deliver the addendum to the Evaluation Report within 30 days after completion of the experimental tests.

3. APPLICABLE FEE

As laid down in Annex IV to Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, two components constitute the fee:

- The first component is intended to support the CRL-FA administrative costs and the costs related to the handling of the reference samples. This first component amounts to EUR 2 000.
- The second component is intended to support the costs of the Rapporteur Laboratory for the scientific evaluation and preparation of the evaluation report. This second component amounts to EUR 4 000.

The applicability of these two components depends on the type of application submitted by the applicant, and determines the total fee to be paid.

As a general rule, the first component is applicable each time that the intended application for authorisation implies that samples must to be sent to the CRL-FA. The second component is applicable each time that an Evaluation Report must be delivered by the CRL-FA.

The decision tree in Annex CRL/III to this document helps to estimate the total fee to be paid in the different cases.

4. SAMPLES

4.1. General

Article 3 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009 stipulates that applicants seeking authorisation for a feed additive, have to send three reference samples of the product concerned to the CRL-FA.

These reference samples must be in the form in which the feed additive is intended to be placed on the market.

In case of "chemically defined flavourings" for which groups of applications are submitted simultaneously under Article 10 (2) of Regulation (EC) No 1831/2003, the reference samples can be in the form of one (or more) mixture of the concerned substances, provided that the mixture is representative of all substances in the group and that they can be analysed using the multi-analyte method(s) described in the technical dossier.

In addition to the reference samples mentioned above, the applicant shall supply also reference standards of the pure active agents in the following cases:

- Zootechnical additives[15] (except micro-organisms);
- Coccidiostats and histomonostats;
- Products produced by genetically modified micro-organisms (GMOs);
- Products for which maximum residue limits (MRLs) are established or are proposed[16].

During the entire duration of the authorisation (10 years) new reference samples must be supplied by the applicant to the CRL-FA to replace those expired, as laid down in Article 3 (3) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

Also, the applicant shall supply additional reference samples, reference standards, feed and/or food test materials upon request of the CRL-FA.

4.2. Requirements

Quantity

The quantity required for each sample should be sufficient to prepare minimum 25 kg and maximum 30 kg of complete feedingstuffs calculated considering the concentration or the activity of the active agent in the product and the maximum dose proposed in the conditions of use. The quantity of the reference standards should be sufficient to allow for 100 analyses and should not be less than 1 gram.

[15] In case of enzymes, a preparation with certified activity shall be submitted as reference standard.

[16] When MRLs are proposed or established for marker residues, reference standards of these marker residues shall be submitted.

Packaging

The container used (bottle, bag, etc.) should not negatively influence the physical/chemical properties of the content and **shall be sealed using a tamper-proof closure that has to be broken in order to open the container, thus revealing irreversibly whether the container has been opened.**

The containers shall preferably be in accordance to the following specifications:

Plastic bottles

High density polyethylene (HDPE) bottles, square cross-section, wide neck (for liquids, powders, granulates) or narrow neck (for liquids), colour white, sized according to the dimensions detailed in the table below:

Capacity (ml)	Approx. dimensions (mm)
50	36x36
100	45x45
250	59x59
350	60x60
500	75x75
750	83x83
1000	85x85

Round bottles with similar capacity and dimensions can also be used.

Closures: screw polypropylene (PP) closures with tamper evident ring and conical seal.

For photosensitive substances, amber (brown) HDPE bottles similar to the white bottles described above shall be used.

Closures: screw polypropylene (PP) closures with tamper evident ring and conical seal, Teflon® cup seal, or polyterafluoro ethylene (PTFE)-coated silicone seal (depending on the physico-chemical characteristics of the additive).

Glass bottles

Whenever possible, glass containers should be avoided. Glass bottles can be used when the additive or its components are incompatible with plastic bottles.

Glass bottle can be transparent or amber coloured depending on the photosensitivity of the components of the additive and shall be preferably square shaped. Specifications are provided in the table below:

Capacity (ml)	Square bottles approx. dimensions (mm)	Round bottles approx. diameter (mm)	DIN-thread
50 -		46	GL 32
100	50x50	56	GL 32 or GL 45
250	64x64	70	GL 45
500	78x78	86	GL 45
1000	94x94	101	GL 45

For hazardous substances (e.g. acids), glass bottles with external plastic coating shall be used in order to avoid leakage in case of damages to the bottle during transport.

Closures: in all cases standardised (GL) screw polypropylene (PP) closures with tamper evident ring and conical seal, Teflon® cup seal, or polytetrafluoro ethylene (PTFE)-coated silicone seal (depending on the compatibility) shall be used.

Aluminium foil bags

Aluminium foil bags thermally sealed can be used when the required sample quantity is small and the other types of containers are considered as not appropriate.

Storage conditions

The intended storage conditions and corresponding shelf life shall be clearly stated on the label (see labelling requirements).

Storage temperature different from room temperature (~ 20 °C) should be considered by the applicant, especially if this extends significantly the shelf life of the product, thus resulting in a less frequent replacement of the samples (see paragraph 4.1).

In such cases, the applicant should demonstrate the stability and establish the shelf life of the product at + 5 °C or at - 30 °C, which are the storage temperatures available at the CRL-FA facilities.

The samples must be delivered to the CRL-FA already at the temperature chosen by the applicant (+ 20 ° C, + 5° C or at - 30° C). In such cases, the applicant shall pay particular attention to shipment conditions especially for frozen samples in order to avoid defrosting during transport.

In no circumstances the samples will be stored by the CRL-FA at a different temperature than that of shipment.

Shipment

Shipping boxes shall be sealed with tamper evident closures.

The box shall carry the indication "Laboratory samples – no commercial value". Hazardous materials must be packed and labelled as required by transport regulations.

Care should be taken to prevent and minimise risk of breakage of fragile samples containers during transport. The applicant is responsible for ensuring appropriate transport conditions (i.e. temperature, humidity) in accordance with the specific requirements for storage of the product, in particular using special transport services.

It is recommended to contact the CRL-FA well in advance in order to notify arrival date/time of refrigerated or frozen samples, specifying the required storage conditions.

Labelling requirements

The provisions laid down in Article 16 of Regulation (EC) No 1831/2003 as regards labelling of feed additives apply by analogy to the corresponding reference samples. Thus, the same label as proposed for purpose of placing the additive on the market shall be used to label the samples.

In particular, the label shall mention:

a) the specific name proposed or given to the additives upon authorisation, preceded by the name of the functional group as proposed or mentioned in the authorisation;

b) the name or business name and the address or registered place of business of the person responsible for the particulars referred to in this Article;

c) the net weight or, in the case of liquid additives either the net volume or the net weight;

d) where appropriate, the approval number of the establishment manufacturing or placing on the market the feed additive or the premixture pursuant to Article 10 of Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene or, as applicable, to Article 5 of Directive 95/69/EC;

e) directions for use, and any safety recommendations regarding the use and, where applicable, the specific requirements proposed or mentioned in the authorisation, including animal species and categories for which the additive is intended;

f) the identification number, if applicable;

g) the batch reference number and date of manufacture.

In addition to the information specified above, the label of an additive belonging to a functional group specified in Annex III of Regulation (EC) No 1831/2003 must bear the information, indicated in that Annex. Namely:

a) Zootechnical additives, coccidiostats and histomonostats:

— the expiry date of the guarantee or the storage life from the date of manufacture,

— the directions for use, and

— the concentration;

b) Enzymes, in addition to the abovementioned indications:

— the specific name of the active component or components in accordance with their enzyme activities, in conformity with the authorisation given,

— the International Union of Biochemistry identification number, and

— instead of concentration: units of activity (units of activity per gram or units of activity per millilitre);

c) Micro-organisms:

— the expiry date of the guarantee or the storage life from the date of manufacture,

— the directions for use,

— the strain identification number, and

— the number of colony-forming units per gram;

d) Nutritional additives:

— the active-substance level, and

— the expiry date of the guarantee of that level or storage life from the date of manufacture;

e) Technological and sensory additives with the exception of flavouring compounds:

— the active substance level;

f) Flavouring compounds:

— the incorporation rate in premixtures.

For "chemically defined flavourings" for which groups of applications are submitted simultaneously under Article 10 (2) of Regulation (EC) No 1831/2003, if the reference samples is in the form of one (or more) mixture of the concerned substances, the above mentioned labelling requiremens may be replaced by the mention **"Mixture of chemically defined flavourings"** followed by the list of the substances included in the mixture.

When, according to Article 3 (1) (a) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, also samples of the reference standards have to be submitted, the label shall mention **"Reference standard"** in addition to the following information:

a) the specific name of the substance/agent;

b) the name or business name and the address of the producer;

c) the net weight or, in the case of liquids either the net volume or the net weight;

d) the concentration (or units of activity per gram or units of activity per millilitre);

e) any safety recommendations;

f) the batch reference number and date of manufacture;

g) the expiry date of the guarantee or the storage life from the date of manufacture.

The check- list provided in Annex CRL/II to this document details the labelling requirements for the different type of samples and additives.

If the labels can not be put directly on the samples containers due to their small size (e.g. vials), it is recommended that the samples are sealed in a plastic (transparent) bag or in plastic container with wide neck and screw closure and the label put on it.

Samples carrying labels not in compliance with these requirements or hand-written labels will be considered as not valid samples and will be disposed by the CRL-FA.

Samples documentation

The following documentation shall complement the samples:

a) Copy of the Application Form (AF) as sent to DG SANCO; or Annex CRL/IV to this Guidance document in case of replacement samples;

b) Where the application concerns a feed additive consisting of or containing micro-organisms, a letter authorising the CRL-FA to access the microbial strain deposited at the internationally recognised culture collection mentioned in point 2.2.1.2.of Annex II of Regulation (EC) No 429/2008, as provided for in Article 3 (1) of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009;

c) A certificate of identification stating that the samples have the characteristics and properties described in the application and that they are suitable to be used as reference samples;

d) Material Safety Data Sheet;

e) Samples Validity checklist (Annex CRL/II);

f) Public summary of the technical dossier

g) Scientific summary of the technical dossier.

5. CONTACTS

Duly completed and signed Declaration Forms have to be submitted **both via e-mail (or fax) and via standard mail** at the following address:

Community Reference Laboratory for Feed Additives
Attn: Mrs. Machteld de Smet
European Commission – Joint Research Centre
Institute for Reference Materials and Measurements
Retieseweg, 111
B- 2440 Geel
Belgium

e-mail: jrc-irmm-crl-feed-additives@ec.europa.eu
fax: +32 (0)14 571 787

Samples have to be submitted to the same address. Please contact the CRL-FA in advance to inform about delivery date and time in case of special shipment conditions (refrigerated/frozen samples).

European Commission

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Abstract
In 2009 the CRL-FA Authorisation submitted a total of twenty four evaluation reports to EFSA and organised the annual workshop attended by twenty seven National Reference Laboratories. This report provides a detailed overview of the yearly activities, thus including a major deliverable of the year 2009: The CRL-FA "Administrative Guidance for Applicants".

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