ANNEX II

GENERAL REQUIREMENTS TO BE SATISFIED BY THE DOSSIER PROVIDED FOR IN ARTICLE 3

2. SECTION II: IDENTITY, CHARACTERISATION AND CONDITIONS OF USE OF THE ADDITIVE; METHODS OF ANALYSIS

The additive has to be fully identified and characterised.

2.1. Identity of the additive

2.1.1. Name of the additive

If appropriate, a proposal for the trade name shall be made for additives linked to a holder of authorisation.

2.1.2. Proposal for classification

A proposal for the classification of an additive for one or more categories and functional groups according to its main functions under Article 6 and Annex I of Regulation (EC) No 1831/2003 shall be made.

Any data from other known uses of the identical active substances or agents (e.g. use in food, human or veterinary medicine, agriculture and industry) must be provided. Any other authorisation as feed or food additive, veterinary drugs or other kind of authorisations of the active substance has to be specified.

2.1.3. Qualitative and quantitative composition (active substance/agent, other components, impurities, batch to batch variation)

The active substance(s)/agent(s) and all other components of the additive shall be listed, giving the proportion by weight in the final product. The qualitative and quantitative batch to batch variation of the active substance(s)/agent(s) shall be determined.

For micro-organisms: the number of viable cells or spores expressed as CFU per gram shall be determined.

For enzymes: each declared (main) activity shall be described and the number of units of each activity in the final product given. Relevant side activities shall be also mentioned. The units of activity shall be defined and preferably as μmoles of product released per minute from the substrate, also indicating the pH and the temperature.

If the active component of the additive is a mixture of active substances or agents, each of which is clearly definable (qualitatively and quantitatively), the active substance(s)/agent(s) components must be described separately and the proportions in the mixture given.

Other mixtures in which the constituents cannot be described by a single chemical formula and/or where not all can be identified shall be characterised by constituent(s) contributing to its activity and/or typical major constituent(s).

Without prejudice to any request of supplementary information made by the Authority according to Article 8(2) of Regulation (EC) No 1831/2003, the applicant may omit the description of other components with no safety concerns other than active substances or agents for additives not within the categories of zootechnical additives, coccidiostats and histomonostats, and not in the scope of Regulation (EC) No 1829/2003. In any case, all studies reported in the dossier must be based on the actual additive requested for the authorisation and may provide information on the other possible different preparations that could be made. An in-house identifier may be

allowed, embedded in third-party documents, and a statement is required to list the identifiers and to confirm that the identifier(s) refers to the formulation(s) for which the request is made.

2.1.4. Purity

The applicant shall identify and quantify chemical and microbial impurities, substances with toxic or other undesirable properties that are not intentionally added and do not contribute to the activity of additive. In addition, for fermentation products, the applicant shall confirm the absence of production organisms in the additive. The protocol used for the routine screening of production batches for contaminants and impurities shall be described.

All the data provided have to support the proposal for a specification of the additive.

Specific requirements depending on the production process, complying with existing Community legislation, are listed below.

2.1.4.1. Additives whose authorisation is linked to a holder of authorisation

For additives whose authorisation is linked to a holder of authorisation, the relevant information related to the specific process used by the manufacturer, based on existing standards used for other related purposes, shall be provided. Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications or specifications from European Community food additive authorisations can be used.

2.1.4.2. Additives whose authorisation is not linked to a holder of authorisation

For feed additives whose authorisation is not linked to a holder of authorisation, existing standards used for other related purposes, or that have specifications for food additives as authorised in the European Community or from JECFA can be used. When such standards are not available, or where relevant to the manufacturing process, at least the following particulars shall be described and their concentrations determined:

— for micro-organisms: microbiological contamination, mycotoxins, heavy metals;
— for fermentation products (not containing micro-organisms as active agents): they shall follow the same requirements as for micro-organism products (see above). The extent to which spent growth medium is incorporated into the final product shall also be indicated.
— for plant derived substances: microbiological and botanical contamination (e.g. castor oil plant, weed seeds, rye ergot in particular), mycotoxins, pesticide contamination, maximum values for solvents and, where appropriate, substances of toxicological concern known to occur in the original plant;
— for animal derived substances: microbiological contamination, heavy metals and maximum values for solvents, where appropriate;
— for mineral substances: heavy metals, dioxins and PCBs;
— for products produced by chemical synthesis and processes: all chemicals used in the synthetic processes and any intermediate products remaining in the final product shall be identified and their concentrations given.

The selection of mycotoxins for analysis shall be made according to the different matrices, where appropriate.

2.1.5. Physical state of each form of the product

For solid preparations data on particle size distribution, particle shape, density, bulk density, dusting potential and the use of processes which affect physical properties shall be provided.

For liquid preparations, data for viscosity and surface tension shall be given. Where additive is intended to be used in water, the solubility or extent of dispersion shall be demonstrated.

2.2. Characterisation of the active substance(s)/agent(s)

2.2.1. Description

A qualitative description of the active substance or agent shall be given. This shall include purity and origin of the substance or agent, plus any other relevant characteristics.

2.2.1.1. Chemical substances

Chemically well-defined substances shall be described by generic name, chemical name according to IUPAC (International Union of Pure and Applied Chemistry) nomenclature, other generic international names and abbreviations and/or Chemical Abstract Service Number (CAS). The structural and molecular formula and molecular weight must be included.

For chemically defined compound used as flavourings, the FLAVIS number in connection with relevant chemical group shall be included. For plant extracts the phytochemical markers must be included.

Mixtures in which the constituents cannot be described by a single chemical formula and/or not all of them can be identified shall be characterised by constituent(s) contributing to its activity and/or typical major constituent(s). Marker compound shall be identified to allow stability to be assessed and to provide a means of traceability.

For enzyme and enzyme preparations, the number and systematic name proposed by the International Union of Biochemistry (IUB) in the most recent edition of 'Enzyme Nomenclature' shall be given for each declared activity. For activities not yet included, a systematic name consistent with the IUB rules of nomenclature shall be used. Trivial names are acceptable provided that they are unambiguous and used consistently throughout the dossier, and they can be clearly related to the systematic name and IUB number at their first mention. The biological origin of each enzyme activity must be given.

The microbial origin of chemical substances produced by fermentation shall also be described (see 2.2.1.2 Micro-organisms).

2.2.1.2. Micro-organisms

For all micro-organisms, whether used as product or as production strain, the origin shall be provided.

For micro-organisms used as a product or as production strain, any history of modification shall be indicated. The name and taxonomic classification of each micro-organism shall be provided, according to the latest published information in the International Codes of Nomenclature (ICN). Microbial strains shall be deposited in an internationally recognised culture collection (preferably in the European Union) and maintained by the culture collection for the authorised life of the additive. A certificate of deposition from the collection, which shall specify the accession number under which the strain is held, must be provided. In addition, all relevant morphological, physiological and molecular characteristics necessary to provide the unique identification of the strain and the means to confirm its genetic stability shall be described. For GMOs the description of the genetic modifications shall be given. The unique identifier for each GMO, as referred in Commission Regulation (EC) No 65/2004 of 14 January 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms, shall be included.

2.2.2. Relevant properties

2.2.2.1. Chemical substances

Description of physical and chemical properties shall be given. Dissociation constant, pKa, electrostatic properties, melting point, boiling point, density, vapour pressure, solubility in water and in organic solvents, K_{ow} and K_d/K_{oc}, mass spectrometry and absorption spectra, NMR data, possible isomers and any other appropriate physical properties shall be provided, where appropriate.

Substance produced *via* fermentation shall be free of antimicrobial activities relevant to the use of antibiotics in humans or animals.

2.2.2.2. Micro-organisms

— Toxins and virulence factors

Toxins or virulence factors shall be demonstrated to be absent or of no concern. Strains of bacteria belonging to a taxonomic group that includes members known to be capable of producing toxins or other virulence factors shall be subject to appropriate tests to demonstrate at a molecular and, if necessary, cellular level the absence of any cause for concern.

For strains of micro-organisms for which there is no history of an apparent safe use and whose biology remains poorly understood, a full package of toxicological studies shall be necessary.

— Antibiotic production and antibiotic resistance

Micro-organisms used as additives or as production strain, shall be free of antibiotic activity or shall not be capable of producing antibiotic substances that are relevant as antibiotics in humans and animals.

Strains of micro-organisms intended for use as additives shall not contribute further to the reservoir of antibiotic resistance genes already present in the gut flora of animals and the environment. Consequently, all strains of bacteria shall be tested for resistance to antibiotics in use in human and veterinary medicine. Where resistance is detected, the genetic basis of the resistance and the likelihood of transfer of resistance to other gut-inhabiting organisms shall be established.

Strains of micro-organisms carrying an acquired resistance to antimicrobial(s) shall not be used as feed additives, unless it can be demonstrated that resistance is a result of chromosomal mutation(s) and it is not transferable.

2.3. Manufacturing process, including any specific processing procedures

To define the critical points of the process that may have an influence on the purity of the active substance/agent(s) or additive a description of the manufacturing process shall be given. A material safety data sheet of chemicals used in the production process shall be provided.

2.3.1. Active substance(s)/agent(s)

A description of the production process (e.g. chemical synthesis, fermentation, cultivation, extraction from organic material or distillation) used in the preparation of the active substance(s)/agent(s) of the additive shall be submitted, if appropriate by way of a flowchart. The composition of the fermentation/cultivation media shall be provided. Purification methods shall be thoroughly described.

For Genetically Modified Micro-organisms (GMMs), used as source of additives and grown under contained conditions, Council Directive 90/219/EC[1] applies. A description of

fermentation processes (culture medium, fermentation condition and downstream processing of the fermentation products) shall be included.

2.3.2. Additive

A detailed description of the manufacturing process of the additive shall be submitted. The key stages in the preparation of the additive including the point(s) of introduction of the active substance(s)/agent(s) and other components, and any subsequent processing steps affecting the additive preparation should be provided, if appropriate by means of a flowchart.

2.4. Physico-chemical and technological properties of the additive

2.4.1. Stability

Stability is generally measured by the analytical follow-up of the active substance(s)/agent(s) or its activity/viability. For enzymes, stability may be defined in terms of loss of catalytic activity; for micro-organisms in terms of loss of viability; for flavouring substances in terms of loss of flavour. For other chemical mixtures/extracts stability may be assessed by monitoring the concentration of one or more appropriate marker substances.

Stability of the additive

The stability of each formulation of the additive, on exposure to different environmental conditions (light, temperature, pH, moisture, oxygen and packing material) shall be studied. Expected shelf-life of the additive as marketed should be based on at least two model situations covering the likely range of use conditions (e.g., 25 ^{o}C, 60 % relative air humidity (HR) and 40 ^{o}C, 75 % HR).

Stability of the additive used in premixtures and feedingstuffs

For additives used in premixtures and in feedingstuffs, with the exception of flavouring compounds, the stability of each formulation of the additive shall be studied under common manufacturing and storage conditions of premixtures and of feedingstuffs. Stability studies in premixtures shall be of least six months' duration. Stability shall be tested preferably with premixtures containing trace elements; otherwise the additive should be labelled as ‘not to be mixed with trace elements’.

Stability studies in feedingstuffs normally shall extend at least for three months. Generally stability shall be checked in mash and pelleted (including the influence of pelleting or other forms of treatment) feed for the main animal species of the claim.

For additives intended to be used in water, the stability of each formulation of the additive has to be studied in water under condition simulating practical use.

Where there is a loss of stability, and where appropriate, potential degradation or decomposition products shall be characterised.

Data shall be provided from analyses that include at least one observation at the beginning and one at the end of the storage period.

Where necessary, studies shall contain the detailed quantitative and qualitative composition of the premixtures or of the feedingstuffs used for the trials.

2.4.2. Homogeneity

The capacity for homogeneous distribution of the feed additive (other than flavouring compounds) in premixtures, feedingstuffs or water must be demonstrated.

2.4.3. Other characteristics

Other characteristics, such as dusting potential, electrostatic properties or dispersability in liquids must be described.

2.4.4. Physico-chemical incompatibilities or interactions

Physico-chemical incompatibilities or interactions that could be expected with feed, carriers, other approved additives, or medicinal products must be shown.

2.5. Conditions of use of the additive

2.5.1. Proposed mode of use in animal nutrition

The animal species or categories, age group or production stage of animals shall be indicated in accordance with the categories listed in Annex IV of this Regulation. Possible contra-indications shall be mentioned. The proposed use, in feed or water shall be defined.

Details of the proposed method of administration and level of inclusion must be provided for premixtures, feedingstuffs or water for drinking. In addition, the proposed dose in the complete feed and the proposed duration of administration and proposed withdrawal period must be provided where appropriate. A justification is required where a particular use of an additive in complementary feedingstuffs is proposed.

2.5.2. Information related to users/workers safety

2.5.2.1. Chemical substances

A material safety data sheet formatted in accordance with the requirements of Commission Directive 91/155/EEC of 5 March 1991 defining and laying down the detailed arrangements for the system of specific information relating to dangerous preparations in implementation of Article 10 of Directive 88/379/EEC[(2)] must be provided. If necessary, measures for the prevention of occupational risks and means of protection during manufacture, handling, use and disposal shall be proposed.

2.5.2.2. Micro-organisms

A classification according to Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC)[(3)] shall be submitted. For micro-organisms not classified in group 1 in this Directive, information shall be provided to customers to allow them to take the relevant protection measures for their workers, as defined in Article 3 (2) of the said Directive.

2.5.2.3. Labelling requirements

Without prejudice to the labelling and packaging provisions laid down in Article 16 of Regulation (EC) No 1831/2003, any specific labelling requirements and, where appropriate, specific conditions for use and handling (including known incompatibilities and contraindications) and instructions for proper use shall be indicated.

2.6. Methods of analysis and reference samples

The methods of analysis shall be submitted in the standard layout as recommended by ISO (i.e. ISO 78-2).

According to Regulation (EC) No 1831/2003 and Regulation (EC) No 378/2005, methods of analysis included in this section shall be evaluated by the CRL. The CRL shall submit to the

Authority an evaluation report indicating whether these methods are suitable to be used for official controls of the feed additive that is the object of the application. The CRL evaluation shall focus on the methods specified in sections 2.6.1 and 2.6.2.

If an MRL has been established for the substance object of the application by Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin[(4)], section 2.6.2 will not be subject to evaluation by the CRL. The applicant shall compile section 2.6.2 providing the same method, information and particulars (including relevant updates) for submission to European Medicines Agency (EMEA) in accordance with Annex V of Regulation (EEC) No 2377/90 and in accordance with 'Notice to Applicants and Guidelines', Volume 8 of the series 'Rules governing medicinal products in the European Union'.

Analytical methods described under 2.6.3 may also be included in the evaluation, if considered necessary by the CRL, the Authority or the Commission.

In accordance with Regulation (EC) No 378/2005, the applicant shall provide reference samples directly to the CRL prior to the evaluation of the technical dossier, and replacement samples before the expiration date.

Applicants shall refer to the detailed guidance provided by the CRL in accordance with Article 12 of Regulation (EC) No 378/2005.

2.6.1. Methods of analysis for the active substance

Detailed characterisation of the qualitative and, where applicable, quantitative analytical method(s) for determining compliance with maximum or minimum proposed levels of the active substance(s)/agent(s) in the additive, premixtures, feedingstuffs and, when appropriate, water, shall be provided.

2.6.1.1. These methods shall meet the same requirements as those for methods of analysis used for official control purpose laid down in Article 11 of Regulation (EC) No 882/2004 In particular they shall meet at least one of the following requirements:

— comply with relevant Community rules (e.g. Community methods of analysis) where they exist;

— comply with internationally recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted, or those agreed in national legislation (e.g. CEN Standard methods);

— are fit for the intended purpose, developed in accordance with scientific protocols and validated in a ring test in accordance with an internationally recognised protocol on collaborative trials (e.g. ISO 5725 or IUPAC); or

— are validated in-house according to international harmonised guidelines for the in-house validation of methods of analysis[(5)] with respect to the characterising parameters mentioned in 2.6.1.2.

2.6.1.2. The detailed characterisation of the method(s) shall include the appropriate characteristics set out in Annex III of Regulation (EC) No 882/2004.

2.6.1.3. Performance characteristics of in-house validated methods shall be verified by testing the method in a second, accredited and independent laboratory. Results of such tests shall be provided together with any other information supporting the transferability of the method to an official control laboratory. For reasons of independence and involvement in the evaluation of the documentation provided by the applicant, where the second laboratory is a laboratory participating in the consortium of National

Reference Laboratories (NRLs) assisting the CRL, as laid down in Regulation (EC) No 378/2005, the laboratory shall send a declaration of interests to the CRL, as soon as the application is received by the CRL, describing the work of the laboratory in the application and shall not participate in the evaluation of the application.

2.6.1.4. The CRL may select appropriate characteristics as mentioned under Annex III of Regulation (EC) No 882/2004 in its evaluation report to the Authority.

2.6.1.5. Performance criteria for methods for specific groups of substances (e.g. enzymes) may be established in the detailed guidance provided by the CRL in accordance with Article 12 of Regulation (EC) No 378/2005.

2.6.2. Methods of analysis for the determination of the residues of the additive or of its metabolites in food

Detailed characterisation of the qualitative and quantitative analytical method(s) for determining the marker residues and/or metabolites of the additive in target tissues and animal products shall be provided.

2.6.2.1. These methods shall meet the same requirements as those for methods of analysis used for official control purposes as laid down in Article 11 of Regulation (EC) No 882/2004. In particular, the methods shall meet at least one of the requirements mentioned in 2.6.1.1.

2.6.2.2. The detailed characterisation of the method(s) shall include the appropriate characteristics as set out in Annex III of Regulation (EC) No 882/2004 and shall take into account the requirements set out in Commission Decision 2002/657/EC[(6)]. The same performance criteria laid down in Commission Decisions laying down analytical methods to be used for detecting certain substances and residues thereof in live animal products according to Council Directive 96/23/EC shall be considered where appropriate.

The limit of quantification (LOQ) for each method must not exceed half of the corresponding MRL and must be validated across a range at least from one-half to two times the MRL.

2.6.2.3. Performance characteristics of in-house validated methods shall be verified by testing the method in a second, accredited and independent laboratory. Results of such tests shall be provided. For reasons of independence and involvement in the evaluation of the documentation provided by the applicant, where the second laboratory is a laboratory participating in the consortium of National Reference Laboratories (NRLs) assisting the CRL, as laid down in Regulation (EC) No 378/2005, the laboratory shall send a declaration of interests to the CRL, as soon as the application is received by the CRL, describing the work of the laboratory in the application and shall not participate in the evaluation of the application.

2.6.2.4. The CRL may select appropriate characteristics from the ones mentioned under point 2.6.2.2 in its evaluation report to the Authority.

2.6.2.5. Performance criteria for methods for specific groups of substances (e.g. enzymes) may be established in the detailed guidance provided by the CRL in accordance with Article 12 of Regulation (EC) No 378/2005.

2.6.3. Methods of the analysis relating to the identity and characterisation of the additive

A description of the methods used for the determination of the characteristics listed under points 2.1.3, 2.1.4, 2.1.5, 2.2.2, 2.4.1, 2.4.2, 2.4.3, and 2.4.4 shall be provided by the applicant.

In accordance with Annex II of Regulation (EC) No 1831/2003 as amended by Regulation (EC) No 378/2005, the methods submitted under this section may also be evaluated if considered relevant by the, the Authority or the Commission for the assessment of the application.

It is recommended that the methods described under this section are internationally recognised. For those methods that are not internationally recognised, the methods have to be fully described. In those cases, studies shall be performed by accredited and independent laboratories and shall be documented according to appropriate quality standards (e.g. GLP in accordance with Directive 2004/10/EC or ISO standards).

Methods for the identification and characterisation of the additive shall meet the same requirements as those for methods of analysis used for official control purposes as laid down in Article 11 of Regulation (EC) No 882/2004, particularly where legal requirements are established (e.g. impurities, undesirable substances).

(**1**) OJ L 117, 8.5.1990, p. 1. Directive as last amended by Commission Decision 2005/174/EC (OJ L 59, 5.3.2005, p. 20).

(**2**) OJ L 76, 22.3.1991, p. 35. Directive as last amended by Directive 2001/58/EC (OJ L 212, 7.8.2001, p. 24).

(**3**) OJ L 262, 17.10.2000, p. 21.

(**4**) OJ L 224, 18.8.1990, p. 1. Regulation as last amended by Commission Regulation (EC) No203/2008 (OJ L 60, 5.3.2008, p. 18).

(**5**) M. Thompson et al.: Harmonized Guidelines For Single Laboratory Validation Of Methods Of Analysis (IUPAC Technical Report) Pure Appl. Chem., Vol. 74, No. 5, pp. 835-855, 2002.

(**6**) OJ L 221, 17.8.2002, p. 8. Decision as last amended by Decision 2004/25/EC (OJ L 6, 10.1.2004, p. 38).

Changes to legislation:
There are outstanding changes not yet made to Commission Regulation (EC) No 429/2008. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.
View outstanding changes

Changes and effects yet to be applied to the whole legislation item and associated provisions

- Signature words omitted by S.I. 2019/654 reg. 81
- Annex 2 s. 4 words substituted by S.I. 2019/654 reg. 83(e)(i) (This amendment not applied to legislation.gov.uk. Reg. 83(e)(i) substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 12(15)(d)(i))
- Annex 2 s. 2 words substituted by S.I. 2019/654, reg. 83(c)(iii) (as substituted) by S.I. 2020/1504 reg. 12(15)(b)(i)
- Annex 2 s. 2 words substituted by S.I. 2019/654, reg. 83(c)(iv) (as substituted) by S.I. 2020/1504 reg. 12(15)(b)(ii)
- Annex 2 s. 2 words substituted by S.I. 2019/654, reg. 83(c)(v) (as substituted) by S.I. 2020/1504 reg. 12(15)(b)(iii)
- Annex 2 s. 3 words substituted by S.I. 2019/654, reg. 83(d)(iii) (as substituted) by S.I. 2020/1504 reg. 12(15)(c)(i)
- Annex 2 s. 3 words substituted by S.I. 2019/654, reg. 83(d)(iv)(aa) (as substituted) by S.I. 2020/1504 reg. 12(15)(c)(ii)
- Annex 2 s. 3 words substituted by S.I. 2019/654, reg. 83(d)(iv)(bb) (as substituted) by S.I. 2020/1504 reg. 12(15)(c)(ii)
- Annex 2 s. 4 words substituted by S.I. 2019/654, reg. 83(e)(i) (as substituted) by S.I. 2020/1504 reg. 12(15)(d)(i)
- Annex 2 s. 4 words substituted in earlier amending provision S.I. 2019/654, reg. 83(e)(ii) by S.I. 2020/1504 reg. 12(15)(d)(ii)