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SCIENTIFIC OPINION

Guidance for the preparation of dossiers for zootechnical additives[1†¥]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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This guidance document follows the structure and definitions of Regulation (EC) No 1831/2003 and its implementing rules (Regulation (EC) No 429/2008). It is intended to assist the applicant in the preparation and the presentation of its application, as foreseen in Article 7.6 of Regulation (EC) No 1831/2003. This document does not substitute for the obligation of an applicant to comply with the requirements of Regulation (EC) No 1831/2003 and its implementing rules.

A zootechnical additive is any additive used to affect favourably the performance of animals in good health or used to affect favourably the environment. The category 'zootechnical additives' is further divided into four functional groups (Annex I of Regulation (EC) No 1831/2003):

(a) digestibility enhancers: substances which, when fed to animals, increase the digestibility of the diet, through action on target feed materials;

(b) gut flora stabilisers: microorganisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora;

(c) substances which favourably affect the environment;

(d) other zootechnical additives.

[1] On request from EFSA, Question No EFSA-Q-2010-01159, adopted on 14 December 2011.

[†] Parts in italics are coming from Regulation (EC) No 429/2008.

[¥] This guidance document replaces the previous EFSA Guidance for the preparation of dossiers for zootechnical additives, adopted in July 2008 (EFSA-Q-2008-403). The following sections have been updated: 2.1.4, 2.1.5, 2.2,2.3, 2.4, 2.5, 3 and 4.

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THE TECHNICAL DOSSIER – GENERAL ASPECTS

The dossiers must enable an assessment to be made of additives based on the current state of knowledge and permit verification of the compliance of these additives with the fundamental principles for authorisation, which are laid down in Article 5 of Regulation (EC) No 1831/2003.

The studies to be submitted and the extent of them will depend on the additive nature, the functional group, the substance itself, the target animals and the conditions of use. The applicant should refer to Regulation (EC) No 429/2008 in order to evaluate which studies and information should be submitted with the application.

Reasons must be given for the omission from the dossier of any data prescribed there.

The dossier shall include detailed reports of all the studies performed, presented in accordance with the numbering system proposed in the Regulation (EC) No 429/2008. The dossier shall include references and copies of all published scientific data mentioned and the copies of any other relevant opinions which have already been produced by any recognised scientific body. Where these studies have already been evaluated by a European scientific body following the legislation in force in the European Union, a reference to the result of the evaluation should be sufficient and a copy should be provided. *Data from studies that have been conducted and published previously or coming from peer review shall clearly refer to the same additive as the one subject to the application for authorisation.*

Studies, including those that have been conducted and published previously or coming from peer review, shall be performed and documented according to appropriate quality standards (e.g. good laboratory practice (GLP) in accordance with Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances or International Organization for Standardization (ISO).

Where in vivo or in vitro studies are carried out outside the European Union, the applicant shall demonstrate that the facilities concerned comply with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (GLP) or ISO standards.

The determination of physico-chemical, toxicological and eco-toxicological properties must be performed in accordance with the methods established by Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, as last amended by Commission Directive 2004/73/EC, or with updated methods recognised by international scientific bodies. The use of methods other than these must be justified.

The studies involving animals should respect the rules on animal welfare laid down by European Union legislation, particularly those listed in Directive 63/2010/EU, and they should not be repeated if not necessary. *The use of in vitro methods or of methods refining or replacing the usual tests using laboratory animals or reducing the number of animals used in these test shall be encouraged. Such methods shall be of the same quality and provide the same level of assurance as the method they aim to replace.*

The description of the methods of analysis in feed or water shall be in conformity with the rules of Good Laboratory Practice as laid down in Directive 2004/10/EC and/or EN ISO/IEC 17025. These methods shall comply with the requirements laid down in Article 11 of Regulation (EC) No 882/2004 of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

Each dossier shall contain a public summary and a scientific detailed summary in order to enable the additive concerned to be identified and characterised, a post-market monitoring proposal and a labelling proposal as referred to in Article 7(3) of Regulation (EC) No 1831/2003.

1 SECTION I: SUMMARY OF THE DOSSIER

1.1 Public summary according to Article 7(3)(h) of Regulation (EC) No 1831/2003

The applicant shall submit a summary indicating the main features of the additive concerned. The summary shall not contain any confidential information and shall be structured as follows:

1.1.1 Contents

a) name of the applicant(s);
b) identification of the additive;
c) method of production and method of analysis;
d) studies on safety and efficacy of the additive;
e) proposed conditions for use; and
f) proposal for post-market monitoring.

1.1.2 Description

a) name and address of the applicant(s)

This information shall be provided in all cases. *When a dossier is submitted by a group of applicants, the name of each of them shall be indicated.*

b) identification of the additive

The identification of the additive shall contain a summary of the information required according to Annex II and III of Regulation (EC) No 429/2008, depending on the type of the feed additive authorisation. In particular: name of the additive, proposed classification by category and functional group, target species/animal categories and doses.

c) method of production and method of analysis

The manufacturing process shall be described.

The general procedures of the analytical methods to be used for the analysis for the official controls of the additive as such, in premixtures, and in feedingstuffs, as required in Annex II and III of Regulation (EC) No 429/2008 shall be described. If appropriate, on the basis of the information submitted, the procedure of the method(s) to be used for the analysis for the official controls of the additives or its metabolites in food of animal origin shall be included.

d) studies on safety and efficacy of the additive

The conclusion regarding the safety and efficacy of the additive based on the different studies performed shall be given. The results of the studies may be included in a tabular form to support the conclusion of the applicant(s). Only studies required according to Annex III of Regulation (EC) No 429/2008 shall be indicated in the summary.

e) proposed conditions for use

The proposal for conditions of use shall be provided by the applicant(s). In particular the applicant shall describe the level of use in feed or water, together with the detailed conditions of use in complementary feedingstuffs. Information is also required where other methods of administration or incorporation in feed or water (e.g., top dressing) *are used. Any specific conditions for use (e.g., incompatibilities), specific labelling requirements and animal species for which the additive is intended shall be described.*

f) proposal for post-market monitoring

1.2 Scientific summary of the dossier

A scientific summary including details of each part of the documents submitted to support the application shall be submitted. This summary shall include the conclusions made by the applicant(s).

The summary must follow the order of Annex II of Regulation (EC) No 429/2008 and address all the different parts with reference to the relevant pages of the dossier.

1.3 List of documents and other particulars

The applicant must identify the number and titles of volumes of documentation submitted in support of the application. A detailed index with reference to volumes and pages shall be added.

1.4 List of parts of the dossier requested to be treated as confidential, where necessary

The list shall make reference to the relevant volumes and pages of the dossier.

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

The additive has to be fully identified and characterised. The studies described in this section must be based on the final product(s) for which authorisation is sought. In-house identifiers should be avoided unless embedded in third-party documents. In this case a statement is required to confirm that the identifier(s) refers to the formulation(s) for which the claim is made.

2.1 Identity of the additive

2.1.1 Name of the additive

The name of the additive (characterisation of the active substance(s) or agent(s) as defined in the subsections 2.2.1.1 and 2.2.1.2) must be given. A proposal for a trade name may be made to be used within the dossier to identify the additive and in the proposal for register entry.

2.1.2 Proposal for classification

A proposal for the classification of an additive for one or more categories[4] *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 and properly referenced.

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. Evidence should be provided by the analysis of at least five production batches that the amount and nature

[4] If the applicant applies for one or more categories in addition to zootechnical additives, reference should be made to the relevant guidance document(s).

of the active substance(s)/agent(s) in the additive specified by the applicant is satisfied in practice.

For microorganisms: the number of viable cells or spores expressed as colony forming units (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 also be mentioned. The units of activity shall be defined preferably as µmoles of reaction product released per minute from the substrate at a specified pH and 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) 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 the constituent(s) contributing to its activity and/or typical major constituent(s).

2.1.4 Purity

The applicant shall identify and quantify microbial and chemical (including residual solvents) impurities, substances with toxic or other undesirable properties that are not intentionally added and do not contribute to the activity of additive. Any substances produced via fermentation should be free of antimicrobial activities relevant to the use of antibiotics in humans or animals. In addition the absence of production organisms in the additive should be confirmed.

The protocol used for the routine screening of production batches for contaminants and impurities should be described and appropriate action levels should be defined.

Monitoring for contaminants and impurities should be consistent with existing legislation (e.g., Directive 2002/32/EC, or specifications from European Union food additive authorisations) and recommendations from internationally recognised sources when these are available (e.g., Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications for enzymes; Commission recommendation on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding). Additional measures should be introduced following the HACCP analysis of the specific process, as necessary.

As a guide the following should be considered as minimum requirements (measured in at least three batches of the additive):

- for microorganisms: microbiological contamination (at least *Salmonella*, enterobacteriaceae, total yeasts and filamentous fungi), and depending on the fermentation media and excipients, mycotoxins,[5] heavy metals (Pb, Hg, Cd) and arsenic.
- for fermentation products (not containing microorganisms as active agents): in addition to the above, the extent to which spent growth medium is incorporated into the final product should also be indicated. For fermentation products produced by genetically modified microorganisms (GMM),

[5] The selection of mycotoxins for analysis should be made according to the different matrices, where appropriate.

identification and quantification of recombinant DNA in the final product should be provided.

- for plant derived substances: microbiological and botanical contamination (e.g., castor oil plant, weed seeds, rye ergot in particular), mycotoxins, dioxins and dioxin-like PCBs, pesticides[6] and, where appropriate, substances of toxicological concern known to occur in the original plant.
- *for animal derived substances: microbiological contamination, heavy metals* and arsenic.
- *for mineral substances:* heavy metals and arsenic, dioxins and dioxin-like PCBs.
- *for products produced by chemical synthesis and processes: chemicals used in the synthetic processes and any intermediate products remaining in the final product shall be identified and their concentrations given.*

The current maximum levels set for residual solvents used in veterinary drugs (VICH guidance GL18) should not be exceeded.

Certificates of analysis indicating the exact values for each measured parameter should be provided. Statements of compliance alone are not considered sufficient. The limit of quantification (LOQ) of the method should be given when the results are expressed as less than a given value.

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. Studies on particle size distribution should provide information on particles of diameters less than 100, 50 and 10 µm (expressed as mean or median in relation to volume). For details, see the technical guidance on user safety.

For liquid preparations, data on vapour pressure, specific weight and viscosity and where the additive is intended to be used in water, solubility or dispersability should be provided.

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 the International Union of Pure and Applied Chemistry (IUPAC) nomenclature, other generic international names and abbreviations and/or Chemical Abstract Service (CAS) number. The structural and molecular formula and molecular weight must be included. Where relevant, data on isomeric forms and accompanying structurally related compounds should be included.

[6] Residues specified under the undesirable substances directive (Directive 2002/32/EC) and any other pesticide residues of potential concern to target animals and/or consumer safety.

For additives of plant origin the information required under section 2.2.2.1 of the guidance for sensory additives/flavouring compounds should be provided. The constituent(s) contributing to the claimed effects should be identified. The phytochemical marker(s) characteristic of the plant of origin 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). A marker compound should be selected which will allow the additive to be identified in the different studies.

For 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 be described (see 2.2.1.2 Microorganisms).

2.2.1.2 Microorganisms

For all microorganisms, whether used as product or as production strain, the origin shall be provided and *any history of modification shall be indicated.* It should be clearly stated whether the microorganism is genetically modified or not within the meaning of the legislation (Directive 2001/18/EC). *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 GMMs the description of the genetic modifications should be given. Applicants are requested to provide data in accordance with Section III (Information requested in applications for GMM and/or their products) of the "Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use". *The unique identifier for each genetically modified organism, 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 and any other relevant physical properties shall be provided where appropriate. For enzymes, the optimum pH and temperature of activity should be provided.

2.2.2.2 Microorganisms

- Toxins and virulence factors

  Toxins or virulence factors shall be demonstrated to be absent or of no concern. Strains of microorganisms 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. As an example on how to assess the potential for toxin production see the technical guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition.

  For strains of microorganisms recognised by EFSA as qualifying for the QPS approach to safety assessment or when its biology is sufficiently well known to allow pathogenic/toxigenic strains to be excluded by direct testing, toxicological studies are normally not required (see 3.2.2).

- Antibiotic production and antibiotic resistance

  Microorganisms used as additives or as a 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 (see technical guidance on microbial studies).

  Strains of microorganisms 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. See technical guidance on antibiotic resistance.

  Strains of microorganisms 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 detailed description of the manufacturing process shall be given.

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 and downstream purification steps) used in the preparation of the active substance(s)/agent(s) of the

additive should be submitted, if appropriate by means of a flowchart. *The composition of the fermentation/cultivation media shall be provided.*

For GMMs used as source of additives and grown under contained conditions, Directive 90/219/EC 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 process steps affecting the additive preparation should be provided, if appropriate by means of a flowchart. A material safety data sheet (MSDS) must be provided for all components of the additive.

2.4 Physical-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) (e.g., mg/kg)*/agent(s)* (e.g., CFU/g) *or its activity* (e.g., units of catalytic activity/kg). For some chemical mixtures/extracts stability may be assessed by monitoring the concentration of one or more appropriate marker substances. Data should be provided from at least three batches of the additive that include at least one observation at the beginning and one at the end of the storage period.

Where there is a loss of stability, measured by the analytical follow-up of the active substance, potential degradation or decomposition products should be characterised, where appropriate.

2.4.1.1 Shelf-life of the additive

The expected shelf-life of the additive as marketed should be proposed, based on at least two model situations covering the likely range of use conditions (e.g., for a solid formulation 25ºC, 60% relative air humidity (RH) and 40ºC, 75% RH; for a liquid preparation, 25ºC and 40ºC). If the additive is distributed in sealed impermeable containers, then requirements for relative humidity can be disregarded.

Stability studies to determine the shelf-life are normally not required for mineral-based products, generally assumed to be stable.

2.4.1.2 Stability of the additive used in premixtures and feedingstuffs

The stability of each formulation of the additive at the recommended inclusion level normally should be studied in feedingstuffs manufactured and stored under common conditions, and if relevant, in premixtures. The quantitative and qualitative composition of the premixtures or the feedingstuffs used for the studies should be given.

Stability studies in feedingstuffs and premixtures should be of at least three and six months' duration, respectively.

Stability should be tested preferably in a premixture containing trace elements; otherwise the additive should be labelled as "not to be mixed with trace elements". Stability in feedingstuffs should be assessed in both mash and further processed feed (e.g., pelleted or extruded, including the influence of the respective processing) for the main animal species of the claim.

2.4.1.3 Stability of the additive used in water or aqueous media

The stability of each formulation of the additive intended to be distributed via the water supply or using aqueous media should be studied under conditions simulating practical use (e.g., environment and water temperature, time) for a minimum duration of 48 h. These data should also take into consideration the presence of excipients that could trigger growth of contaminant microorganisms.

2.4.2 Homogeneity

The capacity for homogeneous distribution of the feed additive in premixtures, feedingstuffs or water must be demonstrated, as appropriate. The same criteria as described under 2.4.1 should be used. As a guide, a minimum of ten sub-samples (10-20 g) from a single batch (of the premixture or feedingstuff) should be analysed and the coefficient of variation calculated. If homogeneity is demonstrated in the final feedingstuff, there is no need to demonstrate homogeneity of mixing at any preceding stages in feed production (including premixtures).

Statistical considerations[7] as a substitute for analytical data from subsamples will not be considered.

For additives intended to be distributed via the water supply or using aqueous media, homogeneity studies are only required when the active substance is not fully soluble/miscible at its proposed concentration of use. In those cases, sampling should take into consideration conditions of use and may require sampling at different locations (where the animal has access to the additive) and time points. Samples from a minimum of ten locations per time point should be analysed and the coefficient of variation calculated.

2.4.3 Other characteristics

Any other relevant characteristics should be described.

2.4.4 Physico-chemical interactions in feed

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

2.5 Conditions of use of the additive

2.5.1 Proposed mode of use in animal nutrition

The proposed use in feed or water shall be defined. *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 Regulation (EC) No 429/2008. Possible contra-indications shall be mentioned.*

[7] For example, Jansen HD. (1992) Mischtechnik im Futtermittelbetrieb. Die Mühle + Mischfuttertechnik. 129 (20), 265-270.

Details of the proposed method of administration and level of inclusion must be provided for premixtures, feedingstuffs or drinking water. In addition, the proposed dose (minimum and maximum) in the complete feedingstuff and the proposed duration of administration and proposed withdrawal period, if any, must be provided. If a particular use in complementary feedingstuffs for some animal species or categories is intended, the (daily) dose should be proposed and justified.

For additives intended to be used in water for drinking, the concentrations derived from feed use should follow the considerations in paragraph 2.3 of the technical guidance on tolerance and efficacy studies.

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[8] 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 as amended by Directive 2001/58/EC 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 Microorganisms

A classification according to Directive 2000/54/EC shall be submitted. For microorganisms not classified in group 1 in this Directive,[9] 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

Methods of analysis to determine the active substance/agent in the additive itself and in premixtures and feedingstuffs as appropriate should be submitted. These should be suitable for the official control of the feed additive. If there are residues of concern, a method of analysis of the active substance and/or its metabolites (including the marker residue) in the relevant tissues/products should be provided.

These methods will be evaluated by the European Union Reference Laboratory (EURL). Details of the requirements specified in the Regulation (EC) No 429/2008. Applicants should also refer to the guidance provided by the EURL.

Methods to determine the identity and the characteristics of the additive (composition of the additive, impurities, physical and chemical properties) should be internationally recognised or otherwise fully described.

[8] Repealed by Regulation (EC) No 1907/2006.
[9] In practice, in the absence of any entries under group 1, this information would be required for all microorganisms.

3 SECTION III: STUDIES CONCERNING THE SAFETY OF THE ADDITIVE

The studies included in this section are intended to permit assessment of:

- *the safety of use of the additive in the target species;*
- *any risk associated with the selection and/or transfer of resistance to antimicrobials and increased persistence and shedding of enteropathogens;*
- *the risks to the consumer of food derived from animals given feedingstuffs containing or treated with the additive or which could result from the consumption of food containing residues of the additive or its metabolites;*
- *the risks from respiratory, other mucosal tissue, eye or cutaneous contact for persons likely to handle the additive as such or as incorporated into premixtures or feedingstuffs; and*
- *the risks of adverse effects on the environment, from the additive itself, or products derived from the additive, either directly and/or excreted by animals.*

Where an additive has multiple active components, each should be separately assessed for safety for consumers and then consideration given to additivity (exclusion of interactions). Alternatively, when the components of a mixture cannot be fully separated (e.g., a plant extract) the complete mixture should be assessed.

No studies concerning the safety of use of the additive for the target animal (Subsection 3.1), for consumers (Subsection 3.2) and for the environment (Subsection 3.4) are required for microorganisms considered by EFSA to qualify for the QPS approach to safety assessment.

No studies concerning the safety for the consumer (Subsection 3.2) and user/worker (Subsection 3.3) are required for additives which are authorised as food additives or approved as components of foodstuffs in the European Union without any restriction.

No studies concerning the safety for the consumer (Subsection 3.2) and environment (Subsection 3.4) are required for additives intended to be used only in pets and other non food-producing animals. Consideration should be given to the safety of the owner.

For additives intended to be used in minor species, see the technical guidance on extrapolation to minor species.

3.1 Studies concerning the safety of use of the additive for the target animals

The studies included in this section are intended to assess:

- *the safety of use of the additive in the target species per se; and*
- *any risk associated with the selection and/or transfer of resistance to antimicrobials and increased persistence and shedding of enteropathogens.*

3.1.1 Tolerance studies for the target species

The aim of the tolerance test is to provide a limited evaluation of short-term toxicity of the additive to the target animals. It is also used to establish a margin of safety, if the additive is consumed at higher doses than recommended.

Tolerance studies in the relevant target species/categories are required for all zootechnical additives except for microorganisms considered by EFSA to qualify for the QPS approach to safety assessment.

All studies reported in this section must be based on the additive described in Section II, except in cases where a concentrated form of the additive is recommended to be tested (e.g., enzymes and microorganisms).

Applicants are encouraged to use, wherever possible, at least a 100-fold overdose in the experimental group and consequently reduce the number of end-points required. When a concentrated form of the additive is needed for this purpose, concentration should be adjusted by reducing the amount of carrier present but the ratio of active agent(s)/substance(s) to the other fermentation products must remain the same as in the final product. *For enzymes, the diet shall provide the appropriate substrate(s).*

For **additives already authorised in food**, refer to the technical guidance for additives already authorised for use in food.

For additives only intended to be used in **pets and other non food-producing animals,** refer to the guidance for pets and other non food-producing animals.

For details on how to perform and report tolerance studies, see the technical guidance on tolerance and efficacy studies in target animals.

3.1.2 Microbial studies

Studies are not required for:

- compounds known or demonstrated not to posses an antimicrobial activity, or whose structure or physical properties preclude antimicrobial activity, at concentrations relevant to feed use.
- microbial additives which consist only of microorganisms considered by EFSA to qualify for the QPS approach to safety assessment.

Where required, studies should demonstrate that the additive does not induce cross-resistance to antibiotics used in human or veterinary medicine or encourage the growth and/or shedding of zoonotic agents.

For those additives that in the tolerance test give an indication of an adverse effect possibly related to digestive tract disturbances, studies on the effects on the target animal gastrointestinal microbiota are required.

For the details see the technical guidance on microbial studies.

3.2 Studies concerning the safety of use of the additive for consumers

The aim is to evaluate the safety of the additive for the consumer and to establish potential residues of the additive or its metabolites in food derived from animals given feed or water containing or treated with the additive. This section consists of a subsection metabolic and residue studies (3.2.1.), toxicological (*in vitro* and *in vivo*) studies (3.2.2) and the assessment of consumer safety (3.2.3).

For additives already authorised in food, refer to the guidance on additives already authorised in food.

For details on how to assess consumer safety, refer to the specific technical guidance on consumer safety.

3.2.1 Metabolic and residue studies

The establishment of the metabolic fate of the additive in the target species is a determinant step in the identification and quantification of the residues in the edible tissues or products derived from the animals given the feed or water containing the additive.

For some additives, depending on their nature or use, it may not always be necessary to carry out metabolic and residues studies.

Metabolic and residue studies are not required if:

- the substance is essentially not absorbed and excreted unchanged (or if transformed in the digestive tract, its metabolites can be demonstrated to be essentially not absorbed); or
- the substance is absorbed in the form of physiological compounds; or
- the active component(s) of the additive consists only of microorganisms or enzymes.

3.2.1.1 Metabolic studies

The purpose of metabolic studies is to evaluate the absorption, distribution, biotransformation and excretion of the additive in the target species and in a laboratory animal, if necessary.

Metabolic studies are not required if the substance is naturally present in significant amounts in food or feedingstuffs or the substance is a normal constituent of body fluids or tissues.

3.2.1.2 Residue studies

Residue studies are required for all substances for which metabolic studies are needed.

Residue studies are required for substances which are a natural constituent of body fluids or tissues or are naturally present in food or feedingstuffs if the additive substantially increases the intake or tissue retention. In such cases, the requirement for residue studies is limited to the comparison of the tissue/product levels in an untreated group and in the group supplemented with the highest dose claimed.

3.2.2 Toxicological studies

The safety of the additive is typically assessed on the basis of the toxicological studies performed *in vitro* and *in vivo* usually on laboratory animals.

Toxicological studies must be carried out with the active substance. If the active substance is present in a fermentation product, the fermentation product should be tested. The fermentation product tested must be identical to that to be used in the commercial product.

Toxicological studies are not required if:

- the substance is absorbed as physiological compound(s); or
- enzymes are produced by microorganisms considered by EFSA to qualify for the QPS approach to safety assessment (or rarely from a commercial strain (lineage) of micro-organism with a substantial history of documented safe use); or
- enzymes are produced by GMMs for which the recipient strain is considered by EFSA to qualify for the QPS approach to safety assessment, and for which the molecular characterisation of the event does not give rise to concern; or
- the micro-organism is considered by EFSA to qualify for the QPS approach to safety assessment or when its biology is sufficiently well known to allow pathogenic/toxigenic strains to be excluded by direct testing;

Toxicological studies are required:

- for microorganisms and their products not exempted above. In this case, genotoxicity/mutagenicity studies and a subchronic (90 day) oral toxicity study should be provided to exclude a potential for the production of toxic metabolites. For microorganisms genotoxicity studies should not be made with living cells as the test item. For microorganisms used as additives and those

used for the production of enzymes, the specific concerns in section 2.2.2.2 should always be addressed, as appropriate.

- for xenobiotic substances (defined as chemicals which are not a natural component of the organism exposed to them), the complete set of toxicological studies described in the guidance for consumer safety is normally required.

Physiological substances whose use results in much higher concentrations than usual in the target organism may be treated as xenobiotics. In these cases, the need for toxicological studies should be considered on a case by case basis, taking into account the level and nature of exposure.

3.2.3 Assessment of consumer safety

Consumer safety is assessed by a comparison of the established health based reference value, such as the Acceptable Daily Intake (ADI) or Tolerable Upper Intake Level (UL) and calculated theoretical intake of the additive or its metabolites from food. For additives without a health based reference value, an estimate of toxicity should be established following 3.2.2.

3.3 Studies concerning the safety of use of the additive for users/workers

Workers can be exposed mainly by inhalation or topical exposure while manufacturing or handling or using the additive.

An assessment of risk to workers shall be included. Experience in the manufacturing plant is often an important source of information in evaluating the risks to workers from exposure to the additive itself by both airborne and topical routes.

Risks to workers shall be assessed in a series of studies using the additive in the form for which the application has been submitted.

Studies on skin irritancy must be performed, and if these give negative results, mucous membrane (e.g., eye) irritancy shall be assessed. Allergenic potential/skin sensitisation potential shall also be assessed. The toxicity data generated to meet consumer safety (see 3.2.2) should be used in the assessment of the systemic toxicity of the additive.

Additives with a high dusting potential or those used under circumstances which could generate aerosols are of particular concern. Data on dusting potential will be used for exposure assessment and this may require information on particle size and content of the active substance in dust. Additives containing enzymes and microorganisms are assumed to be respiratory sensitisers.

The formulation of the product (e.g. micro-encapsulation) may obviate the need for some or all tests. In such cases, appropriate justification shall be provided.

Information on precautionary measures to be taken when handling the additive should be provided (see 2.5.2). *However, use of personal protective devices shall only be regarded as a measure of last resort to protect against any residual risk once control measures are in place. It is preferable, for example, to consider reformulation of the product.*

For details on how to assess user/worker safety, refer to the technical guidance on user safety.

3.4 Studies concerning the safety of use of the additive for the environment

Administration of additives typically occurs over long periods, often involves large groups of animals and the active substance(s) may be excreted to a considerable extent either as the parent compound or its metabolites.

To determine the environmental impact of additives, a stepwise approach shall be followed. All additives have to be assessed through Phase I to identify those additives which do not need further testing. For the other additives a second phase (Phase II) assessment is needed to provide additional information, based upon which further studies may be considered necessary.

The impact on the environment as a result of the Phase I assessment will be considered negligible if:

- the substance/agent is a physiological/natural substance/agent whose use will not result in a substantial increase in concentration in the environment; or
- the additive is intended for non food-producing animals only.

For enzymes produced by genetically modified microorganisms the specific requirements of the "Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use" should be satisfied.

For details on how to assess environmental safety, refer to the technical guidance on environmental risk assessment.

4 SECTION IV: STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

Studies shall demonstrate the efficacy for each proposed use and satisfy at least one of the characteristics set out in Article 5(3) of Regulation (EC) No 1831/2003, according to the functional groups as provided by Article 6 and Annex I of the said Regulation. The effects should be demonstrated in relation to each target animal species/category. Such studies should permit conclusions to be drawn on the efficacy of the additive when used in the European Union. This does not necessarily exclude the reporting of studies made outside the EU.

The studies shall be based on the final product(s) for which authorisation is sought. To avoid confusion, in-house identifiers should be avoided unless embedded in third-party documents. In this case a statement is required to confirm that the identifier(s) refers to the formulation(s) for which the claim is made.

A minimum of three independent *in vivo* studies showing significant effects should be provided to demonstrate efficacy for the relevant target species/categories. These should be carried out at least at two different locations, at least one of which should be in the EU. Efficacy studies should include the lowest dose proposed by the applicant. The minimum content of the additive in feedingstuffs or water (the lowest effective dose expressed as concentration in complete feedingstuffs or water) will then be derived from those dose(s) for which significant effects are demonstrated.

For those additives affecting animal production or performance, long-term efficacy studies should be provided. Depending on the properties of the additive, outcome measures may be based on performance characteristics (e.g., feed efficiency, average daily gain, milk or egg production, carcass composition, herd performance) or reproduction parameters. *Evidence of the mode of action can be provided by short-term*

in vivo or laboratory studies measuring relevant end-points. Such data may be used in support of the claim, but generally will not substitute for long-term efficacy studies.

However, for enzymes which affect the digestibility of non-starch polysaccharides (NSP), phytate phosphorus or protein, short-term (balance) studies can substitute for long-term studies provided that properly defined and specific methods are applied. All studies for demonstration of the efficacy of phytases can be designed as short-term studies provided that digestibility of phytate/total P and partial (e.g., bone ash/P) or total P retention are included as end-points. Ileal digestibility measurements are not encouraged

For other enzymes, two short-term studies could replace two long term studies provided that the following end-points are positively affected:

- NSP-ases/amylases: apparent metabolisable energy (AME) in pigs and nitrogen-corrected AME (AMEn) in poultry, where also the output (eggs) should be taken into consideration.
- Proteases: N retention. The output (eggs/milk) should be included in the balance studies.

For additives affecting animal welfare, affecting the characteristics of animal products and those which directly or indirectly favourably affect the environment, the choice of long-term or short-term studies will depend on the effect claimed and/or the proposed mode of use of the additive.

- For additives affecting welfare, long term studies would be needed to detect changes in morbidity/mortality while short-term studies may be sufficient to measure reduced stress levels as monitored by metabolic indicators.
- For additives which directly or indirectly favourably affect the environment (e.g., reduction of nitrogen or phosphorus excretion, methane production or odour), evidence of efficacy for the target species can be given by short-term efficacy studies

These studies should take into consideration the possibility of an adaptive response to the additive.

For additives seeking authorisation for use in feed and water, a total of three efficacy studies is generally needed to support efficacy. Oral administration routes of additives, via feed or water, are principally considered as bioequivalent. Thus there is no preference for the route of administration of the additive, provided a minimum effective dose can be derived for both routes. The minimum content in feed and in water would be derived from the lowest (daily) intakes of the additive resulting in a significant effect.

If the applicant proposes a dose in water which is lower than the corresponding minimum content authorised/intended for feed use, three efficacy studies should be provided to demonstrate efficacy in water.

If the applicant proposes a dose in water which is higher than the corresponding maximum content recommended/authorised for feed use, additional studies demonstrating the safety of the additive for the target species may be required depending on the margin of safety.

For details on how to perform and report efficacy studies, see the technical guidance on tolerance and efficacy studies in target animals.

Attention shall be paid to known or potential biological or chemical interactions between the additive, other additives and/or veterinary medicines and/or components of the diet, where this is relevant to the efficacy of the additive concerned (e.g., compatibility of microbial additives with coccidiostats and histomonostats or organic acids).

For details on how to perform compatibility studies between microbial additives and other additives showing antimicrobial activity, see the technical guidance on compatibility between zootechnical microbial additives and other additives showing antimicrobial activity.

4.6 Studies on the quality of animal products where this is not the effect claimed

Evidence should be given that the additive does not have a negative effect or other unintended effect on the sensory and nutritional (and if appropriate, hygienic and technological) characteristics of food deriving from animals fed with the additive.

Evidence can be based on physiological/metabolic considerations or given by reference to other scientific literature. Specific studies may be necessary in case of substances for which residue studies are required. An unsupplemented group should be compared with a group receiving the highest dosage proposed for the additive. *The data shall allow statistical evaluation.*

5 SECTION V: POST-MARKET MONITORING PLAN

A post-market monitoring plan is required in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects resulting from the use of the additive on human or animal health or the environment, in accordance with the characteristics of the products concerned.

The design of the monitoring plan shall be detailed on a case-by-case basis and identify who (e.g., applicant, users) will carry out the various tasks that the monitoring plan requires, who is responsible for ensuring that the monitoring plan is set into place and carried out appropriately.

It would generally be sufficient to follow the requirements of the Feed Hygiene Regulation (Regulation (EC) No 183/2005) and Good Manufacturing Practices. The post-market monitoring plan shall in all cases ensure that there is a route by which the competent control authorities, the Commission and the EFSA are informed of any observed adverse effects.