European Food Safety Authority

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**SCIENTIFIC OPINION**

# Guidance for the preparation of dossiers for technological 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 technological additive is any substance added to feed for a technological purpose and which favourably affects the characteristics of feed. The category 'technological additives' is further divided into 13 functional groups (Annex I of Regulation (EC) No 1831/2003):

(a) preservatives: substances or, when applicable, microorganisms which protect feed against deterioration caused by microorganisms or their metabolites;

(b) antioxidants: substances prolonging the storage life of feedingstuffs and feed materials by protecting them against deterioration caused by oxidation;

(c) emulsifiers: substances that make it possible to form or maintain a homogeneous mixture of two or more immiscible phases in feedingstuffs;

(d) stabilisers: substances which make it possible to maintain the physico-chemical state of feedingstuffs;

(e) thickeners: substances which increase the viscosity of feedingstuffs;

(f) gelling agents: substances which give a feedingstuff texture through the formation of a gel;

(g) binders: substances which increase the tendency of particles of feedingstuffs to adhere;

(h) substances for control of radionuclide contamination: substances that suppress absorption of radionucleides or promote their excretion;

(i) anticaking agents: substances that reduce the tendency of individual particles of a feedingstuff to adhere;

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[1] On request from EFSA, Questions No EFSA-Q-2010-00017 and EFSA-Q-2010-00902, 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 technological additives, adopted in July 2008 (EFSA-Q-2008-403). The following sections have been updated: 2, 3 and 4.

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(j) acidity regulators: substances which adjust the pH of feedingstuffs;

(k) silage additives: substances, including enzymes or microorganisms, intended to be incorporated into feed to improve the production of silage;

(l) denaturants: substances which, when used for the manufacture of processed feedingstuffs, allow the identification of the origin of specific food or feed materials.

(m) substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action.[4,5]

[4] This functional group was established by Regulation (EC) No 386/2009 after Regulation (EC) No 429/2008 came into force. Consequently, no requirements for this functional group are included in the implementing rules.

[5] For the purpose of this guidance, substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action, including those that reduce the toxicity of the mycotoxin (by modifying its chemical structure), will be referred to as SRMC (Substances for the Reduction of Mycotoxin Contamination). Those substances that (partially) compensate adverse/toxic effects related to mycotoxins by a direct action in the host organism (e.g., antioxidants, immune stimulators, pharmacological substances) are not considered to belong to this group of additives.

## 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 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 17025:2005. 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* and a labelling proposal as referred to in Article 7(3)(e) of Regulation (EC) No 1831/2003.

A post-market monitoring proposal should be proposed only for those additives which consist of, contain or are produced from genetically modified organisms as required by Article 7(3)(g) 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 should 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 water or feed, 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 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

This part is only required for additives falling within the scope of European Union legislation relating to the marketing of products consisting of, containing or produced from GMOs.

### 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 should 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.* For the majority of technological additives, which are not subject to a specific holder of the authorisation, the paragraphs 2.1.2, 2.1.3, 2.1.4, 2.1.4.2, 2.2, 2.3.1, 2.4.1, 2.4.2, 2.4.4, 2.5, 2.6 apply. For those technological additives subject to a specific holder of the authorisation (i.e., additives falling within the scope of European Union legislation relating to the marketing of products consisting of, containing or produced from GMOs), the whole Section II applies (follow the section II of the guidance for zootechnical additives).

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

For many technological additives there is no distinction between the active substance and 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) should be given.

### 2.1.2 Proposal for classification

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

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. Data from other known uses of the identical active substances or agents also should be provided.

### 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 applicant should provide a specification of the product as it relates to the active substance(s)/agent(s). Evidence should be provided by the analysis of at least five production batches that this specification is satisfied in practice. Certificates of analysis indicating exact values should be attached. Statements of compliance alone are not considered sufficient.

*For microorganisms: 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 given. Relevant side activities shall be also mentioned. The units of activity should be defined preferably as µmoles of reaction product released per minute from the substrate at a specified pH and temperature.

For mineral substances: denomination and specification should follow internationally recognised systems. For SRMC, the mycotoxin binding capacity should be provided.

If the active component 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).*

*Without prejudice to any request for supplementary information made by the EFSA 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 scope of Regulation (EC) No 1829/2003.*

### 2.1.4 Purity

The applicant should identify and quantify microbiological 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 the 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.

*All the data provided have to support the proposal for a specification of the additive.* Evidence should be provided by the analysis of at least three production batches that this specification is

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

satisfied in practice. Certificates of analysis indicating the exact values 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.

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:

- for microorganisms: microbiological contamination (at least *Salmonella*, enterobacteriaceae, total yeasts and filamentous fungi) and, depending on the fermentation media and excipients, mycotoxins,[7] 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), 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,[8] *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: all 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.

### 2.1.5 Physical state of each form of the product

EFSA recommends the provision of dusting potential (triplicate analysis) for solid preparations representative of the form(s) likely to be marketed to allow an assessment of respiratory exposure for users. Depending on the outcome of these studies and the nature of the substance, further investigations (e.g., particle size distribution in dust) may become necessary.

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

The same data should be provided for feed additives already authorised as food additives for which a detailed assessment of user safety was not performed.

[7] The selection of mycotoxins for analysis should be made according to the different matrices, where appropriate.
[8] 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.

## 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 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.

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 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 *).*

#### 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 GMM the description of the genetic modifications shall 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, must 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* (e.g., for clays, X-Ray Diffraction, Differential Thermal Analysis, Infrared Spectroscopy). 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. 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 the biology of the organism is sufficiently well known to allow pathogenic/toxigenic strains to be excluded by direct testing, toxicological studies are not required (see 3.2.2).

- Antibiotic production and antibiotic resistance

  *Microorganisms used as additives or as a production strains, 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. Purification methods shall be thoroughly described.*

*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) or effects (e.g., pellet durability). If specific effects are claimed for a particular form of the additive (e.g., nanoparticles) the stability of that specific form of the additive should be followed. For some chemical mixtures/extracts stability may be assessed by monitoring the concentration of one or more appropriate marker substances.

Stability studies should 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.

Stability studies are normally not required for mineral-based products.

#### 2.4.1.1 Shelf-life of the additive

The expected shelf-life of the additive as marketed should be proposed, based on data from studies performed under the recommended storage conditions, which should be specified. Data should be provided from at least three batches of the additive.

#### 2.4.1.2 Stability of the additive used in premixtures and feedingstuffs

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

Data provided should cover a representative range of feedingstuffs (at least three) relevant to the use of the additive.

Stability of the additive in feedingstuffs should generally be demonstrated by the maintenance of its effects. Although such an approach is possible with substances from functional groups h and m, it may prove more practical to monitor the presence of the active substance(s)/agent(s).

Duration of stability studies in feedingstuffs should reflect the technological role of the additive. If the additive is intended to be used via premixtures a duration of six months should apply.

It should be noted that stability studies may be identical to the required demonstration of efficacy, particularly for functional groups a, b, c, g and i.

Stability studies in feedingstuffs are not required for silage additives.

#### 2.4.1.3 Stability of the additive used in aqueous media

Use in water for drinking is only anticipated for functional groups a, h and m.

The stability 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.

For those silage additives intended for application through an aqueous suspension/solution, short term stability (48 h) should be demonstrated.

### 2.4.2 Homogeneity

Experimental evidence for the capacity for homogeneous distribution of the feed additive in premixtures, feedingstuffs or water is required for functional groups a, b and m (excluding mineral-based additives). The same criteria as described under 2.4.1 should be used. As a guide, a minimum of ten sub-samples 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). For those 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.

Indirect evidence of homogeneity may be provided by efficacy studies for the functional groups c, d, e, f, g, i, j and l.

Statistical considerations[9] as a substitute for analytical data from subsamples will not be considered.

Homogeneity studies are not required for silage additives.

### 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 use

The proposed use and level of inclusion in complete feedingstuffs or water shall be defined. It shall be indicated whether the treated feed is intended for all animal species/categories or whether a restricted list applies. In the later case they should accord with the categories listed in Annex IV of Regulation (EC) No 429/2008. *Possible contra-indications shall be mentioned.*

If a particular use in feed materials and 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.

For SRMC, the target mycotoxin(s) should be specified.

### 2.5.2 Information related to worker safety

#### 2.5.2.1 Chemical substances

*A material safety data sheet formatted in accordance with the requirements of Commission Directive 91/155/EEC[10] 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*

[9] For example. Jansen HD. (1992) Mischtechnik im Futtermittelbetrieb. Die Mühle + Mischfuttertechnik. 129 (20), 265-270.
[10] Repealed by Regulation (EC) No 1907/2006.

*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,*[11] *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 are specified in the Regulation (EC) No 429/2008. Applicants should 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.

For SRMC, evidence must be provided that the use of the additive does not interfere with the analytical determination of mycotoxins in feed.

## 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.

[11] In practice, in the absence of any entries under group 1, this information would be required for all micro-organisms.

In the case of substances for reduction of the contamination of feed by mycotoxins that modify the chemical structure of mycotoxins, the combined effects of both the additive and the resulting metabolite(s)/degradation products(s) on the safety for the target animal and consumer should be considered.

No safety studies are required for substances or microbiological agents which are already approved as additives for the same target species/categories at a comparable use level.

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:

- additives for which no significant amounts of the active substance(s) or relevant metabolites or the active agent(s) are present in the feed at time of feeding.
- microorganisms considered by EFSA to qualify for the QPS approach to safety assessment.[12]
- silage additives where it can be demonstrated that the active substance(s) and agent(s) occur as normal constituents of silage and use of the additive does not substantially increase their concentration compared to silage prepared without use of the additive (i.e. where there is no substantial change in exposure).

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.

## 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.*

*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).

Tolerance studies in the relevant target species/categories are required for **xenobiotic[13] substances**. If the application is for all animal species, tolerance should be tested in at least three major animal species representing different physiologic/metabolic capacities (a monogastric, a ruminant, a poultry or a salmonid).

For **silage additives** for which tolerance studies are required it is usually sufficient to restrict tolerance to a ruminant species, normally the dairy cow. Studies involving other species are required only when the nature of the ensiled material makes it more appropriate for use with non-ruminants or when there are particular concerns when treated silage is used for categories

[12] For SRMC based on QPS micro-organisms, the effects of the metabolites/degradation products on target animals and consumer safety should be studied.

[13] A xenobiotic is a chemical which is not a natural component of the organism exposed to it. Physiological substances which are present in much higher concentrations than normally occur may also be treated as xenobiotics.

other than adult ruminants (e.g., moist corn for pigs or fish silage for fur animals). The tolerance study normally should be made with at least ten-times the maximum recommended application level in the complete feed, or ten times the concentration present in the ensiled material at the time of normal use where this can be conclusively established. Particular consideration should be given to additives containing viable microorganisms and their capacity for survival and multiplication. For silage additives based on enzymes and/or microorganisms, applicants are encouraged to use, wherever possible, at least a 100-fold overdose in the experimental group. The additive should be added to a basal diet and results compared to a negative control with the same diet. The diet may contain a single source of silage prepared without the use of an additive. Comparisons made between animals fed silage prepared with an overdose of the test additive and the same material ensiled without an additive or with another source of ensiled material are discouraged. Results may be confounded by the use of feed material from different fermentations for control and test groups.

In the case of substances for reduction of the contamination of feed by mycotoxins that modify the chemical structure of mycotoxins, the combined effects of both the additive and the resulting metabolite(s)/degradation products(s) on the safety for the target animal needs to be examined in appropriate toxicological studies (see Section 3.2.2). If enzymes/microorganisms are used, only the resulting metabolite(s)/degradation product(s) need to be examined provided that the additive is demonstrated to be safe.

For substances **other than xenobiotics and silage additives**, not already authorised for feed use, the absence of harm to animals at the highest proposed use level should be demonstrated. This demonstration may be limited to one experiment in one target species or laboratory animal (the most sensitive in each case).

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.
- microbial additives if their constituent microorganisms are not present in feedstuffs at time of feeding (e.g., some silage additives).

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.

### 3.1.3 Interactions *in vivo*

Interactions with feed materials, carriers, other approved additives, or medicinal products which could be expected when the additive is ingested must be documented.

For those additives which exert their activity mainly by binding (e.g., clays), there is the possibility that the availability of crucial nutrients could also be affected. So, consideration should be given to the extent to which the supply of nutrients, micronutrients and other additives to the animals could be reduced. It is recognised that it is not practical to consider all possible nutrients/additives. Therefore, it is recommended that apparent digestibility of crude protein, zinc, retinyl or tocopheryl esters, thiamin or pyridoxine and a coccidiostat, in case the additive is intended to be used in poultry/rabbits, are measured. Such studies should be performed with the highest recommended dose of the additive and could be made in the context of a tolerance/efficacy study.

## 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:

- no significant amounts of the active substance(s) or relevant metabolites or the active agent(s) are present in the feed at time of feeding; or
- 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 (see also 3.2.1.2); or
- the active component(s) of the additive consists only of microorganisms or enzymes.

For substances for reduction of the contamination of feed by mycotoxins that modify the chemical structure of mycotoxins, the major mycotoxin metabolites/degradation products (representing more than 10 % of total metabolites) derived from the mycotoxin should be identified (e.g., in *in vitro* studies), preferably at different time points. Any minor metabolite/degradation product of toxicological concern should also be identified. When the use of the substance results in the formation of mycotoxin metabolites/degradation products of toxicological concern, methods for their determination in the appropriate tissues/products need to be provided.

#### 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/products 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:

- no significant amounts of the active substance(s) or relevant metabolites or the active agent(s) are present in the feed at time of feeding; or
- the substance is absorbed as physiological compound(s); or
- the product consists of microorganisms commonly encountered in ensiled materials; 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; or
- chemicals produced by fermentation are separated from the crude fermentation medium and highly purified (as a guide <1% of unidentified material on a dry matter basis).

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 the organism exposed to them 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.

Additional requirements for substances for reduction of the contamination of feed by mycotoxins that modify the chemical structure of mycotoxins are:

- Any major metabolite(s)/degradation products(s) of the mycotoxin should be examined for oral toxicity by comparing their toxicity with that of the parent mycotoxin. The end-points selected should include mycotoxin-specific effects. Depending on the outcome, further metabolism, residue and toxicity studies may be required.
- The genotoxicity of major metabolites/degradation products of the mycotoxin should be assessed.

### 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 toxicologically relevant 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. 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.*

User safety is established on the basis of a final formulation. However, once an active substance/agent has been authorised as a technological additive, different formulations and premixtures can be placed on the market with reference to that authorisation. Consequently, not all forms of the product can be directly tested for user safety. For assessing the safety for the user of technological additives, the active substance(s)/agent(s) is the principal concern provided that other components do not introduce safety issues.

Therefore, assessment of user safety will be based on the available specific studies, the MSDS, and the nature of the active substance(s)/agent(s).

Additives with a high dusting potential or those used under circumstances which could generate aerosols are of particular concern. Any data on dusting potential (see 2.1.5) may be used for exposure assessment. Additives containing enzymes and microorganisms are assumed to be respiratory sensitisers.

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.*

## 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.

For silage additives, the effects of the additive on the production of effluent from clamp or silo during ensiling should be considered, particularly when the silage additive contains cell-wall degrading enzymes. Where the nature of the additive makes an effect on effluent production unlikely, this requirement can be waived. If measurements made in the course of efficacy studies suggest an increase in effluent production then the amount, composition (e.g., N, P) and biological oxygen demand of the effluent should be determined.

## 4 SECTION IV: STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

*Studies shall demonstrate the efficacy for each proposed use.* Such studies must permit the evaluation of the efficacy of the additive according to common feed processing and manufacturing practices in the EU.

*Technological additives are intended to affect the characteristics of feed* (except substances for control of radionuclide contamination and SRMC) *but have generally no direct biological effect on animal production.* Normally, evidence of the efficacy of the additive should be demonstrated using laboratory-based studies by means of appropriate criteria as reflected in recognised acceptable methods, under the intended practical conditions of use in comparison with appropriate control feed. Substances for control of radionuclide contamination and SRMC normally do not affect the characteristics of feed until after their ingestion by the animal.

The studies (at least three tests/subsets) should be designed to cover a representative range of feed materials to which the additive will be applied. Results of each test/subset should be statistically evaluated and differences between groups accepted at $P \leq 0.05$. Non-parametric (one-sided) tests may be necessary when a low number of observations is available, but applicants are encouraged to use sufficient replicates to allow for parametric tests to be performed.

For functional groups a, b, c, g and i, efficacy studies may be used to provide information on stability in feed.

Efficacy will normally be assessed only by *in vitro* studies, with the exception of substances for control of radionuclide contamination and SRMC. *The appropriate end-points are indicated in the following table for the various functional groups.*

**Table 1:** End-points for different technological additives

| Functional group | End-points for demonstration of efficacy |
|---|---|
| Preservatives | Inhibition of microbial growth, particularly that of known pathogenic and spoilage organisms. The period for which a preserving effect is claimed should be demonstrated. |
| Antioxidants | Protection against oxidative damage of key nutrients/components during feed processing and/or storage. The period for which a protecting effect is claimed should be demonstrated. |
| Emulsifiers | Formation/maintenance of stable emulsions of otherwise immiscible or poorly miscible feed ingredients. |
| Stabilisers | Maintenance of the physico-chemical state of feedingstuffs. |
| Thickeners | Viscosity of the feed materials or feedingstuffs. |
| Gelling agents | Formation of a gel resulting in a change in the texture of the feed. |
| Binders | Pellet durability or performance of pellet formation. |
| Substances for control of radionuclides | Evidence of reduced contamination of food of animal origin. |
| Anti-caking agents | Flow ability. The period for which an anti-caking effect is claimed should be demonstrated. |
| Acidity regulators | pH and/or buffering capacity in feedingstuffs. |
| Silage additives | Improved production of silage<br>Inhibition of undesirable microorganisms<br>Reduction of effluents<br>Improved aerobic stability |
| Denaturants | Indelible identification of feed materials. |
| Substances for the reduction of contamination of feed by mycotoxins | Suppression or reduction of absorption of mycotoxins<br>Increased excretion of mycotoxins/reduced deposition in tissues or products |

### Silage additives

For additives intended for the preparation of silage from all (or unspecified) forages, separate tests should be made with one example of each of the following categories;

- Easy to ensile forage: >3% soluble carbohydrates in the fresh material (e.g., whole plant maize, ryegrass, brome grass, sugar beet pulp);
- Moderately difficult to ensile forage: 1.5 – 3.0% soluble carbohydrates in the fresh material (e.g., meadow grass, fescue, wilted alfalfa);
- Difficult to ensile forage: <1.5% soluble carbohydrates in the fresh material (e.g., orchard grass, leguminous plants).

For additives intended for the preparation of silage from specific sub-categories of forage described in terms of dry matter, the dry matter range should be explicitly stated. Three tests should then be made with material representative of the claimed range, where possible using examples of different botanical origin.

Claims restricted to, or including, feedingstuffs other than forages, require tests specific to the particular feedingstuffs. This would include fish ensiled for use with production of fur animals.

All studies should demonstrate efficacy in comparison to a negative control made with the same material for ensiling. Positive controls are optional but may be useful to demonstrate the capacity of the system to detect change. Formic acid at an application rate of approximately 3.5 kg/tonne treated material is commonly used for such purposes in material below 35% dry matter.

As a general guide, all replicate tests should be made with approximately one kg or more of homogeneous fresh material in a closed laboratory silo with the potential to vent gas and drain effluent. The harvesting and preparation of the test material must be similar to normal practice.

Compaction in the silos should be constant across replicates. The duration of the study normally should be 90 days or longer at a constant temperature (recommended range 15-25ºC). Use of a shorter duration must be justified. Sampling may be concentrated during the early stages of fermentation where claims relate to a more rapid fall in pH or acid production, but should continue for the whole experimental period to confirm the persistence of the effect. Changes occurring in the early stages of fermentation must also deliver a significant benefit at the time of use for efficacy to be demonstrated.

Other larger scale test systems (e.g., wrapped bales, field studies) may be used (and on occasions may have to be used) provided that they are consistent with the claims made and meet the general requirement above (including negative controls). Field studies without a negative control (with or without positive controls) may offer supportive information but would not alone allow a conclusion to be drawn on efficacy.

Claims made for silage additives differ and may relate to the preservation process in general, to specific aspects of the preservation process or to the aerobic stability of silage once the clamp/silo has been opened. The observations needed to demonstrate a significant benefit for the lowest dose claimed will differ both in nature and sampling time and frequency. Some claims may require an experimental design which allows replicated silos to be removed from the experiment for sampling at each time point. As a rule measurements of the following parameters should be provided in comparison to the negative control:

- dry matter and calculated dry matter losses (corrected for volatiles);
- pH
- concentration of volatile fatty acids and lactic acid
- concentration of alcohols
- ammonia nitrogen

In addition, other microbiological and chemical parameters should be included as appropriate to substantiate the specific claim made (e.g., numbers of lactate assimilating yeasts, numbers of clostridia, numbers of *Listeria* in silage for sheep, biogenic amines).

A claim for effluent reduction will be judged against the total volume of effluent produced over the entire experimental period taking into account the likely effect on the environment (e.g., ecotoxicity of the effluent, biological oxygen demand). Reduction of effluent production should be demonstrated directly. The capacity of the silo should be of sufficient size to allow effluent to be released with the application of pressure. The duration of the study should normally be 50 days. If a different period is used, this should be justified.

A claim made in relation to improved aerobic stability should be demonstrated in comparison with a negative control. Test material spoiled by butyric fermentation must be avoided. It is recommended that such studies should be made *in vitro* with silage of high dry matter because of greater probability of oxygen penetration and the relatively high content of residual sugars. If the claim is restricted to materials with low dry matter, this should be reflected in the material used for the test.

Aerobic stability studies should be of at least seven days duration after exposure to air and the additive should provide evidence of stability for at least two days longer than that shown by the untreated control. It is recommended that the experiment is made at an ambient temperature of 20ºC, and a rise in temperature of 3ºC or more above background taken as indicative of instability. Temperature measures may be replaced by measurement of $CO_2$ production. Measurement of dry matter loss and direct counts of aerobic spoilage organisms may be used as supportive evidence of improved stability.

**Substances for the reduction of contamination of feed by mycotoxins (SRMC)**

Substances for reduction of the contamination of feed by mycotoxins normally do not affect the characteristics of feed but produce their effects after their ingestion by the animal. Consequently, efficacy can only be fully demonstrated by *in vivo* studies. The dietary concentration of mycotoxin(s) used in such studies should not exceed official or advisory limits.

The studies should be based on the final product(s) for which authorisation is sought. The mycotoxin(s) against which the additive will exert its function and the target species should be specified. The mode of action (suppression or reduction of absorption, promotion of excretion or modification of the mode of action of the mycotoxin) should be declared and demonstrated.

*In vitro* studies are considered as a screening tool for the potential of substances to act as substances for reduction of the contamination of feed by mycotoxins. They may provide also indications on the mode of action of the additive. However, *in vitro* studies do not sufficiently mimic the conditions in the digestive tract, the differences between target animals and their metabolism, and consequently, cannot be used to demonstrate efficacy under practical conditions.

A minimum of three *in vivo* studies showing significant effects should be provided to demonstrate efficacy at the lowest recommended dose. These should be carried out at least at two different locations. Any extrapolation of data obtained with one animal species to other species is limited because of differences in intestinal mycotoxin absorption and potential mycotoxin degradation by the gastro-intestinal microbiota (particularly in the forestomachs) and different maximum contents of mycotoxins in feed. For additives intended to be used in all animal species except fish, studies should be performed in at least three major species, a poultry, a monogastric mammal and a ruminant, in each case including the animal category for which the lowest maximum content of the respective mycotoxin in feed is set in Directive 2002/32/EC or recommended in Commission Recommendation 2006/576/EC (see Table 2). For additives intended to be used in fish, specific studies in fish (preferably salmonids) are required. The efficacy of substances for reduction of the contamination of feed by mycotoxins observed in laboratory animals cannot be normally taken as a basis to conclude on efficacy in target animals.

**Table 2:** Target species/categories that should be included in an application for all animal species

| Mycotoxin(s) against which the additive is intended to act | Species/category |
|---|---|
| Aflatoxin $B_1$ | Dairy cow |
| Deoxynivalenol, Ochratoxin A, Fumonisins B1+B2 | Pig |
| Zearalenone | Piglet or gilt |

The mycotoxin content in feed used in studies should not exceed the values given in Directive 2002/32/EC for aflatoxin $B_1$ and in Commission Recommendation 2006/576/EC for deoxynivalenol, zearalenone, ochratoxin A and fumonisins B1+B2 for complete feedingstuffs for the respective animal species/category. For mycotoxins without a maximum content established at EU level (e.g., T-2 and HT-2), the dietary levels chosen should not exert adverse effects in the target animals.

As a source of mycotoxins, naturally contaminated feed materials are preferred. Alternatively, feed supplemented with mycotoxins could be used, if properly justified. However, because some mycotoxins regularly occur in nature associated with others, diets with more than one added mycotoxin may be used in the relevant studies. In any case, detailed analysis of mycotoxins[14] present in feed should be provided for each trial.

[14] At least aflatoxin $B_1$ and $B_2$, deoxynivalenol, nivalenol, zearalenone, ochratoxin A, fumonisins B1+B2, T-2 and HT-2, and any other for which a claim is made should be determined.

The experimental design of studies performed to assess substances for reduction of the contamination of feed by mycotoxins efficacy against mycotoxins with a maximum content set/recommended should include at least two groups: one group fed the basal contaminated diet as such (control) and the other fed the same basal contaminated diet supplemented with the additive for which authorisation is sought. For mycotoxins without a maximum content set/recommended, and in order to ensure the absence of adverse effects at the levels of mycotoxins used, an additional control group should be included. In this group, the feed should be free of these mycotoxins[15] and have, in general, the same composition as the feed given to the other two groups. The composition of diets should follow in all cases commonly accepted principles for well-balanced diets.

*In vivo* efficacy studies for substances for reduction of the contamination of feed by mycotoxins are considered short-term studies. Any measurement of end-points should not be started before metabolic steady-state of mycotoxin(s) in tissues/products is reached. In any case, the pre-sampling period should not be shorter than seven days. If balance studies are performed, the sampling period (faeces and urine) should be at least five days. Blood samples should also be collected over a five-day period.[16] Tissues should be sampled without withdrawal of the mycotoxin from the diet.

The number of animals/replicates should allow statistical evaluation of the results. For details on how to perform and report efficacy studies, see the technical guidance on tolerance and efficacy studies in target animals.

In general, mycotoxin/metabolites excretion in faeces/urine, concentration in blood/plasma/serum, tissues or products (milk or eggs) or other relevant biomarkers should be taken as end-points for demonstration of efficacy of substances for reduction of the contamination of feed by mycotoxins. The end-points should be selected according to the mycotoxin and target species, and taking into account their relevance (close correlation to exposure) and the availability of sensitive analytical methods validated for the specific matrices. Recommendations on the end-points are given in Table 3.

Zootechnical parameters should be reported but cannot be used for demonstration of efficacy for substances for reduction of the contamination of feed by mycotoxins.

**Table 3:** Most relevant end-points for substances reducing the contamination of feed by mycotoxins (SRMC)

| Mycotoxin(s) against which the SRMC is intended to act | Most relevant end-points |
|---|---|
| Aflatoxin $B_1$ | Aflatoxin $M_1$ in milk/egg yolk |
| Deoxynivalenol | DON/metabolites in blood serum |
| Zearalenone | ZEA + α- and β-zearalenol in plasma<br>Excretion of ZEA/metabolites |
| Ochratoxin A | OTA in kidney (or blood serum) |
| Fumonisins B1+B2 | Sphinganine/sphingosine ratio in blood, plasma or tissues |

For substances for control of radionuclide contamination a similar approach to SRMC should be followed.

### 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

[15] Below or at least close to the limit of detection.
[16] For poultry and small animals, different animals/replicates can be taken for the daily sampling.

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 only for technological additives that are products consisting of, containing or produced from GMOs, 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.* 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.