SCIENTIFIC OPINION

Statement on the establishment of guidelines for the assessment of additives from the functional group 'substances for reduction of the contamination of feed by mycotoxins'[1]

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

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority was asked to deliver technical advice on the guidelines to be followed for the submission of dossiers accompanying applications for authorisation of additives belonging to the functional group of substances for reduction of the contamination of feed by mycotoxins.

Based on additional requirements identified for the assessment of additives falling into the functional group 'substances for reduction of the contamination of feed by mycotoxins', the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) derived a proposal for the modification of Annex III of Regulation (EC) No 429/2008.

KEY WORDS

Mycotoxin, guidelines, technological additives, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2009-00894, adopted on 13 July 2010.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition.

In particular, Article 7(4) of that Regulation lays down that the Commission, having first consulted the Authority, shall establish implementing rules concerning the preparation and the presentation of applications for authorisation.

The Commission, in accordance with Article 6(3) of Regulation (EC) No 1831/2003, has adopted Regulation (EC) No 386/2009 introducing a new functional group of feed additives entitled "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". This additional functional group is established within the existing category of technological additives.

Consequently, the guidelines contained in Regulation (EC) No 429/2008 should be adapted in order to include specific rules for the presentation and preparation of applications for authorisation of additives belonging to the new functional group.

It is understood that the use of such new types of feed additives may not result in an increase of the existing maximum or guidance levels established in the context of Directive 2002/32/EC of the European Parliament and of the Council, but should improve the quality of the feed for animal nutrition which is lawfully on the market, providing additional guarantees for the protection of animal and public health.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Food Safety Authority is asked to give a technical advice on the guidelines to be followed for the submission of the dossier accompanying applications for authorisation of additives belonging to the functional group of substances for reduction of the contamination of feed by mycotoxins, taking into account, among others, the following parameters:

1) Mycotoxins for which the additive is requested and identification of the possible residues, or complexes derived from those products and their effects and stability.
2) Presence and characterisation of possible interactions with, among others, nutrients, coccidiostats or veterinary medicinal products.
3) Evaluation of the efficacy of the additive at the existing maximum or guidance levels of mycotoxins and the different mechanisms of action.

Introduction

Following the establishment of the new functional group of additives within the category of technological additives 'substances for reduction of the contamination of feed by mycotoxins', the European Commission requested technical advice from EFSA for the preparation of guidelines for the assessment of those additives (EFSA-Q-2009-00894).

In preparing the response to the Commission's request, the FEEDAP Panel developed a draft document that considered the additional information which, in its view, would be needed to complete an assessment of additives falling within this functional group, following the provisions of Article 8 of Regulation (EC) No 1831/2003. This document was shared with industry experts in a technical meeting held on 8 June 2010. Note was taken of the comments received from industry and, after further discussion within the Panel, the present revised document was produced. Part I of the present document details only the requirements which are additional to the general elements of Regulation (EC) 429/2008 required for any technological additive. Those *additional* requirements are listed below, following the structure of Annex II of Regulation (EC) No 429/2008. Part II of the present document contains a proposal for the modification of Annex III of Regulation (EC) No 429/2008.

PART I. ADDITIONAL REQUIREMENTS FOR THE ASSESSMENT OF ADDITIVES FALLING INTO THE FUNCTIONAL GROUP 'SUBSTANCES FOR REDUCTION OF THE CONTAMINATION OF FEED BY MYCOTOXINS'

Substances for reduction of the contamination of feed by mycotoxins comprise two different groups of additives: those that can suppress or reduce the absorption and/or promote the excretion of mycotoxins and those that modify their mode of action by modifying the chemical structure of the mycotoxin. Substances that (partially) compensate for adverse/toxic effects related to mycotoxins by a direct action in the host organism (e.g. antioxidants) are not considered to belong to this group of additives.

Most technological additives act directly in feed and consequently their effects can be assessed *in vitro* without reference to the animals consuming the feed. By contrast, the functional groups 'substances for reduction of the contamination of feed by mycotoxins' and 'substances for control of radionuclide contamination' have little or no effect in or on the feed itself until after ingestion by the animal. Consequently, some *in vivo* studies are required for their assessment.

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

2.2.2 Relevant properties

2.2.2.1 Chemical substances

Data from instrumental methods such as X-ray diffraction and differential thermal analysis may be useful for characterising the surface properties of substances for reduction of the contamination of feed by mycotoxins that act by binding (e.g. clays).

2.4 Physico-chemical and technological properties of the additive

2.4.1 Stability

For technological additives, the stability should generally be demonstrated by the maintenance of their effects. Although such an approach is possible with substances for the reduction of the contamination of feed by mycotoxins, it may prove more practical to monitor the presence of the active substance(s)/agent(s).

2.5 Conditions of use of the additive

2.5.1 Proposed use

Some substances for reduction of the contamination of feed by mycotoxins are non-specific in their actions, while some others act only on certain mycotoxins. Therefore, the target mycotoxin(s) should be specified.

2.6 Methods of analysis and reference samples

Feed subject to contamination by mycotoxins is considered to be of merchantable quality only if it meets the maximum or guidance levels established by Directive 2002/32/EC[4] or Commission Recommendation 2006/576/EC.[5] Evidence is needed that the use of the additive does not interfere with the analytical determination of mycotoxins in feed and so compromise the intentions of the Directive/Recommendation.

3 SECTION III: STUDIES CONCERNING THE SAFETY OF THE ADDITIVE

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.

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

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/micro-organisms are used, only the resulting metabolite(s)/degradation product(s) need to be examined provided that the additive is demonstrated to be safe.

3.1.3 Interactions

For the additives that exert their activity mainly by binding (e.g. clays), there is the possibility that the availability of crucial nutrients could also be affected. Consideration should thus 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

3.2.1 Metabolic and residue studies

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.

[4] OJ L 140, 30.5.2002, p. 10.
[5] OJ L 229, 23.8.2006, p. 7.

3.2.2 Toxicological studies

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

- 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.

4 SECTION IV: STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

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. Those 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 (Table 1). 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 1: 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 (DON), Ochratoxin A (OTA), Fumonisins B1+B2	Pig
Zearalenone (ZEA)	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[6] present in feed should be provided for each trial.

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 those mycotoxins[7] 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.[8] 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 2.

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 2: Most relevant end-points for substances reducing the contamination of feed by mycotoxins

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

[6] 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.

[7] Below or at least close to the limit of detection.

[8] For poultry and small animals, different animals/replicates can be taken for the daily sampling.

PART II. PROPOSAL FOR THE MODIFICATION OF ANNEX III OF REGULATION (EC) NO 429/2008

The following is a proposal of the FEEDAP Panel to modify Annex III of Regulation (EC) No 429/2008 to include the requirements for the assessment of additives belonging to the functional group 'substances for reduction of the contamination of feed with mycotoxins'.

PROPOSED ADDITIONS TO '1. TECHNOLOGICAL ADDITIVES'

1.2. Section II: identity, characterisation and conditions of use of the additive; methods of analysis

For 'substances for reduction of the contamination of feed by mycotoxins', in addition to the general requirements listed in Section 2.5.1, the target mycotoxin(s) against which the additive is active shall be specified.

For 'substances for reduction of the contamination of feed by mycotoxins', in addition to the general requirements listed in Section 2.6, evidence must be provided that the use of the additive does not interfere with the analytical determination of mycotoxins in feed.

1.3. Section III: studies concerning the safety of the additive

For '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 shall be considered.

1.3.1.3. Interactions

For additives which exert their activity by binding, evidence shall be provided to assess whether, and to what extent, the supply of nutrients, micronutrients and other additives to the animals can be affected.

1.3.2. Studies concerning the safety of use of the additive for consumers

1.3.2.1. Metabolic and residue studies

For 'substances for reduction of the contamination of feed by mycotoxins' that modify the chemical structure of mycotoxins, the major mycotoxin metabolites/degradation products derived from the mycotoxin shall be identified. 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 shall be provided.

1.3.2.2. Toxicological studies

In addition, for 'substances for reduction of the contamination of feed by mycotoxins' that modify the chemical structure of mycotoxins, any major metabolite(s)/degradation products(s) of the mycotoxin shall 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 (including genotoxicity) studies may be required.

1.4. Section IV: studies concerning the efficacy of the additive

Most technological additives are intended to improve or stabilise the characteristics of feed but have generally no direct biological effect on animal production.

By contrast, the functional groups 'substances for reduction of the contamination of feed by mycotoxins' and 'substances for control of radionuclide contamination' have little or no effect in or on

the feed itself until after ingestion by the animal. Consequently, *in vivo* studies are required for their assessment.

End-points for different technological additives

Functional group	End-points for demonstration of efficacy
(m) Substances for reduction of the contamination of feed by mycotoxins	Suppression or reduction of absorption of mycotoxins Increased excretion of mycotoxins/reduced concentration in plasma, tissues or products

Substances for the reduction of contamination of feed by mycotoxins

The mode of action (suppression or reduction of absorption, promotion of excretion or modification of the mode of action of the mycotoxin) shall be declared and demonstrated. Evidence of the mode of action can be provided by *in vitro* studies.

Efficacy shall be demonstrated in *in vivo* studies (normally short-term) and should be performed in the relevant target species for which the additive is intended.

A minimum of three *in vivo* studies showing significant effects on the relevant end-points shall be provided to demonstrate efficacy independent of the number of target species applied (except fish). The mycotoxin content in feed used in studies shall 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.