



Guidance for the assessment of detoxification processes in feed

EFSA Panel on Contaminants in the Food Chain (CONTAM)

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Guidance for the assessment of detoxification processes in feed

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Abstract

This statement provides scientific guidance on the information needed to support the risk assessment of the detoxification processes applied to products intended for animal feed in line with the acceptability criteria of the Commission Regulation (EU) 2015/786.

KEYWORDS

contaminant, decontamination, detoxification, feed, process, undesirable substance

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BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY EFSA

Background

Directive 2002/32/EC of the European Parliament and of the Council,¹ in its article 3, provides that the use of products intended for animal feed which contain levels of undesirable substances exceeding the maximum levels laid down in Annex I of that Directive cannot be placed in the European market. It is possible to use acceptable detoxification processes on these products in order to conform with the provisions of Annex I of that Directive. Commission Regulation (EU) 2015/786² establishes the acceptability criteria for detoxification processes to ensure that the detoxified feed does not endanger animal and public health and the environment and that the characteristics of the feed are not adversely altered by the detoxification process. The Panel on Contaminants in the food chain (CONTAM Panel) has assessed a series of requests for feed detoxification processes since the entry into force of the Regulation (EU) 2015/786 on 1 July 2017. Frequently the information provided is insufficient for the Panel to come to conclusions if the detoxification process fulfils the criteria set in the legislation and additional clarifications are necessary which prolong the risk assessment process. To help the feed business operators in the preparation and submission of the necessary information for the evaluation of feed detoxification processes according to the Regulation, the CONTAM Panel, in its Plenary meeting on 15 December 2022, identified the need for guidance. The guidance will be provided in a statement addressing the implementation of the criteria for the acceptance of detoxification processes applied to products intended for animal feed, based on the Panel's experience gained during the last years while working under the provisions of the abovementioned Regulation.

Terms of Reference

In view of the above, the European Food Safety Authority (EFSA) asks its CONTAM Panel to provide guidance on the information needed to support the risk assessment of the detoxification processes applied to products intended for animal feed, as provided for in Directive 2002/32/EC of the European Parliament and of the Council and in line with the acceptability criteria of the Regulation (EU) 2015/786.

SCOPE OF THE GUIDANCE

This guidance concerns any detoxification (referring to both detoxification and decontamination) process in line with the Regulation 2015/786, through which an undesirable substance (as listed in Annex I of Directive 2002/32/EC) in non-compliant contaminated feed is (i) on purpose removed, via a physical detoxification process, (ii) broken down or destroyed by a chemical substance into harmless compounds, in a chemical detoxification process, and/or (iii) metabolised or destroyed or deactivated into harmless compounds, in a (micro)biological detoxification process.

1 | INTRODUCTION

1.1 | Legislation

The Directive 2002/32/EC deals with undesirable substances in products intended for animal feed in order to guarantee that these products would not represent a potential danger for human and animal health or the environment, and ensures safe livestock production in the European Union (EU). With this aim, a list of undesirable substances with their maximum admissible levels in different feed products is included in the Annex I of the Directive. Batches of feed products containing substances (metals, mycotoxins, organic pollutants, hydrocyanic acid and insecticides among others mentioned in Annex I) that do not comply with those given limits cannot be used, neither as such nor mixed with other standard compliant batches. The Directive, however, mentions that non-compliant feed could undergo detoxification processes. The acceptability criteria for these processes and resulting products are specified in the Regulation (EU) 2015/786, to ensure that a uniform approach is applied in the EU, and to entrust the scientific assessment to be performed by EFSA on a request from the European Commission (EC). The correct application of these processes should be checked by Member States. Once a detoxification process is deemed to be scientifically sound, it can be carried out only in establishments approved by the local competent authority. In general terms, the detoxification process should be effective, without adversely changing the characteristics of the decontaminated feed and any harmful residue that is removed should be disposed in an appropriate way.

¹Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10–22.

²Commission Regulation (EU) 2015/786 of 19 May 2015 defining acceptability criteria for detoxification processes applied to products intended for animal feed as provided for in Directive 2002/32/EC of the European Parliament and of the Council. OJ L 125, 21.5.2015, p. 10–14.

In parallel, the general rules on feed hygiene, the conditions and arrangements ensuring traceability of feed and the conditions and arrangements for registration and approval of establishments should apply in line with the Regulation (EC) No 183/2005 of the European Parliament and of the Council.³

1.2 | Identification of types of feed and of undesirable substances

Definitions regarding feed products are included in article 2 of the Directive 2002/32/EC while description of the feed materials and of the processes applied in Europe are provided in parts C and B of the Commission Regulation (EU) No 1017/2017.⁴ Feed materials are of different origins, and their purpose is to meet the nutritional requirements of farmed and companion animals. There are a wide variety of feed materials which are used in animal feed from both terrestrial and aquatic environments, for example corn, soybean and cottonseed, and fish meal and fish oil.

While most of the feed products are safe and pose no direct risk to animals or indirect risk to humans from the consumption of animal products, the presence of undesirable substances can occur. They may be accidentally introduced during the manufacturing process or occur naturally in the feed. Therefore, EU legislation (Directive 2002/32/EC) establishes maximum permitted levels for certain undesirable substances in animal feed; products exceeding the limits are considered contaminated materials.

Undesirable substances in feed materials may be broadly classified as follows⁵:

- a. Persistent organic pollutants (POPs), including dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) and polychlorinated biphenyls (PCBs), which are organic pollutants that accumulate in the environment and have been detected in soils, water, plants and animals. Also, other POPs, like perfluoroalkyl substances (PFASs) and brominated flame retardants (BFRs) have become a health concern. For some of these compounds maximum levels are established, or may be established in future.
- b. Toxic elements such as metals and metalloids may get into the feed via soils, fertilisers and additives. For lead, cadmium, mercury, arsenic and fluorine maximum levels are established in the EU.
- c. Mycotoxins are a subset of natural toxins which are secondary metabolites produced by fungi growing on certain agricultural commodities, most commonly on cereals but also on dried fruit, nuts and spices. Aflatoxins, fumonisins, ochratoxins, ergot alkaloids, trichothecenes and zearalenone are the more prevalent mycotoxins in animal feeds. Mycotoxin-producing fungi increases in warm, damp and humid conditions, although different climatic storage conditions may favour different fungi. In cereals, contamination can occur both on standing cereal crops (pre-harvest) and in the stored grain. Contamination of feed materials may also occur during processing and transport.
- d. Natural (endogenous) toxins are present in certain plants and act as defence mechanisms against predators. Common examples of endogenous natural toxins in feed include solanine in potatoes, cyanogenic glycosides in e.g. cassava and linseed, phorbol esters in jatropha, lectins and enzyme inhibitors in soya beans. Pyrrolizidine alkaloids have been detected in honey due to bees collecting nectar from certain toxin producing plants but may also occur because of weeds introduced around the wasp nests during harvesting of crops. The latter has also been shown for tropane alkaloids.

2 | DATA REQUIRED FOR THE EVALUATION OF FEED DETOXIFICATION PROCESSES

2.1 | Description and characterisation of the process

The feed business operator (FBO) should provide a detailed description of the feed detoxification/decontamination process they intend to implement, including a detailed description of the feed material used and information on the type and concentration of the undesirable substances intend to be detoxified. Commonly, detoxification processes are divided into physical, chemical or (micro)biological processes, or any combination of these according to the mechanism underlying the detoxification. Physical detoxifications often utilise adsorption or solvent extraction or irradiation, whereas chemical detoxifications use the chemical reaction with an added chemical agent and (micro)biological detoxification use microorganisms or enzymes. Frequent contaminants of feed are dioxins and dioxin-like PCBs and various mycotoxins, but sometimes even endogenous constituents of the feed material pose a toxicological hazard, such as cyanogenic glycosides in linseed. Depending on the nature of the undesirable substance, a physical, chemical or biological process may be most suitable for detoxification (Huwig et al., 2001). Chemically non-reactive contaminants such as dioxins require a physical detoxification, e.g. adsorption to active carbon, whereas mycotoxins amenable to chemical reactions, such as aflatoxins, can be detoxified by adding ammonia. A case of a combined biological/physical detoxification process is the degradation of the cyanogenic glycosides in linseed by the enzyme β -glycosidase, followed by removal of the formed hydrocyanic acid by heating. For

³Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1–22.

⁴Commission Regulation (EU) 2017/1017 of 15 June 2017 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials, OJ L 159, 21.6.2017.

⁵<https://www.efsa.europa.eu/en/topics/topic/chemical-contaminants-food-feed>

some undesirable substances, e.g. the estrogenic mycotoxin zearalenone, either physical, chemical or biological processes of detoxification have been described (Zinedine et al., 2007).

2.1.1 | Physical detoxification

Physical detoxification methods aim at removing the undesirable substance or converting it to a compound non-harmful or with a lower toxicity than the parent compound by physical means. These comprise adsorption to inorganic or organic materials (e.g. active carbon, minerals, organic polymers), extraction with lipophilic or hydrophilic solvents, thermal treatments (dry or wet) and irradiation (microwaves, ultraviolet (UV) light, ionising irradiation), among others.

If the process is based on adsorption, the kind of adsorbent, its binding capacity, its commercial source, the ratio of adsorbent to contaminated feed, the temperature and pH of the liquid phase, the contact time and the purification of the detoxified feed must be reported. If the undesirable substance is removed by adsorption onto a matrix by e.g. filtration, the sorbent should be removed and disposed of before it becomes saturated. Standard operating procedures need to be in place to ensure that there is no break-through of the undesirable substances.

For the extraction step of the processes all information necessary to unambiguously identify the extractant⁶ must be given in particular for non-common chemicals used in food and feed. Furthermore, its purity and commercial source, the ratio of extractant to contaminated feed, the temperature and pH of the aqueous phase, the extraction duration, the method of extraction (e.g. stirring or shaking) and the removal of the extractant from the detoxified feed need to be described. The removal must be supported by analytical data.

A heat treatment process requires reporting of the temperature and the duration of heating, as well as any additional measures (e.g. addition of water or other liquid, application of vacuum or pressure). Moreover, information on the fate of the undesirable substance should be provided (e.g. possible degradation products).

Irradiation protocols should contain information about the source of the radiation, its wavelength and intensity, and the duration of the irradiation treatment. Further relevant information concerns the water content of the treated feed and the nature of radiolytic degradation products.

2.1.2 | Chemical detoxification

Chemical detoxification methods aim at decreasing the toxicity of an undesirable substance by altering its structure via a chemical reaction. Common reactions are ammoniation (reaction with ammonia) and oxidation (e.g. reaction with ozone or hydrogen peroxide).

Requested information for chemical detoxification processes should include the identity,⁵ purity, source of the chemical(s) applied and analytical method to detect the residues of the added chemical(s), the amount applied in relation to the contaminated feed, the temperature and pH of the reaction mixture, length of reaction time and any method for promoting the chemical reaction (e.g. stirring, pressure, addition of catalyst). It should also be described how the chemical detoxification reaction is stopped, how excess chemical is removed and disposed of, and how the decontaminated feed is purified. Information on the reaction products formed from the undesirable substances should be provided.

2.1.3 | Biological detoxification

Biological detoxifications commonly use microorganisms or enzymes to modify the chemical structure of the undesirable substance (e.g. by metabolism) or to remove it (e.g. by binding to cell wall polysaccharides of bacteria).

For processes using microorganisms, their exact identity, source and purity should be given on a case-by-case basis. The origin and the steps followed to obtain the strain to be used should be indicated. If the strain is genetically modified, according to the definition in Directive 2001/18/EC,⁷ the genetic modification should be described. In general, the following taxonomic information needs to be provided for all microorganisms: genus, species and strain name or code. The microorganism should be taxonomically identified unambiguously at species level based on up-to-date methodologies and current knowledge. For bacterial species information on the antimicrobial susceptibility should be provided including the phenotypic resistance and the presence of antimicrobial resistance genes. For all microorganisms the capacity of producing antimicrobial substances and their toxigenicity and pathogenicity should be investigated.

The preparation of the contaminated feed for the microbial incubation should also be described (e.g. drying, crushing, sterilising). Further requested information concerns the colony forming unit (CFU) used per kg of feed, the pH and composition of the incubation medium and the gas atmosphere (e.g. aerobic, anaerobic, other gases). If illumination is applied (e.g.

⁶Generic name, chemical name according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature, other generic international names and abbreviations and the Chemical Abstract Service (CAS) number and the European Inventory of Existing Commercial chemical Substances number (EINECS), European Community number and European Enzyme Commission number if available. The structural and molecular formula, the open SMILES notation and the molecular weight must be included. Where relevant, the isomeric forms should be given. Information on structurally related substances should be included, when appropriate.

⁷Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC OJ L 106, 17.4.2001, p. 1–39.

with photosynthetic bacteria), the wavelength and intensity of the light should be given. Important information concerns the incubation time and temperature, as well as the purification of the detoxified material.

If enzymes are used for the decontamination process, information on the source (e.g. if originating from microorganisms the requirements above apply), and the production of food enzyme should be provided as well as on the characterisation of the enzyme including its activity and chemical and microbiological purity under relevant conditions for the specific detoxification process. Furthermore, information on the amount/activity of enzyme required per kg product to be treated, the composition of the incubation medium, its pH, the incubation time and temperature, and the final purification of the detoxified feed is requested.

2.2 | Efficacy of the process

Several feed detoxification processes that detoxify feed have been described in the literature. The principal task of the FBO is to demonstrate the efficacy of their application of the process at an established plant for feed to which the process will be applied. The efficacy may initially be demonstrated in a pilot plant, but it is important to show that the process is transferable and reproducible on an industrial scale.

2.2.1 | Level of undesirable substances before and after detoxification

The efficacy of the detoxification process should be tested in at least three batches of the feed to which the process will be applied. For processes intended to be applied to one type of feed, the content of undesirable substance before and after the treatment should be measured in at least three sub-samples of the same batch, making it possible to assess the reduction of the undesirable substance concentrations. Where a range of different feed are being processed, the FBO should ensure that the batches analysed are representative of all the feed that may be subject to the process. In this context a batch is defined as a distinct unit of feed intended to be detoxified. The concentrations of the undesirable substance in the untreated contaminated feed should be above or around the legal limits given in Council Directive 2002/32/EC before detoxification and below those limits following the detoxification process. The FBO should indicate the range of the levels of the contaminated feed intended to be reduced.

2.2.2 | Analysis used for the undesirable substances

The application should be supported by analytical data that can demonstrate the concentrations of the relevant undesirable substance before and after the decontamination process, to prove the effectiveness of the procedure. The analysis should preferably be conducted using a method accredited to ISO 17025, where available. If no accredited method is available for the matrix of the respective feed intended for use, or if there is good justification not to use an accredited method, then the analytical data should be supported with validation data on the matrix to be detoxified or a similar matrix to allow the method performance to be assessed. At least three replicates should be analysed to be able to assess repeatability and reproducibility of the analytical method, and homogeneity of the undesirable substance within the sample. Data should be accompanied with an estimate of measurement uncertainty if a non-accredited procedure is used.

2.2.3 | Co-occurrence of undesirable substances

Undesirable substances may occur as mixtures. It is relevant to consider the extent to which related undesirable substances are removed by the process. For example, dioxins and dioxin-like PCBs may be removed by binding to activated charcoal, but this may not be the case for non-dioxin-like PCBs which are likely to co-occur in contaminated areas. As a result, decontaminated batches may contain relatively high levels of these PCBs that may go unnoticed if not being included in the analysis. This may, for instance, occur when processing fish oil and fishmeal from contaminated areas. In these cases, analytical data on the related undesirable substances should also be provided. Similar may be the case for mycotoxins produced by the same moulds that are responsible for the target compound for the decontamination. For this reason, it is important to provide results for incurred materials and not just spiked materials.

2.3 | Reversibility of the detoxification process

The FBO should consider if the process may be reversible meaning that the levels of the undesirable substance can increase again in the feed or inside the animal.

It is unlikely that a physical process of detoxification, where the undesirable substances are removed, will be reversible and therefore no evidence is required to demonstrate reversibility of physical detoxification processes.

In the case of a chemical or enzymatic process where the degradation products are not removed, these may revert to the target compounds. These can be evaluated in the feed. The applicant should demonstrate that the process is not reversible when the feed is processed, stored or when offered to the animals. In addition, on a case-by-case basis, the irreversibility should be confirmed in the target animals fed with decontaminated material followed by analysis of tissues, milk or eggs. *In vitro* digestion models may be helpful in these studies as a first step.

2.4 | Safety of chemical and (micro)biological agents used

If the FBO demonstrates that the residues of the detoxification agents and their metabolites of concern (physical, chemical or (micro)biological) are not detectable in the detoxified feed, safety data will not be needed. Analytical data to demonstrate the absence of the residues in the detoxified feed can be obtained from the samples used in the efficacy studies with the use of up-to-date analytical methods.

In case residues remain in the feed product, information on the safety of the agents and their metabolites for the animals fed the feed, the consumers of food products from these animals and for the environment is needed.

Regarding the application of physical and chemical processes, residues of the applied chemicals used could be solvents, adsorbants or other chemical agents (e.g. impurities, metabolites) in the detoxified feed. In case legal limits have been set for the chemical agents to be used, the FBO should demonstrate that these will not be exceeded in feed and in food produced from animals consuming this feed. For other chemicals, a basic set of data from toxicological studies may be required to demonstrate the safety of the chemicals for animals and humans (genotoxicity, sub-chronic toxicity studies augmented as appropriate by other studies e.g. chronic, reproduction, carcinogenicity etc. studies, on a case-by-case basis).

The use of strains of microorganisms belonging to a species that qualifies for the QPS approach (EFSA, 2007; EFSA BIOHAZ Panel, 2023) and for which the qualifications were met, are considered safe for the animals, humans and, if relevant, for the environment and therefore no further data are required.

It is noted that microorganisms such as bacterial strains carrying acquired genes that confer resistance to relevant antimicrobial(s) or pathogenic, virulent or toxigenic strains and those capable of producing relevant antimicrobials may represent a risk for the target animals and humans.

Safety data in animal species may be required taking into consideration the identity and the characterisation of the microorganisms and this could include literature information, toxicological tests or studies in the target animals (following for example the principles of the guidance on the safety for the target species in the Feed Additives area [EFSA FEEDAP Panel, 2017]). The safety for the consumers of food products obtained from the animals may also need to be addressed (e.g. with a basic dataset of genotoxicity and sub-chronic oral toxicity study).

For enzymes, if the enzyme is present at the end of the process and/or depending on its nature, the source and the preparation, data on the safety for the animals and the consumers of food products obtained from these animals may be required. For example, a basic set of toxicity studies (genotoxicity and sub-chronic oral toxicity studies) may be relevant.

2.5 | Toxicity of products formed during the detoxification process

In any detoxification process, when degrading undesirable substances without any removal of the degradation products, it is in principle required to examine which degradation products are formed and especially if they could present a risk to the target animal or the consumer due to residues in animal-derived products. Degradation products might be identified using a labelled standard of the target compound added to feed material. Another option is the treatment of a pure standard followed by identification and analysis of the degradation products in the treated commodities. However, degradation products may also react with feed materials. The toxicity of the individual degradation products should be tested.

In practice, it may be not possible to identify degradation products of the undesirable substance(s), especially in the specific matrices. In such cases a more pragmatic approach may be followed by performing appropriate toxicological testing on the product or product extracts before and after the treatment, e.g. genotoxicity evaluation of the extracts of the treated products and/or the identified degradation products. It should be demonstrated by appropriate testing that the process is able to provide a substantial detoxification.

For microbiological based detoxification applications, additional biohazard considerations apply, including the possibility that the microbes used may produce toxins. These should be addressed in accordance with the toxigenicity and pathogenicity requirements of the Section 2.4 of the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018) and section 3.5.3 of the Statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA 2021).

2.6 | Characteristics and nature of the processed feed

The feed detoxification process should not adversely affect the nutritional value of the feed. The FBO should provide evidence that the detoxification process does not compromise the nutritional value of the product. This evidence should include analysis of at least three samples from three batches of a specific feed before and after detoxification with pre- and

post-treatment samples taken from the same batch. For example, such evidence could be provided for the fatty acid composition of fish oil treated with charcoal to remove chlorinated dioxins (EFSA CONTAM Panel, 2017). Depending on the feed, it should be shown that the levels and the profile of essential nutrients are not adversely affected (e.g. fatty acid profile and contents of vitamins for fish oil treated with charcoal).

2.7 | Disposal of waste

Any waste material that is generated from the process (e.g. spent filters, solvents, extracted chemicals) should be documented and in compliance with legal requirements. An assessment of the maximum possible content of the undesirable substances should be made and this needs to be considered when choosing the best method of safe disposal, and it may be necessary to consider spent material as hazardous waste.

Issues related to the potential harm to the environment caused by the agents used or their residues need to be addressed.

ABBREVIATIONS

CAS	Chemical Abstract Service
CFU	colony forming unit
CONTAM	The EFSA Panel on Contaminants in the Food Chain
EINECS	European Inventory of Existing Commercial Chemical Substances
FBO	feed business operator
FEEDAP	The EFSA Panel on Additives and Products or Substances used in Animal Feed
GMM	genetically modified microorganism
GMO	genetically modified organism
ISO	The International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
ML	maximum limit
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-p-dioxin
PCDF	polychlorinated dibenzofuran
PFAS	per- and polyfluoroalkyl substance
POP	persistent organic pollutant
QPS	qualified presumption of safety
SMILES	Simplified Molecular Input Line Entry System
UV	ultraviolet

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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