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Homogeneity Testing of Feed Ingredients
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At Step 4: Public Consultation

HOMOGENEITY TESTING OF FEED INGREDIENTS

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Adopted by the ICCF Steering Committee

The International Cooperation for Convergence of Technical Requirements for the Assessment of Feed Ingredients (ICCF) was launched in 2017 and aims to develop and establish common guidance documents to provide technical recommendations for the assessment of feed ingredients, including new uses of existing feed ingredients.

The founding members of the ICCF include the Canadian Food Inspection Agency (CFIA), the European Commission (DG SANTE), the U.S. Food and Drug Administration (FDA), as well as the American Feed Industry Association (AFIA), the Animal Nutrition Association of Canada (ANAC), the EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA) and the International Feed Industry Federation (IFIF).

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HOMOGENEITY TESTING OF FEED INGREDIENTS

1. INTRODUCTION

1.1. Objective of the Guidance

This document provides guidance regarding the homogeneity testing approaches and data to be included in a pre-market approval or authorization application for feed ingredients.

Considerations in the document are provided for the assessment of feed ingredients throughout the feed chain and using different intended feed matrices. Guidance has been developed with an international team of experts and represents the best practices for the provision of meaningful results.

While this guidance supports the acceptability of the study protocol, applicants are advised to consult the appropriate regulatory authorities or their guidelines during the development phase of new feed ingredients or a new use of an authorized feed ingredient. This will help to determine whether the study described herein is acceptable, or if the study is needed for pre-market assessment.

1.2. Definitions

The following definitions apply solely in the context of this guidance document:

Active substance[1]: Any substance in a feed ingredient that contributes to the intended effect.

Batch: An identified quantity of a feed ingredient or intended matrix having uniform characteristics, with specified limits and being produced from the same cycle of manufacturing production.

Coefficient of variation (CV): A measurement of relative variability. It expresses the standard deviation as a percent of the mean. It is calculated using the formula:

$$\boldsymbol{CV = \frac{SD}{\bar{X}} \times 100\%}$$

SD = standard deviation, $\bar{X}$ = mean

Feed (Feedingstuff)[2]**:** Any single or multiple materials, whether processed, semi-processed or raw, which is intended to be fed directly to animals.

Feed ingredient[2] A component part or constituent of any combination or mixture making up a feed, whether or not it has nutritional value in the animal's diet. Ingredients are of plant, animal, microbial or aquatic origin, or other organic or inorganic substances.

[1] Active substance includes microorganisms that contribute to the intended effect.

[2] Note adapted from Codex Alimentarius, Code of Practice on good animal feeding (CAC/RCP 54-2004)

Feed Supplement: A feed used with another feed to improve the nutritive balance or performance of the total ration and intended to be:
(1) Fed undiluted as a supplement to other feeds; or
(2) Offered free choice with other parts of the ration separately available; or
(3) Further diluted and mixed to produce a complete feed.

Homogeneity: The ability of a feed ingredient to be distributed uniformly throughout its intended matrices.

Intended matrix: The matrix expected to be used in the market to supply the feed ingredient to the animals. It may include the market formulations, premixtures, feeds, feed supplements, and drinking water for animals.

Market formulation: A mixture of one or more feed ingredients and other functional or diluent materials, that is formulated and packaged together to be marketed and incorporated into intended matrices. It is not intended for direct feeding to animals.

Premixture (Premix): A uniform mixture of one or more micro-ingredients/feed ingredients with diluent and/or carrier, not intended for direct feeding to animals. It is used to facilitate uniform dispersion of the micro-ingredients/feed ingredients in a larger mix.

1.3. Scope of the Guidance

This guidance document addresses the homogeneity testing of feed ingredients, when incorporated into its intended matrices.

The types of feed ingredients covered by this guidance in each regulatory jurisdiction are determined by each region's relevant statutes and regulations.

2. GENERAL PRINCIPLES

A homogeneity study should evaluate the uniform distribution of the feed ingredient to demonstrate that the active substance(s) contained in the feed ingredient can be homogeneously distributed under conditions of the proposed use in the intended matrices. For nutritional feed ingredients, this is to ensure the animal's uniform exposure to the feed ingredient and avoid either a nutrient deficiency or overexposure. For feed ingredients having other intended effects in feed, this is to ensure the intended effect can be achieved uniformly. The homogeneity study shall allow the demonstration of the ability of the feed ingredient to be uniformly mixed in the intended matrices, using mixing equipment that is readily available to a feed manufacturer (see **Section 5**).

When the feed ingredient contains more than one active substance, it is not necessary to test each active substance, unless there are reasons to assume that all active substances contained in the feed ingredient would not follow the same distribution pattern. Homogeneity testing should be conducted under the intended conditions of use of the feed ingredient. In particular, the way the feed ingredient is

incorporated in the intended matrix should be considered (e.g., mixing of a solid feed ingredient, spraying of a liquid form feed ingredient).

The homogeneity test should be based on appropriate sampling procedures (see **Section 5**) to ensure the representative samples are tested. The sampling procedures should consider the physical characteristics of the ingredient, the approach to incorporate the feed ingredient into the intended matrices, and the variation of analytical method(s) used. The study should include a minimum of 10 representative samples that are taken from one batch of the intended matrix being tested (see **Section 5**). The concentration/activity of the active substance(s) contained in the feed ingredient in each sample is measured using an appropriate analytical method. The Coefficient of Variation (CV) should be calculated using results from all the samples collected to demonstrate the homogeneity (see **Section f)**). Depending on the use level of feed ingredient in the intended matrix tested, the acceptable CV may vary. For example, if the concentration/activity of the active substance in the intended matrix is very low, such as low parts per million (ppm) or in the parts per billion (ppb) range, the acceptable CV for the tested feed ingredient may be higher than the one of another feed ingredient used at higher level.

Analytical method(s) used in a homogeneity study should be regulatory or internationally accepted methods for the relevant active substance in the intended matrix. In the absence of such methods, the method of analysis should be validated in all the intended matrices in which the homogeneity of the feed ingredient is intended to be tested. The method validation should follow the protocols recommended by international standards or guidance.

3. HOMOGENEITY TESTING

The need for homogeneity testing and how it should be conducted depends on the proposed conditions of use of the feed ingredient, including the directions for incorporation in the intended matrices and the proposed use level in those. When the intended use level of a feed ingredient in a specific feed matrix is a range of levels, the homogeneity test should be conducted using the minimum intended use level.

This section describes the approach to conduct a homogeneity test in each of the intended matrix. If the feed ingredient is to be used in different matrix compositions and the difference in compositions may impact the ability of the feed ingredient to be evenly distributed, the applicant can either test the feed ingredient in each composition or test the feed ingredient in a representative intended matrix suitable for the target animal species. In that case, the choice of the composition tested should be justified.

For example, a feed ingredient is intended to be produced in two different market formulations (liquid and solid). A homogeneity test in each of the solid and liquid market formulation should be considered.

Another example is when a feed ingredient is to be used in premixtures for cattle, swine, and poultry, differing in their composition for each target species. It may be acceptable to choose one

representative premixture composition for cattle, swine and poultry species, respectively, for homogeneity testing. A rationale should be provided to justify the choice of the representative compositions.

3.1. Homogeneous Distribution of the Feed Ingredient in Its Market Formulation

When a feed ingredient is formulated and packaged with other substances (e.g. diluent) prior to being marketed, a homogeneity test should be considered. This will ensure the proper control of the feed ingredient market formulation (e.g., in compliance with the label guarantees) and adequate incorporation in subsequent intended matrices.

A homogeneity test in a feed ingredient market formulation is recommended but may not be necessary when properly justified.

The following elements should be considered when determining whether a homogeneity test is needed for a feed ingredient market formulation:

a) The physical characteristic of the market formulation. For example, it may be more difficult to evenly distribute a feed ingredient in a viscous formulation than in a dry free flowing formulation.
b) The particle size of the feed ingredient and other component(s) used in the formulation. For example, a significant difference in particle size between the feed ingredient and the other components could cause uneven mixing.
c) The inclusion level of the feed ingredient in the formulation. In general, it is more challenging to evenly distribute a feed ingredient when the inclusion level in the market formulation is low.
d) The safety profile of the feed ingredient. For certain feed ingredients, its unique safety profile may warrant a homogeneity test to ensure the uniform distribution in the market formulation. For example, selenium has a very narrow safe and effective working range. The effective concentration of selenium is very close to the lowest concentration at which selenium becomes toxic. For a feed ingredient intended to be used as a selenium source, a homogeneity test may be needed to ensure the even distribution of the ingredient in its market formulation to avoid safety concerns for animals.

When a homogeneity test is needed, it should be conducted at the intended concentration/activity level of the active substance and composition of the final market formulation.

3.2. Homogeneous Distribution of the Feed Ingredient in Premixture

Testing the ability of the feed ingredient to be evenly distributed in a premixture is recommended, when the feed ingredient is intended to be used in a premixture prior to the incorporation into animal feed. To conduct a homogeneity test in premixture, the feed ingredient should be incorporated into the premixture at the intended inclusion level using mixing equipment that is readily available and commonly used in the feed industry (see **Section 5**). The quantitative and qualitative composition of the

premixtures should be provided. The composition of the premixtures should reflect formulations commonly used in the regulatory region in which the approvals will be sought and be representative for the target animal species (e.g. poultry, swine, ruminants, aquatic animals).

3.3. Homogeneous Distribution of the Feed Ingredient in Feed

Testing the ability of the feed ingredient to be evenly distributed in a specific feed matrix is recommended to ensure that the intended effect of the ingredient can be achieved uniformly throughout the feed. To conduct a homogeneity test in feed, the feed ingredient should be incorporated into the feed at the intended inclusion level using mixing equipment that is readily available and commonly used in feed industry (See **Section 5**). The quantitative and qualitative composition of the feed should be provided. The composition of the feed tested should reflect formulations commonly used in the regulatory region in which the approval will be sought and be representative for the target animals (e.g. poultry, swine, ruminant, aquatic animals).

If a feed ingredient is intended to be directly added in feed and a properly conducted homogeneity test demonstrates a homogeneous distribution of the feed ingredient in feed, a test in the premixture according to **Section 3.2** is not necessary.

If adding a feed ingredient directly into the feed could potentially cause a homogeneity issue, for example if the inclusion level of the feed ingredient in feed is too low for the active substance to be accurately analyzed using current available analytical methods, or the physical characteristic of the feed ingredient (e.g. particle size, viscosity) makes it a challenge to evenly distribute it throughout the feed, a pre-dilution of the feed ingredient in a premixture prior to the incorporation in feed should be considered. In that case, the homogeneity of the feed ingredient in the premixture should be demonstrated following the recommendation provided in **Section 3.2** of this guidance.

When a feed ingredient is intended to be used in both mash feed and pelleted feed produced from the same mash feed, a homogeneity test in one form (either mash or pelleted) may be used to support the homogeneity in both mash and pelleted feeds.

For a feed ingredient intended to be used in a liquid form feed supplement, if the feed ingredient is soluble/miscible at the proposed use level, the homogeneity test is generally not necessary. Otherwise, as there is a higher possibility of separation of the feed ingredient, a homogeneity study should be conducted at the intended inclusion level(s) under the conditions simulating practical use. The homogeneity test should also be conducted at two time points, preferably at the time of production and at the end of the proposed shelf life to demonstrate the feed ingredient remains evenly distributed over the anticipated lifetime of the liquid feed supplements. If the CV from the homogeneity test at the end of the proposed shelf life is significantly higher than that from the initial test, or if the visual, physical inspection demonstrate absence of homogeneity, labeling instructions for agitation/recirculation of the liquid feed supplements may be needed to ensure the even distribution of

the feed ingredient over the anticipated lifetime, either at the time of feeding or during the storage period.

3.4. Homogeneous Distribution of the Feed Ingredient in Drinking Water for Animals

For a feed ingredient intended to be administered to animals through drinking water, if the feed ingredient is soluble/miscible in water at the proposed use level, a homogeneity test is generally not necessary. Otherwise, the test should demonstrate that the feed ingredient can be dispersed in water uniformly at the intended inclusion level and under specified water conditions simulating practical use (e.g. pH, mineral contents, water temperature, time, microbial contents, dispersion/suspension of the ingredient). If a dispersion/suspension system is used, the homogeneity test should also demonstrate whether the ingredient is uniformly dispersed over the anticipated lifetime of the drinking water for animals (typically 48 hours). A test at the end of the anticipated lifetime (e.g. 48 hours) is acceptable to demonstrate the homogeneous distribution of the feed ingredient over the anticipated lifetime of drinking water for animals.

In the case where the CV is significantly higher at the end of the anticipated lifetime (48 hours) than at the time of introduction in drinking water for animals, of if the visual, physical inspection demonstrate absence of homogeneity, the label instructions should indicate that the drinking water containing the feed ingredients should be used quickly and agitation/recirculation may be needed to ensure the intended effect.

4. SPECIAL CONSIDERATIONS:

4.1. Silage Ingredient

The homogeneity test for a silage ingredient in its market formulations and in premixture if it is intended to be added in a premixture prior to incorporation into silage should be conducted according to **Sections** 3.1 and 3.2, as appropriate.

For a silage ingredient, a homogeneity test in the silo is normally not necessary. The homogeneous distribution of a silage ingredient in the silo may be supported by demonstration of its intended effect.

4.2. Flavoring Agent

The homogeneity test for a flavoring agent in its market formulation and in premixtures should be conducted according to **Sections** 3.1 and 3.2, as appropriate.

When an analytical method is available for feed and/or drinking water for animals, either to quantitatively determine the concentration of the flavoring substance or, in case of complex flavoring mixture (e.g., crude extract/oil), the predominant compound(s) providing flavoring or the marker compound(s) if the predominant compound(s) cannot be identified, a test should be conducted according to **Section** 3.3 and 3.4, as relevant.

When no analytical method is available to quantitatively determine the concentration of the flavoring agent, or the predominant compound(s) providing flavoring or the marker compound(s) of a crude extract/oil in feed or in drinking water for animals, the applicant should adequately describe the approach/dilution process used to incorporate the flavoring agent in the feed or in drinking water for animals to ensure the proper mixability. In addition, depending on the acceptance of the regional authority, the demonstration of feed palatability, may be used to support the proper mixability of a flavoring agent.

4.3. Colorant[3]

The homogeneity test for a colorant in its market formulation and in premixtures should be conducted according to **Sections 3.1** and **3.2**, as appropriate.

If the intended effect of the colorant is to add or restore color to feed or drinking water or is used in feed for ornamental fish and birds, a homogeneity test in feed and/or water is normally not necessary. For a colorant to be used in feed or drinking water for pigmenting tissues or animal products (e.g. eggs) of food producing animals, a homogeneity test in feed and/or water should be conducted under the proposed conditions of use.

5. SAMPLING FOR A HOMOGENEITY STUDY

A proper homogeneity study should be conducted using at least 10 samples taken from one batch of the intended matrices, using mixing equipment that is readily available and commonly used in the feed industry. Each sample should contain enough material to carry out the necessary analysis. Samples from commercial production batches are preferred to be used in homogeneity studies. If samples from pilot or laboratory scale production are used, the mixing process using pilot or laboratory scale equipment should be comparable with the mixing process of commercial production. A justification should be provided to support that the homogeneity data obtained from the pilot or laboratory scale batches reflect the homogeneity of the commercial production batches.

To ensure that the samples taken are representative of the distribution of the feed ingredient in the batch under evaluation, an appropriate sampling plan should be employed and provided to the regulatory agency. The following are some approaches that can be envisaged. These approaches are applicable to both dry and liquid form of intended matrices including drinking water for animals, as relevant:

[3] In the United States of America, the Center for Veterinary Medicine (CVM) of the U.S. Food and Drug Administration (FDA) approves the food additives used in animal food (including drinking water for animals). However, color additives, including those intended to be used in animal food, are approved by FDA's Center for Food Safety and Applied Nutrition (CFSAN). Please contact CFSAN or visit www.fda.gov for detailed information regarding data and information needed for a color additive approval.

a) Timed sampling during the production process: samples of same amount taken at evenly distributed time intervals over the course of emptying the mixer/blender or at the final production step (e.g. pelleting or extrusion process).
b) Geometric sampling in a bulk container: samples of same amount are taken at different locations within the container. The locations of the sampled points should be evenly distributed throughout the container. The sampling process should avoid unnecessary disturbance to the matrix in the bulk container.
c) Sampling from different containers of the same batch: samples of same amount are taken from at least 10 containers randomly selected throughout the entire batch. If the containers can be traced by production time, samples can also be taken from at least 10 containers with evenly distributed production time intervals.

Sampling equipment must be suitable for taking representative samples from the intended matrix being tested (e.g. grain probe or other standardized particulate matter or liquid sampling device). The sampling equipment should be properly cleaned between each sampling to avoid potential carry-over from one sample to another. Samples taken from each specified location/time point should be the same amount and each sample should be adequate to perform the necessary analyses. Any further mixing among samples must be avoided.

Samples must be properly labelled with the sampled matrix, the expected level of the feed ingredient and the origin of the sample (time or location of the samples depending on the sampling method used).

6. DATA EVALUATION AND STATISTICAL ANALYSIS

The analytical results from all samples should be presented. A CV should be calculated based on the analytical results of all the samples, unless the exclusion of any outlier(s) is properly justified.

It is recommended to take more than 10 samples to address potential outliers identified during analysis. An analytical result should be considered an outlying result only if properly justified, using an appropriate statistical evaluation. When outlying values are detected, these should be reported and should be removed from the calculation of the CV. In any case, the number of samples used for the calculation of the CV cannot be less than 10.

The acceptability of a CV value to demonstrate homogenous distribution of a feed ingredient in its intended matrices depends on several factors. To evaluate the ability of the feed ingredient to be homogeneously mixed in the relevant intended matrices, it is recommended to consider the following elements:

a) The concentration/activity of the active substance contained in the feed ingredient in the intended matrix tested (i.e. for a low concentration/activity, a higher CV might be acceptable)
b) The precision and variability of the analytical method at the concentration tested (i.e. for less precise methods of analysis, a higher CV might be acceptable)
c) The safety profile of the feed ingredients (i.e. if the feed ingredient is well tolerated by the target animals, a higher CV might be acceptable)

7. DATA REPORTING

The homogeneity study report should include a description of the study (including the sampling plan) and all analytical data. The CV should be reported based on the analytical test results.

7.1. The Description of a Homogeneity Study Should Include:

a) Identity of the ingredient under study;
   Note: Documents (e.g. Certificates of Analyses) should be provided to demonstrate the name, batch numbers, manufacturing dates and contents of the feed ingredient under test.
b) Analyte(s) and parameter(s) that are tested for, including the active substance, predominant substance, and marker compound when relevant;
c) Qualitative and quantitative compositions of the intended matrices used in the test;
d) Proposed inclusion levels of ingredient in the intended matrices;
e) Mixing procedure for each tested matrix;
f) Sampling protocol, including sample collection approach, number(s) of samples and sample sizes for each type of intended matrices;
g) Name and address of the testing facility.

7.2. The Analytical Data Should Include:

a) Actual test date
b) Individual analytical result with measurement units for each sample tested with a reference to the batch number
   Note: Original analyst worksheets, spectra, chromatograms, certificates of analyses, charts, or other pertinent information should be submitted to support and verify reported analytical results. The CV calculated with all tested samples should be provided, unless the exclusion of outlier(s) is properly justified (see **Section 6**). When providing instrument/computer printouts, explanations should be included to clarify information such as sample identification, method code, etc. It is recommended to consult the regional regulatory authorities to determine whether the original data are necessary for a specific submission.
c) Description of test method(s)
   Note: If a test method is not a regulatory or internationally accepted method for the intended analysis, method validation information may be needed to support the use of the method in the homogeneity study.
d) Evaluation (e.g. statistical analysis) of the data and summarized data presentation (tables, charts, etc.)
   Note: The data points excluded from the calculation of the CV (i.e., outlier(s)) should be included in the raw data.

8. BIBLIOGRAPHY

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c) European Union Commission Regulation (EC) No 429/2008 of 25 April 2008 on Detailed rules for the Implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as Regards the Preparation and the Presentation of Applications and the Assessment and the Authorization of Feed Additives; Official Journal of the European Union L133/1

d) Universal Feed Assurance Scheme (UFAS) Guidance Sampling and Testing April 2016

e) US Food and Drug Administration, Center for Veterinary Medicine, Guidance for Industry # 221 - Recommendations for Preparation and Submission of Animal Food Additive Petitions, June 2015