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VLOG Carryover Test for Grinding and Mixing Facilities

Guideline for Planning, Implementation and Documentation

Carryover tests are mandatory for grinding and mixing facilities according to VLOG Standard 20.02, Chapter C 6.2, which also lists further information and requirements.

1. Definitions

Test batch: Batch with marker

Follow-up batch: The batch that is checked for carryover. It follows the test batch (after a system purge, if necessary)

System purge: A follow-up batch for "cleansing", which is run after the test batch to reduce the carryover from the test batch in the follow-up batch

Marker (substance): The means of identifying the test batch in the follow-up batch (e.g. a specific DNA, GMO etc.)

2. Objective of the Carryover Test

The purpose of the carryover test is to determine the carryover quantity from the previous batch in the follow-up batch at a grinding and mixing facility.

3. Purpose of the Carryover Test

The test can validate existing operating procedures or identify problem areas. Depending on the result of the test, measures may have to be taken to reduce carryover in order to comply with GMO threshold values in feed under European legislation[1] and the VLOG Standard.

4. Material used for the Test

The test batch and follow-up batch consist of single-component feeds commonly used in the facility.

If a system purge is conducted, it must comprise material that is typically used for system purges at the facility. It must be ensured (e.g. based on analysis) that the follow-up batch or system purge contain no marker substance or that the precise quantity of the marker substance is known and very low.

5. Processes checked by the Carryover Test

A carryover test must either be conducted for all operating processes performed by the facility (e.g. grinding, crushing or mixing) OR cover the mixing/grinding combination with the greatest risk of carryover used by the grinding and mixing facility. In the second case, the carryover test must comprise test batches and

[1] Regulations EC 1829/2003 and 1830/2003 are most relevant in this context. They specify that GMO content exceeding 0.9% must be labelled as genetically modified in all cases. Values between 0.1% and 0.9% require no labelling only if they are accidental and technically unavoidable (i.e. all operational measures to avoid them were exhausted). Values below 0.1% are generally regarded as accidental and technically unavoidable.

subsequently, follow-up batches run in all work equipment of the facility (e.g. mills, mixers and screw conveyors).

6. Identifying the Carryover

If the follow-up batch contains components of the test batch (carryover), they must be identifiable by the method selected. The marker substance and the detection method must be capable of identifying carryover of 0.1% or less.

Examples of suitable procedures/markers:

- **2 different (types) of pure single-component feed** are used: one for the test batch[2] and the other for the follow-up batch (and the system purge, if relevant). The carryover of the first type is then determined in the follow-up batch. **This procedure is recommended by VLOG**[3]. added
- **GMO as a marker**: a GMO single-component feed is used as the test batch, while a non-GMO feed **of the same species** serves as the follow-up batch[4]. The GMO content in the follow-up batch is determined by PCR analysis to calculate the carryover. added

7. Procedure for the Carryover Test

For facilities that operate with complete discharges but not (always) with system purges in VLOG production

1) Process the test batch as usual
2) Perform a normal complete discharge
3) Process the follow-up batch as usual
4) Take a representative sample of the follow-up batch
5) Analyse the sample and evaluate the results

For facilities that operate with system purges but not (always) with complete discharges in VLOG production

1) Process the test batch as usual
2) Perform a system purge with the usual minimum quantity
3) Process the follow-up batch as usual
4) Take a representative sample of the follow-up batch
5) Analyse the sample and evaluate the results

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[2] The species that can be identified with sufficient precision must be agreed upon with the laboratory (e.g. identified by PCR analysis, as microscopic methods generally don't offer satisfactory results in this context). We recommend using soy for the test batch and having a VLOG-recognised laboratory determine the percentage of soy in the follow-up batch.

[3] See Example A), below

[4] A sufficiently large and **concretely known** GMO content in the test batch is necessary for meaningful results (min. 30% GMO). Please note that "GMO feed" normally does not contain 100% GMO, but, at times, significantly less. In this scenario, a VLOG-recognised laboratory must analyse both the test batch and the system purge and follow-up batch for GMOs. See Example B), below.

For facilities that always operate with complete discharges and system purges in VLOG production [5]

1) Process the test batch as usual
2) Perform a system purge with the usual minimum quantity (e.g. in accordance with manufacturer specifications)
3) Perform a normal complete discharge
4) Process the follow-up batch as usual
5) Take a representative sample of the follow-up batch
6) Analyse the sample and evaluate the results

8. Representative Sampling

Since most carryover does not occur evenly during the mixing process, measures must be taken to ensure that the sample is representative. There are two options:

A) Take samples of the same size (min. 400 g each) from the batch at several evenly distributed time intervals. In this process, take one sample at the start and one at the very end[6]. Mix the individual samples well (e.g. in a clean bucket) and take a final sample (min. 400 g) from this aggregate sample.
B) Thoroughly remix the entire follow-up batch after passing through the system (e.g. after it has again been taken up by the system) and then take the sample (min. 400 g).

Place the sample into a suitable container (e.g. a bag) with a clean tool, clearly label the container (e.g. with date and number) and apply a tamper-proof seal.

Note: It is always advisable to take a second sample of the same size and keep it as a reference sample in case another test is required/needed later.

9. Analysis of the Follow-Up Batch and Carryover Calculation

The follow-up batch is analysed for carryover from the test batch to determine the test batch proportion still present in the follow-up batch (based on the identification procedure).

If a test batch with less than 100% of the marker is used (e.g. a feed with 40% soy content instead of pure soy), the soy mass determined in the follow-up batch is extrapolated to a soy content of 100% in the test batch.

Example: Test batch feed with 40% soy mass, follow-up batch wheat without soy. Determined soy mass in wheat: 0.2%.

-> With 100% soy as test batch, the carry-over would be 0.2% x 2.5 = 0.5%.

changed

[5]Carryover tests are not mandatory in such facilities under normal operating conditions according to the VLOG Standard. However, the test may serve as a safeguard in such facilities.

[6] It is advisable to take at least 5 samples from the mixture. The larger the batch, the more samples should be taken. The number of samples has to verify that the final sample represents the whole batch and its state of mixture. The consistent distribution of the marker (substance) has to be assured.

A reserve sample should be taken from the follow-up batch before grinding. If too much of the marker (e.g. soy) is detected after grinding, this retain sample can be tested to see whether it was originally really free of the marker.

changed

10. Evaluation of the Results and Determination of Measures

If the results show that the current operational measures taken at the facility are not sufficient for maximum reduction of carryover (see Section 3.), facility-specific measures must be developed to reduce the carryover to an acceptable level. Such measures may include:

- Introducing a system purge/complete discharge
- Expanding the system purge
- Specifying the maximum GMO content in the mixture that is run before the VLOG mixture
- Changing the scheduling/sequence of processing (e.g. previous application only involves crushing; no GMO content in the previous mixture)
- Etc.

11. Documentation of the Carryover Test

The carryover test must be clearly documented. This includes in particular:

- System (model, **vehicle** registration no.), tester, date

added

- Processes tested (e.g. mixing, grinding, screw conveyor…)
- Description of test batch, follow-up batch and system purge, if any (type, quantity)
- Description of marker (various single-component feeds, GMOs, …)
- Description of **conducted cleaning/rinsing** measures (e.g. system purge, complete discharge)

added

- Test procedure (including variants, if any)
- Analytical method, markers used
- Laboratory/test reports, etc. must be retained
- Results of the carryover test
- Any (corrective) measures taken/developed

12. Specific Example for the Carryover Test

Implementation with the two feed materials soy and cereals (wheat from Europe)

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Summary

In this example, a soy single feed (soy meal) is used as a test batch. Afterwards a complete discharge and a cleaning with a system purge (here: wheat from Europe) take place. Then another soy-free feed material (here: wheat from Europe) passes through the facility as follow-up batch. The wheat of the follow-up batch is sampled representatively before and after grinding. For the time being, only a sample taken after the grinding is tested for soy components to determine the extent of the process carry-over. If necessary, a sample taken before the grinding process is also tested.

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Preparation of the carry-over test

1) 1000 kg of the pure soy feed material[7] and 1000 kg of soy-free wheat[8] are provided.
    - The minimum mixing quantity of the plant specified by the manufacturer must be taken into account in the test for test and follow-up batches. If this is more than 1000 kg, at least the quantity specified by the manufacturer must be used instead of the 1000 kg.
    - Since the determination of the carry-over in this example is calculated via the soy mass, it is not relevant whether the product is genetically modified or not.
2) Soy-free material for the system purge batch is provided in the usual amount (ideally the same material as the following batch, in this case: soy-free wheat)
3) A complete discharge of the system is carried out before the carry-over test

changed, foot-notes added

Carrying out the carryover test

4) Test batch: 1000 kg soy are ground and/or mixed as usual and transported via the conveyor system
5) Complete discharge: an usual complete discharge is carried out
6) System purge: The usual system purge is carried out with the usual amount of wheat
7) **Follow-up batch: Before the milling or mixing process, two representative samples of 500 g each of the 1000 kg of wheat are taken**
8) Follow-up batch: 1000 kg of wheat are ground and/or mixed as usual and transported via the conveyor system ~~**Sampling**~~
9) Follow-up batch: Sampling: Distributed over the blow-out process, 10 individual samples of 500 g each are taken and collected in a container. This collective sample is mixed well and then two final samples of 500 g each are taken.

Changed, added

[7] As an alternative to a pure soya straight feed, a compound feed with a soya content of at least 20% can also be used as a test batch. The exact soya content must be known and taken into account when calculating the carry-over (extrapolation necessary).

[8] In order to ensure that the wheat does not contain soya and thus falsify the result, European wheat from our own cultivation or wheat purchased directly from European producers is recommended. This greatly reduces the risk of mixing with soya (e.g. through storage, transport).

10) The four final samples from step 7) and 9) are clearly labelled. One from each of the two steps is sent to the laboratory. The laboratory is informed about the type of feed and the soy content. The remaining two final samples are kept by the company as retained samples.

changed, added

Evaluation of the carry-over test

11) In the laboratory it is examined (e.g. by PCR analysis) how much soy is contained in the follow-up batch. In individual cases, it may be necessary to test the follow-up batch before grinding/mixing.

12) The carry-over is calculated taking into account the soy content in the test batch.

13) Procedure and results of the carry-over test are documented in writing (see chapter 11. Documentation of the carryover test)

Example of measures from the results

Result of the test: the carryover after complete discharge + system purge is 0.5%. For a follow-up batch of 1,000 kg this corresponds to 5 kg (0.5% multiplied by 1,000 kg= 5 kg).

Goal: GMO carry-over is to be reduced to below 0.1% if technically possible (see European legislation in 3.).

Possible measures:

- Before 1.000 kg of VLOG-mixtures only GMO-containing mixtures with less than 20% of material requiring labelling are mixed[9]
- **The system purge is performed with a larger amount of material**

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[9] Method of calculation: 0.5% is five times the target value of 0.1%. Therefore, if the GMO content is reduced to less than one fifth (<20%), the carryover is brought below 0.1%)