AT-08
Cross-contamination
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<td>Item IV</td>
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<td>Addition of multiplying factors</td>
<td>Item IV</td>
<td>25/04/2013</td>
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<td>Item IV</td>
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I. INTRODUCTION

I.1 Definitions

Cross-contamination:
See definition in document ‘AC-00: Introduction’.

Cross-contamination:
The level of contamination is defined as the quantity of a nutrient or component from a previous batch, expressed in percentage and transferred to the next feed batch (the same volume). The cross-contamination levels can be measured over part of the installation (e.g. until the pellet storage bin) or over the entire installation.

Maximum cross-contamination level:
standard (expressed in levels, e.g. ppm or ppb) determined in the context of the auto control system of the compound feed producer, or producers of pre-mixtures and/or additives. The latter will determine the maximum quantity of a nutrient or component allowed in the feed lot, as a result of the cross-contamination.

Installation’ own cross-contamination level:
This concerns the cross-contamination of the installation for manufacturing compound feed, pre-mixtures and additives. The cross-contamination can be measured using the methods described in this document.

Multiplication factor or Multiplier
The multiplier is intended to take account of an extra cross-contamination in addition to the installation cross-contamination, as a consequence of the processing properties of the additive or (medicated) pre-mixture.

In TNO-report I-96-31006 “Cross-contamination of a number of critical substances in cattle feed and the relationship to their characteristic properties /II/”, you will find a description of the studies having served as a basis in determining the multiplication factors (multipliers). A laboratory method was developed: relative wall adhesion. The cross-contamination characteristics of the additives and (medicated) pre-mixtures using this method, have been tested in a practical compound feed plant.

<table>
<thead>
<tr>
<th>The relative wall adhesion factor according to the established method</th>
<th>Multiplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>1</td>
</tr>
<tr>
<td>1 and &lt; 2</td>
<td>2</td>
</tr>
<tr>
<td>2 and &lt; 3</td>
<td>2,5</td>
</tr>
<tr>
<td>Unknown or &gt; 3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1: determining the multiplier in function of the wall adhesion coefficient

A number of additives and (medicated) pre-mixtures have already been subjected to the wall adhesion test. The available data is used in determining the multiplying factor.

Point IV of this document lists some of these factors.

If there is no data available for determining the multiplier, factor 3 will automatically be assigned.
The actual cross-contamination of additives and cross-contamination of (medicated) pre-mixtures must be calculated in order to establish the number of flushes. This cross-contamination can be calculated by multiplying the installation carry over by the multiplier.

The actual cross-contamination thus calculated can be used to calculate how many flush batches are minimally required to remain under the established residue level.

The indicated additional instructions for use are relating to the production sequence in the installation. It is necessary, in order to guarantee the maximum levels indicated for cross contamination, that additional internal company policies and procedures are established:

- transport and storage sequence;
- intake of pre-mixtures, additives and (medicated) pre-mixtures.

Important remark: The company must check that the maximum cross-contamination levels are not exceeded. If an infringement of these maximum cross-contamination levels are detected, the instructions for use and procedures must be adjusted accordingly.

II. COMPOUND FEED

II.1. General principles relating to the measurement of the cross-contamination

When measuring the cross-contamination of additives and/or (medicated) pre-mixtures in a compound feed installation, one must check, based on the chart and on the actual situation of the production installation, which are the parts that may have relevant cross-contamination.

A starting point in determining the degree of contamination in a company is the knowledge and control of both the internal and external return flows.

**The initial process:**
The purchased additives and (medicated) pre-mixtures may have been contaminated by substances other than the ones mentioned on the label.
The producers of pre-mixtures and additives produce, according to the maximum levels of cross-contamination established for this type of animal feed.
The introduction of undesirable substances into the compound feed through the pre-mixtures or additives, is not considered in the following calculations.

**Cross-contamination points:**
Cross-contamination in compound feed facilities may occur in the following processes:

a. When filling silos with pre-mixtures and/or additives:
   Filling silos or containers with pre-mixtures and/or additives can lead to cross-contamination. From the chart can be verified if there are reasons to assume that potential cross-contamination can be identified. The critical points are common transport systems, locks, separation systems and filters.
   In mechanical transport systems such as Redlers, elevators and screw conveyors, cross-contamination over will always occur, therefore it is advisable to measure this kind of cross-contamination one single time. It is preferable to use methods such as described in II.2 (Methods). Also, sufficiently long draining times (10 minutes) must be respected.
   With the pneumatic filling method, using separate filters per silo, the cross-contamination should not be taken into account. If one filter is used for several silo’s, the filter must be shaken for at least 10 minutes on the same silo as the one used for filling.
   There should be an instruction for bulk order, to prevent undesired mixing

b. Dosing, grinding and mixing line:
The highest cross-contamination of additives and (medicated) pre-mixtures occur during the dosing process (addition of additives / (medicated) pre-mixtures) / (possibly grinding)
mixing / transport and storage of the product in meal form in a finished product cell or in
a pellet silo.
The additives and/or (medicated) pre-mixtures must be added as close as possible to the
mixer. It is important that the measured substances are added at the exact same place as
where the additives and (medicated) pre-mixtures were added.

c. The pressing line:
A significant level of cross-contamination may occur in the pressing line. Cross-
contamination tends to increase with the size of the moulds. Moreover, temporary silos
with stock can be a source of cross-contamination. A point of Attention should be the
return flows, fed back directly into the pellet silo during pelleting;

d. Loading and transport:
A point of attention is the reprocessing of the bulk load sieving. If undesired cross-
contamination from additives and (medicated) pre-mixtures are expected to occur during
storage, loading and transporting of finished products, the company may take the
following measures:
   a. The preparation of a prohibited production (work) sequence;
   b. The reservation of production (work) lines;
   c. Additional measures in the event of product changes;
   d. Manufacturing of feed with additives and (medicated) pre-mixtures on other lines.

Measuring points for cross-contamination:
The main causes of cross-contamination are the dosing/grinding/ mixing and pressing line. For a
reliable conclusion, the following measuring points are important:
1. after mixing, but as close as possible to the mixer in order to measure the output levels of
the mixture;
2. At the inflow of the pellet silo during pellet production, or the finished product silo during
meal production, for measuring the cross-contamination of the dosing/grinding/mixing;
3. At the inflow of the finished product silo during pellet production for measuring the cross-
contamination in the dosing/grinding/mixing and the pressing line.

The cross-contamination of the dosing/grinding/mixing line is calculated by means of measuring
points 1 and 2, as described above. The cross-contamination of the pressing line is the difference
calculated for the entire line from measuring points 1 and 3 on the one hand and the
dosing/grinding/mixing line calculated through means of measuring point 1 and 2 on the other
hand.
The cross-contamination calculated in this way is regarded as the installation's own cross-
contamination.

Determination of the number of flush batches
One of the measures in controlling the risk of cross-contamination (e.g. exceeding of maximum
cross-contamination levels) is flushing the installation with an animal feed batch, preferably from
another manufacturing batch. The amount of product used for cleaning, must be adapted to the
installation.
In order to guarantee adequate cleaning, and at the same time reducing the volume of these flush
batches, it is important to define the number of flush batches required. Below, you will find two
different methods.

a) First method
To determine the number of necessary flush batches, the following formula can be used:

\[ \text{number of flush batches} = \frac{\log\left(\frac{a}{c}\right)}{\log(a+b)} \]

Log = decimal logarithm or common logarithm (basis of 10)
a = Installation's cross-contamination inherent (expressed in decimals (e.g. 5 % = 0,05))
b = multiplier for the additive concerned

c = concentration of the additive in the animal compound feed (mg/kg)

d = maximum level of cross-contamination of the additive concerned in the next animal compound feed (mg/kg)

The result is rounded down to the nearest unit.

b) Second method

It is also possible to use an iterative method. This method consists of calculating successively the theoretical residues left in the installation after every production run.

\[
a \times b \times c < d
\]

The calculation is performed successively, until the actual result is lower than the maximum level of cross-contamination. The number of necessary flush charges will then be calculated. It is important to note that the percentage b is expressed in decimals (e.g. 5 % = 0,05)

c) Using the two methods: example

Assuming an additive X, of which the multiplier is not known (so b = 3) whose concentration in the complete animal feed is 66 mg/kg (= c). The maximum level of cross-contamination in the next production run is 1 mg/kg (= d). The cross-contamination inherent to the installation is 13 % (= a)

First method:

\[
\log \left( \frac{1}{66} \right)
\]

Number of flush charges = \( \frac{-}{\log (0,13*3)} \) = 4,45 rounded down to the nearest unit: 4

Second method:

1. Content of additive in the following charge (first passage): 66 * 0,13 * 3 = 25,74 (result > 1 \( \Rightarrow \) this means that if it concerns the manufacturing of a complete feed, the latter should contain much more that 1mg/kg (in theory 25,74mg/kg))

2. Content of the additive in the next batch (second passage): 25,74 * 0,13 * 3 = 10 (result > 1 \( \Rightarrow \) this means that, for a production of a complete feed, the latter will contain much more than 1 mg/kg (in theory 10 mg/kg))

3. Content of the additive in the next batch (third passage): 10 * 0,13 * 3 = 3,92 (result > 1 \( \Rightarrow \) this means that, for a production of a complete feed, the latter will contain much more than 1 mg/kg (in theory 3,92 mg/kg))

4. Content of the additive in the next batch (fourth passage): 3,92 * 0,13 * 3 = 1,52 (result > 1 \( \Rightarrow \) this means that, for a production of a complete feed, the latter will contain much more than 1 mg/kg (in theory 1,52 mg/kg))

5. Content of the additive in the next batch (fifth passage): 1,52 * 0,13 * 3 = 0,59 (result < 1 \( \Rightarrow \) this means that, for a production of a complete feed, this batch will contain less than 1 mg/kg (in theory 0,59 mg/kg)). This means that the 5th passage may consist of a complete animal feed production. As a result, the other 4 passages are considered flush charges.

Thus, the number of flush batches equals 4 (result identical to the first method).

d) Important remarks

1. In some cases, it may be necessary to consider, the presence of additives in certain components used for manufacturing compound feed. If the additive in its natural state, is also present in certain feed ingredients, the percentage of the additive to be incorporated, is calculated in a way that the sum of the added components and the naturally present elements comply with the expected maximum or minimum levels.
2. In the frame of validation and verification of the HACCP plan, it is recommended occasionally to verify the relevance of the following calculation methods by analyzing the additive residues (either in the last flush batch or in the next production batch).

II.2. Methods

General procedure for testing the cross-contamination in the compound feed production by means of a tracer

The procedure for determining the cross-contamination and the homogeneity of flour blends when preparing compound feeds, using tracers which, depending on their properties, can replace the conventional compound feed additives. The test procedure includes processing two or three successive charges from the same feed mixture (Table 2). To perform a cross-contamination test, the same (most current) type of compound feed is preferred.

When the Micro tracers F or FSS are used, it is recommended to not use feed with high fat contents (e.g. chicken feeds). This decreases the availability of the tracer when determining analytical tracer contents in the laboratory.

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Number of charges</th>
<th>Blank / Tracer</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt carbonate</td>
<td>3</td>
<td>1: blank</td>
<td>charge a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: tracer</td>
<td>charge b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3: blank</td>
<td>charge c</td>
</tr>
<tr>
<td>Micro tracer F or FSS (lake)*</td>
<td>2</td>
<td>1: tracer</td>
<td>charge b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: blank</td>
<td>charge c</td>
</tr>
<tr>
<td>Micro tracer RF Blue (lake)*</td>
<td>2</td>
<td>1: tracer</td>
<td>charge b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: blank</td>
<td>charge c</td>
</tr>
<tr>
<td>Salinomycin Sodium (relative wall adhesion coefficient &lt; 1)</td>
<td>2</td>
<td>1: tracer</td>
<td>charge b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2: blank</td>
<td>charge c</td>
</tr>
</tbody>
</table>

* Micro tracer particles are elementary iron particles, coated with a feed colorant. They are not toxic.

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Number of charges</th>
<th>Blank / Tracer</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Tracer</td>
<td>charge a</td>
<td>charge b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>charge b</td>
<td>charge c</td>
</tr>
</tbody>
</table>

Table 2: Number of charges per test procedure

Micro tracer F: ± 25,000 particles per gram of Micro tracer F. Particles with a size distribution of 150-300 µm

Micro tracer FSS: ± 200,000 particles per gram of Micro tracer FSS. Particles with a size distribution of 75-300 µm

Micro tracer RF: > 2,000,000 particles per gram of Micro tracer RF. Particles with a size distribution of 75-150 µm

Prior to the test procedure, it is recommended to flush the installation. Batch A is used for measuring the “natural” tracer content (= cobalt) in the animal feed. The tracer mixture is added to batch B. The tracer content is determined in the “meal” and “pellets” samples taken from the batch B. Production batch C consists of the same animal feed ‘blank’, without the added tracer mixture. The tracer content is determined in the “meal” and “pellet” samples from this batch. This level gives an idea of the cross-contamination inherent to the installation.

Optionally, one can, prior to batch B, produce an additional batch with a tracer mixture in order to condition the installation. For the Micro tracer F and FSS (lake), the tracer content will be expressed as the “number of Micro tracer particles” in a meal or pellets sample. The number of Micro tracer particles is determined by separating the Micro tracer particles from the other feed particles and by making them visible on a sheet of filter paper. The evaluation of the homogeneity of Micro tracers F and FSS are calculated in a different way (Poisson Statistics, see II.2.1.6.4).
II.2.1.1. Scope.
This method is only intended for internal measuring of the cross-contamination and homogeneity, within the company.

II.2.1.2. Equipment and tools.
At least 50 plastic containers of 500 ml. with cover, or sampling bags of 1L are required.

When using cobalt as a tracer, 4 additional containers or bags must be provided for determining the natural background presence in the feed. In any case, it is recommended to anticipate the use of at least 15 additional containers or bags, so that sampling can continue to be performed until there is a significant decrease in the flow of the selected control point.

II.2.1.3. Company data.
Prior to measuring the cross-contamination, following data must be available:
   a. Type of products (additives, (medicated) pre-mixtures) processed;
   b. Diagram showing the different operations: unloading, storing, dosing, reprocessing, grinding, internal transport and final storage of the products mentioned above;
   c. A clear overview of the internal and external return flows;
   d. A schematic diagram of the production facility, indicating the location where the tracer mixture is added and where the samples are taken.

During the execution of a test procedure, the following is required:
Copies of computer prints for reading purposes (so-called extensive protocol):
   - Composition of feed mixtures;
   - Weight of components required by the computer program (sollwert) as well as the actual weight of the components (istwert) or;
   - If the installation is not automated:
     o The total weight of the charge calculated by adding the amount of its components weighed;
     o Readings of the actual weight charge;
     o For calculating the weight of the charge, it should specify where and how much of the other components are added (e.g. manual adding, liquids)

II.2.1.4. Incorporation of the tracer mixture.
A tracer mixture is added to charge B of the compound feed (see table 3 for properties of the tracer mixture).

Enough of the tracer-based mixture should be added in order to correspond to a dose of 2.0 kg per ton of compound feed. One can assume the weight of the food to be considered, is the weight of the charge as required by the processing computer.

The place where the tracer mixture is added, will depend on the cross-contamination to be measured (see II 1.). The location chosen for adding and sampling are indicated in the schematic diagram of the production facility.
<table>
<thead>
<tr>
<th>Tracer</th>
<th>Tracer mixture</th>
<th>Tracer content in compound feed with tracer mixture added</th>
<th>cross-contamination *</th>
<th>Number of charges</th>
<th>Meal / pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt carbonate</td>
<td>5% cobalt</td>
<td>100 ppm</td>
<td>1%</td>
<td>3</td>
<td>Meal / pellet</td>
</tr>
<tr>
<td>Cobalt carbonate</td>
<td>2.5% cobalt</td>
<td>50 ppm</td>
<td>3%</td>
<td>3</td>
<td>Meal / pellet</td>
</tr>
<tr>
<td>Micro tracer F of FSS (lake)</td>
<td>5% MT</td>
<td>100ppm</td>
<td>1%</td>
<td>2</td>
<td>Meal / pellet</td>
</tr>
<tr>
<td>Micro tracer FSS</td>
<td>0.5% MT</td>
<td>10 ppm</td>
<td>1%</td>
<td>2</td>
<td>Meal / pellet</td>
</tr>
<tr>
<td>Micro tracer RF Lake Blue</td>
<td>12.5% MT</td>
<td>250 ppm</td>
<td>0.5%</td>
<td>2</td>
<td>Meal</td>
</tr>
<tr>
<td>Sodium salinomycin</td>
<td>Not applicable</td>
<td>35 ppm</td>
<td>5%</td>
<td>2</td>
<td>Meal / pellet</td>
</tr>
</tbody>
</table>

* Indicates the minimum cross-contamination percentage measured with this tracer when preparing compound feed. If the cross-contamination percentage measured with this tracer is lower than the percentage mentioned in the table, the cross-contamination percentage from the table is used in the calculations.

Table 3: Tracer mixture properties

After adding the tracer mixture, the mixing time should corresponding to the mixing time used during normal production.

II.2.1.5. Taking and processing of samples.

II.2.1.5.1. Taking samples.

From the previous charge, preceding charge B, the passage time is measured at the predetermined measuring point.

The constant period T between two sample takings at a specific measuring point, will equal the measured passage time at that point, divided by the amount of samples to be taken (plus one) at that precise point. For example, if the time of passage directly after the mixing is 10 minutes and if 20 samples need to be taken, a sample will have to be taken app. every 28.6 seconds (=600 seconds / (20+1)) [3,4].

Samples of at least 500 grams should always be taken.

The following schedule can be used for collecting samples, from which a part of the sampling and/or further processing is voluntary, to gain a better insight.

1. Charge A (without added tracer/if applicable):
   - Minimally 4 samples in or directly after mixing (II 1) (AM1-AM4) (A = charge A; M = mixing installation; 1-4 = sequence of the samples). Moisture content and natural cobalt content of the feed is determined in these samples (4x Cobalt, 4x moisture).

2. Charge B (with added tracer mixture):
   - Minimally 20 samples, in or directly after mixing, divided regularly over the outflow time in order to establish the mean tracer content (BM1-BM20) and the homogeneity level (B = charge B; M = mixing installation; 1-20 = sequence of the samples). The first sample is taken after one period T; samples should continue to be taken until there is a considerable decrease in the flow[4].
In at least 10 samples, the tracer content is analyzed (10 x tracer, 4 x moisture if cobalt is used as a tracer).

- If desired (this is voluntary) at least 10 samples can be taken at a selected control point(s) for determining the cross-contamination, the mean tracer content (BC1-BC10) and homogeneity.

3. Charge C (cross-contamination charge):
   - At least 30 samples, taken at selected cross-contamination check point(s) and distributed regularly over the total running time of the charge through this check point, for determining the level of cross-contamination. The first sample will be taken immediately, the second sample will be taken after one period $T$; samples should be taken until there is a significant decrease in the flow [3] (CC1-CC30). ($C = \text{Charge} ; \ C = \text{check point} ; 1-30 = \text{order of the samples}$)

Subsequently, equal amounts are taken from the 30 samples and mixed. From that mixture 3 subsamples are taken (subsample 1 = CCx = (CC1-CC2); subsample 3 = CCz = (CC25-CC30); subsample 2 = CCy = samples from the middle (CC3-CC24)).

- In case Micro tracer F is used:
  i. Subsample 1 (CCx) is composed of 500 g., taken from the samples CC1 and CC2;
  ii. Subsample 2 (CCy) is composed of 100 g. taken from the samples CC3 to CC24; from which a sample of 1000 gr. is collected;
  iii. Subsample 3 (CCz) is composed of 200 g. taken from the samples CC25 to CC30; from which a sample of 1000 g. is collected.

- In case Micro tracer FSS (100 ppm) is used:
i. Subsample 1 (CCx) is composed of 50 g. taken from the samples CC1 and CC2;

ii. Subsample 2 (CCy) is composed of 50 g. taken from the samples CC3 to CC24 from which a sample of 100 g. is collected;

iii. Subsample 3 (CCz) is made from 50 g., taken from the samples CC25 to CC30; from which a sample of 100 g. is collected.

- In case Micro tracer FSS (10 ppm) is used:
  i. Subsample 1 (CCx) is composed of 500 g., taken from the samples CC1 and CC2;
  ii. Subsample 2 (CCy) is composed of 100 g., taken from the samples CC3 to CC24; from which a sample of 1000 g. is collected;
  iii. Subsample 3 (CCz) is composed of 200 g., taken from each of the samples CC25 to CC30; from which a sample of 1000 g. is collected.

Remark: if more than 30 samples (e.g. n) were taken, subsample 2, made from all samples taken between subsample 1 (CC1-CC2) and subsample 3 (CC[n-5]-CCn). Subsample 2 is made from CC3 to CC [n-6].

With this composition of the subsamples, there will be (with a cross-contamination level of 1% or more) at least 15 particles of Micro tracer F or FSS per filter paper present.

Every subsample is analyzed for tracer contents (3 x tracer; 3 x moisture content in case cobalt is used as a tracer [3]).

- If desired (this is voluntary) minimally 10 samples in or as close as possible to the mixer, divided regularly over the outflow time of the charge (CM1-CM10) (C = Charge; M = Mixer; 1-10 = sequence of the samples).

II.2.1.5.1. Sample handling and destination.

Each pellet sample is ground in a suitable mill.

First the pellet samples from charge A are ground (if applicable), followed by the samples from charge C (cross-contamination level) and finally the samples from charge B. In this way the samples are ground according to a rising order of tracer content.

- Clean the mill after each sample, by using compressed air;
- Clean the mill after each group of samples (from the same charge) both, by means of compressed air, as well as dusting with a medium soft brush, after the relevant components have been installed;
- In the mill, there should not be any cross-contamination of materials from a previous group of samples, transferred to a next group;
- Homogenize each grinding as much as possible and return it to the initial container.

As regards the destination of the samples, the following applies:

1. In case of cobalt as a tracer:
   a) All moisture analysis serve in correcting the results of the cobalt analysis for differences in the moisture level, or they can be recalculated based on dry matter.
   b) The samples AM1-AM4 are analyzed individually. This is particularly important for samples from charge C, because the cobalt levels from charges B and C must be corrected with the ‘natural’ cobalt content in the feed.

2. The samples BM1-BM20 can serve two purposes. They can be used alternately for both purposes. If desired (this is voluntary) half of the samples (e.g. the even numbers) can be used for analyzing the homogeneity of the mixture. To this end all 10 samples need to be analyzed separately.
The other half of the samples (e.g. uneven numbers) can be mixed and, possibly after further reduction of the product, used to determine the mean tracer content of charge B. To this end, at least 1 new sample for analysis is taken from the mixture. The mean tracer content of charge B may also be determined by calculating the average individual result of the 10 samples. (for establishment of the homogeneity).

3. Samples BC1 to BC10 can be used, if desired (voluntary) to get an idea of the extent that the homogeneity level of the product, obtained at the output of the mixer (BM1 to BM10), is maintained during further production and transportation process until the next control point. These samples must be analyzed separately.

4. Samples CM1 to CM10 can be used, if desired (voluntary) to determine if and how much cross-contamination will appear during the trajectory between the mixer and the next sampling point. For the analysis, one can choose from, either analyzing a subsample, or analyzing all 10 samples separately (the cross-contamination pattern and the mean calculation).

5. Samples CC1 to CC30: the tracer content is determined in each of the 3 new subsamples (CCx, CCy, CCz). The mean tracer content (CC1-CC30) is determined by calculating the average of these 3 results as follows [3]:

\[
\frac{CCx \times 2 + CCy \times 22 + CCz \times 6}{30}
\]

Comment: if more than 30 samples (e.g. n) were taken the formula will be as follows :

\[
\frac{CCx \times 2 + CCy \times (n - 8) + CCz \times 6}{n}
\]

Assuming that each of the initial samples are representative for an equal part of the charge, the average cross-contamination can then be calculated directly. If this is not the case, e.g. because of irregular time intervals between sampling, the average must be calculated by taking the actual (=measured) time intervals into account. One can opt for analyzing the samples CC1-CC30 separately, and then afterwards, the mean can be calculated based on the results.

II.2.1.6. Processing the results.
II.2.1.6.1. Corrections in case cobalt is used as a tracer.

All results of the cobalt analysis must first be corrected to levels on dry matter, using the mean results obtained from the corresponding moisture level analysis. For the correction the following formula can be used:

\[
C = \frac{100}{100 - V} \times C1
\]

With:
C = cobalt content based on dry matter, ppm;
V = moisture level of the corresponding group of samples, %;
C1 = measured cobalt content, ppm

Corrected values based on dry matter obtained from charges B and C are, in turn corrected with the 'natural' cobalt content in the feed (determined as the average of values AM1-AM4 calculated on the basis of dry matter).
II.2.1.6.2. Calculation of cross-contamination.

Cross-contamination inherent to the installation (for cobalt based on the corrected values of II.2.1.6.1) is calculated as follows:

The mean tracer content in 30 samples CC from charge C, divided by the mean tracer content of 10 samples BM from charge B, is multiplied by 100 to get the mean percentage of cross-contamination in the charge, immediately following the charge with the added tracer mixture, serving as a model for a pre-mixture with an additive.

The percentage of the level of cross-contamination ($V_s\%$) is now calculated as follows:

$$V_s\% = \frac{\text{mean tracer content of charge C}}{\text{mean tracer content of charge B}} \times 100$$

By graphically representing the results of the tracer analysis of 3 mixed samples of samples CCI-CC30, a cross-contamination pattern is obtained, providing more information than the calculated average.

II.2.1.6.3. Homogeneity of the material.

The coefficient of variation between the analytical company samples is a measure for the homogeneity of the meal mixture, or of the pellets, from which the samples were taken.

The coefficient of variation (VC) is calculated as follows:

$$VC = \frac{\sigma}{\bar{X}}$$

De standard deviation ($\sigma$) is the square root of the variance:

$$\sigma = \sqrt{\frac{1}{N} \times \sum_{i=1}^{j} ni \times (Xi - \bar{X})^2}$$

The variation coefficient is generally expressed in % (= VC*100). If the coefficient of variation equals 9%, This means that the standard deviation is 10% of the average.

II.2.1.6.4. Homogeneity of the material: Micro tracers F en FSS [5]

In order to produce a statistically accurate assessment of the homogeneity of material for Micro tracers F and FSS, a minimum number of 15 particles per filter paper must be present.

This will be possible by analyzing the amount of samples here below:

- F: 40 grams;
- FSS (100 ppm): 4 grams;
- FSS (10ppm): 40 grams

For the evaluation of the homogeneity, the following statistical data is provided:

- Number of Micro tracer particles in the individual samples of charge B
- Mean number of Micro tracer particles in charge B (=X)
- Number of degrees of freedom of the system (= number of analyzed samples - 1)
- The square of the difference between the number of Micro tracer particles determined in separate samples, and the mean number of Micro tracer particles in charge B. the sum of these squares gives S (=S)
- Chi square value (=S/X)
- Probability p in % as an indicator for the homogeneity
- Recovery percentage (recovery)
Based on the established chi square value, and the number of degrees of freedom, the probability will be determined. The obtained values can vary between 0.999 en < 0.0005. These values will indicate the probability of the analyzed mixture, (charge B) corresponding to a perfect mixture. The assessment of the homogeneity is defined as follows:

- If \( p \leq 5\% (0.05) \) then, on the basis of the probability calculation, it can be concluded that the mixture is homogenous. The higher the P-Value, the better the mixture approaches a perfect homogeneity.

- If \( p < 5\% (0.05) \) and \( > 1\% (0.01) \), no clear statistical conclusion can be made. It is recommended to repeat the cross-contamination test.

- If \( p \leq 1\% (0.01) \) then, based on the probability calculation it can be concluded that there is a “significant deviation of the homogenous mixture” (non-homogenous mixture). The causes must be investigated and corrected. A new cross-contamination test must be performed.

Example 1: homogenous mixture

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Number of counted particles x</th>
<th>mean</th>
<th>difference (absolute)</th>
<th>Square of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>50</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>50</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>50</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>50</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>mean x = 50</td>
<td></td>
<td></td>
<td></td>
<td>Sum = S = 68</td>
</tr>
</tbody>
</table>

- Number of samples : \( n = 5 \)
- Chi square value : \( \chi^2 = S / x = 1.4 \quad (68 / 50 = 1.4) \)
- Number of degrees of freedom of the system: \( n - 1 = 4 \)
- From Chi square value and the number of degrees of freedom, the probability can be calculated: between 73,6 (0,736) and 91 % (0.91); see below in table 4

Conclusion: if the calculated probability is higher > 5%, the mixture will be considered as homogenous.
Example 2: non homogenous mixture

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Number of counted particles x</th>
<th>mean,</th>
<th>difference (absolute)</th>
<th>Square of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>53</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>53</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>53</td>
<td>17</td>
<td>289</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>53</td>
<td>18</td>
<td>324</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>53</td>
<td>8</td>
<td>64</td>
</tr>
</tbody>
</table>

mean x = 53  Sum = S = 793

- Number of samples: n = 5
- Chi square value: $\chi^2 = S / x = 14.96$ (793 / 53 = 14.96)
- Number of degrees of freedom of the system: n - 1 = 4
- From Chi square value and the number of degrees of freedom, the probability can be calculated: between 0.5 (0.005) and 0.7 % (0.007); (see in table 4)

Conclusion: if the calculated probability is lower than > 1%, the mixture will therefore be considered as non homogenous.

II.2.1.7. Reporting.

For each group of company samples, the following will be reported:
1. In case cobalt is used as a tracer: the mean moisture content of the group of samples (0.01%);
2. In case cobalt is used as a tracer: the average levels of cobalt, measured and corrected on dry matter, represented in each sample to be analyzed (0.1 ppm at cobalt levels higher than 10 ppm and 0.01 ppm at cobalt levels of 10 ppm or less);
3. The average corrected and measured tracer levels of company samples per group (0.1 ppm at tracer levels of 10 ppm or less);
4. The cross-contamination inherent to the installation, calculated according to the test procedure.

For each group of company samples from charge B, the following will be reported:

5. The variation coefficient between the mean samples (0.01%);
6. Micro tracer F and FSS: the chi square value, the number of freedom degrees of the system, and the probability P in %, provides an indication for the homogeneity.

II.2.1.8. Remarks.

II.2.1.8.1. First sampling point.

After dosing the individual components, a feed mixture will not be homogeneous. Even after grinding the feed materials in a hammer mill, this will only be partly the case. Often finer materials will not pass through the hammer mill, but will be fed directly into the mixing installation. In the mixing installation, a homogeneous mixture may then be expected for the first time. Taking samples directly from the mixer is difficult and potentially dangerous, and is therefore not advisable. To this end the sampling point, behind the mixing installation must be used. In most companies, it will be the outflow silo under the mixing installation.

II.2.1.8.2. Acclimatizing company samples.

Company samples, that cannot be analyzed at a short term, must be stored in a refrigerated space to prevent damage. Before the actual processing, the samples must be taken, a long time in advance, to the lab, to allow acclimatization. This procedure prevents the sample material, from being exposed to any moisture condensation coming from the laboratory's hot air. Such a condensation makes it impossible, to determine the exact moisture levels of the sample (in case cobalt is used as a tracer). A heterogeneous distribution of moisture condensation in sample material, will cause greater dispersion in results of the cobalt analysis.

II.2.1.8.3. In case of Micro tracer RF Blue (lake).

The mean tracer level in charge B, must be between 70 and 110% of the expected tracer level in charge B.

II.2.1.9. Processing of compound feed containing a cobalt based mixture.

To charge B, produced in the frame work of a test procedure, a cobalt mixture is added in a dosage of 2 kg per ton of feed (for a tracer mixture of 2.5 to 5% cobalt). The compound feed will, therefore contain approximately 50 to 100 ppm. of cobalt. The feed must be stored in a separate cell and must not be marketed. It is recommended to dilute the cobalt-based feed, so that the total cobalt concentration in the feed, ultimately intended for marketing, does not exceed 2 ppm. To this end, the cobalt levels already present in the feed material, must be taken into account. The feed from charge C, usually contains only small amounts of cobalt. Given that the cross-contamination level is not known in advance, fairly large deviations in the feed's cobalt levels, are to be taken into consideration.

It is recommended, to store this feed separately, and to dilute it sufficiently. If the compound feed company does not wish to use this feed in any way, then it must be disposed of as chemical waste.

II.2.1.10. Standard prescriptions for the preparation of a tracer mixture, for the company's internal measuring of the cross-contamination.

II.2.1.10.1. Mixture based on Micro-tracers.

See point II.2.1.4
II.2.1.10.2. Mixture based on cobalt carbonate.

**Introduction**
The cobalt mixture used for conducting a test procedure, is prepared by mixing chalk with cobalt carbonate. This will ensure that the cobalt is distributed evenly over the mixture, and that the cobalt mixture qua properties, differ very little from compound feed ingredients.

**Ingredients**
- Chalk: has a well defined quality as carrier;
- cobalt carbonate, at least 99% pure.

**Equipment**
- mixing equipment, suitable for dry products;
- extra tools are needed such as suitable scales for weighing the ingredients.

**Safety measures:**
Working with cobalt, requires wearing a protective mask covering mouth and nose (snout), and plastic gloves for hand protection.

**Preparation of the cobalt mixture:**
The necessary quantities of cobalt carbonate and chalk are weighed. The weighed quantities are mixed during the optimal mixing time. After which, the mixture is divided into quantities of 2.0 kg and tightly closed.

The packaging must indicate the following:
- name and code of the product (cobalt mixture);
- net weight in kg at the time of filling;
- production date;
- the nominal cobalt concentration;
- safety measures.

The sealed packages containing this product, should be stored under conditioned circumstances. Packages are to be opened immediately before use.

The cobalt mixture must meet the following requirements:
- particle size: max. 1% > 0.7 mm; max. 10% > 0.5 mm;

**Monitoring and reporting**
During packaging of the cobalt mixture, 4 samples from each homogenized batch are taken. One sample is used for determining the moisture content, another sample for measuring the particle size distribution, and a third sample for measuring the cobalt content. The last sample is kept as a spare sample.

Each of the thus prepared cobalt mixtures will be reported:
- origin and characteristics of the chalk;
- origin of the cobalt carbonate;
- the quantities used as carrier ,and cobalt salt;
- moisture content of the mixture after homogenization;
- the calculated cobalt content of the cobalt mixture;
- the analyzed cobalt content of the cobalt mixture ;
- particle size distribution of the cobalt mixture.

II.2.1.11 Determination of additive (sodium Salinomycin).

The determination methods of the additives listed, should correspond with the official analytical methods. If measurements are below the detection limit, the value for this limit divided by half is used as a value for the content.
Criteria to which the analysis of the additive must comply
The analysis must have a detection limit of 0.5%, of the regular applied dosage. The absolute error, at the level of cross-contamination in the additive, may not exceed 2% of the practical dose applied in the first three mixtures.

Possible sources for errors
The total error in the determination of the cross-contamination is: influence of detection limit + analytical errors + error in initial content (variance).
The possible error is expressed in percentage of the initially added product:

- 0.5% detection limit;
- 2.0% analytical error;
- 1.0% mixing accuracy (assumed variation coefficient less than 10%);
- total: 3.5%.

Literature:


II.2.2. Testing procedure for determining the cross-contamination in the compound feed production, by using manganese mixtures, respectively rich and poor in protein.

II.2.2.1. Scope.
The testing procedure is developed for establishing the amount of cross-contamination, occurring in compound feed producing companies. The cross-contamination of the major components from the dosing equipment for raw materials, and cross-contamination of components added via pre-mixtures, are determined individually.

By collecting samples from different places in the production process, an insight can be gained into the carry-over of the various parts in the production process (e.g.: grinding/mixing line to the pelletizing bin or press/cooling line).

The method is also useful in determining to what extent the installation can produce a homogenous mixture.

II.2.2.2. Principles of the test procedure.
The test procedure is performed by preparing first a protein and manganese-rich soy mixture, then immediately on the same production line, the manufacturing of a protein and manganese-poor corn mixture. The increase of protein and manganese levels in the corn mixture during the production run is caused by cross-contamination. By relating this increase to the protein and manganese soy mixture, the cross-contamination levels can be calculated.

Taking into account that the protein and manganese levels in the corn based mixture evolve hyperbolically (from high levels at the beginning of the flow to lower levels towards the end), this procedure deserves particular attention.
II.2.2.3. Equipment and tools.

To conduct a test procedure, the following is required:
- a quantity of manganese oxide corresponding to 0.4 % of the usual charge size;
- (possibly) A scoop for taking samples;
- two buckets for collecting a number of subsamples;
- sampling jars or bags, which may contain at least 200 grams of material;
- if the testing procedure for the cross-contamination is performed at two places in the production line, 20 sampling jars will normally be enough (only 14 samples will actually be tested).

II.2.2.4. Company data required.

Prior to measuring the cross-contamination, the following data must be available:
- type of processed products (additives, (medicated) pre-mixtures);
- a diagram showing different operations: unloading, storing, dosing, reprocessing, mixing, internal transport and final storage of the products mentioned above;
- a clear overview of the internal and external return flows;

In addition, the company must provide the following information regarding the location where the testing procedures will be conducted:
- a schematic diagram of the production installation;
- the way in which the soy and corn mixture is composed. It should particularly be indicated, how and where exactly the manganese oxide is added, and how the transport system (if any) used for transporting manganese oxide into the mixing installation, will be flushed, for both the soy an the corn mixture.

II.2.2.5. Implementation of the testing procedure.

II.2.2.5.1. Soy mixture.

a. Preparation of the protein and manganese-rich soy mixture:
The soy based mixture (with normal charge size) consists of 91.8% of soy meal extraction, 4 % fat, 3 % sugar cane molasses, 0.4 % manganese oxide and 0.8 % dicalcium phosphate (or chalk or salt). This mixture is dosed, ground, mixed and pelletized in the usual way. Molasses and fats are added, to obtain a flour with normal physical properties and easy to pelletize. The soy meal extraction may come from multiple dosage silos. The manganese oxide replaces the pre-mixture and should follow the same path as the pre-mixture. The manganese oxide is therefore incorporated via a pre-mixture scale, or manually dosed at the same place as the pre-mixture. This will ensure, that virtually the whole amount of manganese oxide ends up in the pre-mixture scales, or in a temporary storage silo.

The manganese oxide should comply with the following requirements:
- Mn level minimally 50%;
- particle size: 100% should be smaller than 0.2 mm.

Normally chalk salt and/or feed phosphate are also dosed via the same weighing scales, or in a temporary storage silo. This is to avoid or decrease any cross-contamination of components from the pre-mixture, especially when first, the pre-mixture and then the other products are dosed.

For the testing procedure first 0.4 manganese oxide, and then 0.8 % chalk, feed phosphate or salt are added.

After adding the contents from the pre-mixture scales (or temporary storage silo) to the soy mixture, the mixing installation will be operated during the usual mixing time. The mixture is then transported to an empty flour press bunker and pelletized (monitoring). The grinding/mixing line and the pelletizing/cooling line must not, after the soy mixture, be used for anything other than the corn mix.
b. **Monitoring of the soy mixture:**
   Upon loading the soy pellets into the silo for finished products, the last part of the charge is used for taking an overall sample.

II.2.2.5.2. *Corn mixture.*

a. **Manufacturing of the protein and Mn-poor corn mixture:**
   The corn mixture (with the same charge size as the soy mixture) consists of 92.2 % corn, 4 % fat, 3 % cane molasses and 0.8 % dicalcium phosphate (or chalk or salt). If it is not possible to dose 92.2 % corn, a corn/wheat mixture or any other protein-poor mixture may be prepared (monitoring).
   The transport system between the pre-mixture scales (or temporary storage silo) and the mixing installation is flushed with 0.8 % dicalcium phosphate (or salt or chalk).
   The normal mixing time will start once the feed phosphate has been added to the mixture.
   The mixture is then evacuated to the (empty) pelletizing bin (monitoring) and subsequently pelletized (monitoring).

b. **Monitoring of the corn mixture:**
   The following samples are collected from the corn based mixture:
   I. the corn (and possibly wheat) which is used in the composition of the mixture;
   II. six samples from the corn mixture, upon inflow in the pelletizing bin;
   III. six samples from the corn pellets, upon inflow into the silo for finished products.

   The sampling procedure is important for the subsamples II and III. Particularly the first part of the meal or pellets, from this charge will have higher levels of protein and manganese, who will then quickly, decrease to a lower and more constant level. It is therefore important, to monitor the first part of the meal or pellet flow intensively, and to know for which part of the feed these samples are representative.

   The monitoring procedure for taking pellets at the entrance of the silo for finished products, is somewhat similar. Because the total duration is usually longer, the procedure will now be as follows:
   - during the first minute as many subsamples as possible are collected in a bucket; then an overall sample is produced by mixing the subsamples together;
   - during the next minute: ditto;
   - then every minute, a random sample is taken from the flow until the pellet flow stops.
   - record the total duration time, and store six samples, namely: the first three samples and three of the other samples.

II.2.2.5.3. **Processing the soy based mixture in compound feed.**
   The soy mixture with low cross-contamination, has a Mn level of app. 2000 mg/kg. When processing the soy mixture in compound feed, it should be taken into account that the Mn concentration in the compound feed cannot exceed 150 mg/kg.
II.2.2.6. Analyzing of the samples.
In total there are 14 (or possibly 15) samples collected:
- 1 sample of soy pellets (+ Mn) = A;
- 1 sample of corn (pure) (+ possibly wheat) = B;
- 6 samples of corn mix-flour (pelletizing bin) = C (1 to 6);
- 6 samples of corn mix pellets (finished product silo) = D (1 to 6).

All samples are analyzed for crude protein and Manganese (Mn) levels.
To determine whether moisture levels in corn mix meal and corn mix pellets have changed during pelletizing, half of the samples will be analyzed. If moisture levels have changed significantly during pelletizing, then crude protein and Mn levels of the corn mixture pellets, should be adjusted according to the moisture level of the corn flour mixture.

II.2.2.7 Calculating cross-contamination percentages.
From the levels of Crude Protein and Mn in the collected samples, the cross-contamination percentages are calculated.
Assuming the following levels are found:
- Soy pellets: 420 grams of crude protein and 2006 mg Mn/kg;
- pure corn: 86 g RE and 4 mg Mn/kg;
- 6 samples corn mixture (on top of the pelletizing bin):
  - overall sample (0.5 min.) 160 grams RE 400 mg Mn/kg;
  - overall sample (0.5 min.) 100 grams RE 60 mg Mn/kg;
  - random sample 90 grams RE 27 mg Mn/kg;
  - random sample 85 g RE 30 mg Mn/kg;
  - random sample 88 g RE 28 mg Mn/kg;
  - random sample 89 g RE 27 mg Mn/kg;

The total duration of the meal flow in the pelletizing bin = 5.5 min.

For samples 3 to 6, the average level of crude protein is calculated at 88 grams, for Mn at 28 mg Mn/kg.

Expected levels in the corn mixture (92.2 % corn and 3 % molasses with 40 grams of crude protein and 25 mg Mn/kg):
\[
\text{Crude protein} = 0.922 \times 86 + 0.03 \times 40 = 80.5 \text{ gram/kg}
\]
\[
\text{Mn} = 0.922 \times 4 + 0.03 \times 25 = 4.4 \text{ mg/kg}
\]

The average levels of crude protein and Mn in the corn mixture are calculated as follows:
\[
\text{Crude protein} = 0.5/5.5 \times 160 + 0.5/5.5 \times 100 + 4.5/5.5 \times 88 = 95.6 \text{ grams/kg}
\]
\[
\text{Mn} = 0.5/5.5 \times 400 + 0.5/5.5 \times 60 + 4.5/5.5 \times 28 = 64.7 \text{ mg/kg}
\]

Samples 1 and 2 each have a flow time of 0.5 minutes, from a total of 5.5 minutes. For samples 3 to 6, the average level is calculated; its duration is 5.5 - 2 \times 0.5 = 4.5 minutes.

The cross-contamination percentage (Vs%) is now calculated as follows:
\[
Vs\% = \frac{\text{average level in corn mix} - \text{expected level in corn mix}}{\text{average level in soy pellets} - \text{expected level in corn mix}} \times 100
\]

The cross-contamination percentages are then (up to the pelletizing bin):
\[
RE = \frac{95.6 - 80.5}{420 - 80.5} \times 100 = \frac{1510}{339.5} = 4.5\%
\]
\[
Mn = \frac{64.7 - 4.4}{2006 - 4.4} \times 100 = \frac{6030}{2001.6} = 3\%
\]
The cross-contamination percentages at the inlet of the finished product silo, are calculated in the same way.

The cross-contamination percentage of crude protein relates to feed itself, starting from the dosing installation.

The cross-contamination percentage for Mn gives an indication of the cross-contamination of the components from the pre-mixture.

II.2.2.8. Measuring homogeneity.

To determine the extent of the installation producing a homogeneous mixture, at least 10 samples of the Mn-rich soy mixture should be collected and analyzed for Mn. The spreading of the Mn levels in these samples (standard deviation or the difference between the highest and lowest value) provides a homogeneity measurement.

When taking samples from the soy mixture, it is important to sample the entire flow of the mixture. As the exact duration of the meal flow is usually unknown, it is advisable firstly to take a large amount of samples, from which however, only a part (namely 10) will be analyzed.

The homogeneity measure can be obtained at various places in the installation. Samples taken immediately after mixing, will provide an idea of how the mixing installation functions. On the other hand, samples taken elsewhere in the installation (but still after mixing), will normally be less homogeneous than those taken immediately after mixing, and this because the de-mixing and cross-contamination plays an important role. Because the Mn-rich soy mixtures will always be produced after a "normal" compound feed with lower Mn levels, the first samples of the soy mixture ‘contaminated’ with a certain amount of compound feed will thus contain less Mn. The following samples will each time be less contaminated by normal compound feed, and will therefore contain increasingly higher Mn levels.

II.2.2.9 Evaluation of errors.

Table 5 shows the Mn and protein levels which are to be expected in the corn mixture, based on various percentages of cross-contamination. This data is based on levels of 80 grams crude protein, and 5 mg of Mn/kg corn mixture (pure), and on 400 grams of crude protein and 1800 mg of Mn/kg soy mixture.

<table>
<thead>
<tr>
<th>Cross-contamination %</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mn</strong> from basis*</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>from soy</td>
<td>0</td>
<td>18</td>
<td>54</td>
<td>92</td>
<td>180</td>
<td>270</td>
</tr>
<tr>
<td><strong>Crude protein</strong> from basis</td>
<td>80</td>
<td>79,2</td>
<td>77,6</td>
<td>76</td>
<td>72</td>
<td>68</td>
</tr>
<tr>
<td>from soy</td>
<td>0</td>
<td>4</td>
<td>12</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>83,2</td>
<td>89,6</td>
<td>96</td>
<td>112</td>
<td>128</td>
</tr>
</tbody>
</table>

* = effect of dilution is neglected

Table 5: Effect of the cross-contamination percentage on Mn and protein contents of the corn mixture

Based on the analytical precision of the Mn and crude protein determination, an estimation can be made of the accuracy with which the cross-contamination percentage is to be determined.

For the 6 corn samples to be analyzed, is assumed that the average Mn-levels found in 95 % of the cases will be situated between 95 and 105 % of the actual level; for levels <60 mg/kg, the absolute interval equals the interval for 60 mg/kg, thus +/- 3 mg/kg.

The Mn levels in the soy based mixture, established through the analysis, is assumed to deviate by maximum 100 mg/kg from the actual level.
The crude Protein levels, established through analyzing 6 samples of corn based mixtures is assumed in 95 % of the cases to be situated between 99 and 101 % of the actual level, and the level measured by analyzing the soy based mixture, can only deviate from the actual level by maximum 2 %.

Table 6 show the results of the calculations. From the results, it can be concluded that low cross-contamination percentages can be detected with reasonable reliability. For low cross-contamination, the analysis of Mn seem more reliable in comparison to that of crude protein, whereas for high cross-contamination levels, protein analysis give better results in comparison to that of Mn.

<table>
<thead>
<tr>
<th>Cross-contamination level</th>
<th>Corn mix</th>
<th>Calculated</th>
<th>Interval analysis</th>
<th>Cross-contamination % *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td></td>
<td>5 mg/kg</td>
<td>2 - 8 mg/kg</td>
<td>0.16 - 0.18 %</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>20 - 26</td>
<td></td>
<td>0.8 - 1.2</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>56 - 62</td>
<td></td>
<td>2.7 - 3.4</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>90 - 100</td>
<td></td>
<td>4.5 - 5.6</td>
</tr>
<tr>
<td>10</td>
<td>185</td>
<td>176 - 194</td>
<td></td>
<td>9 - 11.1</td>
</tr>
<tr>
<td>15</td>
<td>275</td>
<td>261 - 289</td>
<td></td>
<td>13.5 - 16.7</td>
</tr>
</tbody>
</table>

* on the basis of 1800 mg Mn / kg soy mix (variation 1700 – 1900, at low Mn in corn, …..% is calculated with high Mn in soy, and vice versa).

<table>
<thead>
<tr>
<th>Cross-contamination level</th>
<th>Corn mix</th>
<th>Calculated</th>
<th>Interval analysis</th>
<th>Cross-contamination % *</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE</td>
<td></td>
<td>80 g/kg</td>
<td>79.2 - 80.8 g/kg</td>
<td>-0.25 - 0.25 %</td>
</tr>
<tr>
<td>1</td>
<td>83.2</td>
<td>82.4 - 84.0</td>
<td></td>
<td>0.7 - 1.3</td>
</tr>
<tr>
<td>3</td>
<td>89.6</td>
<td>88.7 - 90.5</td>
<td></td>
<td>2.6 - 3.4</td>
</tr>
<tr>
<td>5</td>
<td>96</td>
<td>95.0 - 97.0</td>
<td></td>
<td>4.5 - 5.5</td>
</tr>
<tr>
<td>10</td>
<td>112</td>
<td>110.9 - 113.1</td>
<td></td>
<td>9.4 - 10.6</td>
</tr>
<tr>
<td>15</td>
<td>128</td>
<td>126.7 - 129.3</td>
<td></td>
<td>14.2 - 15.8</td>
</tr>
</tbody>
</table>

* on the basis of 400 g RE/kg soy mix (variation 392 – 408; at low RE in corn, there is …..% calculated with high RE in soy and vice versa).

Table 6: Effect of the accuracy of the analysis for determining cross-contamination percentage
III. PRE-MIXTURES.

III.1. Instructions for the preparation of pre-mixtures.

The additional instructions, aims at making it possible for compound feed producers, to use pre-mixtures in the production of compound feed, without exceeding the maximum cross-contamination levels.

The cross-contamination of additives will also occur during preparation of the pre-mixtures. To prevent compound feed manufacturers from exceeding the standard norms for additives and medicated pre-mixtures, a basic rule has been established, that no more than 25% of cross-contamination levels in compound feed may come from used pre-mixtures. The maximum cross-contamination levels in pre-mixtures, will be calculated in function of the anticipated pre-mixture dosage in compound feed. The cross-contamination of additives may take place through direct or indirect contamination.

Direct contamination:
Direct contamination is possible through the use of pre-mixtures destined for animal feed, for which a maximum cross-contamination level is established. The producer of pre-mixtures, will ensure through his auto-control system, that no more than 25% of the maximum indicated cross-contamination level in compound feed, should arise from a pre-mixture. For any required flush charges, the relevant multiplier should be taken into account, which has been established for that particular product.

Indirect contamination:
In order to obtain very low levels of additives in pre-mixtures, which have occurred as a result of cross-contamination, the premix manufacturers, must produce a number of flush charges after every production run of pre-mixtures. If this flush charge still contains too high levels of additives, the compound feed producer may encounter problems relating to the establishment of maximum cross-contamination levels, in compound feed.

The problem is as follows: compound feed producers, use these pre-mixtures in compound feed which are also used to flush the production lines. In some cases the additive levels, due to processing the pre-mixture into compound feed, are so high that the maximum cross-contamination levels inherent to the installation of the compound feed company, are exceeded.
Assuming, a reasonably common cross-contamination of 10% coming from compound feed companies, with a multiplication factor of 2, a cross-contamination percentage of 20% must be taken into account.

In other words, the level of each critical additive in every charge must be diluted by a factor 5.

The principle says that a quarter (25 %) of the maximum indicated cross-contamination levels in compound feed, may arise from used pre-mixtures, this means that for pre-mixture producers, all other produced pre-mixtures should take the dilution in question, into consideration. All pre-mixtures must be suitable for use in the production of rinse charges, and the content of these pre-mixtures may be multiplied by a factor 5.

The maximum content for the pre-mixtures when preparing compound feed can only be 1.25 times (5 * 0.25) the maximum indicated cross-contamination level for compound feed.

There will be no exception to this rule, unless there is a certain understanding between the pre-mixture producer and the compound feed producer.

III.2. Testing procedure for measuring cross-contamination in pre-mixture installations.

III.2.1. Measurement points for cross-contamination and methods.

The measuring points for the cross-contamination, and the way of measuring of cross-contamination and homogeneity in the pre-mixture plant, corresponds to the methods described in II. For animal compound feed. Attention must be paid to the tracer to be used, and the tracer levels (see III.2.2).
III.2.2. Tracer used.

For measuring cross-contamination in pre-mixtures, the following tracer substances can be used:

a. cobalt mixtures with a cobalt concentration of minimum 200 mg/kg used in flush charge B. For cobalt concentrations of 2000 mg/kg or more, pure cobalt carbonate can also be used.

b. The additive Sodium Salinomycin with a relative wall adhesion factor <1 in a dosage of minimum 3000 mg/kg in flush charge B.

c. The Microtracer FSS mixture is dosed up to 10 ppm (0.5 % tracer mix to 2 kg/ton of pre-mixture to be incorporated into flush charge B)
IV. MULTIPLYING FACTORS.

These tests have been performed in order to determine, the multiplying factor of some of the commercialized medicated pre-mixtures and additives (see definition point I). These tests are performed by the TNO laboratory, at the request of producers of additives or medicated pre-mixtures (see following list), and according to the relative wall adhesion coefficient method (TNO report I-96-31006).

Companies, placing such medicated premixtures and/or additives on the market, and who are having the result of these tests, may forward them to OVOCOM by email (info@ovocom.be). After evaluation, they will be integrated into this document. Companies shall remain responsible for any information they communicate to OVOCOM as well as any possible updates.

The following tables indicates the most recent data available. As stated in point I of this document, if there is no value available for the multiplying factor of a specific medicated pre-mixture or additive, a value of 3 must be assigned.

### IV.1. Medicated premixtures.

<table>
<thead>
<tr>
<th>Name</th>
<th>Active substance in g/kg</th>
<th>Company’s Name</th>
<th>Registration Number</th>
<th>Relative wall adhesion</th>
<th>Multiplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apravet</td>
<td>Apramycin: 100</td>
<td>HUVEPHARMA NV</td>
<td>BE-V442023</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Aurofac 100 MG/G Granular</td>
<td>Chlortetracycline hydrochloride: 100 g/kg</td>
<td>Pfizer Animal Health SA</td>
<td>BE-V317213</td>
<td>0.13</td>
<td>1</td>
</tr>
<tr>
<td>Dokamox 100 MG/G</td>
<td>Amoxicillin 100 g/kg</td>
<td>Laboratoires SOGEVAL</td>
<td>BE-V377404</td>
<td>0.27</td>
<td>1</td>
</tr>
<tr>
<td>Doxyprex 100 MG/G Premix</td>
<td>Doxycycline hyclate: 100 g/kg</td>
<td>INDUSTRIAL VETERINARIA SA (INVESCA)</td>
<td>BE-V300982</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>Doxyral 10% Premix</td>
<td>Doxycycline hyclate Eq:Doxycycline 10%</td>
<td>Emdoka bvba</td>
<td>BE-V388245</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>Flubenol 5% Premix</td>
<td>Flubendazol: 50 g/kg</td>
<td>Eli Lilly benelux NV Division</td>
<td>BE-V315287 (pot)</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>Panacur 4%</td>
<td>Fenbendazol: 40</td>
<td>Intervet</td>
<td>BE-V362275</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>Pharmasin 20mg/g premix</td>
<td>Tylosine (in the form of tylosin phosphate): 20 g/kg (2%)</td>
<td>HUVEPHARMA NV</td>
<td>FR/V/9830463 9/2010 (1)</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>Pharmasin 100mg/g premix</td>
<td>Tylosine (in the form of tylosin phosphate): 100 g/kg (10%)</td>
<td>HUVEPHARMA NV</td>
<td>BE-V337163 (LDPE paper bags)</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Pharmasin 250mg/g premix</td>
<td>Tylosine (in the form of tylosin phosphate): 250 g/kg (25%)</td>
<td>HUVEPHARMA NV</td>
<td>BE-V337172 (paper bags) BE-V337181 (PE/Alu/PET bags)</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

1 This medicated premixture is not available on the Belgian Market.
<table>
<thead>
<tr>
<th>Name</th>
<th>Active substance in g/kg</th>
<th>Company’s Name</th>
<th>Registration Number</th>
<th>Relative wall adhesion</th>
<th>Multiplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promycine 400</td>
<td>Colistin sulfate: 400000 UI/g</td>
<td>V.M.D. NV</td>
<td>BE-V325552</td>
<td>2</td>
<td>2,5</td>
</tr>
<tr>
<td>Pulmotil 200 Premix</td>
<td>Tilmicosine 200</td>
<td>ELI LILLY</td>
<td>BE-V-V290927 (Multi-layer) BE-V290936 (laminate)</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>Rhemox Premix 100 MG/G</td>
<td>Amoxicilin: 100 g/kg</td>
<td>INDUSTRIAL VETERINARIA SA (INVESA)</td>
<td>BE-V36944 Complex film bags in paper/aluminium /BEBD with sealed closure</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>Suramox 5% premix</td>
<td>Amoxicilin: 50 g/kg (5%)</td>
<td>VIBRAC SA</td>
<td>BE-V375566</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>Tilmovet 40 g/kg premix</td>
<td>Tilmicosine : 40 g/kg (4%)</td>
<td>HUVEPHARMA NV</td>
<td>BE-V321517</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Tilmovet 100 g/kg premix</td>
<td>Tilmicosine : 100 g/kg (10%)</td>
<td>HUVEPHARMA NV</td>
<td>BE-V321526 (paper bag) BE-V321535 (bag with air valve)</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>Tilmovet 200 g/kg premix</td>
<td>Tilmicosine : 200 g/kg (20%)</td>
<td>HUVEPHARMA NV</td>
<td>BE-V321544 (paper bag) BE-V321553 (bag with air valve) BE-V321562 (PET/Alu/PE zak)</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>Tylan 100 VET Premix</td>
<td>Tylosinefosfaat 100</td>
<td>EU LILLY</td>
<td>BE-V308874 (laminaat)</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>Vetmulin 100g/kg</td>
<td>Tiamuline hydrogen fumerate: 100 g/kg = 82 g tiamuline/kg</td>
<td>HUVEPHARMA NV</td>
<td>BE-V333453 (PE paper bag) BE-V333462 (PET/Alu/PE bag)</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>Zincoveto 100%</td>
<td>Zinc oxide 1000g/kg</td>
<td>V.M.D. NV</td>
<td>1030 T3 F20</td>
<td>0.7</td>
<td>1</td>
</tr>
</tbody>
</table>

**IV.2. Additives.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Registration number</th>
<th>Name of the person responsible for the putting into circulation</th>
<th>Relative wall adhesion</th>
<th>Multiplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robenidinehydrochloride 66 g/kg (Cycostat 66 G)</td>
<td>E 758 (5 1758)</td>
<td>Pfizer Ltd.</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>Lasalocide A natrium 15 g/100 g (Avatec 15% cc) (Avatec 150 G)</td>
<td>E 763 (5 1763)</td>
<td>Pfizer Ltd.</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Sodium Salinomycin 120 g/kg (SACOX 12%) (Sacox 120)</td>
<td>E 766</td>
<td>Huvepharma NV</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>Name</td>
<td>Registration number</td>
<td>Name of the person responsible for the putting into circulation</td>
<td>Relative wall adhesion</td>
<td>Multiplier</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>---------------------</td>
<td>------------------------------------------------------------------</td>
<td>------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>microgranulate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Salinomycin (Kokcisan G 120)</td>
<td>E 766</td>
<td>KRKA d.d./AVEVE NV</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Sodium Salinomycin 120 g/kg (Salinomax 120G)</td>
<td>E 766</td>
<td>Pfizer Ltd.</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>Maduramicine-ammonium alfa 1g/100g (Cygro 1%)</td>
<td>E 770</td>
<td>Pfizer Ltd.</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>Maduramicine-ammonium alfa 10 g/kg (Cygro 10G)</td>
<td>5 1 770</td>
<td>Pfizer Ltd.</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Decoquinate 60,6 g/kg (Deccox)</td>
<td>E 756</td>
<td>Pfizer Ltd.</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Diclazuril 0,5 g/100 g (Clinacox 0,5 % Premix)</td>
<td>E 771</td>
<td>Eli Lilly and Company Ltd/Janssen Pharmaceutica NV</td>
<td>2.1</td>
<td>1</td>
</tr>
<tr>
<td>Diclazuril 0,2 g/100 g (Coxiril 0,2 %)</td>
<td></td>
<td>Huvepharma NV</td>
<td>1.0</td>
<td>2</td>
</tr>
<tr>
<td>Halofuginone - hydrobromide 6 g/kg (Stenorol)</td>
<td>E 764</td>
<td>Huvepharma NV</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>Sodium Monensin (Coxidin 200 microGranulate)</td>
<td>5 1 701</td>
<td>Huvepharma NV Belgium</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>Narasin (Monteban G 100)</td>
<td>E 765</td>
<td>Elanco / Eli Lilly</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Sodium Monensin (Elancoban G 200)</td>
<td>E 757</td>
<td>Elanco / Eli Lilly</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>Narasin Nicarbazine (Maxiban G 160)</td>
<td>E 772 (5 1 772)</td>
<td>Elanco / Eli Lilly</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

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