



GMP+ Feed Safety Assurance scheme

Minimum Requirements for Sampling and Analysis

BA

GMP+ BA4

4

Version: January 1st, 2012

EN

© GMP+ International B.V.

All rights reserved. The information in this publication may be consulted on the screen, downloaded and printed as long as this is done for your own, non-commercial use. For other desired uses, prior written permission should be obtained from the GMP+ International B.V.

Stadhoudersplantsoen 12
2517 JL The Hague
The Netherlands

Tel: +31 (0)70 370 86 70
Fax: +31 (0)70 370 86 71

info@gmplus.org
www.gmplus.org

History of the document

Revision no. - Date of approval	Amendment	Concerns	Final implementation date
0.0 / 10-2009	New minimum frequencies for sampling and analysis (table)	2.3 Table of feed materials	20-10-2010
0.1 / 09-2010	Transfer of the document from PDV to GMP+ International	Entire document	01-01-2011
	Requirement for sending in analysis results via monitoring in FSD	Section 2.2.2	01-01-2011
	Inconsistency about methods and laboratories removed.	Various places	01-01-2011
0.2 / 05-2011	Extra requirements added for monitoring of different types of fat	2.4	01-06-2011
0.3/ 09-2011	Introduction has been updated	1.1; 1.2	01-01-2012
	Update and clarification of some monitoring requirements of feed materials	2.2.2	01-01-2012
	Remarks on the tabel in 2.3 have been updated to give more clarity	2.3	01-01-2012
	Specific monitoring on insecticides has been removed as it is covered by Pesticides	2.3	01-01-2012

INDEX

1	INTRODUCTION	5
1.1	GENERAL	5
1.2	STRUCTURE OF THE GMP+ FEED SAFETY ASSURANCE SCHEME	5
PART A: IN-COMPANY SAMPLING AND ANALYSIS		7
1	INTRODUCTION	7
2	SAMPLING AND ANALYSIS OF FEED MATERIALS	8
2.1	GENERAL	8
2.2	UNDESIRABLE SUBSTANCES PER FEED MATERIALS	8
2.3	TABLE OF FEED MATERIALS	11
2.4	MONITORING OF FATS AND OILS ON DIOXIN AND DIOXIN LIKE PCB'S	33
3	SAMPLING AND ANALYSIS OF COMPOUND FEEDS	39
3.1	PROTOCOLS RELATING TO SALMONELLA-SAMPLING AND ANALYSIS	39
3.2	PROTOCOL P1: SAMPLING AND ANALYSIS OF SALMONELLA AND ENTEROBACTERIACEAE IN FEEDS FOR POULTRY	40
3.3	PROTOCOL 2: SAMPLING AND ANALYSIS FOR SALMONELLA AND ENTEROBACTERIACEAE IN COMPOUND FEEDS INTENDED FOR PIGS, CATTLE AND OTHER ANIMAL SPECIES (WITH THE EXCEPTION OF POULTRY)	47
3.4	PROTOCOL P4: SAMPLING AND ANALYSIS OF SALMONELLA-CRITICAL FEED MATERIALS (RAW MATERIALS)	49
4	OTHER SAMPLING AND ANALYSIS PROTOCOLS	52
4.1	PROTOCOL P6: SAMPLING AND ANALYSIS AFLATOXIN B1	52
4.2	PROTOCOL P7: SAMPLING AND ANALYSIS ANIMAL PROTEIN	54
APPENDIX 1: PROTOCOL FOR THE SEROLOGICAL CLASSIFICATION OF SALMONELLA		55
PART B: PROTOCOLS FOR THE MEASUREMENT OF CARRY-OVER		56
1	INTRODUCTION	56
2	METHODS FOR MEASURING CARRY-OVER	57
2.1	GENERAL BASIC PRINCIPLES WITH RESPECT TO THE MEASUREMENT OF CARRY-OVER	57
2.2	PROCESS ACCURACY CONTROL PROCEDURE WITH COBALT (REFERENCE METHOD) 61	
2.3	TESTING PROCEDURE FOR CARRY-OVER IN COMPOUND FEED PREPARATION USING COBALT MIXES	73

2.4	TESTING PROCEDURE FOR THE CARRY-OVER IN COMPOUND FEED MIXING USING A MIX OF MANGANATE AND A PROTEIN-RICH AND A PROTEIN-POOR MIX	78
2.5	TESTING PROCEDURE FOR THE MEASUREMENT OF CARRY-OVER IN PREMIX AND ADDITIVES INSTALLATIONS	84
2.6	CHECKING PROCEDURE FOR THE PROCESS ACCURACY OF COMPOUND FEED WITH MICRO TRACERS	85
2.7	CONTROL PROCEDURE FOR THE MEASUREMENT OF CARRY-OVER USING MICRO- TRACERS BY WEIGHING	95
2.8	CONTROL PROCEDURE FOR THE MEASUREMENT OF CARRY-OVER IN ANIMAL FEED PREPARATION USING METHYL VIOLET	97

1 Introduction

1.1 General

The GMP+ Feed Safety Assurance Scheme (GMP+ FSA scheme) was initiated and developed in 1992 by the Dutch feed industry in response to various more or less serious incidents involving contamination in feed materials. Although it started as a national scheme, it has developed to become an international scheme that is managed by GMP+ International in collaboration with various international stakeholders.

The GMP+ FSA scheme is a complete scheme for the assurance of feed safety in all the links of the feed chain. Demonstrable assurance of feed safety is a 'license to sell' in many countries and markets and participation in the GMP+ FSA scheme can facilitate this excellently.

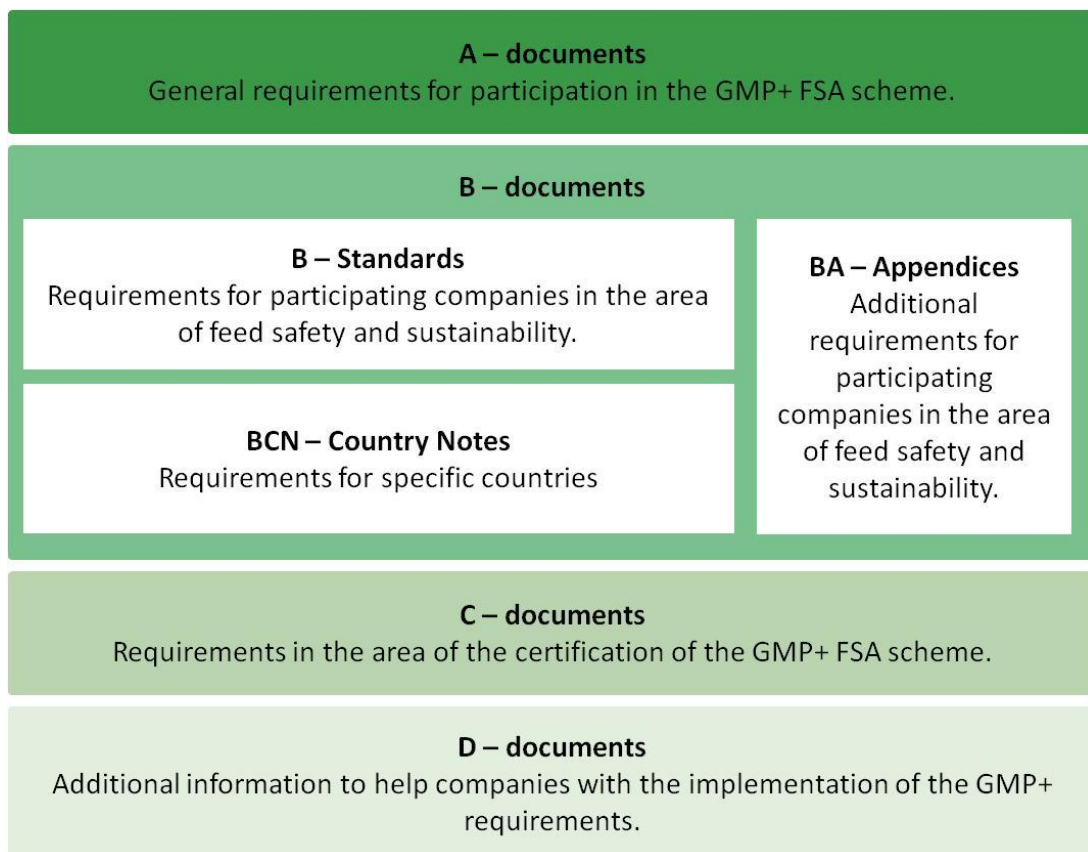
The basic principle of the GMP+ FSA scheme is that the feed chain is part of the food production chain. Proper quality assurance of feed safety throughout the feed chain has a high priority. It is important that companies take their responsibilities in this respect by responding in a proper and convincing way to the need for safe feed materials in the food production chain.

Based on needs in practice, multiple components have been integrated into the GMP+ FSA scheme, such as requirements for the quality management system (ISO 9001), HACCP, product standards, traceability, monitoring, prerequisites programmes, chain approach and the Early Warning System.

Together with the GMP+ partners, GMP+ International transparently sets clear requirements so that feed safety is guaranteed and certification bodies are able to carry out GMP+ certification independently. GMP+ International supports the GMP+ participants with useful and practical information by way of its various databases, newsletters, Q&A lists and seminars.

1.2 Structure of the GMP+ Feed Safety Assurance scheme

The documents within the GMP+ FSA scheme are subdivided into a number of series. The next page shows a schematic representation of the contents of the GMP+ FSA scheme:



All these documents are available via the website of GMP+ International (www.gmpplus.org).

This document is referred to as GMP+ BA4 *Minimum Requirements for Sampling and Analysis* and is part of the GMP+ FSA scheme.

PART A: IN-COMPANY SAMPLING AND ANALYSIS

1 Introduction

In various GMP+ standards it is required that a participant must draw up and implement a sampling and analysis plan.

The nature and intensity of the sampling and analysis is to a great degree determined by the results of the hazard analysis carried out by the participant. This analysis includes in any event the delivered products and raw materials, their own production or creation process and also the feeds which are finally delivered.

An important part of the sampling and analysis is the chemical analysis of the samples. This may also include the monitoring which is carried out within the framework of HACCP. For further information please refer to the HACCP manual.

In Chapter 2 of Part A of this appendix GMP+ BA4 *Minimum Requirements for Sampling and Analysis*, there are requirements for feed materials with respect to sampling and analysis for a number of (undesirable) substances. Chapter 3 and 4 contain sampling and analysis requirements for a number of types of compound feeds. These sampling and analysis requirements relate to the carrying out of chemical analyses.

In the drawing up and implementation of the sampling and analysis plan, the participant should include at least the following requirements if they are relevant. The sampling and analysis is aimed at providing a transparent basis in the sampling and analysis plan which the company must draw up. It is not a requirement in all cases that sampling and analysis must take place for undesirable substances for which norms have been established in the feed legislation. Every company must, of course, comply with the statutory requirements.

If no minimum sampling and analysis requirement has been established then a company may itself determine how often it analyses in order to show that the safety of the feed material is controlled and/or that there is compliance with the legal norm.

2 Sampling and analysis of feed materials

2.1 General

The participant who produces, trades, treats or processes the feed material must include and implement at least the following sampling and analysis of feed materials in his sampling and analysis plan.

2.2 Undesirable substances per feed materials

Section 2.3 includes a table of feed materials. This table is based on the Feed Safety Database (FSD). If the participant produces, trades, treats or processes a feed material then he must analyse the indicated undesirable substances.

2.2.1 Frequency

The frequency of analysis (on a yearly basis) is calculated using the following formula

$$\text{Frequency} = \frac{\sqrt{\text{Volume}}}{100} * \text{'chance'} * \text{'seriousness'}$$

Variable	Explanation
Frequency	The number of samples to be inspected (on a yearly basis) in which the undesirable substance is examined
Volume	<p>Volume in tons of feed materials per year. In principle, the number of samples to be analysed is based on the quantity of feed material which is produced, traded, treated or processed. As the quantity of feed material increases, the number of samples per ton will decrease.</p> <p>Kilograms must be assumed for some feed materials for which, on a yearly basis, only a small quantity is produced, traded or processed. This will be indicated for the feed material in question.</p>
Chance	<p>The standard value for chance is 1. The participant may raise or lower this value if reasons are given. The following considerations may apply to this:</p> <ol style="list-style-type: none">History: see also belowSeasonal influencesPossibility of recontamination. This applies in particular to microbiological parameters.New source / new suppliersHave there been recent incidents. <p>The participant may only select a chance value which is less than one on the basis of (historical) analysis data. The following applies:</p> <ol style="list-style-type: none">Historical data should be representative which means The data must demonstrably relate to the feed material that is being processed, traded or produced. <p>The historic data which is considered as representative may</p>

Variable	Explanation
----------	-------------

differ per undesirable substance.

For some undesirable substances the data for an area can be considered to be representative while, for other undesirable substances, only analysis data for the same production location is representative.

- b. Data from GMP+ International's Feed Safety Database may also be used in determining the sampling and analysis frequency if the participant can show that, in addition to the two requirements above, he participates in the Database of Undesirable Substances.

It is up to the participant to show that the chance value can be lowered. In the event of good historic data for the last 12 months, the chance value may be lowered to 0.5 and in the event of good historic results for the last 24 months, the chance value may be lowered to 0.25.

seriousness This factor expresses the degree of harmfulness of an undesirable substance. For the value for seriousness use has been made as follows of what is in the Feed Safety Database:

Seriousness is great	factor 5
Seriousness is moderate	factor 3
Seriousness is small	factor 1

This leads to the following factors

Undesirable substance	Value
Heavy metals	5
Pesticides	5
Insecticides	5
Feed medicines	5
Mycotoxins	5
Salmonella	5
Fungi	3
Animal components	5
Dioxin	5
Nitrites	5

The established values are all high. This seems logical as these are risky undesirable substances.

2.2.2 Other requirements and remarks

- a. A participant may make use of representative analysis results from manufacturers or suppliers from whom he receives feed materials. This particularly applies to analysis results for undesirable substances where the level no longer changes in theory, such as heavy metals, aflatoxin, pesticides, dioxin. This is not possible for microbiological undesirable substances where recontamination can occur.
- b. Calculated frequencies should always be rounded upwards. The minimum frequency is 1.

- c. Calculation of the monitoring frequency of liquid or moist feed can be based on 88% dry matter content
- d. In the event of seasonal and/or incidental products then a sample is taken at the start of production from the first batch or crop. The established inspection frequency is then maintained from then on.
- e. Following the determination of Salmonella in feed materials, classification (serological and phage type) will take place. The protocol applies as included in appendix I.
- f. The participant must comply, with respect to sampling, analysis and analysis methods, corrective actions, reporting of results, etc., with the requirements of the GMP+ standards. The analyses which are required within the framework of section 2 of GMP+ BA4 *Minimum Requirements for Sampling and Analysis* must be carried out by an ISO 17025 laboratory accredited for the test in question unless this is not reasonably possible.
- g. If a feed material is not included in the following table in § 2.3 then the participant must himself determine and carry out the sampling and analysis for undesirable substances.
- h. It is permissible for participants to carry out their sampling and analysis requirements collective (in a collective plan) and for them to have this plan approved. The following requirements apply with respect to this option:
 - 1. The scope of the plan must be established ('which feed materials are included') and which companies are participating.
 - 2. The collective plan must be representative for the feed materials which the manufacturers produce, trade, treat and / or process. Its representativeness must be supported.
 - 3. The collective plan must comply with the above GMP+ requirements and with the other relevant GMP+ requirements.
 - 4. All the participating companies will obtain all the relevant sampling and analysis results.
 - 5. Approval of this plan (by GMP+ International) means that the participating companies do not in theory have to be audited on this item. The auditor is, of course, free to establish whether the individual company complies with the sampling and analysis instructions for feed materials which are not in the collective plan (such as compound feeds) and to find out what the company has done with the analysis results obtained.
- i. A participant is permitted to deviate from these minimum sampling and analyses requirements. This must be supported per parameter per product.
- j. The results of the analysis should be provided at least once per month to GMP+ International via monitoring in the Feed Safety Database.

2.3 Table of feed materials

Some remarks to the table:

- **Heavy metals:**
 - o ~~Without a footnote the following heavy metals are included:~~ Meant are Arsenic and Cadmium. If a **extra mark** (1), 2) or 3)) is given, then this is meant as additional to be monitored:
 - 1) Fluorine
 - 2) Mercury
 - 3) Lead
- **Veterinary Medical Products:**
 - o CAP (Chloramphenicol), only for (milk)powders from countries where CAP is permitted as a feed medicine.
- **Mycotoxins:**
 - o Monitor for aflatoxins in maize and maize byproducts from all sources except the USA and the EEC
 - o Monitor for aflatoxins in palm kernel and palm kernel byproducts from all known sources with the exception of Indonesia and Malaysia
- **Microbiological:**
 - o Salmonella, only at a pH greater than > 5; in the event of acidification / drying / pelleting sampling and analysis for Salmonella is not necessary.
 - o Clostridia, only sulphite reducing
- **Other undesirable substances:** January 1st, 2012
 - o Dioxin, if there is direct artificial drying with a fuel other than (natural) gas.
 - o See for dioxin in fats and oils also section 2.4.

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Microbiological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
(Sugar-)cane bagasse											
(Sugar-)cane molasses	X ¹⁾										
Ammonium chloride											
Animal fat		X								See 2.4	
Auction fruit											
Auction vegetables											
Baking yeast, cell walls								X			
Barley							X	X			
Barley flakes							X	X			
Barley flakes, peeled								X			
Barley middlings							X	X			
Barley mill byproduct							X	X			
Barley, crushed							X	X			
Barley, crushed, cleaned							X	X			
Barley, heat treated							X	X			
Barley, peeled							X	X			
Bean protein											
Bean protein soluble											
Bean pulp											
Beet molasses											
Beet seed											
Beet seed clew		X									
Beet tail ends											
Blue poppy seed											

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Bread flour											
Bread(remains)								X	X	X	
Brewer's grains								X			
Brewer's yeast, dried								X			
Brewer's yeast, liquid								X			
Buckwheat		X		X	X	X	X				
Buckwheat feed meal		X		X	X	X	X	X		X	
Cacao husks		X		X		X		X			
Calcareous marine algae	X										
Calcium carbonate	X										
Calcium chloride											
Calcium chloride, coated											
Calcium magnesium carbonate	X										
Calcium magnesium phosphate	X										
Calcium phosphate	X										
Calcium sodium phosphate	X										
Canary seed											
Carob pods	X										
Carob pods powder	X										
Carrot flakes											
Carrot peelings steamed											
Carrot pieces, liquid											
Carrots	X										
Category: butter, butter oil and butter concentrate (dairy origin)			X					X			

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Category: casein and caseinates			X					X			
Category: cheese and (melted) cheese products								X			
Category: dairy evaporated & condensed			X					X			
Category: drinking milk (products)			X					X			
Category: powdered milk (products)			X					X			
Category: whey and whey products			X					X			
Centrifuge shot											
Chicory fructose syrup											
Chicory inulin											
Chicory pulp, dried											
Chicory pulp, pressed											
Chicory roots											
Chicory roots, not pulled	X										
Chicory roots, pulled	X										
Cider yeast											
Citrus peelings, moistures rich	X ^{2) 3)}			X	X	X	X				
Citrus pulp	X ^{2) 3)}	X		X	X	X	X			X	
Cocoa bean expeller		X		X		X		X			
Cocoa bean, extracted		X		X		X		X			
Cocoa butter fatty acids		X		X		X				See 2.4	
Coconut distillate		X		X						See 2.4	
Coconut expeller		X		X				X			
Coconut fatty acids		X		X						See 2.4	
Coconut fatty acids distillate		X		X						See 2.4	

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Coconut oil, crude		X		X						See 2.4	
Coconut oil, refined				X						See 2.4	
Coconut, extracted		X		X				X		See 2.4	
Coffee –Skin-Pellets	X			X	X	X	X				
Confectionary											
Confectionary residues											
Confectionary syrup											
Cookies / pastry								X	X	X	
Co-product of the processing of alcohol-water mixture.											
Corn steep liquor		X		X	X	X	X			X	
Cotton seed		X		X	X		X				
Cotton seed expeller		X		X	X		X	X			
Cristobalite	X ²⁾³⁾									X	
Cristobalite meal	X ²⁾³⁾									X	
Distiller's wash					X	X	X	X			
Dextrose, dried											
Dicalcium phosphate	X										
Dough (remains)											
Dried filtrate milk alternatives soybased											
Egg mixture								X			
Egg powder								X			
Egg powder, defatted								X			
Eggshells, heat treated								X	X	X	
Fatty acid mixture from chemically refining										See 2.4	

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
of various vegetable oils											
Feed beans		X						X			
Feed beans, heat treated											
Feed beer								X			
Feed potatoes	X									X	
Fish meal , treated	X ^{2) 3)}							X		X	
Fish oil	X ^{2) 3)}	X								See 2.4	
Fish oil, salmon	X ^{2) 3)}	X								See 2.4	
Fish protein concentrate, salmon											
Flax chaff		X						X			
Flour / meal from the bakery industry					X	X		X			
Foodstuffs produced for human consumption.											
Fruit and vegetables, fresh	X ³⁾	X						X		X	
Fruit juice concentrate								X			
Fruit juice, fresh								X			
Fruit pulp								X			
Fruit retentate								X			
Gelatin concentrate											
Gelatine											
Globin powder								X			
Glucose											
Glycerine, crude											
Grain distillers, fresh					X	X	X	X			
Grass hay					X						

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Grass meal	X			X				X		X	
Grass seed chaff	X	X						X		X	
Grass seeds	X 1)2)3)	X						X			
Grass silage	X			X	X					X	
Grit											
Groundnut expeller		X		X	X	X	X	X			
Groundnut fatty acid distillate		X		X	X	X	X			See 2.4	
Groundnut fatty acids		X		X	X	X	X			See 2.4	
Groundnut husks		X		X	X	X	X	X		X	
Groundnut oil, crude		X		X	X	X	X			See 2.4	
Groundnut oil, refined										See 2.4	
Groundnut screenings		X		X	X	X	X	X			
Groundnut, extracted		X		X	X	X	X	X			
Groundnuts		X		X	X	X	X				
Haemoglobin powder											
Hempseed		X								X	
Horsebeans		X						X			
Ice cream industry co product			X					X			
Lactitol											
Lactose			X					X			
Lard		X								X	
Lentils		X						X			
Lignocellulose											
Linseed		X									

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Linseed expeller		X						X			
Linseed oil, crude		X								See 2.4	
Linseed oil, refined										See 2.4	
Linseed, extracted		X						X			
Lucerne (alfalfa), artificially dried	X			X				X		X	
Lucerne (alfalfa), sundried	X							X		X	
Lucerne meal (pellet), sundried								X		X	
Lucerne meal (pellet), artificially dried				X				X		X	
Lupins		X						X		X	
Magnesite	X										
Magnesite, caustic calcinated	X										
Magnesium acetate	X										
Magnesium calcite	X										
Magnesium chloride											
Magnesium hydroxide	X										
Magnesium oxide	X										
Magnesium oxide, caustic	X										
Magnesium phosphate	X										
Magnesium sulphate 0H ₂ O	X										
Magnesium sulphate 7H ₂ O	X										
Maize		X		X	X	X	X	X		X	
Maize bran		X		X	X	X	X	X		X	
Maize cob silage		X		X	X	X	X			X	
Maize fatty acid distillate		X		X		X				See 2.4	
Maize fatty acids		X		X		X				See 2.4	

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Maize feed meal		X		X	X	X	X	X		X	
Maize flakes, heat treated		X		X	X	X	X	X		X	
Maize flour		X		X	X	X	X	X		X	
Maize germ bran		X		X	X	X	X	X		X	
Maize germ extracted		X		X	X	X	X	X		X	
Maize germs		X		X	X	X	X	X		X	
Maize germs expeller		X		X	X	X	X	X		X	
Maize glutenmeal		X		X	X	X	X	X		X	
Maize grits		X		X	X	X	X	X		X	
Maize meal, heat treated		X		X	X	X	X	X		X	
Maize midlings		X		X	X	X	X	X		X	
Maize oil, crude		X		X	X	X	X			See 2.4	
Maize oil, refined		X		X	X	X	X			See 2.4	
Maize screenings		X		X	X	X	X	X		X	
Maize starch		X		X		X	X			X	
Maize, broken		X			X	X	X	X		X	
Maize, chopped		X		X	X	X	X			X	
Maize, heat treated		X		X	X	X	X			X	
Maize: Corn Cob Mix		X		X	X	X	X			X	
Maizeglutenfeed		X		X	X	X	X	X		X	
Malt					X	X	X	X			
Malt culms					X	X	X	X			
Malting pellets					x	X	X	X			
Milk protein concentrate			X					X			
Milk, permeated			X					X			

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Millet											
Mono ammonium phosphate						X		X		X	
Mono-calcium phosphate	X										
Mono-dicalcium phosphate	X										
Mono-dicalcium sodium phosphate	X										
Mono-sodium phosphate	X										
Mushrooms, dried and milled	X ³⁾	X						X			
Niger seeds		X									
Oatmeal							X	X			
Oats							X	X		X	
Oats, clipped							X	X			
Oats, crushed							X	X			
Oats, heat treated										X	
Oats, husked							X	X			
Oats, husked and cut							X	X			
Ocara		X									
Onion fat crumbs											
Onion juice											
Onion pulp											
Onions, fried											
Palatinoses molasses											
Palm fat, hardened refined										See 2.4	
Palm fat, transesterfied		X								See 2.4	
Palm fatty acid distillates		X								See 2.4	
Palm fatty acids		X								See 2.4	

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Palm fatty acids, pure		X								See 2.4	
Palm kernel fat, hardened refined										See 2.4	
Palm kernel fat, transesterfied		X								See 2.4	
Palm kernel fatty acids		X								See 2.4	
Palm kernel fatty acids distillate		X								See 2.4	
Palm kernel fatty acids, pure		X								See 2.4	
Palm kernel oil olein fraction		X								See 2.4	
Palm kernel oil, chemically refined										See 2.4	
Palm kernel oil, crude		X								See 2.4	
Palm kernel oil, physically refined										See 2.4	
Palm kernels expellers		X		X				X			
Palm kernels, extracted		X		X				X			
Palm oil fatty acids, calcium soaps		X								See 2.4	
Palm oil olein fraction		X								See 2.4	
Palm oil stearine fraction		X								See 2.4	
Palm oil, chemically refined										See 2.4	
Palm oil, crude		X								See 2.4	
Palm oil, physically refined										See 2.4	
Parsley stalks											
Pastry											
Pastry with dairy filling								X			
Pea flakes, heat-treated	X										
Pea protein	X										
Pea protein solution	X										
Pea pulp	X										

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Microbiological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Pea starch	X										
Peanutbutter				X							
Pearl barley						X		X			
Peas	X	X						X			
Peatl barley flakes						X		X			
Permeated whey			X					X			X
Permeated whey, poor of milk sugar			X					X			X
Plasma powder								X			
Poppy seed		X									
Potassium carbonate	X										
Potassium chloride											
Potato crisps											
Potato cuttings, raw											
Potato fat crumbs											
Potato feed starch											
Potato feed starch, heat treated										X	
Potato flakes											
Potato fruit juice, concentrated											
Potato peelings	X									X	
Potato peelings, steamed											
Potato peelings, steamed, silage											
Potato product, pre fried											
Potato protein											
Potato protein, fermentative treated											
Potato pulp, dried										X	

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Potato pulp, pressed											
Potato scrapings	X									X	
Potato starch											
Potato starch, heat treated										X	
Potatoes, mashed											
Potatoes, raw	X									X	
Potatoes, steam peeled											
Poultry bloodmeal								X			
Powder cellulose											
Protein hydrolysate of porcine mucosa fluid bed dried											
Protein hydrolysate of porcine mucosa, liquid								X			
Protein hydrolysate of porcine mucosa, spraydried											
Protein slop of production protein hydrolysates											
Rape fatty acids		X								See 2.4	
Rape fatty acids distillate		X								See 2.4	
Rape oil, crude partially degummed		X								See 2.4	
Rape oil, crude, non-degummed		X								See 2.4	
Rape oil, extracted crude		X								See 2.4	
Rape oil, pressed, crude		X								See 2.4	
Rape oil, refined										See 2.4	
Rape seed expeller		X						X			
Rape seed, extracted, stable (treated with		X						X			

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
CH ₂ O											
Rape seed, extracted, stable (treated with reducing sugars)											
Rapeseed		X									
Rapeseed cleanings		X									
Rapeseed lecithin		X								See 2.4	
Rapeseed olein fraction										See 2.4	
Rapeseed screenings		X									
Rapeseed stearin fraction										See 2.4	
Rapeseed, extracted		X						X			
Residue product sugar- and sweets industry											
Residue yeast											
Rice feed meal				X		X		X			
Rice flakes, heat-treated				X		X		X			
Rice protein concentrate				X		X		X			
Rice solubles				X		X		X			
Rice starch				X		X		X			
Rice water				X		X		X			
Rice, broken											
Rice, crude				X		X		X		X	
Rice, decorticated, polished				X		X		X			
Rice, heat-treated				X		X		X		X	
Rock salt (Sodium chloride)	X										
Rye (bran) grits						X		X			
Rye bran						X		X			
Rye feed						X		X			

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatox.B	Deoxyn.	Ochrat.			Zea	Salmonella
Rye feed flower							X	X			
Rye feed meal							X	X			
Rye flakes							X	X			
Rye							X	X		X	
Rye, heat treated							X	X		X	
Safflower fatty acids, pure		X						X		See 2.4	
Safflower fatty acids, undistilled in refined vegetable oil										See 2.4	
Safflower oil, refined										See 2.4	
Safflower seed		X									
Safflowermeal extracted		X						X			
Safflowermeal, expeller		X						X			
Salty snacks											
Sauces											
Sauces slop											
Seaweed meal, dried and milled	X ³⁾	X						X		X	
Sesame-seed		X						X			
Sesame-seed expeller		X						X			
Sesame-seed, extracted		X						X			
Shea nuts		X		X				X			
Shea olein fraction	X	X		X				X		See 2.4	
Shea stearin fraction		X		X						See 2.4	
Sheanut butter, crude	X	X		X						See 2.4	
Sheanut butter, refined				X						See 2.4	
Shrimp meal	X ²⁾³⁾									X	

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Sodium bicarbonate	X										
Sodium calcium magnesium phosphate	X										
Sodium carbonate	X										
Sodium chloride	X										
Sorghum					X			X		X	
Sorghum, heat treated					X			X		X	
Sorghum flakes , heat-treated					X			X			
Soya bean fat, crude hardened low in nickel		X								See 2.4	
Soya bean fat, hardened refined										See 2.4	
Soya bean husks		X					X	X			
Soya bean husks, heat-treated		X					X				
Soya bean lecithin oil mixture, water containing		X								See 2.4	
Soya bean lecithin, native		X								See 2.4	
Soya bean oil sediment		X								See 2.4	
Soya bean oil, chemically refined										See 2.4	
Soya bean oil, crude degummed		X								See 2.4	
Soya bean oil, crude not degummed		X								See 2.4	
Soya bean oil, crude partially degummed		X								See 2.4	
Soya bean oil, physically refined										See 2.4	
Soya beans, heat-treated		X						X			
Soya beans, heat-treated and dehulled		X						X			
Soya beans, heat-treated, dehulled and flaked		X						X			
Soya beans, raw		X						X			

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatox.B	Deoxyn.	Ochrat.			Zea	Salmonella
Soya cheese / Tofu		X						X			
Soya expeller		X						X			
Soya fatty acid distillate		X								See 2.4	
Soya fatty acid distillates and soy distillates, technically inevitable mixture		X								See 2.4	
Soya fatty acids		X								See 2.4	
Soya fibre		X						X			
Soya filtrate		X						X		X	
Soya flakes, extracted		X						X			
Soya paste		X						X			
Soya solubles		X									
Soya velasses		X						X			
Soya, extracted		X						X			
Soya, extracted, stable (treated with CH ₂ O)		X						X			
Soya, extracted, stable (treated with reducing sugars)											
Soya-protein concentrate from enzymatic treatment		X						X			
Soya-protein concentrate from ethanol extraction		X						X			
Spelt					X	X		X		X	
Spelthulls					X	X		X			
Stomachgrit											
Straw, in bags	X ³⁾	X			X			X			
Sugar											
Sugar beet pulp, dried										X	

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Microbiological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
Sugar beet pulp, pressed											
Sugar water											
Sunflower fatty acid distillate		X								See 2.4	
Sunflower fatty acids		X								See 2.4	
Sunflower lecithin, native		X								See 2.4	
Sunflower oil, crude, non-degummed		X								See 2.4	
Sunflower oil, neutralised, bleached		X								See 2.4	
Sunflower oil, partially degummed		X								See 2.4	
Sunflower oil, refined										See 2.4	
Sunflower olein fraction										See 2.4	
Sunflower seed		X						X			
Sunflower seed cleanings		X									
Sunflower seed expellers		X						X			
Sunflower seed screenings		X						X			
Sunflower seed, extracted		X						X			
Sunflower stearin fraction								X			
Tanalbin											
Tapioca											
Tapioca starch											
Tricalcium phosphate								X			
Triticale						X	X	X		X	
Tulip bulbs, forced on hydroculture	X	X		X	X	X	X	X			
Vacuüm salt (sodium chloride)	X										
Vegetable and fruit steam peelings	X ³⁾	X									
Vegetables and fruit by flow from the proc-	X ³⁾	X						X			

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Microbiological	Animal Components	Others	
				CAP	Aflatoxin.B	Deoxyn.	Ochrat.			Zea	Salmonella
essing of fresh vegetables and fruit											
Vegetables and fruit condensation steam from the processing of fresh vegetables and fruit	X ³⁾	X								X	
Vetches		X						X			
Vinasses (beet-, cane-), alcohol production											
Vinasses (beet-, cane-), amino and organic acids production											
Vinasses (beet, cane), yeast production											
Water, spring-											
Water, surface-											
Water, tap-											
Weeds, raider under controlled circumstances	X ³⁾							X		X	
Wheat		X			X	X		X			
Wheat and wheat bran, malted and fermented											
Wheat bran		X			X	X		X			
Wheat bran grits		X			X	X		X			
Wheat feed flour		X			X	X		X			
Wheat feed meal		X			X	X		X			
Wheat flakes		X			X	X		X			
Wheat flour		X			X	X		X			
Wheat germ expeller											
Wheat germ extracted		X			X	X		X			
Wheat germs		X			X	X		X			

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatox.B	Deoxyn.	Ochrat.			Zea	Salmonella
Wheat gluten		X			X	X		X			
Wheat gluten feed		X			X	X		X			
Wheat gluten feed, liquid											
Wheat grits		X			X	X		X			
Wheat protein, hydrolysed		X			X	X					
Wheat screenings											
Wheat starch with added CaCl ₂ , heat treated		X				X					
Wheat starch, dried		X			X	X		X			
Wheat starch, liquid		X			X	X					
Wheat sugar syrup											
Wheat yeast concentrate		X			X	X		X			
Wheat, crushed		X			X	X		X			
Wheat, heat treated		X			X	X	X				
Wheatgerm oil, crude		X			X	X					
Whey			X					X			X
Whey concentrate			X					X			X
Whey final syrup			X					X			X
Whey minerals											
Whey powder			X					X			X
Whey powder, (partially) desugared and possibly demineralised			X					X			X
Whey protein concentrate			X					X			X
Whey protein isolate											
yeast cell walls											

Product in the Feed Safety Database	Heavy metals	Pesticides	Veterinary Medical Products	Mycotoxins				Micro-biological	Animal Components	Others	
				CAP	Aflatox.B	Deoxyn.	Ochrat.			Zea	Salmonella
Yeast, dried, inactivated											

Land animal products

The following products are not all in the Feed Safety Database but may be manufactured, traded, treated or processed by GMP+ certified companies.

No.	Land animal products	Pesticides (fat soluble)	Dioxin*	Salmonella	Enterobacteriaceae
1	Bone meal			X	X
2	Blood meal			X	X
3	Animal meal			X	X
4	Duck meal			X	X
5	Gelatine (in FSD)				
6	Hairmeal			X	X
7	Greaves meal			X	X
8	Chicken meal			X	X
9	Rabbit meal			X	X
10	Lamb meal			X	X
11	Poultry blood meal (in FSD)			X	X
12	Poultry meat meal			X	X
13	Feather meal			X	X
14	Animal fat (organs) (in FSD)	X	See 2.4		
15	Animal fat (bones) (in FSD)		See 2.4		
16	Meat bone meal			X	X
17	Meat meal			X	X

2.4 Monitoring of fats and oils on dioxin and dioxin like PCB's

Scope

The next tables in this section give the minimum monitoring requirements for the oils and fats products from oil seed processing, oil refining, animal fat processing and fat blending used as feedstock for feed, including monitoring requirements for imported oils & fats that are directly sold to the feed industry.

Sampling

Sampling must be in compliance with the general GMP+ requirements. For sampling fats and oils several sampling techniques and procedures are available. Samples must represent the lot/batch. The samples must be taken from homogeneous and clearly identified lots.

Analysis

Analysis of levels of dioxins and dioxin-like PCBs must be performed by a laboratory accredited by ISO17025 or GMP+ B10 with dioxin/dioxin like PCB's in oil, fats and fatty acids/distillates as scope.

The laboratory must use an officially recognized method of analysis¹. The certificate of analysis must indicate clearly the results of both dioxin and dioxin like PCB's. The level of both of these contaminants may not exceed the maximum residue levels (see GMP+ BA1 *Product standard*).

The results must be provided within one month to GMP+ International's Feed Safety Database via the procedure prescribed by GMP+ International.

¹ For Europe, refer to Directive 2002/70/EC (as amended by Directive 2005/7/EC) establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feedingstuffs and as laid down in the Directive on dioxin screening (Directive 2009/152).

Monitoring frequency

The minimum frequency for monitoring depends on the type of fat/oil, and is indicated in every table as follows:

Category	1	2	3	4
	Not allowed for feed. See also GMP+ BA3 'Minimum requirements Negative List'	Products which require further processing before use in the food/feed chain	Product for use in feed	Product for use in feed
Monitoring frequency		Dioxin possible: 100% monitoring with a positive release	Dioxin possible: 100% monitoring, but positive release not necessary	Dioxin highly unlikely: once every three months

Remarks:

- Positive release: Product must be delivered with a certificate of analysis
- Positive release not necessary: Product may be delivered. Customer must be informed about the analytical results as soon as results are available.
- There must be a clear link between the delivered batch and the certificate of analysis.
- Further processing means refining or fragmentation. Blending is not considered as a permitted further processing step.
- Any cleaning step for removing dioxin must be validated, controlled and certified according to the general GMP+ requirements, and must also be in compliance with legal requirements. A cleaning step must be considered as production and be certified as such ('production of feed material').
- The company that decides to clean is responsible that the cleaning step (either carried out by himself or by another, qualified company) is carried out correctly and successfully. He must inform his buyer about this to avoid doubt about the status of the product.
- In the following tables sometimes products are marked as forbidden. In principle, these products should not be mentioned here but for reasons of transparency they are still in the tables. These products are also registered in GMP+ BA3 'Minimum requirements for Negative List'.
- In Table 1 'Crushing of oil seeds and refining of oils, including imported products', there is a difference in the monitoring frequency between products from chemical and physical refining. Suppliers must therefore inform their customers about the refining step to avoid doubt about the monitoring frequency which is applied.
- The monitoring applies also for imported fats and oils.
- In appendix 6 of GMP+ BA10 *Minimum Requirements for Purchase* also monitoring requirements for palm(pit)oil products are laid down. If applicable, the participant must also comply with these requirements.

1 Crushing of oil seeds and refining of oils, including imported products																	
Processes and products ^a	Description	Palm	Palm kernel	Rape seed	Soya bean	Sunflower seed	Coconut ^b	Groundnut	Linseed	Maize	Shea kernel	Safflower	Sesame	Walnut	Cottonseed	Castor bean	Other Oil
Pressing and extraction																	
Crude oil/fat	Oils and fats from pressing/extraction	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	2
Degumming																	
(partially) degummed oil/fat	Oil treated to remove gums/lecithin			4	4	4		4	4			4	4	4	4	4	4
Lecithin				4	4	4		4	4			4	4	4	4	4	4
Storage																	
Tank bottom	Viscous, solid remains on the bottom of a tank	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Physical refining																	
Refined oil/fat and other edible products	Oils/fats treated to remove colour, odour and off taste	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Fatty acid distillates	Distillate from deodorization of physical refining	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Chemical refining																	
Refined oil/fat and other edible products	Oils/fats treated to remove colour, odour and off taste	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Soap stock and fatty acids/acid oils	Caustic soda refining and soap stock splitting	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	2
Distillates	Distillate from deodorization after chemical refining	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

a In case of mixing of products (for instance fatty acids from physical and chemical refining), the monitoring regime of the fraction with the highest monitoring frequency applies

b Based on actual monitoring results, low levels of dioxin could be present in crude coconut oil. The source can be environmental pollution. Further, a potential source of dioxin can be the drying of copra (the dried meat of the coconut from which the coconut oil is obtained). See also GMP+ BA3 'Minimum requirements Negative List'.

2 Animal fat production, including imported products	Animal fats from land animals						Fish oil
	Tallow	Lard	Pig fat	Ruminant fat	Poultry fat	Animal fat (multispecies)	Fish oil
Processes and product							
Fat processing							
Fat processors edible fats and oils (Regulation (EC) 853/2004)	4	4			4		
Animal by-product fats and oils (Regulation (EC) 1069/2009)			4	4	4	4	
Chemical refining							
Fatty acids & soap stocks	4	4	4	4	4	4	
Distillates from deodorization after chemical refining	3	3	3	3	3	3	
Physical refining							
Fatty acid distillates	3	3	3	3	3	3	
Gelatine production							
Fat from gelatine production	4	4	4	4	4	4	3
Fish oil processing							
Crude fish oil							2
Refined fish oil ^a							2
Soapstock and fatty acids from fish oil ^b							2

a In case the crude oil from which the refined fish oil originated is tested for dioxin within the legal limits, no additional tested is needed since no concentration takes place during the refining process.

b In case the crude oil from which the soap stocks and fatty acids originated is tested for dioxin within the legal limits, no additional tested is needed since no concentration takes place into the soap stocks and fatty acids.

3 Oleochemical processing & bio-diesel production, including imported products	Fats from vegetable or animal origin used as raw material for oleochemical or bio-diesel production						
	Used Cooking Oil	Palm oil	Rapeseed oil	Soya bean oil	Animal oils and fats allowed for food or feed	Category 1 of 2 Animal Fats	Other oils and fats
Processes and product							
Oleochemical production							
By products	1	2	2	2	2	1	2
Biodiesel production							
Fatty acids with methyl esters (fatty matter) ^a	1	1	1	1	1	1	2
Glycerol ^{b,c}	1	4	4	4	4	1	2

a Fatty acids with methyl esters (also called fatty matter) collected after methanol recovery at biodiesel production are prohibited for feed purposes since lipophile additives used in biodiesel production concentrate in the fatty acids.

b Glycerol from biodiesel, produced from raw material that is approved for animal feed is allowed for use in feed.

c Glycerol from biodiesel, produced from raw material forbidden to use in animal feed (like UCO) are forbidden to use in feed.

4 Fat blending	Mixtures of oils/fats and/or products thereof		
Processes and product	Vegetable mixture	Animal mixture	Vegetable/ animal mixture
Mixing of lots			
Mixing of lots that all have dioxin analysis and 100% separation between feed/food and technical products. On the location dedicated piping and tanking are used	4	4	4
Mixing of fats with at least one of the lots missing dioxin analysis	2	2	2

3 Sampling and analysis of compound feeds

3.1 Protocols relating to Salmonella-sampling and analysis

In the following protocols include requirements with respect to the monitoring for and analysis of Salmonella and Enterobacteriaceae in compound feeds for poultry, pigs, cattle and other animals.

All the data from this program is stored in the Feed Safety Database and is accessible to those providing information, those being the feed materials suppliers and also the compound feed manufacturers.

Classification of Salmonella-positive samples

As in the determination of Salmonella in feed materials there will be classification (serological type and possibly phage type). The protocol applies as included in appendix I. The poultry feeds, cattle feeds and pig feeds should be fully classified.

3.2 Protocol P1: Sampling and analysis of Salmonella and enterobacteriaceae in feeds for poultry

1. Target group

Manufacturers of poultry compound feeds intended for delivery to livestock holders.

2. Products

Compound feeds intended for poultry.

3. General additional requirements.

If a Salmonella-positive result is obtained then this should be classified in accordance with appendix I.

4. Inspection frequency

The following situations are distinguished with respect to the animal feeds supplied to poultry farmers

- 4.1 Technologically-treated poultry compound feeds
 - A) which are delivered as such
 - B) which are delivered together with separate feed materials
- 4.2 Non-technologically-treated poultry compound feeds
- 4.3 Final product check

Depending on the situation, requirements will be established for the entry check, production process control, and control in the logistical process. The frequency of inspection is dependent on previously obtained inspection results.

4.1 Technologically-treated compound feeds

Poultry feeds should be supplied Salmonella-free.

4.1.A. For producers of technologically-treated poultry feeds (for example pressing, acidification, etc.) the following requirements apply:

1. The compound feed manufacturer shows by way of an entero reduction test under which conditions the entero reduction is at least a factor 1000. These conditions should be used as set-up parameters for the production of treated poultry feed. The entero reduction test should be carried out at least twice per year. The compound feed manufacturer must be able to demonstrate that these set-up parameters are used in the production of poultry feeds. This applies from the beginning to the end of production.
2. Each company has its own responsibility and specifies the critical points for its own business situation and determines a minimum sampling plan. A sampling process diagram should be part of the sampling plan. This shows the critical points for the process control.

The producer should apply process control at those points which are critical with respect to possible recontamination with Salmonella, including

- a. Coolers, inside where there are possible condensation sites
- b. Air supply from the cooler at places where the air is sucked in
- c. Each point in the production line after the press where recontamination of the product by, for example, dust, enzymes, wheat may occur.
- d. Inside of the ready product silo on the top.
- e. Each point after the production line where recontamination can occur such as open places, loading.
- f. Transport of the ready product to the client.

A representative number of samples should be taken and examined from the critical points mentioned above with a minimum of 10 per production line.

3. With respect to sampling the sampling protocol applies (where applicable) as specified in § 6 of this Protocol P1. Where this is not possible (because of dust, means of transport, for example) use may also be made of the sponge/swabbing method where a minimum of 200 cm² is taken (spinged/swabbed).
4. The critical points must be examined for Salmonella. The frequency of inspection must be once per month and if this is negative for a half year then the frequency can be reduced to once per two months. In the event of a positive finding analysis must be done again once per month for at least half a year. The positive samples must be classified.
5. In the event of contamination corrective measures will be taken immediately until there is demonstrable compliance with the norms.
6. At the request of the poultry farmer the research data related to the above will be made available to him or her.

- 4.1.B. For producers of technologically-treated poultry feeds with separate mixed feed materials the following requirements for separately mixed feed materials apply in addition to the requirements with respect to production of technologically-treated poultry feeds (see section 4.2.A).
1. Only 'non-Salmonella-critical' feed materials may be mixed separately for Salmonella-critical feed materials see GMP+ BA4 *Minimum Requirements for Sampling and Analysis*, (appendix 3.5 Protocol 4).
 2. Any contamination which could possibly occur during reception, transport and storage of these (=non-Salmonella-critical) feed materials must be prevented. The critical points where recontamination with Salmonella can occur must be checked monthly for this². These critical points are also indicated in the process diagram (see section A2). These include as a minimum the reception of feed materials, internal transport and storage (= logistical process).
 3. A representative number of samples should be taken and examined from the critical points mentioned above with a minimum of 3.
 4. The critical points must be examined for Salmonella. The frequency of inspection must be once per month and if this is negative for a half year then the frequency can be reduced to once per two months. In the event of a positive finding analysis must be done again once per month for at least half a year. The positive samples must be classified.
 5. In the event of contamination corrective measures will be taken immediately until there is demonstrable compliance with the norms.
 6. At the request of the poultry farmer the research data related to the above will be made available to him or her.
- 4.1.C. For companies with an annual production of poultry feeds of up to 7,500 tons per year

A company with a lesser annual production (<7500 tons of poultry feeds) may decide to comply with the requirements of this section instead of the relevant requirements with respect to process control in section 4.2A and section 4.2B.

It has been established that for an annual production of poultry feed of 7,500 tons or less, a company should carry out a process check 4x per year (or per production batch) where a sample is taken at 5 critical points. A mix sample may then be made up from these 5 samples and then analysed. The relevant ISO instructions apply with respect to the pooling of samples. This means a total of about 4 analyses per year.

If this results in a positive outcome then 5 samples should then be analysed again separately in order to trace the contamination.

If the mix sample is negative then it can also serve as end product sample.

² This relates to a number of extra critical points in the logistical process in addition to the critical points in the production process specified in section A2.

4.2 Technologically-untreated compound feeds

Poultry feeds should be supplied Salmonella-free.

The following requirements apply with respect to the entry check for feed materials:

1. The compound feed manufacturer will make the following distinction in feed materials in the production of technologically-untreated poultry feed:
 - non-Salmonella-critical feed materials can be processed without an analysis of the batch in question being availableSalmonella-critical feed materials (see GMP+ BA04 *Minimum Requirements for Sampling and Analysis*) can only be processed if the batch in question, after sampling and analysis, appears to be Salmonella-free on the responsibility of the compound feed manufacturer
 - a. As an exception to this Salmonella-critical feed materials may also be processed with an analysis result for the batch in question not being available if it is made demonstrable that the feed material in question is from a specific manufacturer (=origin) and/or has undergone a specific treatment and therefore complies with the norm 'non-Salmonella-critical'. Before this exception clause can be used at least 10 consecutive deliveries must be Salmonella-negative.
 - b. After this, every 5th batch must be sampled and analysed with a
 - c. negative result. In the event of a positive result each batch must again be
 - d. sampled and analysed until 10 consecutive deliveries
 - e. are found to be Salmonella-negative.
2. Method of sampling of feed materials:
 - a. Salmonella-critical and non-Salmonella-critical feed materials are both sampled in the manner described in § 6 of this protocol P1.
 - b. Sampling is done on the responsibility of the compound feed manufacturer. (N.B. the sampling may take place elsewhere, for example during the loading of the feed material)
 - c. For batches of up to 100 tons, at least 1 sample is taken and for batches of more than 100 tons at least 5 samples are taken. For the latter a mix sample may be made for the analysis.

The following requirements apply with respect to the *process control* during the production of poultry feeds:

3. Each company has its own responsibility and specifies the (representative) critical points for its own business situation and determines a minimum sampling plan. A sampling process diagram should be part of the sampling plan. This shows the critical points for the process control.

The critical points in the production process for recontamination of *Salmonella* may, for example, be:

- a. Internal transport from the intake point
- b. Each point in the production line after the grinder/mixer where recontamination of the product by, for example, dust, enzymes, wheat may occur.
- c. Inside of the ready product silo on the top.
- d. Each point after the production line where recontamination can occur such as open places, loading.
- e. Transport of the ready product to the client.

A representative number of samples should be taken from the critical locations in the production process and these should be examined for the presence of *Salmonella* with a minimum of 5 per production line.

4. With respect to sampling (where applicable) the sampling protocol applies as specified in § 6 of this Protocol P1. Where the necessary quantity of sampling material (dust and residues of feeds) can not be obtained (because of dust, means of transport, for example) use may also be made of the sponge/swabbing method where a minimum of 200 cm² is taken (sponged/swabbed).
5. The frequency of examination for these critical points must be once per month and if this is negative for a half year then the frequency can be reduced to once per two months. The critical points must be examined for *Salmonella*. In the event of a positive finding, sampling and analysis must be done again once per month for at least half a year. The positive samples must be classified in accordance with appendix 1.
6. In the event of contamination immediate corrective measures will be taken until there is demonstrable compliance with the norms.
7. At the request of the poultry farmer the examination data related to the above will be made available to him or her by the compound feed manufacturer.
8. A company with a lesser annual production (<7500 tons of poultry feeds) may decide to comply with the requirements of section 4.2C instead of the relevant requirements with respect to process control in this section 3.

4.3 Poultry compound feeds (end products)

The sampling and analysis of the distinguishable types of end product must be done in accordance with the minimum frequency (per company unit) indicated in the table below.

Type of compound feed	Minimum inspection frequency, calculated per 24-ton delivery
Top breeding ³	1 in 2 batches (50%)
Raising increase ⁴	1 in 5 batches (20%)
Breeding ³	1 in 10 batches (10%)
Broilers	1 in 20 batches (5%)
Laying-hens and breeding hens	1 in 20 batches (5%)
Raising breeding turkeys	1 in 5 batches (20%)
Breeding turkeys	1 in 10 batches (10%)
Meat turkeys	1 in 30 batches (3 1/3%)

5. Additional corrective measures in the event of a Salmonella-positive result

-

6. Sampling method

The samples of end product for process control on the basis of Enterobacteriaceae must be taken at a point that is as close as possible before loading the bulk container (or the filling of the sacks). The size of the samples to be taken is at least 60 grams, sufficient to compose a sample and a duplicate sample of 25 grams each.

The samples of compound feed should be taken from the product flow at a point as close as possible before the loading of the bulk container (or the filling of the sacks), or, in the event of process control, as close as possible to the critical point in the process.

7. Analysis method

The method recorded in the Product Board Animal Feed documentation bundle “Inspection Methods” (www.pdv.nl).

The analysis will be carried out by a laboratory certified under the GMP+ FSA scheme for the determination of Salmonella or by an equivalent laboratory. See GMP+ BA10 *Minimum Requirements for Purchasing*

³ meat and egg sectors, respectively

⁴ If, during an uninterrupted period of 2 years inspection of the type of feed in question, no Salmonella-positive sample is found then a minimum sampling frequency may be used of 1 in 30 batches (3 1/3%) .

8. Reporting analysis results

8.1 The Feed Safety Database

The results of the determinations should be provided at least once per month to GMP+ International's Feed Safety Database via the procedure prescribed by GMP+ International. <http://dos.gmpplus.org/>.

8.2 Certification Body

For every observation of *Salmonella enteritidis* (S.e.) and *Salmonella typhimurium* (S.t.) in compound feed for the egg sector there should be immediate consultation with the certification body about the effectiveness of the previous measure.

3.3 Protocol 2: Sampling and analysis for Salmonella and enterobacteriaceae in compound feeds intended for pigs, cattle and other animal species (with the exception of poultry)

1. Target group

Manufacturers of other compound feeds including manufacturers of mixes of wet by-products than those intended for poultry.

2. Products

Other compound feeds than those intended for poultry (including the mixes of other wet by-products).

3. General additional requirements

If a Salmonella-positive result is obtained then this should be classified in accordance with appendix I.

4. Inspection frequency

The inspection of the distinguishable types of end product must be done in accordance with the minimum frequency (per company unit) indicated below. This depends on the treatment the product has had.

4.1 Salmonella reduction treatment

In the event of Salmonella-reducing treatment, testing for Enterobacteriaceae and/or Salmonella must be carried out.

4.1.1 Salmonella

If it is decided to test for Salmonella then the test should take place as follows; Samples should be taken-compound feeds for analysis for Salmonella. The following table clarifies the number of samples to be taken.

Annual production of compound feed for other types of animal than poultry by business unit (for wet mixes, the quantities of dry matter)	Number of samples per quarter
up to 2,000 tons	2
up to 4,000 tons	2
up to 6,000 tons	3
up to 8,000 tons	4
up to 10,000 tons	5
up to 20,000 tons	10
up to 30,000 tons	15
up to 40,000 tons	20
more than 40000 tons	25

4.1.2 Enterobacteriaceae

If testing for Enterobacteriaceae has been opted for then this must be done per production line on which Salmonella-reducing treatment is carried out, through:

- sampling and analysis twice a year at the critical points in the production process in order to determine the course of the level of Enterobacteriaceae to test the production process (thermal treatment);
- 5 samples per quarter of end product per line and analysis of these samples.

In addition, at least twice a year, sampling and analysis for Salmonella must take place at critical points in the production process.

4.2 No Salmonella-reducing treatment

If no Salmonella-reduction treatment takes place then there should be an inspection as intended in § 4.1.1.

4.3 Wet compound feeds

As a replacement for Salmonella testing, the participant can also carry out tests on pH or temperature. The participant should take at least one sample per quarter, per product and have it tested.

In the event of the pH being measured and there is compliance with the maximum pH as specified in GMP+ BA01 Product Standards, then sampling and analysis for Salmonella is not mandatory.

5. Additional corrective measures in the event of a Salmonella-positive result

If a sample of end product is found to be Salmonella-positive then sampling and analysis for Salmonella should be carried out at critical points in the production process.

6. Sampling method

The samples of compound feed should be taken from the product flow at a point as close as possible before the loading of the bulk container (or the filling of the sacks), or, in the event of process control, as close as possible to the critical point in the process. The samples of end product for process control on the basis of Enterobacteriaceae must be taken at a point that is as close as possible before loading the bulk container (or the filling of the sacks). The quantity of the samples to be taken is at least 60 grams, sufficient to compose a sample and a duplicate sample of 25 grams each.

7. Analysis method

~~The method recorded in the Product Board Animal Feed documentation bundle “Inspection Methods” (www.pdv.nl).~~

The analysis will be carried out by a laboratory certified under the GMP+ FSA scheme for the determination of Salmonella or by an equivalent laboratory. See GMP+ BA10 Minimum Requirements for Purchasing

8. Reporting analysis results

The results of the determinations should be provided at least once per month to GMP+ International’s Feed Safety Database via the procedure prescribed by GMP+ International.

<http://dos.gmpplus.org/>

3.4 Protocol P4: Sampling and analysis of Salmonella-critical feed materials (raw materials)

Introduction

On the basis of the sampling and analyses data for the 'output check' from producers / importers / shipping agents of feed materials and the 'input check' of the GMP+-certified compound feed manufacturers GMP+ International maintains the following list of Salmonella-critical feed materials.

Salmonella-critical feed materials

There are currently no feed materials assessed as Salmonella-critical.

3.4.1 Protocol 4A: Sampling and analysis of Salmonella-critical feed materials

1. Target group

Producers of Salmonella-critical feed materials

2. Products

Salmonella-critical feed materials.

Each year, the report "Plan to control Salmonella in the feed sector" is used to determine which feed materials are Salmonella-critical.

3. General additional requirements

At the production location there should be a list showing the following details:

- a. number of vehicles loaded
- b. the quantity delivered per ship
- c. which vehicles were sampled
- d. the number of samples per ship
- e. date of sending samples to the laboratory
- f. results (and the classification if Salmonella-positive).

This list will be filed and made available on request to the inspector of the supervising body.

If a Salmonella-positive result is obtained then this should be classified in accordance with appendix I.

4. Inspection frequency

For each production location at least one sample per delivery day will be examined during loading (from the factory) for the presence of Salmonella.

5. Additional corrective measures

-

6. Sampling method

Per production location a sample of at least 25 grams will be taken per vehicle of the first delivery of the day and then of every fourth vehicle delivery. If ships are being loaded then a sample should be taken per 500 tons or part thereof.

The sample material will be scooped from the product flow during loading and will be packed in sterile sample pots. The manufacturer sends the samples within 2 working days of the sample being taken and gives the laboratory the order to make a mix sample of the material and to have it analysed.

7. Analysis method

~~The method recorded in the Product Board Animal Feed documentation bundle “Inspection Methods” (www.pdv.nl).~~

The analysis will be carried out by a laboratory certified under the GMP+ FSA scheme for the determination of Salmonella or by an equivalent laboratory. See GMP+ BA10 Minimum Requirements for Purchasing

8. Reporting analysis results

The results of the determinations should be provided at least once per month to GMP+ International’s Feed Safety Database via the procedure prescribed by GMP+ International. <http://dos.gmpplus.org/>

3.4.2 “Bonus/penalty” requirements with respect to the sampling and analysis of Salmonella-critical feed materials

A producer of a Salmonella-critical feed material must comply with the minimum sampling and analysis established in the protocol in question. A producer can, however, on the basis of demonstrably good sampling and analysis results be eligible for a decrease in the sampling and analysis frequency. The producer should comply with the following requirements:

- a. The producer has in the previous year complied with all the Salmonella sampling and analysis obligations as specified in GMP+ BA04 *Minimum Requirements for Sampling and Analysis*, Protocol P4. This means that it has complied with the frequency of sampling and analysis and has sent the analysis results to the Feed Safety Database in accordance with requirements.
- b. The Salmonella incidence of the feed material in question has in the previous 4 quarters been lower than 3% per quarter on the basis of regular sampling and analysis in which:
 1. the Salmonella incidence of 3% relates to end product control ex-factory;
 2. The Salmonella incidence of 3% relates to all Salmonellas (all serological classifications)
 3. the Salmonella incidence is calculated on the basis of the prescribed frequency of sampling in GMP+ BA04 *Minimum Requirements for Sampling and Analysis*, Protocol P4.
- c. The producer has in the previous year carried out a proper process control in which all the critical points in the process have been made clear and proper control measures have been taken (in accordance with the HACCP system).

If the producer complies with the established requirements (items a to c) then instead of the prescribed minimum mandatory sampling and analysis he may make do with the following sampling and analysis frequency:

The producer carries out Salmonella sampling and analysis on the basis of in company HACCP.

The minimum sampling frequency is determined via the system detailed in Chapter 2 of GMP+ BA4 *Minimum Requirements for Sampling and Analysis* using the following formula:

$$\text{Freq.} = \frac{\sqrt{\text{Production volume}}}{100} * 1 * 5 * 5.$$

For an explanation of this formula see Appendix 2 of GMP+ BA4 *Minimum Requirements for Sampling and Analysis*. In the above formula a decision has been made on a factor 1 for the history and for a factor 5 for the seriousness.

This formula is derived from a general formula which takes into account the production annual volume and in which a correction factor can be applied for the history, the chance of recontamination and the seriousness.

- a. If the cause of a higher Salmonella incidence than 3% (of end products) in a quarter is to be found in one incident then the producer may make do with the monitoring as specified in point a. There is an incident if the Salmonella incidence of end products after the observation of the incident
 1. Is higher for a maximum of one month (30 days) than 3% and
 2. More than 1 positive result is found within 14 days.
- b. Per two successive quarters only 1 incident may occur.
- c. If the producer has a Salmonella incidence in two successive quarters 3% of end products (which is not the result of one incident) then the producer must inform his certification body of the measures taken.
- d. If the producer does not comply with items a. to d. then for a period of at least one year he should carry out Salmonella sampling and analysis as prescribed in GMP+ BA04 *Minimum Requirements for Sampling and Analysis* for the Salmonella-critical feed material in question.

4 Other sampling and analysis protocols

4.1 Protocol P6: Sampling and analysis Aflatoxin B1

1. Target group

Compound feed manufacturers and suppliers of feed materials for dairy cattle.

2. Products

Feed materials for dairy cattle or for the preparation of compound feeds for dairy cattle.

3. General additional requirements

-

4. Inspection frequency

The following sampling and analysis schedule must be used for testing for Aflatoxin B1 in feed materials for dairy cattle and for the manufacturing of compound feeds for dairy cattle. No minimum testing frequency has been established for the producers not included in the following table and the exempted sources specified here.

A participant which delivers the following feed materials in single form to dairy farmers must provide the dairy farmer with the analysis certificate of the said (origin) batch, or of the testing based on his own sampling.

A participant which delivers compound feeds for dairy cattle must upon purchase or receipt of the following feed materials have an analysis certificate, supplied by the supplier of the said (origin) batch, or of the testing on the basis of his own sampling.

Feed materials class 1	All batches must be tested, whereby the analysis must concern (origin) batches of no more than 500 tons
	The following come into this category: <ol style="list-style-type: none">1. Ground nut flakes and scraps, all origins2. Kapok seed flakes, all origins3. Cotton seed flakes and scraps, all origins4. Coconut (by-)products, all originsMaize and maize by-products, all origins except USA and5. EEC6. Palm kernels and palm kernel by-products, unknown origin7. Safflower seed scraps, all origins

Feed materials class 2	All batches must be tested, whereby the analysis must concern (origin) batches of no more than 3,000 tonnes
	The following come into this category: <ol style="list-style-type: none">1. Palm kernels and palm kernel by-products, all known origins except Indonesia and Malaysia2. Rice by-products, all origins

5. Additional corrective measures in the event of deviations

-

6. Sampling method

-

7. Analysis method

~~The method recorded in the Product Board Animal Feed documentation bundle “Inspection Methods” (www.pdv.nl).~~

The analysis will be carried out by a laboratory certified under the GMP+ FSA scheme for the determination of Salmonella or by an equivalent laboratory. See GMP+ BA10 *Minimum Requirements for Purchasing*

8. Provision of results

The results of the determinations should be provided at least once per month to GMP+ International’s Feed Safety Database via the procedure prescribed by GMP+ International.

<http://dos.gmpplus.org/>

4.2 Protocol P7: Sampling and analysis Animal Protein

1. Target group

Manufacturers of compound feeds including wet mixes for ruminants.

2. Products

Compound feeds including wet mixes for ruminants.

3. General additional requirements

-

4. Inspection frequency

The following numbers of samples from feeds for ruminants must be taken for the microscopic tests for the presence of tissue proteins from mammals.

Inspection table per production location for BSE control

Production in tons per year	Samples / Quarter
< 5,000	1
5,000 < < 10,000	1
10,000 < < 20,000	2
20,000 < < 30,000	2
30,000 < < 40,000	2
>40,000	3

5. Additional corrective measures in the event of the norm being exceeded

In accordance with animal feed legislation.

6. Sampling method

-

7. Analysis method

~~The method recorded in the Product Board Animal Feed documentation bundle "Inspection Methods" (www.pdv.nl).~~

The analysis will be carried out by a laboratory certified under the GMP+ FSA scheme for the determination of Salmonella or by an equivalent laboratory. See GMP+ BA10 *Minimum Requirements for Purchasing*

8. Provision of results

The results of the determinations should be provided at least once per month to GMP+ International's Feed Safety Database via the procedure prescribed by GMP+ International. <http://dos.gmpplus.org/>

APPENDIX 1: PROTOCOL FOR THE SEROLOGICAL CLASSIFICATION OF SALMONELLA

Participants in the GMP+ FSA scheme for the animal feed sector are obliged have Salmonella-positive samples of feeds or feed materials classified.

The poultry feeds, cattle feeds and pig feeds should be fully classified. The feed materials should be classified for the serotypes Enteritidis, Typhimurium, Infantis, Virchow, Hadar, Java and Agona. The serological classification should be carried out by the RIVM or by GMP+ B10 *Laboratory Testing* certified for the serological classification of Salmonella or accredited for ISO 17025 (for Salmonella classification). The costs of the classification will be charged to the (animal feed) company.

The purpose of this classification is to establish more accurately any relationship among Salmonella types in feed materials, the compound feeds produced from them, live animals which eat these feeds and also animal products. It is an aid in investigating the possible cause of Salmonella contamination in a subsequent link in the chain.

The procedure is as follows:

- a. New companies participating will report once only to the RIVM at telephone number 030-2742126.
- b. The RIVM will then send you a transmission medium as quickly as possible including packaging material. This is the standard RIVM packaging with white/pink forms. These forms must be replaced by the green forms for the animal feed project. These forms will be sent to newly registered companies separate from the packaging material.
- c. The packaging material and the new transmission medium will be returned to the sender after each submission. The green forms can be requested each time by telephone at telephone number 030-2742126. The participants who regularly submit a green form to the RIVM must from today also order these forms by telephone.
- d. The green RIVM form should be fully completed and sent to the RIVM together with the identified salmonella culture. The form should contain the following details:
 1. Name/address/place of the sender;
 2. Company ordering the sampling of the product (possibly in code form);
 3. Type of feed or fodder from which the salmonella was isolated;
 4. Country of origin of the feed.

For the first consignment the technique for isolating Salmonella should be specified once and also any future changes in the technique used.

PART B: PROTOCOLS FOR THE MEASUREMENT OF CARRY-OVER

1 Introduction

Section 6.7.1.5 of GMP+ standard B1 *Production, Trade and Services* requires, among other things that a participant who processes E⁵additives or veterinary medical products must measure the carry-over of the installation. This is necessary to be able to control the residue levels of additives and veterinary medical products as laid down in GMP+ BA1 *Product Standards* (see section 7.7 of GMP+ B1 *Production, Trade and Services*).

During measurement the participant must make use of the protocols in this part of the appendix.

The reporting on the carry-over inspection must comply with further conditions. See below for a description of the methods. (see chapter 2, section: Inspection report)

N.B. In anticipation of a review of the carry-over methods, it is permissible for companies to deviate from the method laid down as long as the principle is not affected and there is a real probability that equivalent results will be obtained.

⁵ By E-additives is meant those additives which are included in Group E (Regulation EU 1831/2003, Article 6, subsection 1e).

2 Methods for measuring carry-over

2.1 General basic principles with respect to the measurement of carry-over

When measuring the carry-over of additives in an installation there must be a prior examination using the diagram and the actual situation in the factory of which parts of the factory may be relevant for carry-over.

A basic principle in determining carry-over in a company is that the degree of carry-over as a result of return flows is known and is controlled.

Carry-over points

Carry-over in a (compound feed) factory may occur in the following processes.

1. The filling of premix silos

The filling of the premix silos may be the cause of carry-over. The diagram can be used to find out whether there are reasons to suppose that carry-over occurs here. Critical points are common transport systems, chutes, separation systems and filters.

In mechanical transports such as mass transports, elevators and screw conveyors, carry-over always occurs and it is sensible to measure this carry-over. Also, sufficiently long idle times (10 minutes) should be taken into account.

For the pneumatic filling method with separate filters for each silo, no account needs to be taken of carry-over. If there is a common filter then the filter must, for at least 10 minutes after unloading, be knocked on the same silo as that in which the filling took place.

There should be an instruction for the dumping sequence so that undesired mixing does not take place.

In this situation it must be certain that unacceptable residue levels (see GMP+ BA01 *Product Standards*, part B) no longer occur.

2. Dosage, grinding and mixing line

The greatest amount of carry-over of additives and veterinary medical products occurs in the dosage process (addition of additives or veterinary medical products) / (possibly grinding) / mixing / transport and storage of the product in meal form in a finished product cell or a pressed meal cell.

The place where premixes are added should be as close to the mixer as possible. It is important that the measured substance is added at the same place as where the additive and veterinary medical products were added.

3. **Press line**

A considerable amount of carry-over can occur in the press line. The carry-over increases as the press moulds are bigger. In addition, interim bunkers containing stocks can be a source of carry-over.

An item for attention is the return flows which are brought back directly into the pressed meal silo during pelletising.

4. **Loading and transport**

During storage, loading and transport of a finished product there will only be carry-over of any importance for highly critical additives and veterinary medical products (for example nicarbazine and sulfa-veterinary medical products). In these cases a mandatory working sequence should be used.

An item for attention is the processing of the sievings from the bulk load. Possible processing of such sievings must at least comply with the animal feed legislation and must therefore be processed in a careful and controlled fashion. Any sievings of medicated feed may not be reprocessed.

If the undesired carry-over of critical additives and veterinary medical products may be expected then company may take the following measures:

1. the drawing up of a mandatory production (working) sequence
2. additional measures in the event of product changes
3. the production of feeds with critical additives and veterinary medical products on another line
4. switching to less critical agents.

Measurement points for carry-over

The major causes of carry-over are the dosage / grinding / mixing line and the press line. The carry-over should be known if both feeds with critical additives and veterinary medical products as feeds with a maximum carry-over level are produced on these lines. In order to establish this reliably the following measurement points are important:

After the mixer, but as close as possible to the mixer for the measurement of the output content of the mixer:

- a. at the entry to the pressed meal cell in grain production or the finished product cell in meal production, for the measurement of the carry-over on the dosage / grinding / mixing line
- b. at the entry to the finished product cell in grain production for the measurement of carry-over on the press line.

Carry-over which is determined in this way is considered to be the installation carry-over.

Possible measurement substances

For the sake of reliability it is important to choose a measurement substance which can also be analysed properly at low levels. The following measurement substances are permitted. An indication is also given of to what degree of accuracy these means can be used to determine the carry-over in an installation.

Method	Chapter	Lower limit ⁶ of carry-over inspection accuracy in % ¹⁾
Cobalt chloride 100 ppm	2.2	1
Cobalt sulphate		
- 100 ppm	2.3.1	1
- 50 ppm	2.3.2	3
- 25 ppm	2.3.3	5
Protein/Manganate	2.4	See the table in 2.4
FSS-Lake 100ppm	2.6	1
F-Lake 100 ppm	2.6	1
FSS-Lake 10 ppm	2.6	1
RF microtracer (by way of weighing)	2.7	1
Methyl violet	2.8	1

¹⁾ Chapter 2.5 includes a method for the measurement of the carry-over for the production system for premixes and feed additives

Inspection report

Good reporting on the inspection is important to be able to apply the results unambiguously when determining measures and during supervision of the correct implementation. This should be based on a well thought out and properly described protocol which has been talked through in advance with those who will implement it and on a careful implementation of this protocol. At least the following items should therefore be laid down.

1. date
2. who is responsible for the carry-over inspection
3. description of the method used
4. a plan of the installation with an indication of
 - a. grinding, mixing and press lines which were inspected
 - b. the place where the measured substance was added
 - c. sampling points
5. the number and size of the samples
6. the sampling time interval
7. analysis results
8. proper calculation of the carry-over
9. any sample pre-handling such as grinding, homogenisation, splitting and/or putting together

⁶ The lower carry-over limit is the carry-over percentage on which, using the method applied, a reliable statement can still be made. If the carry-over percentage is lower then at least the carry-over percentage stated here should be used.

New measurement substances

New measurement substances will be admitted on the basis of examination where there has been validation with respect to the reference method (Cobalt method). The validation report must contain at least the following elements:

- a. Name and address details of the submitter and inspection agency
- b. Motivation/problem description
- c. Characteristics with respect to the
 1. Animal feed installation to be used (including mixer/press installation/cooler)
 2. The reference measurement substances and the measurement substances to be examined
 3. Sampling plan for the samples to be taken in the various flush batches
 4. Sample preparation in the laboratory
 5. Analysis methods to be used
 6. Statistical methods to be used
- d. Analysis results
- e. Statistical processing of the analysis results
- f. Conclusions
- g. References

The report may be submitted for assessment by an expert panel to GMP+ International.

2.2 Process accuracy control procedure with cobalt (reference method)

1. FIELD OF APPLICATION

This testing procedure or method for the determining of the uniformity of meals and grains may be used on the usual premixes and mixes of ground compound feed raw materials in compound feed companies.

The method can also be used to obtain an indication of the carry-over which occurs in compound feed raw materials.

2. DEFINITIONS

Product installation: A product installation is an installation which is suitable for the preparation of compound feeds.

Cobalt mix: Cobalt mix is a mixture of wheat grits and Cobalt chloride hexahydrate in such proportions that the cobalt level in the cobalt mix is a minimum of 5% and a maximum of 6% and is prepared in accordance with the applicable standard working instructions as incorporated in § 17 of this inspection procedure.

3. PRINCIPLE

The control procedure for the determination of the degree of uniformity of meal mixes in the preparation of compound feeds makes use of a cobalt mix which, with respect to its properties, can replace the usual compound feed additives.

The control procedure includes the processing of three batches from the same feed mix. The first batch flushes the production installation and serves to determine the "natural" cobalt level in the feed in question. The cobalt mix (see section 2) is added to the second batch. The cobalt level of samples of meal and grains from the second batch is determined. The third production batch consists of the bare feed without the cobalt mix. The cobalt level of the meal and grain samples from this batch is also determined. This level gives a picture of the carry-over which is taking place in the production installation.

The cobalt content of the samples taken is determined using atomic absorption spectrometry (AAS) after heat destruction of the analysis sample at 550 degrees Celsius.

4. EQUIPMENT AND TOOLS

The following are required for the carrying out of the control procedure:

- a. 110 plastic pots with lids with a size of 500 ml for saving the samples of meal and grains
- b. a plastic scoop for taking the samples.

The number of pots specified is required if samples of meal are taken at one point in the production installation and samples of grains are taken at another point. For each extra sampling point 48 pots of 500 ml extra are needed.

There must be a laboratory which is able to determine cobalt level using atomic absorption spectrometry. Appointments should be made in good time with this laboratory for analyses to be carried soon after the samples are taken.

5. COMPANY DETAILS REQUIRED

The following will be requested in advance from a compound feed company at which a control procedure is to be carried out:

- a. a block diagram of the production installation in which it can be indicated during the implementation where the cobalt mix has been added and where samples are taken.

The following will be requested during the implementation of the control procedure:

- b. the computer prints or copies of them which show:
 1. the composition of the feed mix
 2. the batch weight requested by the computer, and
 3. the actual batch weight

or, if there is no computerisation:

1. the composition of the feed mix
2. the calculated batch weight from the sum of the quantities weighed per component
3. the read-out of the actual batch weight.

The following will be requested to be able to calculate the batch weight for the mixer and the grain press:

- c. where and how much molasses, vinasse and other liquid ingredients added to the main flow of the feed, and
- d. where and how much fats, etc., are added to the main flow. The requested addition points are shown in the block diagram.

6. ADDITION OF THE COBALT MIX

A cobalt mix (see section 2) is added to the second batch of compound feed with a nominal cobalt level of at least 5% and maximum 6%.

The place where the cobalt mix is added depends on the carry-over path to be measured (see section 7.1). The place selected for the addition and for sampling should be shown in the block diagram for the product installation.

Add as much cobalt mix as corresponds to a dosage of 2.0 kg per ton of compound feed. The batch weight requested by the process computer may be assumed.

7. TAKING AND HANDLING SAMPLES

7.1 Company samples

7.1.1 Taking the samples

During the implementation of the control procedure in a compound feed company samples are taken at locations agreed in advance:

- a. after the mixer but as close as possible to the mixer (see 13.1)
- b. from the entrance to the finished product silo in the event of meal production or a pressed meal silo
- c. from the entrance to the finished product silo in the event of grain production
- d. another desired end point for the determination of the relevant carry-over path

If the meal or grain flow is not reachable at the desired locations then suitable openings should be made in consultation with the company.

Meal production

From the first batch only samples of meal immediately after the mixer are taken these being 10 samples for cobalt determination and another 4 samples for a fluid determination.

From the second batch 20 samples of meal (immediately after the mixer) and 20 samples of meal of 500 ml (from the input to the finished product silo) and 4 samples of meal (input to the finished product cell) are taken for the determination of fluid.

From the third batch 20 samples of meal (immediately after the mixer) and 20 samples of grains of 500 ml (from the input of the finished product cell) are taken for cobalt determination and another 4 samples of meal (after the mixer) and 4 samples of grains (input to the finished product cell) for the determination of fluid.

Grain production

From the first batch only samples of meal immediately after the mixer are taken these being 10 samples for cobalt determination and another 4 samples for a fluid determination.

From the second batch 20 samples of meal (immediately after the mixer) and 20 samples of grains of 500 ml (from the input of the finished product cell) are taken for cobalt determination and another 4 samples of meal (immediately after the mixer) and 4 samples of grains (input to the finished product cell) for the determination of fluid.

From the third batch 20 samples of meal (immediately after the mixer) and 20 samples of grains of 500 ml (from the input of the finished product cell) are taken for cobalt determination and another 4 samples of meal (immediately after the mixer) and 4 samples of grains (from the finished product cell) for the determination of fluid.

If a split is desired with respect to the carry-over by the dosage/grinding/mixing line on the one hand and the press line on the other hand then during the second and third batches another 20 samples of meal for cobalt determination and 4 samples of meal for fluid determination should be taken at the input to the pressed meal silo. The method of working is identical to the method for meal production.

Sample pots

All sample pots are provided with a sample code before the start of the production of the first batch of feed. Once the meal and/or grains flow starts for the batch to be inspected then 20 samples of meal and 20 samples of grains of 500ml are taken spread as well as possible over the duration of the batch. The sample pots must be filled up to the edge to avoid de-mixing (in the case of meal samples) as much as possible.

N. B.: It is very important that the samples are taken spread as well as possible over the duration of the batch in connection with the samples being representative of the batch as a whole.

7.1.2 Sample handling

Each meal and grain sample is ground in a suitable grinder. 90% of the result must pass through a 1.00 mm sieve and 50% must pass through a 0.50 mm sieve. Use sieves with round holes. Do not grind the samples finer than is necessary in order to avoid as much as possible the grinder heating up.

First grind the meal and grains samples from the first batch and then those from the third batch (carry-over batch) and finally the second batch of feed. In this way the samples are ground in ascending order of their cobalt level.

Clean the grinder after each sample using compressed air.

Clean the grinder after each group of 24 samples using both compressed air and, after disassembly of the relevant parts, by brushing clean with a brush which is not too soft. There may be no carry-over of material from the previous group of samples.

Homogenise each grinding as much as possible and then place it back in the original pot.

7.1.3 Storage of company samples

Company samples which are not inspected within a week of being taken should be stored in a refrigerated area at a temperature of 35 degrees Celsius.

7.2 Analysis of samples

The samples to be inspected which have been stored in a refrigerated area should be transferred at least 16 hours before the start of the inspection to the place where the inspection will take place. The sample packaging may not be opened during this period (see section 13.2). Act as indicated below once the specified period has elapsed.

Homogenise the mix to be inspected in the sample pot as much as possible by stirring it with a spoon or spatula.

From the company sample take 2 analysis samples of the desired amount. Carry out the cobalt determination for both of the samples.

8. DETERMINATION OF THE FLUID LEVEL

The operational sample taken for the determination of fluid level is used for two analysis samples.

The fluid level is determined in the analysis samples in accordance with the method laid down in the documentation bundle "Animal Feed Inspection Methods" from the Product Board Animal Feed (www.pdv.nl) or in accordance with the instructions in NEN 3332.

9. DETERMINATION OF THE COBALT LEVEL

9.1 Principle of cobalt determination

The determination of the cobalt level is done with the help of atomic absorption spectrometry (AAS) after heat destruction of the analysis sample measured by a filter of 240.7 nanometers after injection of this solution into the flame of the equipment.

A calibration graph can be made with the help of previously made solutions with an accurately known cobalt content. The extinctions measured in the analysis samples are converted into cobalt levels. The cobalt levels are expressed in parts per million (ppm).

The cobalt contents assigned to the analysis samples are corrected for the "natural" cobalt content determined in the samples of meal from the first production batch.

9.2 Standard samples

In the working instruction for the carrying out of the cobalt determination using atomic absorption spectrometry includes the inclusion of standard samples with a known cobalt content in each series of analysis samples. These standard samples serve as a check on the measured cobalt level.

9.3 Non-standard results

If the cobalt level of two analysis samples from the company sample deviates by more than 5% of the average measured values then two new analysis samples should be taken from the company sample and inspected (see 13.3).

10. PROCESSING OF THE RESULTS

10.1 Non-standard results

The results of the cobalt determinations in the compound feed from the three production batches will be assessed for deviations in as far as these are company samples of which more than two determinations have been done. In such cases a selection is made from the available results for the sample company sample of the two results with the least differences between them. These two results are then also included in the calculations. This avoids an analysis of variance with unequal degrees of freedom having to be carried out.

After the addition of the cobalt mix to the feed in the second batch the cobalt level in the first samples to be taken will be lower than in the subsequent samples [2]. This is because of a degree of carry-over from a bare floor from the first to the second batch of feed.

This may not be neglected in the determination of uniformity of the feed from the second batch. Although not statistically exact, the cobalt levels of the samples from the second batch are not assessed for a non-standard, average level of the results but they are all used for the calculation of the empiric coefficient of variation of the uniformity. That which was stated in the first sentence of this section does, however, continue to apply. The fact that the spread of the average results for the twenty samples is not "normal" but somewhat distorted is ignored.

An opposite effect is seen in the samples from the third batch of feed. Now the samples show a relatively high cobalt level as a result of carry-over of feed containing cobalt from the second to the third batch [2]. Normally the spread of the cobalt levels in the samples from the third batch is considerably more distorted than in the second batch. It is for this reason that the results of cobalt level determination in samples from the third batch are not checked for deviations. There is also no calculation of an empiric coefficient of variation for uniformity and it is enough to make a graph of the average cobalt level per sample against the sample number. In as far as the samples are properly representative for the whole batch which means they have been properly spread over the total duration, the average carry-over of cobalt can be calculated either in absolute terms or as a percentage of the level in batch two.

10.2 Conversion on the dry substance

The measured cobalt contents apply for the analysis samples or the operational samples with the existing fluid content (product basis). In order to be able to work further with the cobalt levels they should all be related to the dry substance.

Use the following formula for this conversion:

$$C = \frac{100}{100 - V} \times C1$$

Where

- C = the cobalt content on the basis of dry substance in ppm
V = the fluid level of the group of operational samples involved in %
C1 = the measured cobalt level on product basis in ppm.

The measured cobalt levels for dry substance will be decreased by the “natural” cobalt level for dry substance in the bare floor from the first batch. The cobalt levels corrected in this way for dry substance will be used for the further processing of the results.

10.3 The carry-over

The carry-over for the installation is calculated as follows in accordance with this control procedure per measurement point.

The average cobalt level for dry substance in the group of company samples from the third batch divided by the average cobalt level for dry substance in the group of company samples from the second batch. By multiplying this figure by 100 the average carry-over percentage can be calculated.

10.4 The analysis of variance

The measured, corrected cobalt levels on the basis of dry substance from the samples in the second batch are used as elements in an analysis of variance. The results for meal and for grains are analysed separately.

In this analysis of variance the following sources of variation are distinguished:

- a. the differences between the repetitions within the company samples, and
- b. the differences between the sample averages from one group of company samples.

The results of the variation analysis are:

- a. the standard deviation between repetitions (or within samples)
- b. the standard deviation between sample averages (or between samples)
- c. the average cobalt level per analysis sample
- d. the average cobalt level per group of operational samples
- e. the number of degrees of freedom associated with each of the standard deviations

The calculated standard deviations are converted to empiric coefficients of variation by multiplying the standard deviation by 100 and then dividing the product by the average cobalt level of the group of company samples. The empiric coefficient of variation calculated in this way between samples is a measure of the uniformity achieved at the measuring point.

This conversion is necessary because the standard deviation is greatly dependent on the cobalt level in the groups of operational samples.

The arithmetic implementation of the analysis of variance can be found in detail in nearly any manual on mathematical statistics. See, for example, [1].

The cobalt levels of the analysis samples from the third batch are shown in graph form against the number of the sample. These cobalt levels are not suitable for an analysis of variance because they can vary enormously and are usually not spread normally. The average cobalt level in the third batch can be calculated as specified in 10.3.

11. REPORTING

The following is reported for each group of company samples:

- a. the average fluid content for the group of company samples (0.01%)
- b. the average of the corrected measured cobalt levels on the basis of dry substance from each of the analysis samples (0.1 ppm at cobalt levels higher than 10 ppm and 0.01 ppm at cobalt levels of 10 ppm or less)
- c. the average of the corrected measured cobalt levels of the company samples per group (0.1 ppm at cobalt levels higher than 10 ppm and 0.01 ppm at cobalt levels of 10 ppm or less)
- d. the calculated carry-over of the installation in accordance with the control procedure.

A report is also made via each group of company samples from the first and second batches of feed of the following:

- a. the standard deviations between repetitions (0.0001 ppm)
- b. the standard deviation between sample averages (0.0001 ppm)
- c. the number of degrees of freedom associated with the standard deviations as intended in 4. and 5.
- d. the empiric coefficient of variation between repetitions (0.01 %)
- e. the empiric coefficient of variation between sample averages (0.01%)

12. ASSESSMENT OF THE RESULTS

12.1 Repeatability of the cobalt determination

The empiric coefficient of variation between repetitions is a measure of the repeatability of the cobalt determination including sample treatment. The empiric coefficient of variation between repetitions amounts in properly conducted determinations to about 3 - 4% [2]. If the empiric coefficient of variation is greater then the implementation of the cobalt determination should be examined further.

The repeatability (r) is a factor 2.83 higher and therefore roughly amounts to 8.5 – 11.3%. This means that in the implementation of a determination in duplicate by the same analyst with the same equipment, on one in 20 case a difference is found between the two results which is greater than the value given for repeatability (r).

12.2 Uniformity of the material

The empiric coefficient of variation between sample averages is a measure of the uniformity of the meal mix or the grains from which the company samples have been taken. Statistically the group of company samples is not homogenous if the standard deviation between sample averages exceeds the standard deviation between repetitions by more than a given factor (F test). In very small standard deviations between repetitions this leads to a non-uniform mix although there is not yet any reason on technical grounds.

13. REMARKS:

13.1 First sample point

A feed mix is not uniform after the dosage of the various components. Even after the grinding of the raw materials in the hammer mill this is only partly the case. Often finer raw materials are led around the hammer mill and carried straight to the mixer. A uniform feed mix may therefore only be expected for the first time in the mixer. Taking samples directly from the mixer is difficult and may be dangerous and is certainly not recommended. The sample point after the mixer should therefore be used. In most companies this will be the outflow of the bunker under the mixer.

13.2 Acclimatisation of company samples

Company samples which can not be examined in the short term should be stored in a refrigerated area to prevent decay. These samples must be brought to the area where the inspection will actually take place well in advance. This allows the company sample to reach the temperature of the laboratory. This method of working prevents sample material from being exposed to condensation from the warmer air in the laboratory. Condensation makes it impossible to determine the correct fluid content of the sample. A non-homogenous distribution of the condensed fluid in the sample material will also cause a greater spread of the results of the cobalt determination.

13.3 Non-standard results of cobalt determinations

If two cobalt determinations from the analysis samples from the same company sample differ by more than 5% in value then two new analysis samples must be tested.

This procedure usually results in one of the four results being rejected. In addition to company samples with results of two analysis samples there are also samples with three or sometimes four non-deviating results. This makes the implementation of the analysis of variance difficult. Statisticians have developed methods of calculation to replace more than two valid results with two results which contribute in the same way to the variance of the results.

As a judgement on whether or not a mix is uniform rests on a technological agreement on the limit value for the empiric coefficient of variation, it has been decided to simplify the method.

From the set of three or four results of which one (or two) are deviating, the deviating results are rejected.

If three valid results remain then the two results with the least difference between them are used. In this way the variance analysis consists of two company samples each with two repetitions.

14. SAFETY

The control procedure is usually carried out in practice in a compound feed company.

For those who carry out the control procedure in a compound feed company the following safety rules apply:

- a. the operatives will make themselves aware before the start of the work of the safety instructions which apply in the compound feed company
- b. during their stay in the compound feed company the operatives are bound to follow the safety instructions of the compound feed company
- c. during the adding of the premix containing cobalt to the main flow of feed protective gloves and a respiratory protector in the form of a nose covering is to be worn.

15. PROCESSING OF COMPOUND FEED CONTAINING COBALT

The mix containing cobalt is added to the second batch of feed produced for the control procedure at a dosage of 2 kg per ton of feed. The compound feed will then contain about 100 ppm of cobalt. This feed should be stored in a separate cell and may not be traded.

It is recommended that the feed containing cobalt is diluted such that the cobalt concentration in the final feed intended for trading amounts to no more than 2 ppm. Account should be taken when doing this of the cobalt level already present in the raw materials.

The feed from the third batch usually contains only slight amounts of cobalt. As the degree of carry-over is not known in advance, account must be taken of fairly large deviations in the cobalt level of this feed. It is advisable also 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 treated as chemical waste and handled and removed as such.

16. LITERATURE

- a. Snedecor, G.W. and W.G. Cochran
Statistical Methods
6th Edition, 1969
The Iowa State University Press, Ames, Iowa, U.S.A.
- b. Nieman, W., J. Hulshoff, A.J. Vooijs and H. Beumer
De bestaande mate van kwaliteitszorg in de mengvoedersector
Part II: Onderzoek naar de procesnauwkeurigheid bij de verwerking van toevoegingsmiddelen in drie pilotbedrijven met behulp van een kobalt-houdende premix.

17. STANDARD INSTRUCTION FOR THE PREPARATION OF COBALT MIX

Introduction

The cobalt mix for the carrying out of the control procedure is prepared wet from wheat grits and cobalt chloride hexahydrate. This ensures that the cobalt is well distributed over the cobalt mix and that the cobalt mix does not differ much with respect to its characteristics from the compound feed.

Ingredients

- a. wheat grits, well defined quality, as bearer
- b. cobalt chloride hexahydrate, minimum 99% pure
- c. water of at least mains water quality

Equipment

- a. mixing equipment, suitable for dry and wet products, for example the Nauta mixer with clump breaker
- b. equipment for spraying under pressure (compressed air)
- c. drying equipment with forced ventilation
- d. grinding equipment including a high-revolutions grinder
- e. sieving equipment.

Safety measures

When working with cobalt, especially when spraying, grinding and sieving, mouth and nose protection should be used and suitable gloves of synthetic material should be worn.

Preparation of the cobalt mix

The required amounts of cobalt chloride hexahydrate and wheat grits are weighed. The cobalt chloride hexahydrate is dissolved in about twice the amount of water. The mix is slightly warmed if necessary (max. 50 °C) until a clear solution is obtained. The solution is transferred into the pressure vessel of the spraying equipment. The weighed wheat grits are put into the mixer, the mixer is then started and the pressure vessel is put under pressure (c. 2 – 2.5 bar). The supply to the sprayer in the mixer is opened so that the solution is atomised. Once the cobalt chloride hexahydrate solution has been completely atomised, possibly in two or more steps depending on the volume of the pressure vessel, all the equipment which was used three times for the preparation of the cobalt solution and the atomisation must be flushed with a suitable amount of water. The wet cobalt mix is mixed for a further 15 minutes.

After this the mixer is emptied as much as possible and the mixture is dried for 24 hours at c. 60 °C dried?

The dried material is ground with a high-speed grinder (for example a pin crusher) and then sieved with a mesh of maximum 500 µm. The residue from the sieving can be ground again and sieved again with the same sieve.

That which falls through is put together, homogenised in a mixer and hermetically packed, preferably in a quantity which is suitable for immediate use in the testing procedure (i.e. 2 kg/ton).

The packaging states:

- a. the name of the product (cobalt mix)
- b. filled weight
- c. production date and batch and report number
- d. the nominal cobalt concentration
- e. the sequence number of the packaging in the batch
- f. safety measures.

Account must be taken of the fact that the dried cobalt mix is to some extent hygroscopic. It is advisable to work in a dry environment with the least possible exposure to air.

Sampling and reporting

A minimum of four samples are taken from each homogenised batch during the packaging of the cobalt mix. Two of these are intended for a moisture determination and one for the determination of the particle size distribution while at least one is kept as a reserve sample.

The report on the cobalt mix prepared in this way will contain at least:

- a. the origin and characterisation of the wheat grits
- b. the origin and purity of the cobalt chloride hexahydrate
- c. the quantity of carrier, cobalt salt and water used
- d. the average moisture content of the mix after homogenisation
- e. the calculated cobalt level of the cobalt mix
- f. the particle size distribution of the cobalt mix.

2.3 Testing procedure for carry-over in compound feed preparation using cobalt mixes

This chapter describes a number of alternative procedures for in-company measurement of carry-over using cobalt tracer. These are a simplification of the reference method described in Chapter 2.2

On the one hand it is a procedure in which the number of samples to be taken and analysed can be considerably reduced to that which is strictly necessary for a reliable measurement of carry-over. This particularly limits analysis costs. The company is of course free to take and analyse more samples in order to gain more insight into the process accuracy of the installation.

On the other hand, two procedures are involved in which the cobalt level is lowered by a factor 2 to 4 respectively. This limits the problems of responsible processing of the batch of feed to which the cobalt has been added. It also limits, however, the sensitivity of the method. Very low to relatively low carry-over levels (< 3%, resp. < 5%) are not properly measured with this.

For in-company measurement of carry-over with a reduced cobalt level use may be made of both the reference method specified in chapter 2.2 and the above-mentioned procedure with a reduced number of samples.

For the inspection procedures specified in both chapter 2.3.1 and chapter 2.3.2 a mix based on cobalt sulphate may be used instead of the cobalt mix defined in § 17 in chapter 2.2. The mix on the basis of cobalt sulphate should be prepared in accordance with the standard instructions in chapter 2.3.4.

2.3.1 Modification of the reference method with cobalt for the in-company measurement of carry-over of 1% and more in compound feed mixing (reduced number of samples).

Both the reference method (see chapter 2.2) and this modified procedure can be used to measure a carry-over of 1% or more in the preparation of mixed feeds. Essential in this is the minimum content of 5% cobalt in the cobalt mix to be used and the subsequent content of at least 100 ppm in the feed mix to which the cobalt mix is added.

This description indicates where and in what regard the reference method (chapter 2.2) may be modified for in-company measurement of carry-over. For the sake of simplicity the numbering of chapter 2.2 will be used. Parts of the reference method which are not mentioned remain unchanged in theory or only subject to minor, obvious amendments.

1. FIELD OF APPLICATION

This method is only intended for in-company measurement of carry-over.

2. EQUIPMENT AND TOOLS

At least 46 plastic pots of 50 ml with a lid or plastic sample bags of 1 litre are required.

3. TAKING AND HANDLING SAMPLES

3.1 Taking samples

The following schedule can be used when taking samples in which part of the sampling and/or further handling is voluntary if it is desired to obtain more insight.

- a. After the first batch (without added cobalt):
 1. at least 4 samples at the selected control point for carry-over. Preferably after the cooler for the determination of the natural cobalt level in the feed (KAC1 – KAC4)
 2. at least 4 samples at the same control point for the determination of the moisture level (VAC1 – VAC4).
- b. After the second batch (with added cobalt mix):
 1. at least 10 samples, as close as possible after the mixer and regularly distributed over the outflow of a batch for the determination of the average cobalt level (KBM1 – KBM10). Possibly (this is voluntary) 20 samples may be taken (see section 7.2.3)
 2. at least 4 samples at the same point for the determination of the moisture level (VBM1 – VBM4)
 3. possibly (this is voluntary) 10 samples at the specified control point(s) for carry-over for the determination of the average cobalt level (KBC1 – KBC10).
- c. After the third batch (carry-over batch)
 1. possibly (this is voluntary) 10 samples as close as possible after the mixer, regularly distributed over the outflow of a batch (KCM1 – KCM10)
 2. 20 samples at the specified control point(s) for carry-over, regularly distributed over the total duration of the batch at this point for the determination of the degree of carry-over (KCC1 – KCC20)
 3. at least 4 samples at the same point for the determination of the moisture level (VCC1 – VCC4).

3.2 Sample handling and destination

The technical sample handling (grinding, sequence, etc.) remains as described in chapter 2.2. The following applies with respect to the destination of the samples.

- a. All moisture samples have the function that the results of the cobalt analyses for differences in moisture content may be corrected or recalculated for dry substance.
- b. The samples KAC1 – KAC4 are analysed individually in duplicate. This is – especially for the third batch – of great importance because the cobalt levels in batches two and three must be corrected for the “natural” cobalt level in the feed.

- c. Samples KBM1 – KBM10 can serve two purposes. Each sample can if desired be split into a 'a' and a 'b' sample, or, if 20 samples are taken instead of 10 (see section 7.1.2), these can be used in turn or each can be split for one purpose or another.
- d. Possibly (this is voluntary), one half of the samples can now be used to determine the uniformity of the mix. To do this the 10 (or 20) samples must each be analysed separately in duplicate.
- e. A mix can be made of the other half of the 10 or 20 samples possibly after further reduction of the product which is used to determine the average cobalt level of the second batch. To do this at least two new samples are taken from the mix in which the cobalt level and the moisture level are analysed in duplicate. Naturally, the average cobalt level of batch two may also be determined by averaging the individual duplicate results of the 10 or 20 samples.
- f. Using samples KBC1 – KBC190 an impression can be obtained (this is voluntary) of the extent to which the uniformity obtained immediately after mixing (KBM1 – KBM10) in the subsequent production and transport process is maintained up to the control point for carry-over. These samples must each be separately analysed in duplicate.
- g. Using samples KCM1 – KCM10 a determination may possibly be made (this is voluntary) the extent to which carry-over is already occurring in the path up to the sampling point immediately after the mixer. For the analysis a choice can be made to analyse a mix sample (analysis of two samples in duplicate for the average carry-over), or of all ten samples separately in duplicate (carry-over pattern and calculation of the average).
- h. The samples KCC1 – KCC20 may be mixed two at a time, thus KCC1 + 2, KCC3 + 4 etc., after which in each of the 10 new samples in duplicate the cobalt level is determined. Assuming that each of the original samples is representative for an equivalent part of the batch, the average carry-over can be directly calculated. If it is known that this is not the case, for example because of irregular time intervals between sampling, the weighted average, related to the actual time intervals, is calculated.
- i. It may also be decided to analyse each of the samples KCC1 – KCC separately and then to calculate the average as described above.

4. PROCESSING OF THE RESULTS

4.1 Variance analysis

In this simplified implementation the results are only suitable to a limited extent for statistical analysis.

In as far as there are measurement series with analyses carried out in duplicate, it is advisable in any event to calculate via a variation analysis the empiric coefficient of variation between repetitions per measurement series.

In as far as there are measurement series for which in an ideal case the results must have the same value (uniformity), an analysis of variance must be carried out with which both the empiric coefficients of variation between samples as well as between repetitions is calculated.

This applies in particular to the sample series KAC1, KAC4 and possibly for KBM1 – KBM10, KCB1 – KBC10 and KCM1 – KCM10 in as far as one takes samples

from these series, individually analyses them and is interested in the degree of uniformity.

4.2 Calculation of carry-over

All cobalt levels are corrected in advance using the average results of the corresponding moisture determinations for dry substance. The carry-over for the installation is now calculated as follows on the basis of the corrected values:

the average cobalt level in the 20 samples KCC from the third batch minus the average cobalt level in the 4 samples KAC from the first batch, divided by the average cobalt level from the 10 samples KBM from the second batch, also minus the average cobalt level in the 4 samples KAC from the first batch. By multiplying the result by 100 an average percentage carry-over in the batch immediately following the batch to which the cobalt mix was added as a model for a premix with additive can be calculated.

By displaying the results of the cobalt analyses in the samples KCC1 – KCC20 (corrected for the average of KAC1 – KAC4) in graphic form, a carry-over pattern is obtained which gives in principle more information than the calculated average.

4.2.1 Modification of the measurement methods with cobalt for the in-company measurement of carry-over of 3% and more in compound feed mixing

For the in-company measurement of carry-over of 3% or more, either the testing procedure described in chapter 2.2 or the modified procedure described in chapter 2.3.1 is used. Use is made of a cobalt content in the cobalt mix as specified in section 2 of chapter 2.2 of minimum 2.5%. This realises a level of about 50 mg/kg in the second batch of feed in the testing procedure.

4.2.2 Modification of the measurement methods with cobalt for the in-company measurement of carry-over of 5% and more in compound feed handling

For the in-company measurement of carry-over of 5% or more, either the testing procedure described in chapter 2.2 or the modified procedure described above in chapter 2.3.1 is used. Use is made of a cobalt content in the cobalt mix as specified in section 2 of chapter 2.2 of minimum 1.25%. This realises a level of about 25 mg/kg in the second batch of feed in the testing procedure.

Literature

1. Beumer, H.; Nieman, W.. Toetsingsprocedure procesnauwkeurigheid met behulp van kobalt. Consequenties van een lager kobaltniveau. CKD werkgroep Toetsingsprocedure procesnauwkeurigheid May 1992, ref. 630.95/0168/Bm-Hb.

4.2.3 Standard instruction for the preparation of a cobalt sulphate mix for the in-company measurement of carry-over

Introduction

The cobalt mix for the carrying out of the testing procedure is prepared via dry mixing from wheat grits, wheat red dog and cobalt sulphate. This ensures that the cobalt is well distributed over the cobalt mix and that the cobalt mix does not differ much with respect to its characteristics from the compound feed.

Ingredients

- a. wheat grits and wheat red dog, well defined quality, as bearer

- b. cobalt sulphate heptahydrate, minimum 98% pure

Equipment

mixing equipment, suitable for dry products, such as Planet mixer.

Also needed as tools are, among others, suitable balances for weighing the ingredients.

Safety measures

When working with cobalt, mouth and nose protection should be used and suitable gloves of synthetic material should be worn.

Preparation of the cobalt mix

The required amounts of cobalt sulphate heptahydrate, wheat grits and wheat red dog are weighed.

The weighed quantities are mixed in a Planet mixer for 15 minutes. The mix is then measured into buckets of 2.0 kg and properly closed off with a lid.

The packaging states:

- a. name and code of the product (cobalt mix)
- b. filled weight in kg
- c. date of production
- d. the nominal cobalt concentration
- e. the sequence number of the packaging in the batch
- f. safety measures.

The closed buckets should be stored under air-conditioned conditions. Open the packaging immediately before use.

The cobalt mix should comply with the following requirements:

- a. particle size: maximum 1% > 0.7 mm; maximum 10% > 0.5 mm
- b. cobalt level: at least 4.5%

Sampling and reporting

Four 4 samples are taken from each homogenised batch during the packaging of the cobalt mix. Of these 1 is intended for moisture determination, 1 for the determination of particle size distribution and 1 for the determination of cobalt, while 1 is kept as a reserve sample.

The report on the cobalt mix prepared in this way will contain at least:

- a. the origin and characterisation of the wheat grits
- b. the origin and characterisation of the wheat red dog
- c. the origin of the cobalt sulphate heptahydrate
- d. the amount of carrier and cobalt salt used
- e. the moisture content of the mix after homogenisation
- f. the calculated cobalt level of the cobalt mix
- g. the analysed cobalt level of the cobalt mix
- h. the particle size distribution of the cobalt mix.

2.4 Testing procedure for the carry-over in compound feed mixing using a mix of manganate and a protein-rich and a protein-poor mix

1. APPLICATION AREA

The testing procedure was developed for the determination of the carry-over which occurs in compound feed production companies. The carry-over of large components from the batching equipment for raw materials and the carry-over of the components which are added via the premixes are determined separately.

By collecting the samples which have been taken for the carry-over inspection at various different places in the production process, insight can be obtained into the carry-over in components of the production process (for example: grinding / mixing line to pressed meal bunker or the press / cooling line). The method is also suitable for the determination of the extent to which uniform mixes can be produced using the installation (see item 9).

2. DEFINITIONS

Carry-over

Carry-over means that part of the previous batch of feed remains in the production and transport system and gets into the following batches.

Carry-over level

The carry-over level is defined as the amount of a nutrient or component from a previous batch, expressed as a percentage, which gets into the following batch of feed (of the same size). The carry-over level can be measured for a section of the installation (for example the pressed meal bunkers) or for the whole installation.

3. PRINCIPLE OF THE TESTING PROCEDURE

The testing procedure is carried out by first fabricating a protein and Mn-rich Soya mix and immediately afterwards by fabricating a protein and Mn-poor mix on the same production line. The increase in the protein and Mn level of the maize mix during the running of the production line is caused by carry-over. By relating this increase to the protein and Mn level of the Soya mix, the carry-over level can be calculated.

Because the protein and manganese content of the maize mix progresses hyperbolically (from high levels at the beginning of the flow to lower levels afterwards), the sampling procedure must be given particular attention.

4. EQUIPMENT AND TOOLS

The following are required for the carrying out of the testing procedure:

- a. a quantity of manganese oxide corresponding to 0.4% of the usual batch size
- b. (possibly) a scoop for taking samples
- c. two buckets to be able to collect a number of sub-samples
- d. sample pots or bags which can hold at least 200 grams of material. If the carry-over inspection is carried out at two places in the production line then 20 sample pots will usually be enough (only 14 samples will actually be tested).

5. COMPANY DETAILS REQUIRED

The following must be known about the company where the testing procedure will be carried out:

- a. the block diagram of the production installation
- b. the way in which the Soya and maize mix is put together. An exact indication should in particular be given of how and where the manganese oxide is added and how any transport system for the manganese oxide to the mixer is flushed both for the Soya mix and for the maize mix.

6. IMPLEMENTATION OF THE TESTING PROCEDURE

6.1.a. Fabrication of the protein and Mn-rich Soya mix

The Soya mix (with the usual batch size) consists of 92% Soya meal, 4% fat, 3% cane molasses, 0.4% manganese oxide and 0.8% dicalcium phosphate (or chalk or salt). This mixture is batched, ground, mixed and pelletised in the usual way. Molasses and fat are added to obtain a meal with normal physical characteristics which can be pelletised properly. The Soya meal may come from more than one batching silo.

The manganese oxide comes instead of the premix and should take the same route as the premix. The manganese oxide is therefore batched into the premix weighing machine or dumping pit.

The batching should be carried out such that the manganese oxide comes virtually fully to the bottom of the premix weighing machine or dumping pit.

The manganese oxide should comply with the following requirements:

- a. Mn level at least 50%
- b. particle size: 100% should be smaller than 0.2 mm.

Normally, chalk, salt and/or feed phosphate is batched via the same weighing machine or dump pit. Because of this the carry-over of components from the premix will be less especially when first the premix and only then the other products are batched.

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

Once the content of the premix weighing machine (or the dumping pit) has been added to the Soya mix in the mixer, the normal mixing time is carried out. The mix is then removed to an empty pressed meal bunker and pelletised (sample).

The grinding/mixing line and the press/cooling line may not be used for anything other than the maize mix after the Soya mix.

6.1.b. Sampling of the Soya mix

When unloading the Soya pellets in the finished product silo a good mix sample is taken from the last part of the batch.

6.2.a. Fabrication of the protein and Mn-poor maize mix

The maize mix (with the same batch size as the Soya mix) consists of 92% maize, 4% fat, 3% cane molasses and 0.8% dicalcium phosphate (or chalk or salt). If it is not possible to batch 92% maize then a maize/wheat mix or another protein-poor mix may be put together (sample).

The transport system between the premix weighing machine (or dumping pit) and the mixer is flushed with 0.8% dicalcium phosphate (or salt or chalk).

The mixing time starts once the feed phosphate has been added to the mix. The mix is then removed to the (empty) pressed meal bunker (sample) and then pelletised (sample).

6.2.b. Sampling of the maize mix

The following samples of the maize mix are collected:

- a. the maize (and possibly the wheat) which is used for the composition of the mix
- b. six samples from the maize mix at the inflow to the pressed meal bunker
- c. six samples from the maize mix at the inflow to the final product silo.

The sampling procedure is important for the samples in II and III. In particular the first part of the meal or the pellets from the batch will have higher levels of protein and manganese which will then decrease relatively quickly to a lower and more constant level. It is therefore important to sample the first part of the meal or pellet flow intensively and to know to which part of the feed these samples relate.

The sampling procedure at the inflow to the pressed meal bunker (which usually lasts 3 to 5 minutes) is as follows:

- a. during the first 30 seconds as many sub-samples as possible are collected in a bucket; a mix sample is made from these
- b. for the second 30 seconds: idem
- c. then every 30 seconds a random sample from the flow is collected until the meal flow stops.

The total running time of the meal flow is noted and 6 samples are kept, namely the three which were taken first and three of the other samples.

The sampling of the pellets at the inflow to the finished product silo takes place in the same way. Because the total duration is usually somewhat longer the procedure is now as follows:

- a. during the first minute as many sub-samples as possible are collected in a bucket; a mix sample is made from these
- b. during the second minute: idem
- c. then every minute a random sample from the flow is collected until the pellet flow stops.
- d. (If the pellet flow is not continuous then the "real" duration should be used.)

Note the total duration here as well and keep six samples, namely the three which were taken first and three of the other samples.

6.3 Processing of the Soya mix in compound feed

At low carry-over levels the Soya mix has a Mn level of c. 2,000 mg/kg. In the processing of this Soya mix in compound feed account should be taken of the fact that the Mn level of compound feed may be a maximum of 250 mg/kg.

7. THE ANALYSIS OF THE SAMPLES

In total there are 14 (or possibly 15) samples collected:

- 1 sample of Soya pellets (+ Mn) = A
- 1 sample of maize (pure) (+ possible wheat) = B
- 6 samples of maize mix meal (pressed meal bunker) = C (1 to 6)
- 6 samples of maize mix meal (finished product silo) = D (1 to 6)

All samples are analysed for RE and Mn.

Half of the samples of maize meal mix and maize mix pellets are analysed for moisture; this is in order to find out whether the moisture content has changed during pelletising. If the moisture content has clearly changed during pelletising then the RE and Mn levels of the maize mix pellets should be corrected for the moisture content of the maize mix meal.

8. THE CALCULATION OF THE CARRY-OVER PERCENTAGES

The carry-over percentages can be calculated from the levels of RE and Mn in the samples taken. Suppose that the following levels are found:

Soya pellets: 420 grams RE and 2,006 mg Mn/kg

Pure maize: 86 grams RE and 4 mg Mn/kg

samples maize mix (above the pressed meal bunker)

1.	mix sample (0.5 min.)	160 grams RE and	400 mg Mn/kg
2.	mix sample (0.5 min.)	100 grams RE and	60 mg Mn/kg
3.	random sample	90 gram	and 27 mg
4.	random sample	85 grams (avg. 88)	and 30 mg (avg. 28)
5.	random sample	88 gram	and 28 mg
6.	random sample	89 gram	and 27 mg

The total duration of the meal flow in the pressed meal bunker = 5.5 min.

Expected levels of maize mix (92% maize and 3% molasses with 40 grams RE and 25 mg Mn/kg):

$$\begin{aligned} \text{RE} &= 0,92^* 86 + 0,03^* 40 = 80,3 \text{ gram/kg} \\ \text{Mn} &= 0,92^* 4 + 0,03^* 25 = 4,4 \text{ mg/kg} \end{aligned}$$

The average levels of RE and Mn in the maize mix are calculated as follows:

$$\begin{aligned} \text{RE} &= 0,5/5,5^* 160 + 0,5/5,5^* 100 + 4,5/5,5^* 88 = 95.6 \text{ grams/kg} \\ \text{Mn} &= 0,5/5,5^* 400 + 0,5/5,5^* 60 + 4,5/5,5^* 28 = 64.7 \text{ mg/kg} \end{aligned}$$

(samples 1 and 2 each have a duration of 0.5 minutes from a total duration of 5.5 minutes.

For samples 3 to 6 the average level is calculated; the duration of this is $5.5 - 2 \times 0.5 = 4.5$ minutes).

The carry-over percentage (Vs-%) is now calculated as follows:

$$\text{Vs-\%} = \frac{\text{avg. level in maize mix} - \text{expected level in maize mix}}{\text{avg. level in Soya pellets} - \text{expected level in maize mix}} \times 100$$

The carry-over percentages are then (up to the pressed meal bunker)

$$\text{for RE} = \frac{95,6 - 80,3}{420 - 80,3} \times \frac{1.530}{339,7} \times 100 = \frac{15,3}{339,7} = 4.5\%$$

$$\text{and for Mn} = \frac{64,7 - 4,4}{2.006 - 4,4} \times \frac{6.030}{2.001,6} \times 100 = \frac{60,3}{2.001,6} = 3\%$$

The carry-over percentages at the inflow to the finished product cell are calculated in the same way.

The carry-over percentage of the RE relates to the feed as such, from the batching equipment.

The carry-over percentage for the Mn gives an indication of the carry-over of components from the premix.

9. THE MEASUREMENT OF UNIFORMITY

In order to determine the extent to which the installation produces uniform mixes, at least 10 samples should be collected from the Mn-rich Soya mix and analysed for Mn. The spread of the Mn levels of these samples (standard deviation or the difference between the highest and lowest value) is a measure of uniformity.

When taking the samples from the Soya mix one should ensure that the whole flow of the mix is sampled. Because it is often not known exactly how long the meal flow will last, it is desirable in the first instance to take a generous number of samples of which only a part (namely 10) need to be tested.

The uniformity test may be carried out at many places in the installation. If the samples are taken immediately after the mixer then a good picture is obtained of the functioning of the mixer.

If, on the other hand, samples are taken at other places in the installation (but after the mixer) then the uniformity will generally be less than immediately after the mixer.

This is because in this case de-mixing and carry over also play a role. Because the Mn-rich Soya mix is always produced after a "normal" compound feed with much lower Mn levels, the first samples of the Soya mix will be contaminated with a certain amount of compound feed and will therefore contain less Mn. The subsequent samples will be contaminated with less and less normal compound feed and will have higher and higher Mn levels.

10. ERRORS DISCUSSION

Table 1 shows which Mn and protein levels are to be expected in the maize mix at the various carry-over percentages, assuming 80 grams RE and 5 mg Mn/kg maize mix (pure) and 400 gram RE and 1,800 mg Mn/kg Soya mix.

Table 1 Effect of carry-over percentage on Mn and protein level of the maize mix.						
Carry-over %	0	1	3	5	10	15
MN from basis*	5	5	5	5	5	5
From Soya	0	18	54	92	180	270
	5	23	59	95	185	275
* effect of thinning discounted						
RE from basis	80	79,2	77,6	76	72	68
From Soya	0	4	12	20	40	60
	80	83,2	89,6	96	112	128

On the basis of the analysis accuracy of the Mn and RE determination an estimate can be made of the accuracy with which the carry-over percentage can be determined.

For the 6 maize samples to be tested it is assumed that the average Mn-level found in 95% of the cases will lie between 95 and 105% of the actual level; for levels < 60 mg/kg the absolute interval is made equal to the interval for 60 mg/kg, thus +/- 3 mg/kg.

For the Soya mix it is assumed that the Mn level found in the analysis will deviate by a maximum of 100 mg/kg from the actual level.

For the protein it is assumed that the average level found for the 6 maize samples will in 95% of cases lie between 99 and 101% of the actual level and that the level found for the Soya mix will deviate by a maximum of 2% from the actual level.

The results of the calculations are shown in Table 2.

It may be concluded that low carry-over percentages can be determined fairly reliably. For low carry-over levels Mn seems to comply better than the RE; at high carry-over levels, on the other hand, the protein gives better results than the Mn.

Table 2: Effect of the analysis accuracy on the carry-over percentage to be established				
		Maize mix		
Carry-over level		Calculated	Interval analysis	Carry-over percentage*
Mn	0	5 mg/kg	2 - 8 mg/kg	0,16 - 0,18%
	1	23	20 - 26	0,8 - 1,2
	3	59	56 - 62	2,7 - 3,4
	5	95	90 - 100	4,5 - 5,6
	10	185	176 - 194	9 - 11,1
	15	275	261 - 289	13,5 - 16,7
On the basis of 1800 mg Mn/kg Soya mix (variation 1700-1900, at low Mn in maize there is a calculation of high Mn in Soya, and vice versa).				
		Calculated	Interval analysis	Carry-over %*
RE	0	80 g/kg	79.2 - 80.8 g/kg	- 0,25 - 0,25
	1	83,2	82,4 - 84,0	0,7 - 1,3
	3	89,6	88,7 - 90,5	2,6 - 3,4
	5	96	95,0 - 97,0	4,5 - 5,5
	10	112	110,9 - 113,1	9,4 - 10,6
	15	128	126,7 - 129,3	14,2 - 15,8
On the basis of 400 g RE/kg Soya mix (variation 392-408, at low RE in maize there is a calculation of high RE in Soya, and vice versa).				

2.5 Testing procedure for the measurement of carry-over in premix and additives installations

1. SYSTEM

The method of measurement of carry-over in premix and additives installations corresponds as far as the systematics are concerned to Chapters 2.2 to 2.4.

2. CARRY-OVER PROCESS

- a. The carry-over process to be measured relates to the point where the additives and/or animal veterinary products are added to the bulk vehicle load or the bag filling.
- b. Measurement of the carry-over should be carried out for each production line in the installation.
- c. The measurement should be carried out with a quantity of mix which is equal to the smallest batch which in practice may be produced on the production line in question.

3. TRACER SUBSTANCE TO BE USED

The following tracer substance can be used for the measurement of carry-over: cobalt mixes in accordance with Chapter 2.2 or 2.3.4 with a cobalt concentration of at least 200 mg/kg. At cobalt concentrations of 2,000 mg/kg or more use may also be made of pure cobalt sulphate. In addition the microtracers FSS-Lake and F-Lake and methyl violet can be used in the dosage of 10 mg/kg. Otherwise there should be compliance with Chapter 2.3.4.

4. DETERMINATION OF CARRY-OVER

The measurement of carry-over is done by taking the mix in which the carry-over occurs into consideration as a whole. This means that the average level in this mix is the departure point for determining the carry-over. The carry-over is measured as follows:

- a. mix the whole mix again
- b. take and analyse 5 samples from this mix (V1 to V6). The average level is calculated from this
- c. The carry-over is measured as follows:

$$\frac{\text{(average quantity in mix in which carry-over occurs)}}{\text{(batching in previous mix from which there is carry-over)}} \times 100\%$$

2.6 Checking procedure for the process accuracy of compound feed with micro tracers

1. FIELD OF APPLICATION

This testing procedure or method for the determining of the homogeneity of meals and grains may be used on the usual premixes and mixes of ground compound feed raw materials in compound feed companies.

The method can also be used to obtain an indication of the carry-over percentage which occurs in compound feed raw materials.

2. DEFINITIONS

Production plant: A production plant is an installation which is suitable for the preparation of compound feeds.

Microtracer mix: For the testing of a compound feed the microtracer mix contains 4 kg feed lime or wheat grits and 100 g microtracer. Therefore 100 g microtracer is mixed with 1 t compound feed, which corresponds to a mixing accuracy of 1:10 000. For the testing of a premix the microtracer mix contains 4 kg feed lime or wheat grits and 10 g microtracer. Therefore 10 g microtracer is mixed with 1 t compound feed, which corresponds to a mixing accuracy of 1:100 000.

3. PRINCIPLE

So-called microtracers are used as a measuring substance. These are elementary iron particles which are coated with a feed colourant in order to be able to count the colour points in the analysis. An average number of particles per mg is indicated in the analysis certificate for the microtracer used. For the microtracer particles it is a case of particle distribution thus the average number of particles varies depending on the microtracer batch. In order to determine the number of particles in question in the test a microtracer mix is produced in which the average number of particles is determined exactly for the microtracer used (see section 17).

Two different microtracers are suitable for the homogeneity and carry-over analysis. These are distinguished by their particle size and therefore the number of particles per mg. Microtracer F consists of particles with a size distribution of 150 – 300 µm and have been used for some time in the feed industry. The somewhat finer microtracer FSS with a size distribution of 75 – 150 µm was specially developed for chicken feeds to decrease the test quantity used.

The required accuracy for the determination of carry-over of 1% is achieved in both microtracer F and FSS. In order to achieve a statistically accurate assessment, a minimum number of 15 particles must be present per filter. Only then can an accurate assessment of homogeneity be made for the first production batch.

Method	Average number of particles per milligram [mg]	Test quantity for the assessment of homogeneity [g]	Average expected number of particles in the tested quantity	Test quantity for determination of carry-over [g]	Accuracy of the carry-over examination in %	Average expected number of particles in the tested quantity
FSS-Lake 100 ppm	200	2	40	200	1	40
F-Lake 100 ppm	25	20	50	2000	1	50
FSS-Lake 10 ppm	200	25	50	2500	1	50

Table 1:

The control procedure for the determination of the degree of homogeneity of meal mixes in the preparation of compound feeds makes use of a microtrace mix which, with respect to its properties, can replace the usual compound feed additives.

The control procedure includes the processing of two batches from the same feed mix. The microtrace mix (see section 2) is added to the first batch. The number of particles of microtracer in the samples of meal and grain from the first batch of feed is then determined. The second production batch consists of the bare feed without the microtracer mix. The microtracer level of the meal and grain samples from this batch is also determined. This level gives a picture of the carry-over which is taking place in the production installation.

The number of particles of microtracer in the samples which were taken is determined by separating the microtracer particles from the other feed particles using a rotary detector and by using the feed colourant to make the separate microtracer particles visible on a sheet of filter paper.

4. EQUIPMENT AND TOOLS

The following are required for the carrying out of the testing procedure:

- a. 40 plastic sample bags for keeping the samples of meal and grain each with a capacity of twice the quantity of the sample (see table 2)
- b. 40 plastic sample bags for keeping the samples of meal and grain each with a capacity of twice the quantity of the sample (see table 2)
- c. one small and one large plastic scoop for taking the samples.

The number of bags specified is required if production plant samples of meal are taken at one point in the production installation and samples of grains are taken at another point. For each subsequent sampling point 40 bags extra are needed.

Method	Sample quantity to be taken from production batch 1 for the determination of homogeneity	Sample quantity to be taken from production batch 2 for the determination of carry-over
FSS-Lake 100 ppm	≥ 4 g	≥ 400 g
F-Lake 100 ppm	≥ 40 g	≥ 4,000 g
FSS-Lake 10 ppm	≥ 50 g	≥ 5,000 g

Table 2:

A laboratory must be available where microtracer analyses can be done. Appointments should be made in good time with this laboratory for analyses to be carried soon after the samples are taken.

5. COMPANY DETAILS REQUIRED

The following will be requested in advance from a compound feed company at which a control procedure is to be carried out:

- a. a block diagram of the production installation in which it can be indicated during the implementation where the microtracer mix has been added and where samples are taken.

The following will be requested during the implementation of the control procedure:

- b. the computer prints or copies of them which show:
 1. the composition of the feed mix
 2. the batch weight requested by the computer, and
 3. the actual batch weight
 4. or, if there is no computerisation:
 5. the composition of the feed mix
 6. the calculated batch weight This weight is obtained by adding the weights of the components
 7. the read-out of the actual batch weight.

The following will be requested to be able to calculate the batch weight for the mixer and the grain press:

- c. where and how much molasses, vinasse and other liquid ingredients added to the main flow of the feed, and
- d. where and how much fats, etc., are added to the main flow. The requested addition points are shown in the block diagram.

6. ADDITION OF THE MICROTRACER MIX

The microtrace mix (see section 2) is added to the first batch. The place where the microtrace mix is added depends on the carry-over path to be measured (see 7.1). The place where the addition of the microtrace mix must be done should be indicated in the block diagram for the production plant.

The place where the addition of the microtrace mix must be done should be indicated in the block diagram for the production plant. The batch weight requested by the process computer may be assumed.

7. TAKING AND HANDLING SAMPLES

7.1 Analysis samples

7.1.1 Taking the samples

During the implementation of the control procedure in a compound feed company samples are taken at locations agreed in advance:

- a. after the mixer but as close as possible to the mixer (see section 13.1)
- b. from the entrance to the finished product silo in the event of meal production or a pressed meal silo
- c. from the entrance to the finished product silo in the event of grain production
- d. another desired end point for the determination of the relevant carry-over path

If the meal or grain flow is not reachable at the desired locations then suitable openings should be made in consultation with the company.

Twenty samples are taken each time per sampling point. The statistical certainty is increased through the rise in the number of samples. The increase in the number of samples from 30 to 40 is, however, voluntary.

Meal production

From the first batch 20 samples of meal (immediately after the mixer) and 20 samples of meal (from the input to the finished product silo) are taken for the micro-tracer analysis (the sample quantity to be taken, see table 2).

From the second batch 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the micro-tracer analysis (the sample quantity to be taken, see table 2).

Grain production

From the first batch 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the micro-tracer analysis.

From the second batch of feed 20 samples of meal (immediately after the mixer) and 20 samples of grain (from the input to the finished product silo) are taken for the microtracer analysis (the sample quantity to be taken, see table 2).

If a split is desired with respect to the carry-over by the dosage/grinding/mixing line on the one hand and the press line on the other hand then during the first and second batches another 20 samples of meal for microtracer determination should be taken at the input to the pressed meal silo. The method of working is identical to the method for meal production.

Sample bags

All sample bags are provided with a sample code before the start of the production of the first batch of feed. The sample bags must be filled up to the edge and sealed air-tight to avoid de-mixing (in the case of meal samples) as much as possible.

Sampling

- a. Production batch: Once the meal and/or grains flow starts for the batch to be inspected then 20 samples of meal and 20 samples of grains are taken spread as well as possible over the duration of the batch.
- b. Production batch: Due to the irregular distribution to be expected of the microtracer particles in the carry-over batch (in the beginning very high numbers of microtracer particles and at the end very low numbers of microtracer particles) the sampling is done in a different way. The first three samples are continuously collected in a large collection container. The first sample represents the sampling time from 0 to 0.5 min, the second sample 0.5 to 1.0 minutes and the third sample 1.0 to 1.5 minutes in the feed flow. A sample is taken from each of these three collection samples via sampling splitting (quartering method). The other samples are taken as random samples every 0.5 minutes. For a total duration of the feed of 10 minutes there will be 20 samples collected of which the first three are collective samples and the other 17 are individual samples. For lesser durations the sampling intervals must be modified accordingly.

N. B.: It is very important that the samples are taken spread as well as possible over the duration of the batch in connection with the samples being representative of the batch as a whole.

7.1.2 Preparation of the samples

Each meal and grain sample is ground in a suitable grinder.

First grind the samples of meal and grain from the second batch (carry-over batch) and then those from the first batch. This ensures that the samples are ground in ascending order of their microtracer level.

Clean the grinder after each sample using compressed air.

Clean the grinder after each group of 20 samples using both compressed air and, after disassembly of the relevant parts, by brushing clean with a brush which is not too soft. There may be no carry-over of material from the previous group of samples.

Homogenise each grinding as much as possible and then place it back in the original bag.

7.1.3 Storage of analysis samples

Analysis samples which will not be tested within a week of being taken should be stored dry.

7.2 Analysis of samples

The sample packaging may not be opened during this period (see 13.2).

Homogenise the mix to be inspected in the sample bag as much as possible by stirring it with a spoon or spatula.

A sample of the desired size is taken from the sample to be analysed and subjected to a microtracer analysis.

7.3 Archiving

The filters with the colour points from the individual microtracer particles must be archived. A minimum archiving period of 1 year is suitable. The filter sheets can, however, be retained for more than 10 years.

8. DETERMINATION OF THE MICROTRACER PARTICLES

The microtracer particles from a sample are isolated because of their magnetic properties by way of filtering through a rotary detector with a rotary magnet. Other magnetic particles are also filtered out at the same time. The identification of the microtracer particles takes place by way of a bonding colouring agent which causes a chromatographic effect (= colour point) on a filter sheet after treatment with a developer. In order to make the colour points visible the filter is dampened with the developer, the microtracer particles are transferred quantitatively to the filter sheet and the colour development is stopped by then laying the filter sheet on a heated plate.

Other magnetic particles do not develop colour points and are removed from the filter sheet with a brush. The colour points developed on the filter sheet are counted. The microtracer level is indicated as the number of particles per gram of sample.

9. PROCESSING OF THE RESULTS

9.1 Non-standard results

After the addition of the microtracer mix to the feed in the first batch the microtracer level in the first samples to be taken will be lower than in the subsequent samples. This is because of a degree of carry-over of bare feed from the feed batch prior to the batch with microtracer.

An opposite effect is seen in the samples from the second batch of feed. Now the first samples show a relatively high microtracer level as a result of carry-over of feed containing microtracer from the second to the third batch. Normally the spread of the microtracer levels in the samples from the third batch is considerably more distorted than in the second batch. There is also no calculation of a probability for homogeneity and it is enough to make a graph of the average microtracer level per sample against the sample number. In as far as the samples are properly representative for the whole batch which means they have been properly spread over the total duration, the average carry-over of microtracer can be calculated as a percentage of the microtracer level in batch one.

9.2 The carry-over

The carry-over for the installation is calculated as follows in accordance with this control procedure per measurement point.

The average microtracer level of the analysis samples from the second batch divided by the average microtracer level on the basis of dry matter from the analysis samples from the second batch. By multiplying this figure by 100 the average carry-over percentage can be calculated.

9.3 The test for homogeneity

The following statistical data will be determined for the evaluation:

- average number of particles
- standard deviation for the number of particles
- χ^2 (chi squared) – value
- Probability p in % as an indication of the homogeneity
- Microtracer recovery percentage in %.

The probability is determined using the determined chi squared value and the number of degrees of freedom (see table 3). Values between 0.999 and < 0.0005 can be found. The assessment of the homogeneity is recorded by definition. The probability is calculated using an Excel table.

χ^2	1	2	3	4	5	6	7	8	9
1	.317	.607	.801	.910	.963	.986	.995	.998	.999
2	.157	.368	.572	.736	.849	.920	.960	.981	.991
3	.083	.223	.392	.558	.700	.809	.885	.934	.964
4	.046	.135	.261	.406	.549	.677	.780	.857	.911
5	.025	.082	.172	.287	.416	.544	.660	.758	.834
6	.014	.050	.112	.199	.306	.423	.540	.647	.740
7	.008	.030	.072	.136	.221	.321	.429	.537	.637
8	.005	.018	.046	.092	.156	.238	.333	.433	.534
9	.003	.011	.029	.061	.109	.174	.253	.342	.437
10	.002	.007	.019	.040	.075	.125	.189	.265	.350
11	.001	.004	.012	.027	.051	.088	.139	.202	.276
12	.001	.002	.007	.017	.035	.062	.101	.151	.213
13	**	.002	.005	.011	.023	.043	.072	.112	.163
14	**	.001	.003	.007	.016	.030	.051	.082	.122
15	**	.001	.002	.005	.010	.020	.036	.059	.091

Table 3: Table for the determination of probability, horizontal: number of degrees of freedom, vertical: chi squared values

10. REPORTING

The following is reported for each group of feed samples:

- For the calculation of the homogeneity of the first batch of compound feed, the average number of microtracer particles in whole numbers
- For the calculation of the homogeneity of the first batch of compound feed, the number of degrees of freedom of the system Number of analysed samples n-1
- for the calculation of the homogeneity in the first batch of compound feed, the chi squared value (calculated from the empiric coefficient of variation for the analysed samples times the number of data divided by the average number of particles in the analysed samples)

- d. from the number of degrees of freedom and the chi squared value, the probability as a percentage of the analysed samples $[(\text{Chiwert (chi squared; degree of freedom)} \times 100) \times 100]$
- e. the calculated recovery percentage of the microtracer particles in the first batch of feed in relation to the number of microtracer particles in the added microtracer mix
- f. The calculated carry-over in the installation from the number of microtracer particles in the second batch of feed in relation to the number of microtracer particles in the first batch

11. ASSESSMENT OF THE RESULTS

Homogeneity of the material

The calculated probability as a percentage is a measure for the homogeneity of the meal mix or grains in question from which the samples were taken. The probability indicates how probable it is that the tested sampled corresponds to a perfect mix.

If the value found in the test is identical with a probability of more than 5 % (0.05) then it may be assumed on the basis of the probability calculation that there is a "perfect mix".

If the value found in the test is identical with a probability of between 1% and 5% (0.01 to 0.05) then it may be assumed on the basis of the probability calculation that there is a "probable significant deviation from a perfect mix". This refers to a borderline case about which no unambiguous statement can be made. The test must be repeated.

If the value found in the test is identical with a probability of less than 1% then it may be assumed on the basis of the probability calculation that there is a "probable significant deviation from a perfect mix".

A key feature of the poisson distribution is that when there is a "perfect mix" the standard deviation of a test series must be (on average) equal to the square root of the average.

Two examples follow of the calculation of a homogenous and a non-homogenous mix.

Example 1: Homogeneous mix

Sample number	Number of particles counted, x	Average m	Difference $x_n - d_n$	Square of difference d_n^2
1	47	50	3	9
2	53	50	3	9
3	45	50	5	25
4	55	50	5	25
5	50	50	0	0
Average x=50			Sum $d_n^2 = S = 68$	

Table 4: Example of the calculation for a homogenous mix

Number of samples: $n=5$
 Chi squared value χ^2 : $S: x = 1$ ($68: 50 = 1.4$)
 Table values from table 3:
 horizontal: $n - 1 = 4$
 vertical: 1
 calculated probability: 0.910
 calculated probability in %: 91.0%

Result: The calculated probability is greater than 5 %; there is therefore a homogenous mix.

Example 2: Non-homogenous mix

Sample number	Number of particles counted, x	Average m	Difference $x_n - d_n$	Square of difference d_n^2
1	43	53	10	100
2	57	53	4	16
3	70	53	17	289
4	35	53	18	324
5	61	53	8	64
Average $x=53$			Sum $d_n^2=S=793$	

Table 5: Example of the calculation for a non-homogenous mix

Number of samples: $n=5$
 Chi squared value χ^2 : $S: x = 15$ ($793: 53 = 15$)
 Table values from table 3:
 horizontal: $n - 1 = 4$
 vertical: 15
 calculated probability: 0.005
 calculated probability in %: 0.5%

Result: The calculated probability is less than 1 %; there is therefore a non-homogenous mix.

12. NOTES

12.1 First sampling point

A feed mix is not homogenous after the dosage of the various components. Even after the grinding of the raw materials in the hammer mill this is only partly the case. Often finer raw materials are led around the hammer mill and carried straight to the mixer. A homogenous feed mix may therefore only be expected for the first time in the mixer. Taking samples directly from the mixer is difficult and may be dangerous and is certainly not recommended. The sampling should therefore be done after the mixer. In most companies this will be the outflow of the bunker under the mixer.

12.2 Storage of the samples

Samples which can not be examined in the short term should be stored in a dry area to retain sufficient free-flow for the test.

13. SAFETY

The control procedure is usually carried out in practice in a compound feed company.

For those who carry out the control procedure in a compound feed company the following safety rules apply:

- a. the operatives will make themselves aware before the start of the work of the safety instructions which apply in the compound feed company
- b. during their stay in the compound feed company the operatives are bound to follow the safety instructions of the compound feed company

14. PROCESSING OF COMPOUND FEED CONTAINING MICROTRACER

No special instructions.

15. LITERATURE

1. The use of Microtracers to determine Completeness of Mix

The use of microtracers for the determination of the homogeneity of mixes
David A. Eisenberg, President of Micro Tracers, Inc. of San Francisco

2. Mix with Confidence

Safe mixing

David A. Eisenberg, President of Micro Tracers, Inc. of San Francisco
International Milling Flour&Feed, June 1994

2.7 Control procedure for the measurement of carry-over using microtracers by weighing

1. FIELD OF APPLICATION

See 2.6 Control procedure for the measurement of carry-over using microtracers

2. DEFINITIONS

Production plant: A production plant is an installation which is suitable for the preparation of compound feeds.

Microtracer mix: For the testing of a compound feed the microtracer mix contains 4 kg feed lime or wheat grits and 500 g microtracer. Therefore 500 g microtracer is mixed with 1 ton of compound feed, which corresponds to a mixing accuracy of 1: 2000.

3. PRINCIPLE

Use will be made for the measuring substance of the so-called RF microtracer (elementary iron particles). With an average number of particles of 1,000,000 per gram. For the microtracer particles it is a case of particle distribution; the average number of particles varies depending on the microtracer batch. In order to determine the number of particles in question in the test a microtracer mix is produced in which the average number of particles is determined exactly for the microtracer used.

The number of particles of microtracer in the samples which were taken is determined by separating the microtracer particles from the other feed particles using a rotary detector. The sample should be led twice over the rotary detector for this.

Once the sample has passed the magnet then the excess product is brushed from the filter with a brush, do this accurately and with a rotating magnet. Remove the filter from the magnet and transfer and return the microtracer in a tared copper weighing boat.

NB 1: In order to correct for "factory iron" at least three blank samples will be measured. In the final calculation there should be a correction for the average of the blank measurements.

NB 2: When adding the microtracer mix there should be 500 gram/ ton added. The sample size should be 300-500 grams.

4. EQUIPMENT AND TOOLS

See 2.6 Control procedure for the measurement of carry-over using microtracers

5. COMPANY DETAILS REQUIRED

See 2.6 Control procedure for the measurement of carry-over using microtracers

6. ADDITION OF THE MICROTRACER MIX

See 2.6 Control procedure for the measurement of carry-over using microtracers

7. TAKING AND HANDLING SAMPLES

See 2.6 Control procedure for the measurement of carry-over using microtracers

8. DETERMINATION OF THE MICROTRACER PARTICLES

By way of double filtration using a rotation detector with a rotary magnet the micro-tracer particles from a sample are isolated because of their magnetic properties. Other magnetic particles are also filtered out at the same time. The identification of the microtracer particles is done by weighing.

NB 1: In order to correct for “factory iron” at least three blank samples will be measured. In the final calculation there should be a correction for the average of the blank measurements.

NB 2: When adding the microtracer mix there should be 500 gram/ ton added. The sample size should be 300-500 grams.

9. PROCESSING OF THE RESULTS

See section 2.6 Control procedure for the measurement of carry-over using micro-tracers

10 REPORTING

See section 2.6 Control procedure for the measurement of carry-over using micro-tracers

11. ASSESSMENT OF THE RESULTS

See section 2.6 Control procedure for the measurement of carry-over using micro-tracers

12. REMARKS

See section 2.6 Control procedure for the measurement of carry-over using micro-tracers

13. SAFETY

See section 2.6 Control procedure for the measurement of carry-over using micro-tracers

14. PROCESSING OF COMPOUND FEED CONTAINING MICROTRACER

See section 2.6 Control procedure for the measurement of carry-over using micro-tracers

2.8 Control procedure for the measurement of carry-over in animal feed preparation using methyl violet

This text will be added later