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Definitions

Batch:	A quantity of charges produced consecutively without interruption by products with a different composition in terms of ingredients. Also called a lot.
Charge:	A quantity of feed in the mixer with the same composition and the same production number. Two or more charges compose a batch or a lot.
Final product cell or compound feeding stuff silo:	Containers, silos or compartments in which the feed is collected and stored before being loaded onto the truck and transported to the livestock farms.
Heterogeneity:	The extent to which constituents are non-uniformly distributed throughout the entire lot. The opposite of homogeneity.
Homogeneity:	The extent to which constituents are uniformly distributed throughout the entire lot. The opposite of heterogeneity.
Lot:	A quantity of charges produced consecutively without interruption by products with a different composition in terms of ingredients. Also called a batch.
Uniform sampling procedure:	Sampling method with quantitative requirements for control of substances or products uniformly distributed throughout the feed as described in Annex 1, number 5 of Regulation (EC) No 152/2009.
Non-uniform sampling procedure:	Sampling method with quantitative requirements for control of substances or products non-uniformly distributed throughout the feed as described in Annex 1, number 5 of Regulation (EC) No 152/2009.
Flushing charge:	The first charge of feed produced after a charge of feed containing coccidiostats.

Summary

Coccidiostats are additives that are intended to kill protozoa or inhibit their growth. They are mainly used as additives in feed for broilers, rearing hens and turkeys. Feed manufacturers may produce a broad range of feeds within one production plant and therefor different types of products may have to be manufactured after each other in the same production line. Carry-over may occur; unavoidable traces of a product remain in the production line and end up in another feed product. Flushing charges are a critical point in feed manufacturing. In Directive 2002/32/EC[1] and Commission Regulation (EC) No 152/2009 the sampling procedure for carry-over of coccidiostats is not clearly specified. Also, several possibilities for interpretation of the analysis results seem to be applicable.

The objective of this study is to give recommendations to the Dutch Ministry of Economic Affairs and the Dutch Food and Consumer Product Safety Authority on the interpretation of the EU legislation with respect to sampling and control of carry-over of coccidiostats in feeds in the Netherlands. To this end, relevant EU legislation has been listed and it has been examined how the Netherlands and seven other EU Member States interpret the law regarding sampling and evaluation of analytical results of the flushing charge.

For control of carry-over of coccidiostats in feed it is recommended that the samples should be taken as close to the destination as possible (preferably at the offloading, or alternatively in the product stream of a final product cell (i.e. containers, silos, compartments)). This corresponds to the interpretation of the EU Member States surveyed.

Corresponding to Regulation (EC) No 178/2002 a batch could be defined as the quantity of a uniform feed that is supplied to one farmer. Five out of six EU Member States consider a batch to be the feed with the same lot number of the producer. Six Member States surveyed indicated that it is permissible to add different charges of the same composition to a final product cell, if traceable and homogenous as required by Regulation (EC) No 183/2005.

Five EU Member States surveyed use the uniform sampling procedure as described in Regulation (EC) No 152/2009. In this report, as a case study, the homogeneity of one flushing charge of salinomycin has been evaluated according to the homogeneity criteria by OVOCOM (2009). It was concluded that this flushing charge has to be considered as inhomogeneous. The inhomogeneity did not change by any mixing due to overflows. It is therefore recommended to take into account the inhomogeneity of the coccidiostat when sampling the flushing charge. The preferred procedure seems to be the sampling protocol described in Regulation (EC) No 152/2009 for 'undesirable substances or products likely to be distributed non-uniformly throughout the feed'. For the application of this sampling procedure, it is recommended to evaluate the coccidiostats level of each final sample to the legal limit(s) according to Directive 2002/32/EC. Five of six EU Member States surveyed did not have suggestions for the interpretation of results of analysis.

Technical and organizational measures may prevent or minimize cross-contamination and human errors (like actions during milling). According to five of the six EU Member States it is legal to use a feed material (e.g. cereal grains), which is used for inclusion in a consecutively produced feed containing the same coccidiostat (at additive level) for flushing. It is unclear what measures include dilution as described in the Directive 2002/32/EC and, therefore, it is recommended to make agreements on EU-level.

[1] The legal acts quoted refer, where applicable, to the latest amended version.

1 Introduction

Coccidiosis is a parasitic infection which primarily occurs in poultry and cannot be transmitted to humans. The disease is caused by different Eimeria varieties, which spend a significant part of their life-cycle intracellularly in the intestinal wall of poultry. Coccidiosis is spread from one animal to another by Eimeria oocysts in the faeces of infected chicks (Anadón & Martínez-Larrañaga, 1999; Van den Ban, Aarts, Bokma-Bakker, Bouwmeester & Jansman, 2005). Infections can lead to economic losses due to reduced growth and feed conversion or reduction in egg production among poultry (although coccidiosis primarily occurs in young broilers) (Peek & Landman, 2003; Vermeulen, Schaap & Schetters, 2001). Risk factors which play a role in the prevention of coccidiosis on farms primarily relate to hygiene measures and the hygiene status of the farm (Graat *et a*l., 1998). The use of coccidiostats is the most common strategy for controlling coccidiosis in the poultry meat sector in the EU (Van den Ban *et al*., 2005). Coccidiostats are agents designed to kill protozoans or inhibit their growth (Regulation (EC) No 124/2009).

In the EU, coccidiostats are usually applied as an additive in animal feed, falling under the category of 'coccidiostats and histomonostats' (Regulation (EC) No 1831/2003). This category of substances comprises the polyether ionophore antibiotics (lasalocid sodium, narasin, salinomycin sodium, monensin sodium, semduramicin sodium, maduramicin ammonium) and various chemotherapeutic agents (robenidine hydrochloride, decoquinate, halofuginone hydrobromide, nicarbazin and diclazuril) (Anadón & Martínez-Larrañaga, 1999). Regulation (EC) No 1831/2003 lays down specific conditions for use, such as target animal species or categories for which the coccidiostats are intended and the associated concentration range. Usually, these are feeds for broilers, rearing hens and turkeys, but sometimes also rabbits.

In principle, all coccidiostats are effective for the goal in question, but resistance can reduce their effectiveness. For this reason, rotation and 'shuttle' programmes are used (Jeurissen & Veldman, 2002). Rotation involves alternating the use of two or more agents at intervals of several months. In a 'shuttle' programme, the use of an agent in the starter feed is alternated with a different agent in the growth feed during the growth of a group of chicks (Van den Ban *et al*., 2005).

1.1 Carry-over during compound feed production

Compound feed is produced in various process steps. Figure 1 represents compound feed production in schematic form. Trucks supply ingredients which are stored in ingredients silos. These ingredients are weighed and ground. Subsequently they are mixed with additives and, if applicable, veterinary medicines (usually in the form of premixes) in a mixer. The homogenised mixture is compressed into pellets in multiple press lines. The pellets are cooled and stored in compound feeding stuff silos. Besides pellets, meal is also produced which is stored in compound feed silos immediately after mixing. Finally, the compound feed is loaded into different compartments of trucks for transportation to the livestock farms.

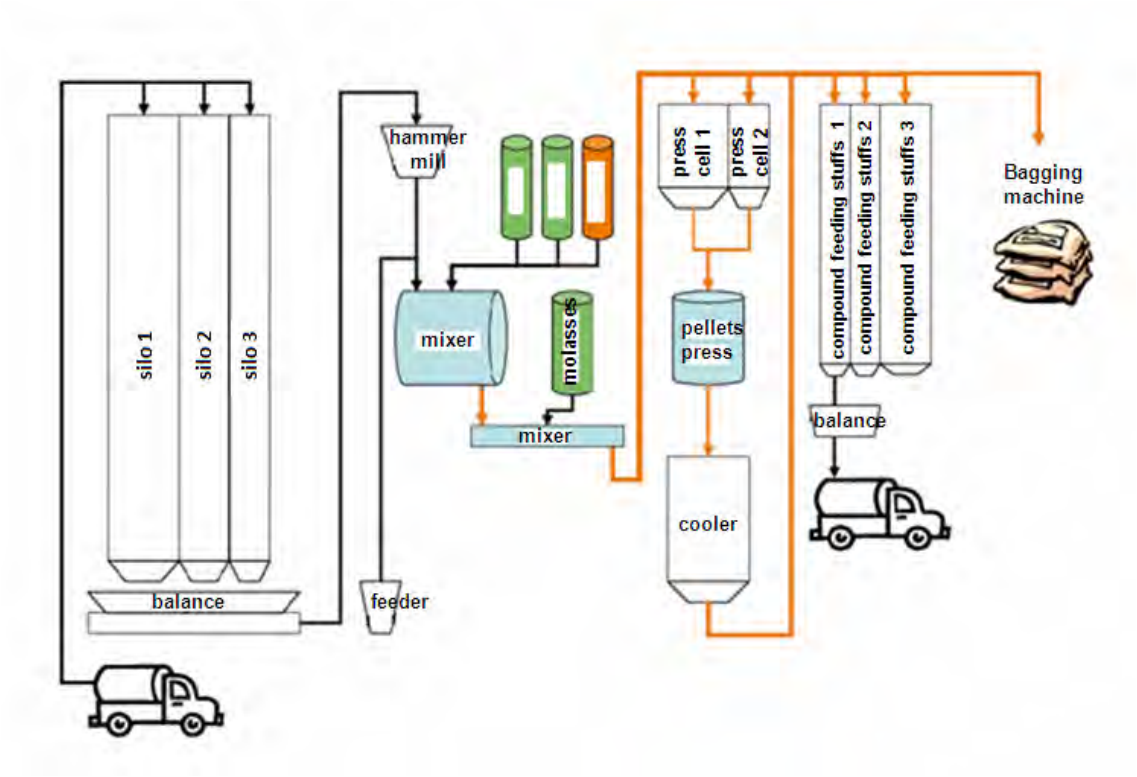

Figure 1 *Schematic representation of compound feed production (Zuidema et al., 2010).*

Animal feed producers usually produce several (different) compound feeds in one and the same installation, with various products being produced on the same production line one after the other. In practice, it is technically unavoidable that traces of one product are present in the production line when starting production of a following (different) compound feed. This transfer from one production batch to another is known as 'carry-over'. The production batch in which this contamination occurs is called the 'flushing charge'. This is a charge of compound feed or feed intended to remove any residual quantities of the previous batch from the system (GMP+International, 2010). Flushing charges are a critical point in the animal feed industry (and industry in general), because they are sometimes inadequate to remove residues of ingredients used previously (Van der Roest *et al.*, 2004; Vis *et al.*, 2003).

Carry-over occurs in the process steps of dosing (adding additives/veterinary medicines), grinding, mixing, transport, compression and cooling, and to a lesser extent in the storage of the product in a final product cell (i.e. containers, silos or compartments in which the feed is collected and stored before being loaded onto the truck and transported to the livestock farms) (GMP+International, 2011a; Hooglugt *et al.*, 2012; Zuidema *et al.*, 2010).

Carry-over may be reduced through modifications to the installation (Kennedy, Smyth, Hewitt & McEvoy, 1998). From research, Strauch (2002) concludes that carry-over is primarily the result of machinery or plant-specific features, and to a much lesser degree by the materials handled. In particular, the point at which additives are introduced, incorrect product flows and particular system parts such as the screw transporter and elevator have a major impact on the extent of carry-over. In installations with dead corners, flushing charges do not help to reduce carry-over. Modern mixing systems with sealing valves and good fluid application systems produce little carry-over caused by the installation (Strauch, 2002).

Other critical points for the incidence of carry-over are the return flows which are transported directly into the compressed meal silo during pelleting, and the processing of the portion of the bulk consignment removed in sieving (GMP+International, 2011a).

1.2 Homogeneity of flushing charges

The homogeneity of a lot is defined as the extent to which constituents are uniformly distributed throughout the entire lot. A perfectly homogenous sample is distributed completely uniformly throughout the whole: each subsample has identical properties (Berendsen & De Jong, 2010). Lightly contaminated untreated lots are mostly heterogeneously distributed, whereas highly contaminated treated lots are mostly relatively homogenously distributed. However, in the case of a treated product lot consisting of various sub-lots of different origins, this lot will also be heterogeneous and should be treated as such (Berendsen & De Jong, 2010).

In flushing charges, in many cases a particular constituent is only introduced after the mixer (GMP+International, 2011a; Hooglugt *et al.*, 2012; Strauch, 2002). This results in a high concentration of that constituent in the first part of the flushing charge, after which the concentration quickly drops. It may be expected that such a batch will be inhomogeneous. The degree of inhomogeneity of a flushing charge is dependent on the ingredients of the flushing charge itself, the substances to be flushed out, and the 'flushability' of the installation. If the ingredient used in the batch flushes easily, the substance to be removed flushes out easily, and the installation is easily 'flushable,' the concentration will be very high at the start and will drop quickly (fast flush). Conversely, in the case of poor 'flushability' (slow flush), the concentration will drop gradually.

There are different profiles for flushing charges (see Figure 2). In a properly functioning installation, the substances to be flushed can leave the installation quickly, which means that the first samples will contain a high concentration. If the first part of this 'fast flush' is separated off, the rest of the flushing charge will be homogenous. A 'slow flush' is the result of an installation functioning less effectively (for example, as a result of dead corners). In this case, the concentration of the contaminant to be flushed out is at the same (excessive) level throughout the flushing charge, and is therefore homogenous. In some cases, the product clings to the inside of the installation before suddenly being dislodged as a result of the opening and closing of valves. At that moment, the high concentration of 'contaminant to be flushed' will occur at another point, for example in the middle of the flushing charge ('flush with dislodged product') (Hooglugt *et al.*, 2012).

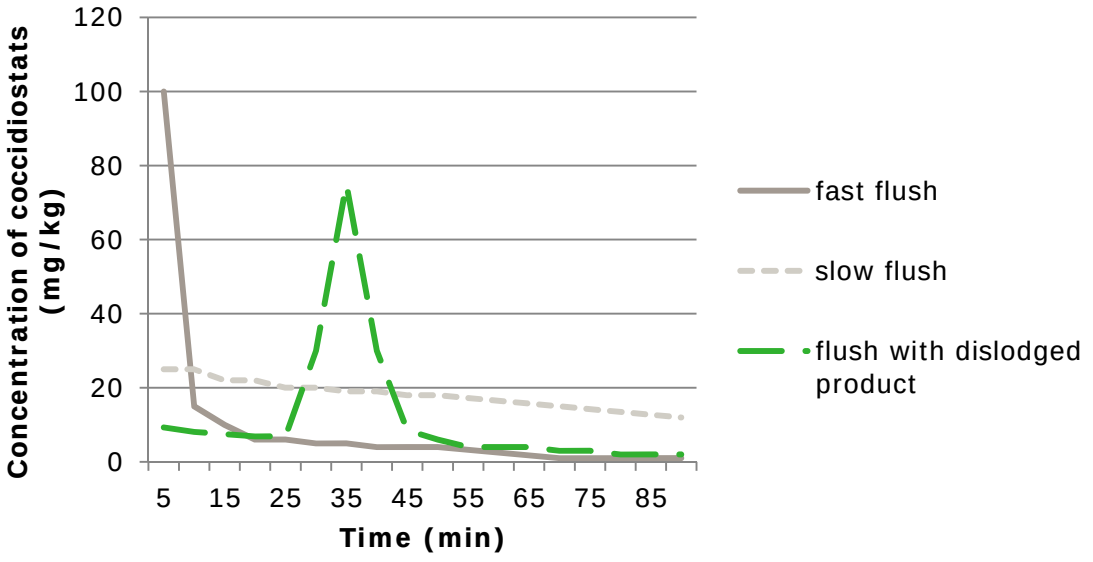

Figure 2 *Examples of concentration over time in different flushing charges.*

If it is known that a contaminant is distributed completely homogenously within a batch, the sampling procedure should be subject to different requirements compared to inhomogeneous distribution of the contaminant within the batch (Berendsen & De Jong, 2010). Regulation (EC) No 152/2009 describes a sampling procedure for both homogenously and heterogeneously distributed substances or products in

animal feed (i.e. not specifically for compound feeds). The procedure has consequences for the number of samples which need to be taken and analysed, and for the evaluation of the results.

1.3 Carry-over of coccidiostats

In the production of compound feeds containing a dosed coccidiostat, carry-over can result in residues of the coccidiostat in the flushing charge. EFSA has formulated scientific opinions to chart the risks of this carry-over of coccidiostats in 'non-target animal feeds'. Here, 'non-target animal feeds' are defined as feeds for which the use of coccidiostats is not permitted, such as feeds for animal species or categories not mentioned in the registration of the additive. The scientific recommendations reveal that EFSA believes the presence of coccidiostats in non-target animal feeds resulting from unavoidable carry-over will probably not have an adverse impact on animal health and that the health risk to consumers is negligible. Based on these risk assessments, the EC has set maximum permitted carry-over percentages for coccidiostats in non-target animal feeds in Directive 2009/8/EC, as equally described in the latest amended version of Directive 2002/32/EC[2]. A distinction is drawn between 'feeds for less sensitive non-target animal species,' which are subject to a 3% standard (of the permitted maximum concentration), and 'feeds for sensitive non-target animal species and withdrawal feeds which are used in the period before slaughter,' which are subject to a 1% standard. Directive 2002/32/EC additionally states that a percentage of 1% should also be considered for cross-contamination of other feeds for target animal species, to which no coccidiostats are added, and for non-target animal feeds for 'continuously food-producing animals,' such as dairy cows and laying hens, if it has been shown that transmission from animal feeds to foodstuffs of animal origin is possible. In addition, maximum concentrations for coccidiostats in food have been set to protect public health (Regulation (EC) No 124/2009), and waiting periods for slaughter have been laid down in order to prevent residues of coccidiostats in meat (Commissie-van-de-Europese-Gemeenschappen, 2008).

According to the Undesirable Substances Directive 2002/32/EC, it is not permitted to mix flushing charges which fail to meet the standards, into other animal feeds (with or without another coccidiostat). The literal sentence in this Directive is: 'Member States shall prescribe that products intended for animal feed containing levels of an undesirable substance that exceed the maximum level fixed in Annex 1 may not be mixed for dilution purposes with the same, or other, products intended for animal feed.'

GMP+ provides limited scope for the use of internal return flows: preferably in the batch from which they originated, and otherwise in a subsequent batch of the same type of feed. In this case, it must be specified in which storage locations the return flows are stored, which return products are included in which products, and at which percentages this may occur. Records must show which quantities of return product are processed in which batches (for each type of feed) (GMP+International, 2011b). The document does not specify whether the internal return flows may also be used for flushing charges of coccidiostats.

In compound feed enterprises which have an in-house carry-over percentage of 3% (or 1%) or more, the following strategies may be used to reduce the carry-over percentage:

1. Setting up a compulsory production (work) sequence (GMP+International, 2011a).
2. Supplementary measures when changing products (GMP+International, 2011a), such as separating off part of the flushing charge.
3. Producing feeds containing critical additives and veterinary medicines on a different line (GMP+International, 2011a).
4. Switching to less critical substances (GMP+International, 2011a).
5. Producing a feed with coccidiostats from multiple batches, with the final batch being the flushing charge. For example, the last four tonnes of a sixteen tonne production run with salinomycin is used as a flushing charge and mixed with the other twelve tonnes.

[2] The legal acts quoted in this report refer, where applicable, to the latest amended version.

6. The flushing charge of the meal mixing line is compressed in a clean press line, and a clean batch from the meal mixing line is used to flush the press line.
7. Cleaning the installation by flushing with an animal feed. This feed can subsequently be destroyed or used in an equivalent coccidiostat feed containing the same coccidiostat dosage.
8. Dosing outside the existing line. Coccidiostats can be added outside the existing line with the help of a fine dosing device on the truck. The required quantity of coccidiostat is added during the transfer of the feed from the truck to the silo at the livestock farm. Since July 2007, it has been possible to apply for certification of fine dosing devices in Belgium. GMP+ certified businesses must comply with document 'AT-13: Procedure for the use of a fine dosing device'. The fine dosing device must be designed in such a way that the formation of residues as a result of carry-over is negligible (OVOCOM, 2008).

In the Animal Feed National Monitoring Programme, the Dutch Food and Consumer Product Safety Authority (NVWA) takes a substantial number of samples in order to monitor compliance with the European standards. However, Directive 2002/32/EC and the Sampling and Analysis Methods regulation (Regulation (EC) No 152/2009) do not state clearly in what way the sampling should be carried out. In particular, the sampling location, sampling procedure and interpretation of the analysis results leave room for different interpretations. Among the EU Member States, it was found that divergent approaches are used with respect to unavoidable carry-over (Regulation (EC) No 124/2009).

1.4 Objective

The objective of this study is to give recommendations to the Dutch Ministry of Economic Affairs and the Dutch Food and Consumer Product Safety Authority on the interpretation of the EU legislation with respect to sampling and control of carry-over of coccidiostats in compound animal feed in the Netherlands. To this end, the relevant EU legislation is catalogued and the interpretation of the current legislation with respect to sampling and analysis results in the Netherlands and the surrounding EU Member States is examined. Based on the information collected, recommendations are made which can be used to improve the sampling and control strategy relating to the carry-over of coccidiostats in compound feeds in the Netherlands in accordance with the EU legislation.

2 Materials and methods

2.1 Homogeneity of coccidiostats in flushing charges

In 2009, the NVWA took samples during the production of one flushing charge. This flushing charge was produced following a compound feed containing the additive level salinomycin. From a flushing charge of 20,000 kg of withdrawal feed for broilers containing salinomycin, a total of eleven samples were taken at intervals of five minutes, and after 45 minutes two samples at an interval of 20 minutes. The homogeneity test described in the GMP document of OVOCOM, Belgium 'BT-08 Batch B Part B; Homogeneity and carry-over' (OVOCOM, 2009), was used to analyse whether this flushing charge should be regarded as homogenous or inhomogeneous. In this calculation, the samples/subsamples within a batch are analysed and the variation coefficient (VC) of the results calculated. If the VC ≥ 12%, the batch is non-homogenous. If the VC ≤ 8%, the batch is homogenous. Between 8% and 12%, the batch is acceptably homogenous (OVOCOM, 2009).

In this way, the homogeneity of the flushing charge is determined a) at the end of the press line and b) after mixing as a result of pouring the batch into a silo at the compound feed factory, filling the bulk transporter, pouring the feed into a silo at a farm, and transportation from the silo to the feed through of the animal in question. No models or practical data are available for the mixing or segregation occurring during pouring and transportation. In order to simulate the mixing occurring as a result of pouring, the following assumptions have been made:

- The processes of pouring and transportation will involve a certain degree of mixing of the fractions within the flushing charge. This study assumes 22.5% or 45% (two-way) mixing between fractions within the flushing charge, with the mixing taking place according to a more or less normal distribution (Figure 3).
- In making the calculations, it has been assumed that the container into which the batch is being poured is empty. That is to say, no mixing with the 'head' and 'tail' of the flushing charge.

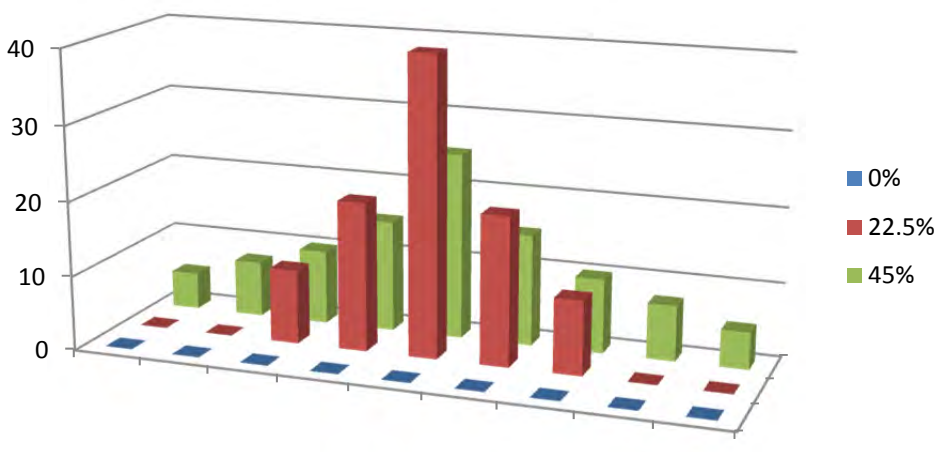

Figure 3 *Normal distribution of mixing at 22.5% and 45%.*

2.2 Survey

2.2.1 Preparation

An inventory was made of the EU legislation relating to the sampling and monitoring of carry-over of coccidiostats in compound animal feeds in order to catalogue where different interpretations of the EU legislation are possible. In addition, based on face-to-face interviews with NVWA inspectors, the current sampling practice in the Netherlands was charted. With the help of this information and a supplementary literature review on carry-over, sampling and legislation, a questionnaire was drawn up in order to catalogue how the EU legislation relating to sampling to detect carry-over of coccidiostats in compound feeds is interpreted in the EU Member States surrounding the Netherlands (see Annex 1 for the questionnaire).

2.2.2 Organisation of survey and processing of the results

The questionnaire was presented by email to enforcement agencies in eleven EU Member States which a) have a trading relationship with the Netherlands and/or b) in which, based on expert knowledge, it was expected that there would be a range of interpretations of the EU legislation. The responses were then used to analyse how the EU legislation is interpreted by the Netherlands and other EU Member States in terms of sampling, monitoring and analysis results.

3 Results

3.1 Homogeneity

Figure 4 shows the time-concentration profile of the flushing charge (for salinomycin). The salinomycin concentration in the flushing charge drops from approximately 17 mg/kg to approximately 4 mg/kg in 80 minutes.

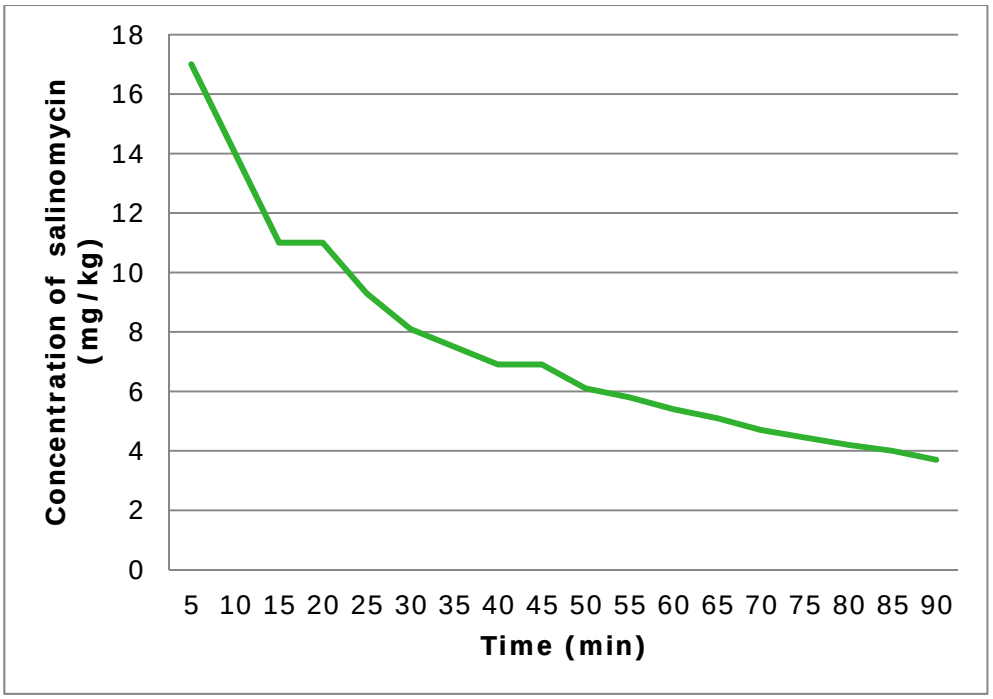

Figure 4 *Concentration of salinomycin in samples of a flushing charge taken over time at the end of the press line.*

When the above flushing charge was tested for homogeneity using the OVOCOM method, it was qualified as inhomogeneous. The variation coefficient (VC) was 49.1%, which is clearly above the upper limit of 12%. These data represent the results of chemical analyses of samples taken at the end of the press line.

If we assume that mixing occurs as a result of pouring and transportation, according to the OVOCOM test, the charges created after three pourings (Figure 5, 22.5% and 45%) are still inhomogeneous: at 22.5%, VC = 36.0%, and at 45%, VC = 30.9%.

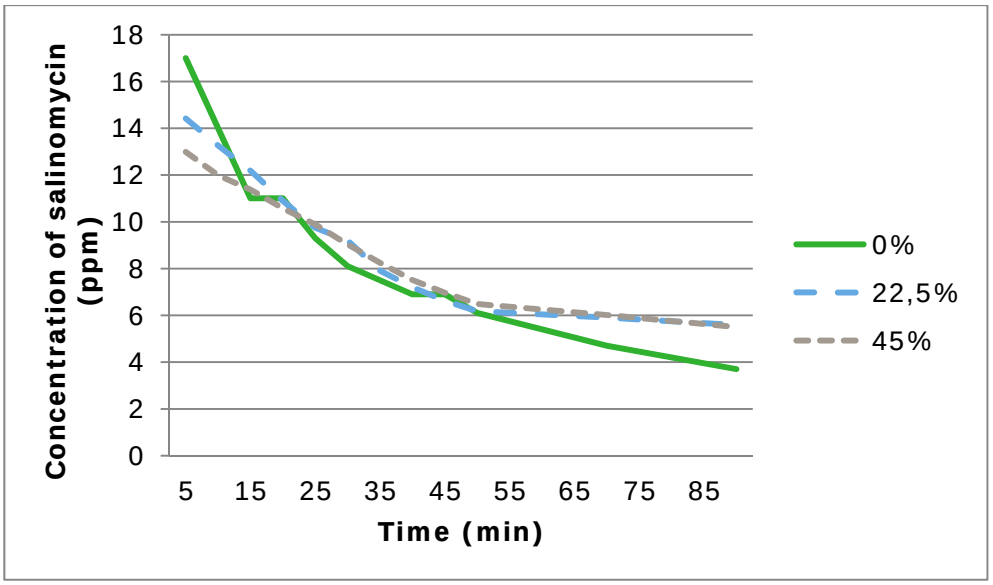

Figure 5 *Concentration of salinomycin (in ppm) in a flushing charge over time, without and with mixing after three pourings during the production process.*

3.2 Results of the survey

3.2.1 Response to the survey

The questionnaire was answered by eight enforcement agencies from eight EU countries: the Netherlands (NVWA), Belgium (FPS + FAVV/AFSCA), Luxembourg (ASTA), Germany (LANUV-NRW), Denmark (FVST), Italy (Ministry of Health, DGSA, office VII animal nutrition), Spain (MARM), and Poland (GIW) (see Annex 2). The response was 73% (eight responses received out of eleven enforcement agencies contacted). The respondents were experts in the field of animal feed inspection holding the posts of inspector or policy staff member. In one country, parts of the questionnaire were completed by three different experts. In the Netherlands, the entire questionnaire was answered twice.

3.2.2 Use and addition methods of coccidiostats

The statutory basis for the sampling and monitoring of carry-over in animal feed comprises General Food Law Regulation (EC) No 178/2002; Feed Hygiene Regulation (EC) No 183/2005; Official Control Regulation (EC) No 882/2004; and, in the Netherlands, the Animal Act. Relevant requirements for the sampling and inspection of carry-over of coccidiostats in animal feed are stated in the Undesirable Substances Directive 2002/32/EC; Regulation (EC) No 767/2009; and Sampling and Analysis Methods Regulation (EC) No 152/2009. This directive and these regulations set out specific rules for the maximum concentration of coccidiostats, the carry-over percentage, measures to prevent carry-over, sampling methods, and the interpretation of analysis results. For specific aspects of the EU legislation, different interpretations are possible. In this chapter, the elements of the legislation which are unclear and the interpretations used in the Netherlands and seven other EU Member States are described in terms of the sample, the sampling location, the sampling procedure, the measurement of carry-over and measures to prevent carry-over.

3.2.2.1 The Netherlands

Based on the NVWA's response to the survey, the following coccidiostats are added to compound feed in the Netherlands: lasalocid sodium, narasin, salinomycin sodium, monensin sodium, semduramicin sodium, maduramicin ammonium alpha, robenidine hydrochloride, decoquinate, halofuginone hydrobromide, nicarbazin and diclazuril. The compound feed to which coccidiostats are added is in the form of both meal and pellets.

Coccidiostats are added in the premix, before the hammer mill or in the mixer in the animal feed producer's factory. In some cases, an extra mixer may be fitted to the truck, which is used to add coccidiostats immediately before loading. It is not permitted to add coccidiostats outside the factory if the company's licence only covers the building.

3.2.2.2 EU Member States

Compound feed containing coccidiostats is produced in six of the seven other participating EU Member States; in one Member State (EU4), no compound feed with coccidiostats is produced, and this respondent therefore did not answer the remainder of the questionnaire. Not all coccidiostats are added in every Member State. Lasalocid sodium, narasin, salinomycin sodium, monensin sodium, nicarbazin and diclazuril are used in all six EU Member States and robenidine hydrochloride in five out of six Member States. In addition, semduramicin sodium, maduramicin ammonium alpha and decoquinate are added in four Member States. Halofuginone hydrobromide is used in three of the six Member States.

Six Member States indicated that the use of different coccidiostats in the same compound feed is not permitted in principle, except where a mixture is registered in the additives register. The compound feed of five Member States is produced in the form of both meal and pellets; one Member State (EU1) only produces pellets.

Coccidiostats are added in five EU Member States in the premix or through the feeder pipe, in any case just before or in the mixer (see Table 1). According to four EU Member States (the Netherlands, EU3, EU5, EU7) it is not permitted to add coccidiostats outside the factory (for example during loading) under Regulation (EC) No 183/2005, which lays down requirements for animal feed hygiene. One EU Member State (EU 6) indicated that coccidiostats are permitted outside the factory. In that case, the truck must be certified in accordance with Regulation (EC) No 183/2005. Another Member State (EU1) indicated that livestock farmers can also be certified in accordance with article 10, 1, c of Regulation (EC) No 183/2005, although this is not the practice in their country.

Table 1

Point at which coccidiostats are added in the Netherlands and six other EU countries.

Point of addition	Netherlands	EU1	EU2	EU3	EU5	EU6	EU7
Feeder pipe	x		x		x	x	
Premix	x	x	x	x	x	x	
Outside the factory (loading, farm)	Not permitted	Permitted when licensed in accordance with Regulation (EC) No 183/2005	Permitted when licensed in accordance with Regulation (EC) No 183/2005	Not permitted	Not permitted	Permitted when licensed in accordance with Regulation (EC) No 183/2005	Not permitted

3.2.3 Sampling of coccidiostat carry-over

Regulation (EC) No 124/2009 states that divergent approaches are currently taken with respect to unavoidable carry-over in the Member States. The Sampling and Analysis Methods regulation (Regulation (EC) No 152/2009) does not clearly state how and where the sampling is to be carried out. In addition, it is essential to determine which sampling procedure applies to flushing charges and coccidiostats. In this paragraph, the interpretation of the EU legislation relating to sampling by different EU member states is described.

3.2.3.1 Unclear elements of EU legislation

Definitions of a lot are given in Regulation (EC) No 767/2009 and Regulation (EC) No 152/2009. In Regulation (EC) No 767/2009, a lot is defined as an identifiable quantity of animal feed for which common characteristics have been determined, such as origin, type, packaging type, packer, shipper or labelling. In the case of a production process, the definition of the lot is a unit of production by a

company which uses uniform parameters in its production or a number of such units which are produced immediately after each other and are stored together. This definition describes a batch or lot.

According to Regulation (EC) No 152/2009, a lot is defined as a quantity of a product which forms a unit, and which is assumed to have uniform properties. This definition focuses on the sampled part. These are broad definitions which may be applied to both the quantity of animal feed delivered to the livestock farm and the quantity produced in the factory. In addition, a lot can consist of different charges or a flushing charge, possibly mixed in the press line or in the collecting silos.

3.2.3.2 The Netherlands

In the Netherlands, the NVWA is responsible for enforcing the legislation with regard to carry-over of coccidiostats in compound feed. In the Animal Feed National Plan 2010, the NVWA includes a substantial number of samples for the monitoring of compliance with maximum permitted carry-over concentrations of coccidiostats in animal feeds for non-target animals.
The NVWA aims to take samples of flushing charges. However, this is not always possible, because it requires that a flushing charge is being produced just when the inspector is present. In addition, in closed systems it is not always clear when and from which press line the feed is coming. The flows in the factory are also difficult to follow. The head of the flushing charge with the highest concentration of coccidiostats is often not identifiable.

The definition of a lot is interpreted differently by NVWA staff, depending on the place of sampling. For the NVWA, for the purposes of analysing contamination with coccidiostats, a lot is the quantity of the same animal feed delivered to a livestock farm. Usually, the truck goes to a single livestock farm. If the truck delivers to three different livestock farms, the feed is contained in three separate compartments. These are regarded as three separate lots. A different definition is used for the factory, with the lot being identified with the lot number. If the ingredients are the same and are mixed together, a single lot number is assigned, and this is therefore one lot. This lot may consist of different batches. For example, five charges with the same composition may be produced in the mixer, distributed across several press lines. The entire lot of five charges (with a single lot number) may then be loaded into ten bulk transporters capable of holding 30 tonnes each.
In the collection silo, the same product from different lot numbers may also be collected. In this case, samples are therefore taken of different lots from the factory when loading.

3.2.3.3 EU Member States

Four of the six EU Member States select random charges for sampling (see Table 2). These can be both ordinary charges and flushing charges. Of the six, one member state (EU2) indicated that the inspector samples flushing charges if possible, or end products following the production of charges containing coccidiostats.

One member state (EU6) indicated that the flushing charges are always sampled and sometimes also ordinary charges. The sampling is intended to determine whether the samples comply with Directive 2002/32/EC. Another member state (EU5) indicated that it only takes samples from an ordinary charge of an end product.

Five EU Member States regard a lot as being all animal feeds with the same lot number from a producer (see Table 2). One member state (EU2) regards a lot as being all the feeds with the same composition which are produced consecutively. A producer may split up feed from one lot into a larger number of lots.

Table 2

Different interpretations relating to sampling by enforcement agencies in the Netherlands and six other EU countries.

Lot	Netherlands	EU1	EU2	EU3	EU5	EU6	EU7
Sampling	Random	Random	Random; where possible flushing charge, end product or charges	Random	End product	Not random. Flushing charge, in addition sometimes ordinary charges	Random
Lot	Factory: - Feed with the same lot number from the producer; - Feed that is produced in the mixer at the same time; - Feed with the same composition, produced consecutively Legal: - Destination (livestock farm), possibly a compartment of the truck	Feed with the same lot number from the producer	Feed with the same composition, produced consecutively, possibly split between a larger number of lots	Feed with the same lot number from the producer	Feed with the same lot number from the producer	Feed with the same lot number from the producer	Feed with the same lot number from the producer

3.2.4 Sampling locations

Samples can be taken either inside or outside the factory. However, Regulation (EC) No 152/2009 does not describe at which locations samples should be taken.

The final product cell can be sampled in different ways: the entire container or part of the container; in the product flow or in the static product; at the top, in the middle of the container taken vertically, or at the bottom of the container; at the side or on the middle of the container taken horizontally. The method for sampling silos is not described in the EU legislation.

3.2.4.1 The Netherlands

In the Netherlands, samples were formerly taken in the factory. Currently, the samples are taken when loading the truck, in line with the method in Belgium. The advantage of this method is that the charge which ends up at the livestock farm is sampled and that no further processing in the factory occurs. The analysis results of the NVWA show that fewer carry-over infringements are observed in sampling when loading compared to sampling in the factory. High concentrations appear to be reduced by mixing during the production process.

The feed is poured into the truck, where it can mix further. Each truck has four to five compartments. These can be filled with different types of feed, or with a single type. Loading is a fast process: for sampling purposes, the inspector makes an estimate of how many minutes it will take to load a charge of one type. This time is then divided up equally in order to take samples. If the same feed is loaded into multiple compartments, one sample is taken from each compartment.
If this is not possible because the bulk carrier is hermetically sealed, a sample is taken before the collection in the compound feeding stuff silos (i.e. final product cells). In these silos, funnelling occurs, which means it is not certain whether a sample is being taken of the intended batch. In some cases, the silos are fitted with automatic sampling equipment, with samples being taken between the coater and the compound feeding stuff silo. The silos sometimes have a free fall with a grille, allowing mixing to occur. In the case of meal, samples can also be taken in the mixer; in the case of pellets, after the cooler.

If a final product cell is being sampled, which is not usual, samples are taken from above at various points or during the filling or emptying of the silo. A part of the silo's contents may also be emptied into a collection bin for sampling. An average sample of the entire lot is then taken.

It is difficult to see which press line the animal feed is coming from; the inspector relies on the information from the compound feed producer. If samples cannot be taken in the correct manner for practical reasons, they are designated as preliminary non-official samples.
The most critical lines for carry-over of coccidiostats are lines in which animal feed with various types of coccidiostats are produced. This results in mixing different coccidiostats.

According to the NVWA inspectors, the maximum values for unavoidable carry-over of coccidiostats to non-target animal feeds in Directive 2002/32/EC relate to the feed delivered to the livestock farm.

3.2.4.2 EU Member States

The sampling locations are dependent on the facilities in the factory and the production of meal or pellets. Table 3 shows the different interpretations in terms of sampling locations. In both meal and pellets, samples are preferably taken when loading (truck (n=3), at the bagging machine (n=5), when weighing (n=3)), before the final product cells (n=2), and after the mixer (n=1). Specifically for meal, samples are also taken in the mixer (n=1). Specifically for pellets, samples are also taken after the cooler (n=3) and after the press line (n=1).
One EU Member State (EU2) indicated that the sampling to determine the concentration of coccidiostats in compound feeds for target animals should preferably take place when bagging, loading the truck or shortly before entering the end product silo. Sampling in order to determine the concentration of coccidiostat residues in non-target animal feeds should preferably take place when bagging or loading the truck.
If the preferred location is not possible due to the design of the installation, an alternative location must be used for sampling. One EU Member State (EU3) specified after the unloading of the truck at the livestock farm as an alternative sampling location. Three other Member States (EU5, EU6 and EU7) indicated that, as an alternative, samples should be taken from the end product at the end of production (in silos, truck or packaged animal feed).

Every EU Member State in this study uses final product cells. The six Member States indicated that it is permissible to add different charges with the same composition to a final product cell. One Member State (EU1) added as a condition that traceability must be assured and documented, and that the feed must be homogenous as required by Regulation (EC) No 183/2005, Annex 1.
Another Member State (EU2) indicated that a single lot (i.e. a quantity of charges produced consecutively without interruption by products with a different composition in terms of ingredients) should preferably be stored in the same final product cell, but that multiple lots with the same formula could also be stored in the same end product silo. Another Member State (EU6) indicated that the case for doing so must be recorded in the HACCP plan.

Three EU Member States indicated that samples of a final product cell can best be taken from the product flow (during bagging, loading, transportation to the final product cell). One Member State (EU2) indicated that a silo can only be representatively sampled if it is being pumped around or emptied (thus samples are again taken from the product flow) and that taking a sample at a single point in the silo is not advisable. Two Member States gave no reply regarding the sampling method for the final product cell. One EU Member State (EU6) indicated that the sampling method used for the final product cell depends on the facilities of the compound feed company. Another Member State (EU5) indicated that samples may only be taken from the end product, and that flushing charges or feed are sampled by the compound feed producers during interim steps in the process in order to verify the HACCP plan.

Directive 2002/32/EC lays down the maximum values for unavoidable carry-over of coccidiostats to non-target animal feeds. According to the interpretation of five EU Member States, these standards are meant for the feed delivered to the livestock farm, i.e. the feed brought onto the market in

accordance with Regulation (EC) No 178/2002. One Member State (EU3) also includes the feed after the compression process, after mixing or after cooling.

Table 3

Different interpretations relating to sampling locations by enforcement agencies in the Netherlands and six other EU countries.

Sampling locations	Netherlands	EU1	EU2	EU3	EU5	EU6	EU7
Location	Loading	Loading	Loading. Before final product cell, in mixer, after cooler	After cooler	Loading. Before final product cell	Loading, after mixer, after cooler	Loading
Silo	Top at various points; in product flow during filling or emptying of the silo; part of the silo's contents in a collection bin	Whole and part of final product cell; in product flow and in static product; at the bottom in the middle; side in the middle	In product flow (during bagging, loading or transport to final product cell)	Part of final product cell; in product flow; at the bottom in the middle of the container	Official samples: regular charges of end product. Verification of HACCP plan: flushing charge or feed during production process by producers	Depending on facilities	In product flow
Different charges with the same composition in final product cell	Yes	Yes, provided traceable and documented, and homogenous	Yes, preferably one lot but multiple lots are also possible	Yes	Yes	Yes	Yes

3.2.5 Sampling procedure: uniformity of the lot

3.2.5.1 Unclear elements of EU legislation

Regulation (EC) No 152/2009 lays down a sampling procedure for a uniform and non-uniform distribution of substances in animal feed. The minimum number of samples and the minimum mass are dependent on the distribution (uniform or non-uniform) and the lot size. Because the first hundreds of kilos of flushing feed produced contain much higher concentrations of coccidiostats (see paragraph 1.2) than the final tonnes, the sampling method used is extremely critical. It is not clear in the legislation whether the uniform or the non-uniform sampling procedure applies to carry-over and specifically to flushing charges.

3.2.5.2 The Netherlands

The NVWA has drawn up an instruction which describes the procedure on which the sampling by the compound feed producer must be based. These sampling procedures are set out in the VWA instruction on sampling, storing, transport and transfer (MON-01, BUI02 and BUI03). The intervention policy is described in the VWA general intervention policy (IB01).

The sampling is carried out in accordance with the uniform sampling procedure set out in Regulation (EC) No 152/2009 (see Table 4), with one final sample being analysed. No foundation was given.

If a non-uniform sampling procedure is being carried out, according to the inspectors, the level of each end sample must comply with the maximum concentrations laid down in Directive 2002/32/EC.

If only part of a charge can be sampled, which part of the quantity produced has been sampled is specified. This is a preliminary non-official sample, the results of which cannot be used for

enforcement purposes. Another inspector indicated that if more than 50% of a charge is sampled, the samples taken are then representing the entire charge.

3.2.5.3 EU Member States

Five EU Member States use the uniform sampling procedure for taking samples in order to determine the carry-over of coccidiostats (see Table 4). Of those, one Member State (EU5) uses the uniform sampling procedure for all feed additives. The reason for this is that the most important source of carry-over is the mixer, which it claims means the carry-over will be uniform. This Member State indicated that the non-uniform sampling procedure would generate more representative results, but is harder to perform. Another Member State (EU6) also cited the perceived difficulty in practice of sampling using the non-uniform sampling procedure as its reason for using the uniform sampling procedure.
One Member State (EU1) uses both the uniform and the non-uniform sampling procedure. If a sample is taken in order to approve or reject a batch, the non-uniform sampling procedure is used. The average concentration of coccidiostats in the end samples is calculated. The average concentration may not exceed the maximum concentration in accordance with Directive 2002/32/EC. If the sampling is performed as a check on the monitoring plan of the compound feed producers, the uniform sampling procedure is used. If the maximum concentrations are exceeded, the compound feed producer must demonstrate that the entire batch is below the maximum concentration, or that it is being used for a following batch of animal feed containing coccidiostats. If the compound feed producer fails to conduct monitoring procedures, the inspecting authority can impose penalties in accordance with Regulation (EC) No 183/2005.
Three Member States were unable to provide explanations for their choice of the uniform sampling procedure.

Five EU Member States indicated that in some cases, the entire charge is not sampled, for example due to the large quantity of animal feed in the charge or the high speed of production. The analysis results from incomplete lots are interpreted differently (see Table 4). In one Member State (EU1), this situation always occurs and the results are used to check the monitoring plans of the compound feed producers, whose responsibility it is to monitor the concentration in the entire charge. Three Member States indicated that the results are regarded as being representative for the entire charge. One Member State (EU7) interprets only the sampled charge.
One Member State (EU6) never faces this situation. Assuming that a lot may consist of a number of different charges, a charge can always be sampled.

Table 4

Different interpretations in terms of sampling procedures of enforcement agencies in the Netherlands and six other EU countries.

Sampling procedure	Netherlands	EU1	EU2	EU3	EU5	EU6	EU7
Uniform	x	x As check of procedures of animal feed producers	x	x	x All additives. Largest carry-over in the mixer, therefore uniform distribution	x	x
Non-uniform		x When the sample has been taken to approve or reject			More represen-tative results but harder to carry out	Sampling more difficult. Besides distribution, concentration of coccidio-stats in flushing charge is important. Producer must know how installation works and what the distribution over time is.	
Evaluation of results	The level of coccidiostats in each individual end sample must meet the levels stated in 2002/32/EC	The average quantity of coccidiostats in final samples is calculated and must comply with the levels stated in 2002/32/EC					
Sampling of part of the charge	Specified if part of the charge is sampled (preliminary non-official sample)	Always part sampled as check on animal feed producers	Samples as represen-tative as possible; interpretation as if sampling was completely represen-tative	Results are represen-tative for the entire charge	In case of uniform distribution, the result is regarded as represen-tative for the entire charge	A lot may consist of various charges. For this reason, a charge may be sampled completely.	Results are only interpreted for the sampled charge

3.2.6 Measurement and measures to prevent carry-over of coccidiostats

Directive 2002/32/EC contains maximum permitted carry-over concentrations of coccidiostats in animal feeds for non-target animals. With due regard for the application of good production practices, the maximum levels of unavoidable carry-over of coccidiostats or histomonostats in nontarget feed should be established following the ALARA (As Low As Reasonably Achievable) principle. For the purpose of enabling the feed manufacturer to manage the above mentioned unavoidable carry-over, a carry-over rate of approximately 3 % compared to the authorised maximum content should be considered as regards feed for less sensitive non-target animal species. For feed intended to sensitive non-target animal species and 'withdrawal feed', i.e. feed used for the period before slaughter, a carry-over rate of approximately 1 % compared to the authorised maximum content should be retained. The carry-over rate of 1 % should also be considered for cross-contamination of other feed for target species to which no coccidiostats or histomonostats are added, and as regards non-target feed for 'continuous food-producing animals', such as dairy cows or laying hens, where there is evidence of transfer from feed to food of animal origin. If feed materials are fed directly to the animals

or if complementary feedingstuffs are used, their use in a daily ration should not lead to the animal being exposed to a higher levels of a coccidiostat or histomonostat than the corresponding maximum levels of exposure where only complete feeding stuffs are used in a daily ration.

The following paragraphs describe the results of how the extent of carry-over is measured and how the different Member States interpret the EU legislation in terms of measures to prevent carry-over.

3.2.6.1 Measuring carry-over

Unclear elements of EU legislation
The EU legislation imposes no obligation to determine the installation-specific carry-over percentage. GMP+ does require this every two years, specifying different methods to measure the extent of carry-over and homogeneity in the compound feed factory with the help of tracers (such as cobalt chloride, methyl violet) (GMP+ International, 2011a). There are doubts as to whether these tracers can be used to determine carry-over and homogeneity of coccidiostats.

The Netherlands
During an inspection, the NVWA looks at the results of the carry-over determination of the milling/mixing line and the press lines which each GMP+ certified compound feed company is required to perform. The carry-over from the milling/mixing line and each press line are added up to obtain the total carry-over. If the carry-over of the milling/mixing line is high, the total carry-over may nevertheless be acceptable because the flushing charge is then sent through clean press lines. The NVWA does not collect any data on carry-over results; these remain with the company.
The Food and Veterinary Office (FVO) has noted that it is unclear whether the substances used for the carry-over determination (Mn-proteins, tracers) are comparable with coccidiostats. For this reason, it states that the carry-over determination should be performed using coccidiostats. Moreover, during the determination, the production quantity is kept the same, which is not the case in practice. One NVWA inspector believes that the tracers are not representative of the carry-over of coccidiostats due to the differences in density and adhesion/cohesion between the various coccidiostats and tracers.

In order to prevent carry-over and contamination of compound feed, the product sequence is taken into account. The companies work in accordance with a particular product sequence based on a contamination table. This table is drawn up by an individual company in response to carry-over tests in accordance with GMP+ (GMP+ BA1; GMP+ B1). Monitoring is conducted by GMP+ International, but NVWA also evaluates the results.

EU Member States
Four EU Member States believe that tracers are representative for the measurement of coccidiostat carry-over. Of those, one Member State indicated that the method has been evaluated by a scientific committee and that the enforcement agency recommends that producers measure carry-over as part of their HACCP plans. Another Member State indicated that the compound feed producer is responsible for selecting a tracer which is representative for the (coccidiostat) carry-over.

One EU Member State regards the tracers as not being representative because physical-chemical factors/adhesion properties of additives and premixes vary with respect to the tracers. For this reason, carry-over is determined using analytical methods which determine the quantity of the specific coccidiostat present in accordance with Regulation (EC) No 183/2005, Annex 2 and/or art. 6.2. The results from the use of tracers can be used as supplementary evidence.

One EU Member State had no experience with the measurement of carry-over using tracers.

3.2.6.2 Measures to combat carry-over

Unclear elements of EU legislation
The Undesirable Substances Directive 2002/32/EC states that products intended for animal feed containing concentrations of an undesirable substance that exceed the maximum concentration laid down may not be mixed for dilution purposes with the same, or other, products intended for animal feed. This directive further stipulates that undesirable substances may exclusively occur under the

conditions laid down in this directive in products intended for feeding animals and may not be used for feeding animals in any other way.

Annex II of Feed Hygiene Regulation (EC) No 183/2005 specifies that technical and organisational measures must be taken in order to prevent cross-contamination and human error in production or limit them as much as possible. Sufficient appropriate resources must be available to perform checks during the production process. However, it is unclear which measures may be taken in the factory to prevent carry-over.

The Netherlands
According to the NVWA, the following measures are legal (numbered in accordance with question 14 on the questionnaire, Annex 1):
- B. Flushing with a feed material (e.g. grains) which is used for a feed that contains the same coccidiostat (at additive level).
- I. Flushing with a feedstuff (e.g. grains), which is subsequently destroyed.
- J. Taking measures within the installation or the process to limit carry-over to < 1%, such as sealing valves, application systems for fluids, product sequence.

According to the NVWA, the following measures are illegal:
- D. Flushing with a feed material which is used for a feed that contains a different coccidiostat (at additive level).
- E. Flushing with an animal feed not containing coccidiostats which is used for a feed that contains a different coccidiostat (at additive level).
- G. Flushing with an animal feed that contains a different coccidiostat (at additive level).

There were differences of interpretations among the Dutch respondents about the legality of the following measures:
- A. Mixing a flushing charge into a comparable feed which contains no coccidiostats.
- C. Flushing with an animal feed not containing coccidiostats, resulting in feed with a low concentration of the coccidiostat. The flushing charge is subsequently used for a larger quantity of animal feed containing the same coccidiostat (at additive level). For example: the last four tonnes of a sixteen tonne production run with salinomycin is used as a flushing charge and mixed with the other twelve tonnes. The premix is added to the twelve tonnes in a higher dosage, so that the end product has the right concentration. The issue here is whether the homogeneity of the coccidiostat can be guaranteed.
- F. Flushing with an animal feed with the same coccidiostat (at additive level).
- K. Adding coccidiostats outside the existing line. The issue here is whether the homogeneity of the coccidiostat can be guaranteed.
- H. Using different flushing charges for the milling/mixing line and the press line, resulting in double the volume of the flushing charge.

None of the inspectors gave reasons for their opinions on legality.

EU Member States
In line with the view in the Netherlands, five EU Member States indicated that the following measure is legal:
- B. Flushing with a feed material (e.g. grains) which is used as an ingredient for a feed that contains the same coccidiostat (at additive level).

One Member State (EU5) proposed a different legal measure: the combined use of successive production charges and flushing charges. It also recommended that specific components of a factory be kept extra clean. This is the responsibility of the compound feed producer.

According to five EU Member States, the following measures are illegal:
- A. Mixing a flushing charge into a comparable feed which contains no coccidiostats. The reason given by three EU Member States is that Directive 2002/32/EC does not permit the mixing of a feed containing an undesirable substance into an end product which does not contain the same

undesirable substance. In addition, the charges may not be diluted (article 5). This contrasts with the Netherlands, where there was a difference of opinion between the inspectors.

- D. Flushing with a feed material which is used for a feed that contains a different coccidiostat (at additive level). The reason given is that Directive 2002/32/EC does not permit the mixing of a feed containing an undesirable substance into an end product which does not contain the same undesirable substance. In addition, it was stated that there is a risk that the maximum levels of undesirable substances laid down in Directive 2002/32/EC will be exceeded.
- E. Flushing with a feed not containing coccidiostats which is used for a feed that contains a different coccidiostat (at additive level). The same reasons were given as for the previous measure.
- G. Flushing with a feed that contains a different coccidiostat (at additive level). The same reasons were given as for the previous measures. Another Member State (EU2) indicated that this is illegal, except where the maximum levels set by Directive 2002/32/EC are not exceeded. In addition, the production of a compound feed is not regarded by this Member State as a flushing charge.

As in the Netherlands, there were differences of interpretations among the six Member States about the legality of the following measures:

- C. Flushing with an animal feed not containing coccidiostats, resulting in feed with a low concentration of the coccidiostat. The flushing charge is subsequently used for a larger quantity of animal feed containing the same coccidiostat (at additive level) (n=3 legal, n=2 illegal). For example (see 3.2.6.2.2): the last four tonnes of a sixteen tonne production run containing salinomycin is used as a flushing charge and mixed with the other twelve tonnes. The premix is added to the twelve tonnes in a higher dosage, so that the end product has the right concentration. Two Member States (EU5 and EU6) gave as their reason that this measure is not permitted because the homogeneity of the coccidiostat cannot be guaranteed. One Member State (EU2) regards the measure as permissible because the producer is responsible for a homogenous product.
- F. Flushing with an animal feed containing the same coccidiostat (at additive level) (n=2 legal, n=3 illegal). One Member State (EU2) regards this situation as legal because this is not a flushing charge. Another Member State (EU6) indicated that this is theoretically legal, but that it can lead to further cross-contamination if animal feed is produced in the form of meal.
- H. Using different flushing charges for the milling/mixing line and the press line, resulting in double the volume of the flushing charge (n=1 legal, n=2 illegal). None of the Member States gave their reasoning for this position. One Member State (EU6) felt this was practically not feasible.

One EU Member State (EU1) did not answer the question about legal measures because it had not yet defined any illegal measures. Its compound feed manufacturers must demonstrate that they comply with the legislation, and that their quality management systems are effective, documented and implemented. Another Member State (EU6) did answer the questions, but indicated that it has no specific rules for which flushing charges are legal or illegal. The criterion used is what is necessary to prevent the risk of carry-over such as to comply with the EU legislation. The measures will be discussed with the inspection agencies at national and regional level.

4 Recommendations

On the basis of the information collected from the literature review and the questionnaire among a number of neighbouring EU Member States, a number of recommendations can be made which may be used to improve the sampling and control strategies regarding the carry-over of coccidiostats in compound feeds in the Netherlands which complies with the EU legislation.

4.1 Lot and sampling location

For enforcement of the carry-over of coccidiostats in animal feed, it is recommended that the samples be taken as close as possible to the final destination (for example, at loading). The advantage of choosing such a location is that at this point, the measurement will be most representative of the feed the animals receive. These values may not exceed the maximum quantities of coccidiostats in animal feeds intended for application to non-target animals (as listed in the Undesirable Substances Directive 2002/32/EC). This corresponds with the interpretation of the EU Member States studied. According to five EU Member States' interpretations of Regulation (EC) No 178/2002, the maximum standards are intended for the feed supplied to the livestock farm, i.e. the feed which is brought onto the market. A lot should therefore be defined as the quantity of a same animal feed which is supplied to a livestock farm.

Regulation (EC) No 152/2009 does not specify at which locations samples should be taken. If it is technically not possible to sample when the truck is loaded, as an alternative a final product cell could be sampled, preferably at the moment when the silo is emptied or loaded. Static product is harder to sample representatively.

4.2 Sampling procedure: uniformity of the lot

Most of the EU countries studied use the uniform sampling procedure described in Regulation (EC) No 152/2009, but were not able to provide reasons for their choice. However, the evaluation of a salinomycin flushing charge clearly shows that a flushing charge must be regarded as being inhomogeneous. Any mixing as a result of pouring does not change this. Although this is the result of one sample, this is a general picture and it is expected that no change will occur with a larger sample. It is therefore recommended when sampling a flushing charge to take the inhomogeneity of the coccidiostat in the flushing charge into account. The sampling protocol set out in Regulation (EC) No 152/2009 for 'undesirable substances or products likely to be distributed non-uniformly throughout the feed' would appear to be the appropriate procedure for doing so.

In the case of a non-uniform sampling procedure, it is recommended that the concentration of each end sample be checked against the maximum concentrations specified in Directive 2002/32/EC. Most of the EU Member States studied made no suggestions regarding this procedure. One EU country calculates the average concentration of coccidiostats in the final samples in order to approve or reject a batch. The average concentration may not exceed the maximum level in accordance with Directive 2002/32/EC.

4.3 Measures to combat carry-over of coccidiostats

The Undesirable Substances Directive 2002/32/EC states that animal feed containing an excessive concentration of an undesirable substance may not be mixed for dilution purposes with the same product or with other products intended for feeding animals. It is unclear which measures fall under dilution, and it is therefore recommended that agreements be made about these measures at EU level.

It should be noted that the control strategy for the enforcement of dilution will have to be different from the strategy for the enforcement of carry-over: samples will have to be taken in the factory in order to be able to analyse the process.

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Annex 1 Questionnaire

Sampling and inspection strategy for carry-over of coccidiostats in compound feed

Cross-contamination of coccidiostats in feed manufacturing companies is a recognised problem. After the preparation of a compound feed with coccidiostats, contamination of subsequent production is quite common. Cross-contamination of coccidiostats is regulated by EU- legislation. EU Regulation (EG) No 8/2009 (2002/32/EG) describes maximum cross-contamination levels of coccidiostats in feed, and Regulation (EG) No 152/2009 describes the methods of sampling and analysis for the official control of feed.

The Dutch new Food and Consumer Product Safety Authority (represented by Mr. Drs. R.G. Herbes) and Dutch Ministry of Economic Affairs, Agriculture and Innovation (represented by Drs. E. Deckers) have asked RIKILT – Institute for Food safety (part of Wageningen University and Research centre) to advise on the sampling and inspection strategy for carry-over of coccidiostats in feed. The objective of this questionnaire is to study the sampling strategies, and the interpretation and implementation of 2009/8/EG in the other EU member states.

We highly appreciate your assistance to fill in and return the questionnaire before October 7th, 2011 to marjolein.vanderspiegel@wur.nl. The results of this questionnaire will be treated confidentially. A brief report of anonymous results will be provided as an expression of gratitude for your assistance. If you have any questions regarding the questionnaire, please do not hesitate to contact me.

Note on definitions:

1. In this questionnaire the quantity of feed in the mixer with the same composition and the same production number is called a charge.
2. The first charge of feed produced after a charge with coccidiostats is a called a 'flushing feed charge' in this questionnaire.
3. All questions below are related to carry-over of coccidiostats and not to other additives or antibiotics.
4. Feed manufacturers in our questionnaire include both independent feed manufacturing companies and feed mills of integrated poultry producers.

Respondent

Name:	Click here to enter text.
Function:	Click here to enter text.
Organisation:	Click here to enter text.
Country:	Click here to enter text.
Email address:	Click here to enter text.

Questionnaire Sampling and inspection strategy

Feed

1. Coccidiostats can be included in (compound) feeds. Do feed manufacturers in your country produce coccidiostat containing feed?

   ☐ Yes
   ☐ No ---> Thank you for your cooperation. Please send the questionnaire.

2. Several coccidiostats can be used in feed:
   a. Which coccidiostats are added to feed in your country?

      ☐ lasalocid sodium
      ☐ narasin
      ☐ salinomycin sodium
      ☐ monensin sodium
      ☐ semduramicin sodium
      ☐ maduramicin ammonium alpha
      ☐ robenidine hydrochloride
      ☐ decoquinat
      ☐ halofuginone hydrobromide
      ☐ nicarbazin
      ☐ diclazuril
      ☐ Other: Click here to enter text.

   b. Is it allowed that more than one coccidiostat is included in the same charge of feed?

      ☐ Yes
      ☐ No

   c. In which type of feed products are coccidiostats used?

      ☐ Meal
      ☐ Pellets

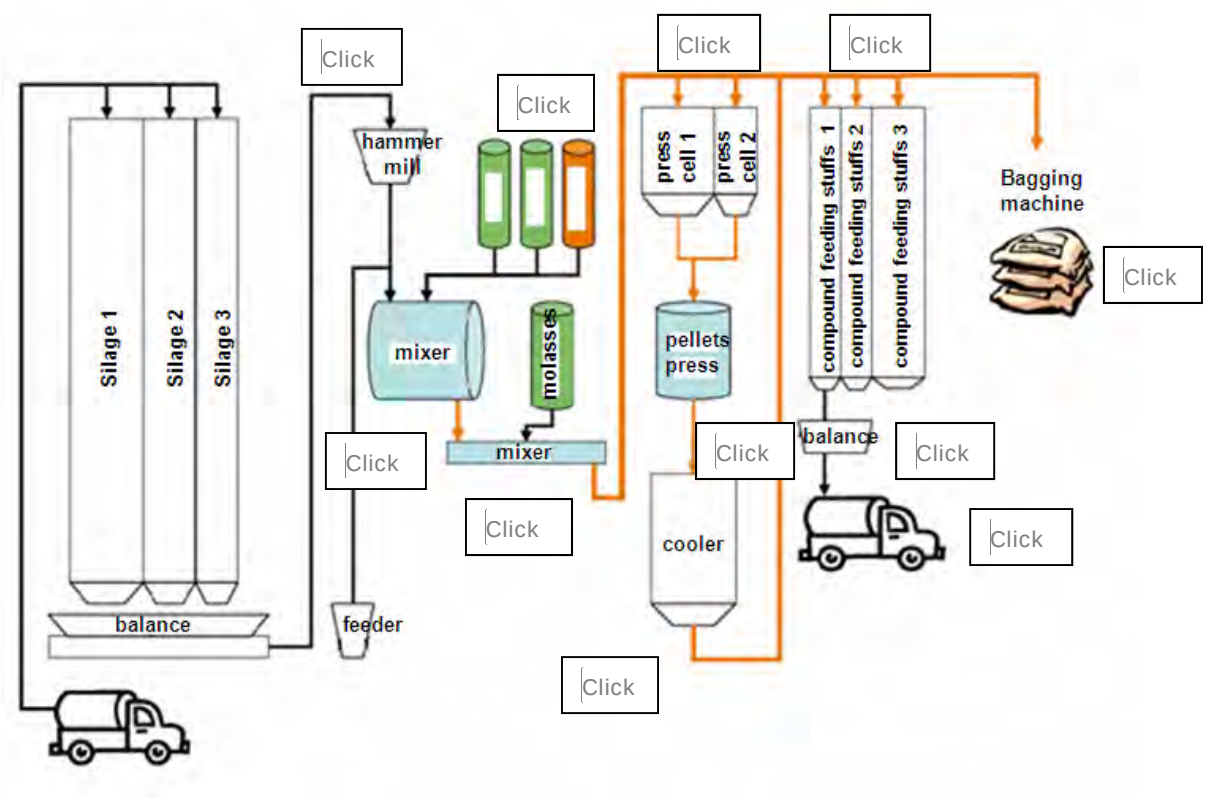
Sampling location

3. Is adding of coccidiostats outside the factory (e.g. dosing at the truck) allowed?

   ☐ Yes

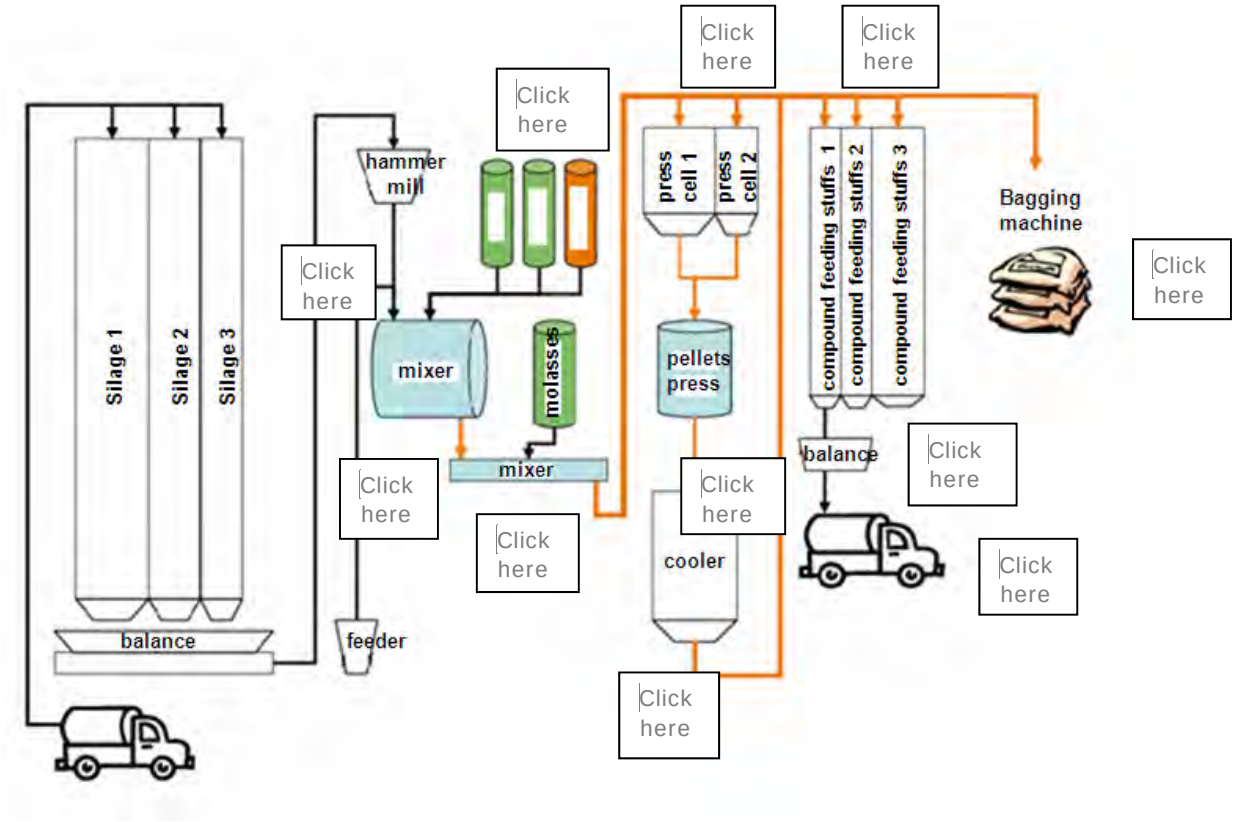
   ☐ No

4. The next figure shows a process description of a feed manufacturer. Could you indicate in this figure where (at which point in the production line) coccidiostats are added? Specify the locations for meal (M) and pellets (P) separately.



Click here to enter text.

5. Could you indicate in the next figure where samples for the official inspection of coccidiostats are usually taken? Specify the locations for meal (M) and pellets (P) separately.



Click here to enter text.

6. What is an alternative sampling location when sampling at the preferred official location is not possible due to design of equipment or production lines? Please specify the locations for meal (M_a) and pellets (P_a) separately in the figure above.

Click here to enter text.

7. Final product cells are defined here as containers, silos or compartments in which the complete feed is collected and stored before loading the truck and shipment to the farmers.

   a. Are final product cells used in your country?

      ☐ Yes

      ☐ No

   b. Is it allowed to add different charges of (flushing) feed (with the same composition) in one final product cell?

      ☐ Yes

      ☐ No

   Explanation: Click here to enter text.

   c. How do you take samples from these final product cells? Specify for meal (M) and pellets (P):

* Location in the cell:
  - ☐ Whole final product cell Choose an item.
  - ☐ Part of final product cell Choose an item.

* Process:
  - ☐ In the product flow Choose an item.
  - ☐ Stationary product Choose an item.

* Length:
  - ☐ Top of the cell Choose an item.
  - ☐ Middle of the cell Choose an item.
  - ☐ Bottom of the cell Choose an item.

* Width:
  - ☐ Side of the cell Choose an item.
  - ☐ Middle of the cell Choose an item.

Click here to enter text.

8. EU Regulation (EG) No 8/2009 (2002/32/EG) describes maximum cross-contamination levels of coccidiostats in feed. According to your national interpretation, for which feed is this level meant?

   ☐ Feed to be delivered to the farmer
   ☐ Feed after the pelleting process
   ☐ Feed after mixing
   ☐ Feed after cooling
   ☐ Other: Click here to enter text.

Sample

9. Which feed charges are selected for sampling?

   ☐ Flushing feed charges
   ☐ Random charges (could be regular charge or flushing feed charge)
   ☐ Other: Click here to enter text.

10. During inspection the batch of feed assumed to contain coccidiostats is sampled. According to Regulation EC (No) 767/2009 a 'batch' or 'lot' means an identifiable quantity of feed determined to have common characteristics, such as origin, variety, type of packaging, packer, consignor or labelling, and, in the case of a production process, a unit of production from a single plant using uniform production parameters or a number of such units, when produced in continuous order and stored together. According to Regulation EC (No) 152/2009 a 'sampled portion' means a quantity of product constituting a unit, and having characteristics presumed to be uniform.

    Please explain what a batch means during sampling of potential coccidiostats contaminated feed, according to the interpretation in your country:

    ☐ All feed with the same lot number of the producer
    ☐ All feed produced in the mixer at the same time
    ☐ All feed with the same composition produced in a sequence
    ☐ All feed with the same composition not produced in a sequence
    ☐ All feed with the same composition going to the same farmer
    ☐ Other: Click here to enter text.

Sampling procedure

11. Commission Regulation (EG) 152/2009 describes a sampling procedure for uniform and non-uniform distributed substances throughout the feed.

    a. Are samples for carry-over of coccidiostats taken according to the uniform and non-uniform sampling procedure?

    ☐ Uniform

    ☐ Non-uniform

    b. Why do you consider that sampling of carry-over of coccidiostats should be conducted according to the uniform or non-uniform sampling procedure?

    Click here to enter text.

    c. In case your country uses the non-uniform sampling procedure, how do you evaluate the results according to 2009/8/EC?

    ☐ The coccidiostatic content in each individual final sample should meet the levels mentioned in 2009/8/EC

    ☐ The average coccidiostatic content of the final samples is calculated and this should meet the levels mentioned in 2009/8/EC

    ☐ Only if the coccidiostatic content in all end samples is above the legal limit(s) of 2009/8/EC, the charge is evaluated as 'not compliant'.

    ☐ Other: Click here to enter text.

12. In some cases it is possible that not the whole charge of feed can be sampled, for instance due to a large quantity of feed or a high production speed.

    a. Does it occur in your country that not the whole charge can be sampled?

    ☐ Yes

    ☐ No

    b. In case that only a part of the charge can be sampled, how would you interpret the results?

    Click here to enter text.

Carry-over

13. Several tracers are used for measurement of the carry-over percentage in the feed factory (e.g., cobalt chloride, methylviolet). Do you think these tracers represent the carry-over of coccidiostats?

    ☐ Yes

    ☐ No

    Explanation: Click here to enter text.

14. Several measures can be taken in the production process to reduce carry-over of coccidiostats. Could you indicate which of the following procedures of operators of feed establishments are legal or illegal in your country?

Measure	Legal	Illegal	Explanation
A. Mixing of the flushing feed charge in an equivalent blank (i.e. free of coccidiostats) feed product	Click here to enter text.	Click here to enter text.	Click here to enter text.
B. Flushing with a feed material (e.g. cereal grains), which is used for inclusion in a feed containing the same coccidiostatic (at additive level)	Click here to enter text.	Click here to enter text.	Click here to enter text.
C. Flushing with a blank feed (feedingstuffs), which is used to produce a larger amount of feed containing the same coccidiostatic (at additive level). For example, the last 4 tons of a 16 ton production with salinomycine are used as flushing feed and mixed with the previous 12 tons. The premix is added to the 12 tonnes in a higher dosage, so that the final feed has the right concentration.	Click here to enter text.	Click here to enter text.	Click here to enter text.
D. Flushing with a feed material, which is used for a feed containing another coccidiostat (at additive level)	Click here to enter text.	Click here to enter text.	Click here to enter text.
E. Flushing with a blank feed, which is used for a feed containing another coccidiostat (at additive level)	Click here to enter text.	Click here to enter text.	Click here to enter text.
F. Flushing with a feed containing the same coccidiostatic (at additive level)	Click here to enter text.	Click here to enter text.	Click here to enter text.
G. Flushing with a feed containing another coccidiostatic (at additive level)	Click here to enter text.	Click here to enter text.	Click here to enter text.
H. Use of different flushing feeds for the grinding/mixing line and pelleting line resulting in double volume of flushing feed	Click here to enter text.	Click here to enter text.	Click here to enter text.
I. Other: Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Thank you for your cooperation by filling in this questionnaire. We kindly ask you to send the questionnaire before October 7th, 2011 to marjolein.vanderspiegel@wur.nl.

Remarks

Click here to enter text.

Annex 2 Enforcement agencies

The following enforcement agencies answered the questionnaire:

The Netherlands:	Dutch Food and Consumer Product Safety Authority (NVWA) – 2 respondents
Belgium:	Federal Public Service Health (FPS) - Food Chain Safety and Environment + Federaal Agentschap voor de Veiligheid van de Voedselketen / Agence fédérale pour la sécurité de la chaîne alimentaire (FAVV/AFSCA)
Denmark:	Danish Veterinary and Food Administration (FVST)
Germany:	Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV-NRW)
Italy:	Ministry of Health, General Directorate of Protection and Animal Health (DGSA), office VII animal nutrition
Luxembourg:	Administration des Services Techniques de l'Agriculture (ASTA)
Poland:	General Veterinary Inspectorate (GIW)
Spain:	Ministerio de Medio Ambiente y Medio Rural y Marino (MARM), Subdirección general de conservación de recursos y alimentación animla – 3 experts, 1 respondent

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RIKILT report 2013.014

RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

The mission of Wageningen UR (University & Research centre) is 'To explore the potential of nature to improve the quality of life'. Within Wageningen UR, nine specialised research institutes of the DLO Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment. With approximately 30 locations, 6,000 members of staff and 9,000 students, Wageningen UR is one of the leading organisations in its domain worldwide. The integral approach to problems and the cooperation between the various disciplines are at the heart of the unique Wageningen Approach.

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