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Cereals and cereal products — Sampling studies

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Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 338, *Cereal and cereal products*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document presents the results of three groups of studies which results have been used to elaborate ISO 24333

These studies have been managed by United Kingdom in May 2003, by France in 2004-2005 for the first one and 2006-2007 for the second one and Germany in 2008.

Cereals and cereal products — Sampling studies

1 Scope

This document presents the description and the results of the three studies conducted by United Kingdom, France and Germany related to grain sampling in order to define a harmonized sampling protocol for official controls.

These results had been used to draft ISO 24333.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Context

European directives for official controls of some contaminants such as mycotoxins required methods for sampling and analysis. In order to harmonize sampling procedures necessary for these analysis and to determine the best way to prepare a homogenous and representative laboratory sample, studies had been conducted by United Kingdom, France and Germany.

The results of these 3 studies are presented in this report.

5 Study n°1: extract from "Grain sampling and assessment: sampling grain in lorries" – Project report n° 339"

5.1 General

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This is the final report of a 13-month project that started in May 2003. The work was funded by HGCA (project 2955).

5.2 Context

This two-year programme was made at the request of HGCA to improve and standardise grain sampling and analysis across the UK cereals industry.

The first phase of the programme was to develop and validate protocols suitable for collecting samples of grain on UK farms at harvest time and to train farmers in their use. The second part was to examine approaches to the collection of samples during storage and to compare the results obtained, wherever possible, with data collected as the store was filled. During the course of earlier sampling work, there was strong interest expressed in the mechanics and effectiveness of sampling loads of grain in lorries. In addition, some of the work done during storage involved sampling grain as it left the store in lorries. This showed up the limitations of some approaches to lorry sampling and highlighted the need for more information. An assessment of lorry sampling was therefore, added and is reported here.

Almost all grain is sampled as it is delivered to end users to confirm its quality and to ensure that contractual obligations are met. This sampling takes the form of collecting one or more samples from a lorry-load on arrival. The equipment used and method of sampling varies between end users and there are no data to show if these variations may cause bias in the representativeness of the sample and, therefore, in the results of quality analysis.

The key aim of end-user sampling is to ensure that the quality of the grain is suitable for its intended use. Therefore, sampling is done before the load is tipped and this limits access to the surface of the load. This access is further constrained by food safety and HSE legislation that prevent the sampler from walking on the load.

A limited assessment of the practicalities of sampling lorries was done in 1992 (HGCA Project Report 79) in which the effects of method of sampling, number and position of sample points, the methods of loading lorries were considered. The wheat sampled was low-grade feed material with a low specific weight and a high level of fine material so was not representative of other grades. The results suggested that loading lorries with a front loader or from a hopper had no effect on the distribution of the quality characteristics within a load. Small differences were noted in the mean values for specific weight between automatic sampling using a Samplex CS90 and a manual spear but overall variability of the grain meant that these differences were not significant. There was significant variability in the results obtained at individual points with either method although this variability was random and not associated with any part of the load. Fine material appeared to be very difficult to measure. At the time, this work was undertaken there were no restrictions limiting access to the surface of the grain so that a widely disbursed pattern of sampling points could be used with the manual sampling. The conclusions from this work were that it was extremely unwise to base an assessment of lorry load of grain on a single sample and that more work was needed to confirm the results and to assesses other grades of grain. The aim of this project was to establish if there are any inherent problems with the sampling of grain for the determination of quality characteristics in lorries at the point of intake and to establish recommendations in the form of a protocol for the sampling of grain under these conditions.

Grain was sampled using automated systems (Samplex CS90) and manual spearing to see if the method of sampling influenced the grain quality measurements. A key part of the process was to assess the influence that the number of samples taken from each load had on the likely accuracy of the results. Samples were collected at 4 different locations; on two occasions 10 lorries were sampled and on two occasions 8 lorries were sampled. At three locations, CS90 samplers were used and 8 samples were withdrawn from each load and at the other location samples were taken manually with a multi-compartmented spear with 5 samples being taken from each lorry. A comparison of different ways of sample handling was obtained by comparing the individual results from the 8 samples against an analysis of samples withdrawn from a composite sample formed from 8 samples. The latter method reflects more accurately the procedure followed at most stores.

Results indicated that there were no statistically significant differences between results from the individual samples or from the composite samples. Monte Carlo simulation of the impacts of using 2, 3, 5 or 8 samples per load revealed that the greater the number of samples used the greater the reliability of the result and the more likely it was to represent the true mean of the load. It was noted that automatic sampling equipment can no longer sample the entire length of a trailer and this could cause problems with obtaining the ideal sample. Manual sampling also had severe limitations due to the lack of safe access for sampling of trailers.

A sampling protocol for lorries is presented which emphasises the need for 8 samples to be taken from each load in order to get a good representation of the quality of the entire load.

5.3 Studies conducted and objectives

The study was conducted to assess the effectiveness of different approaches to sampling loads of grain in lorries.

The specific objectives were:

— To assess if the method of collecting samples influences grain quality measurement;

— To assess if the number and position of sampling points influences grain quality measurement;

— To provide guidelines for sampling lorries giving reliable information about grain quality.

5.4 Methodology

5.4.1 Conducting tests

5.4.1.1 Collection of data relating to current sampling practice

In November 2002 the HGCA circulated a questionnaire to commercial grain stores and end-users of grain requesting information about their methods of analysis and methods of intake sampling. The information that was collected was used to assist with the design of the assessment of lorry sampling.

5.4.1.2 Sample collection

5.4.1.2.1 Store 1

The work was done at a store specialising in the storage of malting barley. Lorries were loaded with malting barley, variety Pearl, of a quality representative of that delivered to central storage from farms. The lorries, all 28 t articulated units, were loaded with a front loader fitted with a 2 t bucket.

Ten loads were sampled over a 2-day period. Sampling was done using the store's Samplex CS90 automated vacuum sampler. Initially, it had been expected to re-programme the CS90 to take 10 samples/load in a pre-set pattern. However, observation of the method of operation and sampling pattern achieved by the CS90 suggested that there was no advantage in using more than the 8 points provided by one of the standard sampler programmes.

During the setting up and initial testing of the CS90, the slide on the sample spear was opened to its maximum to increase the sample size. The system was set to collect grain only during the withdrawal as is recommended by the manufacturer for granular materials.

Each of the eight points was sampled three times. On the first occasion, individual samples were held separately. During the second and third samplings, all samples were bulked into single batches. One of these bulk samples was held as a composite sample and the other was used to provide samples of 1, 2 and 3 litres (small medium and large) collected at random with a 1-litre jug.

A small sub-sample from each of the individual samples was tested on the spot by store staff for screenings and germinative capacity. Screenings were tested by sieving a 100 g sub-sample with a motorised shaker fitted with a 2,25 mm mesh screen for 2 minutes. The germinative capacity was tested using the standard tetrazolium test.

5.4.1.2.2 Store 2

Work was done at a commercial store during the normal out-loading of feed wheat. The lorries were loaded from an on-floor bulk using a front-bucket loader and were sampled as they left the store. Normal sampling practice was to collect a single spear sample/load using a manual, multi-compartmented spear of about 1,7 m in length. Access to the load was via a small sampling platform that only allowed samples to be taken from less than half the length of the loaded trailer and from only one side of the load.

For the purposes of this investigation, 5 sample points were used for each load and the lorry was moved forward during the sampling process so that access to the whole length of the load could be obtained. However, it was not practical to turn the lorry round to give access to both sides of the load. Manual sampling meant that there was, inevitably, some variation in the exact location of the sample points between loads. Three spear samples were collected at each point. The first was held as an individual sample, the second bulked to form a composite sample and the third bulked to give a sample from which three random samples (small, medium and large) could be taken without mixing.

Ten lorry loads were sampled over a two-day period.

5.4.1.2.3 Store 3

The assessments were made at a commercial store during the normal out-loading of milling wheat. Samples were collected using a Samplex CS90 but without the automatic option. Therefore the spear had to be controlled manually by the operator and this meant that there was considerable variation in the positioning of sample points between loads. A further constraint on sampling was that the CS90 was positioned at one end of the weighbridge thus limiting access to just one-half of the load. The slide on the sampler was fully open and grain was collected only as the spear withdrew.

The lorries were loaded using a bucket loader from a 2 000 t batch of wheat stored on-floor. Only a limited number of loads were dispatched each day and time constraints meant that only 8 loads were sampled during this assessment.

Three samples were collected from 8 sample points in each load. The first was held as an individual sample, the second bulked to form a composite sample and the third bulked to give a sample from which three random samples (small, medium and large) could be taken without mixing. The 8 points were arranged in a 3, 2, 3 pattern with the two samples being taken from the centre line and each row of 3 taken down the sides of the load (see Figure 1). The location of the sampler and the position of the lorry on the weighbridge meant that the samples always came from the front half of the load.

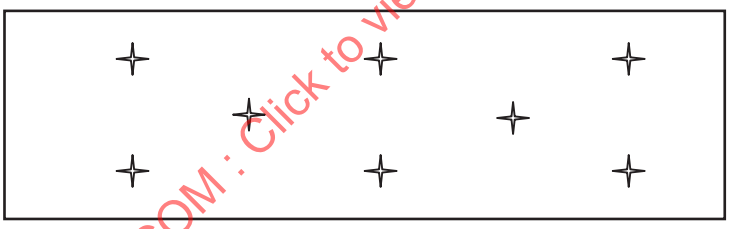


Figure 1 — Arrangement of sampling points used by CS90 sampler

Data was supplied by the storekeeper giving the store's own assessment of the quality of the batch of grain as measured when the store was filled.

5.4.1.2.4 Store 4

Loads of milling wheat delivered to a large flour mill were sampled with a Samplex automatic CS90. This work was done some months after the earlier assessments and it was decided that the collection of extra samples to make up composite batches was not justified. Eight samples were collected from eight loads of wheat over a 10 d period. For technical reasons, the collection of samples had to be done by mill staff so the exact details of the points sampled are not known.

5.4.1.3 Assessment of samples

Samples had to be transported to the testing laboratory and some additional delays occurred between the collection and assessment of samples. However, samples that could not be analysed within 48 h were stored in a freezer at -16 °C to minimise changes in the properties of the grain. These samples were allowed to return to ambient temperature before testing. The exceptions to this were samples collected from store 4.

These were collected over a 10 d period and a further 4 d elapsed before the samples arrived for testing. During this period, the samples were not held under controlled temperature conditions.

Individual samples were tested separately, as were the three different sizes of random sample. However, in the case of the medium and large samples, these were divided by coning and quartering to give the correct volume of grain for assessment. The composite samples were also mixed and then divided by coning and quartering. Five sub-samples of the composites were tested from Store1 but only three sub-samples were tested from stores 2 and 3.

As a first step in the assessment process, the screenings in each sample were measured by manual sieving. Each sample was weighed and then sieved for 30 s using a 2,5 mm slotted sieve for wheat or a 2,25 mm slotted sieve for barley. The sievings were weighed and the percentage calculated. The weights of the individual samples gave an indication of the variation in the size of sample collected on each occasion.

After sieving, the properties of each sample were assessed using a Foss Infratec Grain Analyser 1241 GA-TWM[1)]. The machine used official calibrations as provided by the NIR network and measured moisture content, specific mass, protein in the case of wheat, or nitrogen in the case of barley and made an assessment of hardness of wheat.

In addition, some of the samples of wheat from stores 2 and 3 were sent to NIAB for assessment of Hagberg Falling Number. Complete sets of individual samples from 5 loads, together with a single composite sample were tested from Store 3. Complete sets of individual samples and a single composite from 4 loads were tested from Store 2. The testing was done using standard methodology and each result was the mean of two determinations.

5.4.1.4 Estimating the reliability of sampling

The impact of the number of samples taken on the reliability of the result obtained from those samples was assessed by determining the mean and standard deviation for the results of the 8 samples taken from each lorry. This information was used to define a probability curve for a normal distribution for each of the lorries. A Monte Carlo simulation was then run to sample from either 2, 3, 5 or 8 of these distributions depending on the sampling regime to be simulated. The simulation was run for a total of 100 000 trials. From this result, cumulative probability distribution curves were obtained and these were used to estimate the confidence limits for different numbers of samples for a given margin of error. Thus, the confidence interval for sampling 2, 3, 5, or 8 times for a known deviation from the mean for the different quality parameters could be produced. The final figure shows the probability of the confidence interval actually covering the mean value.

5.4.2 Results and conclusions

5.4.2.1 Sample analysis results

5.4.2.1.1 Collection of data relating to current sampling practice

As part of the initiative to standardise grain testing a survey of laboratory practice was undertaken and this included a set of questions on the collection of samples from lorry-loads of grain. This provided information from a range of commercial premises receiving, handling or processing grain about current lorry sampling practices. The response showed that there was no common industry-wide approach. The most frequently used equipment was the Samplex CS90 or other unspecified Samplex units (49 %) followed by manual sampling (37 %). The number of samples collected per load ranged between 1 and 10 and the mass of grain collected varied between 0,4 kg and 11 kg.

1) Foss Infratec Grain Analyser 1241 GA-TWM is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of thisproduct.

5.4.2.1.2 Observations on the sampling

5.4.2.1.2.1 Store 1

During the assessment, it became apparent that the reach of the CS90 sampling arm and the size of current articulated trailers resulted in parts of the load being inaccessible unless the lorry was moved. Of the total 11 m length of the trailer, up to 2,5 m at the front and 2,5 m at the rear of the trailer were not accessible to the sampler. Therefore, up to 40 % of the grain could not be sampled (see Figure 2).

The weight of individual samples collected varied considerably. The size of sample was related to the depth of grain at the point of sampling, the greater the depth, the larger the sample. The bucket loading method led to peaks and troughs in the loaded grain and this, in turn produced variation in sample size (see Figure 2 and Table 1).

Each individual sample was sucked from the sample spear to the laboratory and was collected in a cyclone. There was obvious separation of fine material and grains during this process (see Figure 3).

Figure 2 — Collection of samples from lorry-loads of barley with a Samplex CS90

Figure 3 — Individual samples collected in the cyclone showing the separation of fine material

5.4.2.1.2.2 Store 2

The collection of samples was limited to one side of the load only and the exact location of the sample points varied between loads because the position of the lorries in relation to the sampling platform varied. The depth of the grain in the trailers varied as at Store 1. However, the grain was always deep enough to permit the manual spear to be inserted to its full depth so there was much less variation in the weight of sample (see Table 1). Conversely, the spear was usually not long enough to reach to the bottom of the load. The process of collecting 5 samples/load was time consuming and physically

demanding. Moving the lorry to allow access to the whole length of the load could not be considered as a practical approach in most situations.

5.4.2.1.2.3 Store 3

The manual control of the insertion of the sample probe was imprecise because the probe tended to swing back and forth as it was moved between sample points. The swing was exaggerated by the effects of wind. The position of CS90 meant that only the front half of each load could be sampled.

The size of individual samples was related to depth of grain in the trailer and this varied within and between loads.

5.4.2.1.2.4 Store 4

The Samplex CS90 was situated some distance from the laboratory and, during normal sampling in which 8 samples were collected/load, the samples were accumulated at the CS90 and then conveyed by suction to the laboratory. The bulked samples were then passed through a mixer/divider to produce a working sample for analysis. Therefore, during normal sampling procedures, it was not possible to obtain samples from individual positions within a load. For the purposes of this investigation, 8 loads were sampled so that each of the eight individual samples/load was conveyed to the laboratory and collected before the next sample was taken. This was a time consuming and disruptive process so that it was only possible to sample a single load/day.

5.4.2.1.3 Mass of samples collected

The masses of the individual samples collected from the loads at each store are summarised in Table 1.

The wide variation in the weights of samples both between and within loads found at the two stores where grain was sampled with a CS90 relates to the variation in the depth of grain at different parts of the load. The variation did not occur with the manual spear because, irrespective of the depth of grain, the spear could always be inserted to its full length.

5.4.2.2 Analysis of the quality parameters of the grain

The results of the quality analysis done using the Foss Infratec Grain Analyser are given in Tables 2, 3, 4 and 5. The results of assessing the level of fine material (screenings) are also given. For stores 2 and 3 the amount of fine material was established by the project staff using a manual sieve but data for the additional tests done by store staff at Store 1 using a mechanical shaker are also included.

The storekeeper at Store 3 provided the project with the results of intake sampling of the store used for filling the loads sampled as part of this project. Obviously, the grain sampled during the project (~250 t) represented a very small part of the 4 500 t contained in the store. However, values are given in Table 5 that allow some comparisons to be made with the results obtained by sampling loads leaving the store after about 10 months storage (see Table 4). The intake sampling procedure used at the Store 3 was to collect samples from three points from each load and to mix these samples to give a composite that was used for analysis. The analyses were done using a Foss Infratec that was calibrated to the same standards as applied to the instrument used during the project. A summary of the storekeeper's analysis results is given in Table 6.

Table 1 — Maximum, minimum and mean masses of the batches of individual samples collected at each store. There were 8 individual samples at stores 1 3 and 4, and 5 individual samples at store 2

		Mass g		
Store	**Load**	**Max.**	**Min.**	**Mean**
Barley Sampled with automatic CS90	1	738,7	476,8	604,2
	2	725,4	615,2	670,6
	3	808,5	469,1	654,5
	4	826,4	425,0	649,4
	5	761,9	437,4	604,9
	6	721,6	487,3	610,5
	7	816,1	510,3	677,0
	8	732,6	464,4	625,2
	9	737,3	471,1	623,1
	10	787,9	424,9	638,7
Wheat sampled manually	1	480,7	378,2	452,3
	2	541,3	418,0	471,2
	3	485,5	468,8	478,1
	4	479,3	368,6	443,4
	5	474,4	406,7	455,1
	6	481,4	464,2	476,3
	7	457,4	389,0	432,3
	8	467,4	410,0	445,4
	9	462,6	445,8	451,9
	10	463,8	436,3	450,6
Wheat Sampled with non-automatic CS90	1	1062,5	470,1	824,6
	2	790,8	458,9	560,5
	3	760,2	760,2	760,2
	4	730,9	427,6	538,2
	5	846,5	390,8	599,1
	6	799,6	466,0	605,3
	7	765,5	117,6	487,3
	8	736,5	490,2	612,3
Wheat sampled with automatic CS90	1	633,9	449,2	525,2
	2	673,0	552,3	597,0
	3	627,6	483,7	571,0
	4	922,8	566,6	642,7
	5	611,4	483,3	546,1
	6	641,5	571,6	604,0
	7	663,0	552,9	606,0
	8	616,1	59,60	470,1

Table 2 — Maximum, minimum and mean values for the analysis of the eight individual barley samples collected at Store 1

	Nitrogen (DM)	Moisture %	Volumic mass	Fines %	
Load 1				**Store**	**Project**
Maximum	1,77	14,4	69,1	4,6	2,9
Minimum	1,74	13,8	64,8	3,2	1,7
Mean	1,79	14,0	67,7	3,6	2,4
Load 2					
Maximum	1,77	14,4	69,0	3,9	2,6
Minimum	1,72	13,7	68,1	3,1	2,1
Mean	1,78	14,0	68,5	3,6	2,3
Load 3					
Maximum	1,75	14,1	69,5	4,3	3,2
Minimum	1,72	13,8	67,9	2,9	1,6
Mean	1,77	13,9	68,8	3,6	2,2
Load 4					
Maximum	1,76	14,3	70,0	3,6	2,8
Minimum	1,75	13,8	68,4	2,9	1,7
Mean	1,78	14,0	69,1	3,4	2,0
Load 5					
Maximum	1,76	14,3	69,7	5,2	2,9
Minimum	1,72	13,8	68,2	3,3	1,9
Mean	1,79	14,1	69,2	4,2	2,4
Load 6					
Maximum	1,75	14,1	69,1	5,6	3,3
Minimum	1,72	13,8	68,3	4,3	2,0
Mean	1,78	14,0	68,7	4,8	2,7
Load 7					
Maximum	1,75	14,3	69,2	6,4	2,9
Minimum	1,74	14,0	68,5	4,2	1,9
Mean	1,77	14,2	68,7	5,1	2,3
Load 8					
Maximum	1,76	15,0	69,5	5,5	3,4
Minimum	1,75	13,7	68,5	3,4	2,1
Mean	1,78	14,2	68,9	4,4	2,6
Load 9					
Maximum	1,76	14,1	69,4	4,4	3,4
Minimum	1,72	13,8	68,5	3,7	2,0
Mean	1,78	14,0	68,9	4,0	2,6
Load 10					
Maximum	1,74	14,5	69,0	5,4	3,5
Minimum	1,60	13,8	68,0	3,0	1,9
Mean	1,78	14,1	68,6	4,5	2,5

Table 3 — Maximum, minimum and mean values for the analysis of the five individual wheat samples collected at Store 2

	Protein DM %	Moisture %	Hardness	Volumic mass	Fines %
Load 1					
Maximum	11,2	13,7	41,4	76,3	2,9
Minimum	10,8	13,4	37,1	72,9	1,8
Mean	11,0	13,5	39,0	75,1	2,4
Load 2					
Maximum	11,2	14,7	40,5	75,0	2,2
Minimum	10,9	13,8	34,1	72,1	1,7
Mean	11,0	14,2	38,0	73,9	1,9
Load 3					
Maximum	11,1	13,9	43,0	76,3	2,4
Minimum	10,7	13,7	35,6	75,2	1,9
Mean	10,9	13,7	38,0	75,7	2,2
Load 4					
Maximum	11,3	14,1	37,9	74,9	2,1
Minimum	10,7	13,8	31,6	73,7	1,7
Mean	11,0	13,9	35,4	74,4	1,8
Load 5					
Maximum	11,1	14,2	38,4	75,5	2,1
Minimum	10,6	13,5	34,3	73,3	1,6
Mean	10,9	13,9	36,8	74,8	1,9
Load 6					
Maximum	11,2	14,0	35,6	76,3	2,9
Minimum	10,8	13,3	28,7	74,8	2,0
Mean	11,0	13,6	33,0	75,8	2,4
Load 7					
Maximum	11,1	14,9	36,5	75,2	2,5
Minimum	10,5	14,3	29,3	74,4	1,7
Mean	10,8	14,6	32,4	74,9	2,1
Load 8					
Maximum	10,6	14,5	31,7	75,4	2,2
Minimum	10,2	14,3	24,1	74,5	1,8
Mean	10,4	14,4	28,6	75,0	2,0
Load 9					
Maximum	11,0	14,8	37,2	75,6	2,3
Minimum	9,9	14,5	16,5	73,8	1,9
Mean	10,6	14,7	29,2	74,6	2,1
Load 10					
Maximum	11,6	15,7	40,5	75,5	2,1
Minimum	10,9	14,6	16,6	73,5	1,4
Mean	11,2	15,0	33,7	74,8	1,8

Table 4 — Maximum, minimum and mean values for the analysis of the eight individual wheat samples collected at Store 3

	Protein DM	Moisture %	Hardness	Volumic mass	Fines %
Load 1					
Maximum	14,0	14,3	62,7	82,6	1,4
Minimum	13,7	14,1	55,9	82,0	0,5
Mean	13,9	14,1	58,5	82,4	0,8
Load 2					
Maximum	14,0	14,1	59,5	82,5	1,3
Minimum	13,8	14,0	55,9	81,8	1,1
Mean	13,9	14,0	57,8	82,1	1,2
Load 3					
Maximum	14,2	14,0	58,4	82,6	1,4
Minimum	13,7	13,9	53,1	81,4	1,0
Mean	13,9	14,0	55,7	82,2	1,2
Load 4					
Maximum	14,1	14,0	59,5	82,9	1,2
Minimum	13,8	13,9	54,7	82,4	0,9
Mean	14,0	14,0	57,5	82,7	1,0
Load 5					
Maximum	14,3	14,1	77,3	83,2	1,2
Minimum	14,0	13,8	53,8	82,8	0,6
Mean	14,1	13,9	61,2	83,0	1,0
Load 6					
Maximum	14,1	14,1	59,5	83,0	1,2
Minimum	13,8	13,9	55,8	82,2	0,7
Mean	13,9	14,0	57,3	82,7	0,8
Load 7					
Maximum	13,9	14,1	58,7	82,8	1,2
Minimum	13,7	14,0	56,5	82,0	0,8
Mean	13,8	14,0	57,4	82,4	0,9
Load 8					
Maximum	13,9	14,0	58,9	82,9	1,3
Minimum	13,8	13,9	53,7	81,8	0,9
Mean	13,9	14,0	57,2	82,5	1,2

Table 5 — Maximum, minimum and mean values for the analysis of the eight individual wheat samples collected at Store 4

The results of the store's own analysis, based on a composite sample made up from 8 individual probe samples, are given as Mill data.

	Protein (DM)	Moisture %	Hardness	Volumic mass	Fines %
Load 1					
Maximum	13,0	12,4	68,9	77,7	3,5
Minimum	12,7	12,1	61,9	76,5	1,9
Mean	12,8	12,3	65,2	77,0	2,63
Mill data	13,3	11,9	6	77,6	3,1
Load 2					
Maximum	11,8	14,1	40,5	76,8	4,2
Minimum	11,6	13,7	31,3	75	3,4
Mean	11,7	13,9	36,3	76,0	3,88
Mill data	11,8	14	2	76,8	4,9
Load 3					
Maximum	13,9	13,0	51,4	80,6	4,2
Minimum	13,4	12,6	42,2	79,5	3,4
Mean	13,7	12,7	47,0	80,1	3,88
Mill data	13,7	13,3	6	80,7	5,1
Load 4					
Maximum	12,4	13,4	46,2	79,7	1,9
Minimum	12,0	12,8	33,8	77,8	1
Mean	12,2	13,2	41,5	79,0	1,35
Mill data	12,6	13,6	2	79,5	2
Load 5					
Maximum	12,5	15,0	28,1	78,4	2,8
Minimum	12,1	14,7	21,5	75,9	1,8
Mean	12,3	14,9	24,50	77,3	2,23
Mill data	12,1	15	2	77,6	3,2
Load 6					
Maximum	12,5	12,8	48	78,4	2,1
Minimum	12,2	12,5	39,2	76,6	1,9
Mean	12,3	12,6	43,2	77,8	1,95
Mill data	12,5	12,8	2	79,1	3,2
Load 7					
Maximum	13,0	12,2	55,1	81,6	2,3
Minimum	12,5	12,0	41,2	80,1	2
Mean	12,7	12,1	47,2	81,0	2,14
Mill data	12,8	12,2	5	81,1	3,2
Load 8					
Maximum	11,3	13,2	38	77,5	2,2
Minimum	11,03	13,1	29	75,9	1,6
Mean	11,2	13,2	34,6	76,7	1,88
Mill data	11,4	13,4	2	77,6	2,8

Table 6 — Maximum, minimum and mean values for the analysis of the samples taken by the storekeeper during intake at Store 3

Figures in brackets are the mean value of the 8 loads sampled coming out of store.

	Protein (DM)	Moisture %	Volumic mass	Fines %
Maximum	14,0 (14,1)	16,0 (14,1)	82,0 (82,8)	4,3 (1,3)
Minimum	10,9 (13,8)	12,1 (13,9)	74,1 (82,1)	0,1 (0,8)
Mean	12,5 (13,9)	14,1 (14,0)	78,1 (82,5)	2,2 (1,0)

A summary of the analyses of the Falling Number of some samples from Stores 2 and 3 are given in Table 7. Three replicates of each sample were tested and the mean values used in the calculation of maxima, minima and means. These data show that there was important variation between the values obtained from the individual samples. However, it must be appreciated that the variability (commonly, ±4 s to 6 s) between replicates of the same sample *i.e.* measurement variation, could account for up to 20 % of the variation between samples.

Table 7 — Results of falling number assessments on individual samples from some loads at stores 2 and 3

	Load				
Store 2	3	5	6	7	8
Maximum	229	226	220	245	205
Minimum	168	183	172	185	181
Mean	198	203	200	218	194
Store 3	5	6	9	10	
Maximum	183	164	193	186	
Minimum	152	131	163	122	
Mean	166	147	175	168	

The tests on the germinative capacity done on barley samples from Store 1 showed very little variability between samples with all values being 99 % or 100 %.

At store 4 (a flour mill), normal practice was to collect 8 samples with an automatic Samplex CS 90 probe, mix these samples and then divide the resulting composite sample to give an analytical sample. The results obtained by the mill using their method of sampling and analysis are given in Table 5. In most cases comparisons can be made but the mill used a different method of assessing Hardness so these results cannot be compared.

5.4.2.3 Estimating the sampling reliability

The results of the Monte Carlo simulations are given in Table 8 and show the improvement in sampling reliability gained from taking more samples. The use of 8 samples reflects the number of samples that most operators take when using a CS90 automated sampler to sample from trucks on intake.

The ranges for each quality measurement were selected on the basis that they represented relatively small variation about the mean value.

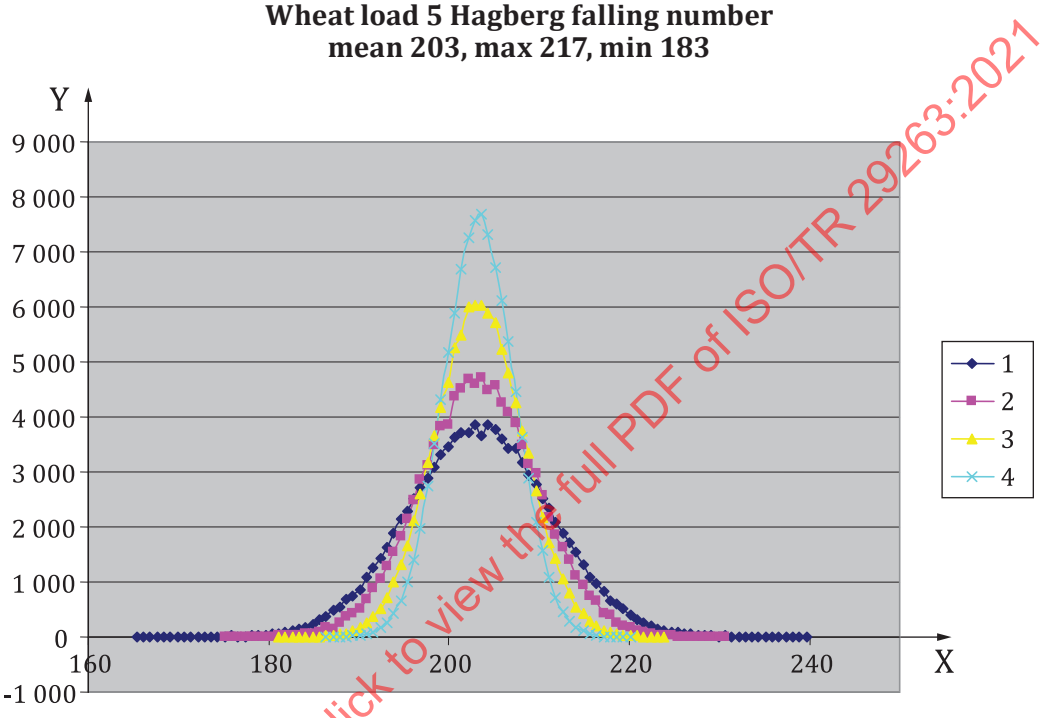
Table 8 — Impact of taking greater number of samples on the confidence limits for a given level of variation about the mean value (1 sur 2)

Wheat Hagberg Falling Number (see Figure 4) Mean = 203 Maximum = 217 Minimum = 183 using a range of 10 seconds either side of mean (193-213) the following confidence limits apply *i.e.* the probability of this interval covering the mean	
2 samples	79 %
3 samples	88 %
5 samples	96 %
amples	99 %
Wheat Specific Weight Mean = 82,04 Maximum = 82,6 Minimum of 81,6 using a range of 0,4 kg either side of mean (81,64-82,44) the following confidence limits apply *i.e.* the probability of this interval covering the mean	
2 samples	77 %
3 samples	85 %
5 samples	94 %
8 samples	98 %
Wheat Moisture Content Mean = 13,93 Maximum = 14,06 Minimum = 13,87 using a range of 0,1 % either side of mean (13,83-14,03) the following confidence limits apply *i.e.* the probability of this interval covering the mean	
2 samples	69 %
3 samples	79 %
5 samples	90 %
8 samples	96 %
Wheat Protein Mean = 14,02 Maximum = 14,24 Minimum = 13,79 using a range of 0,1 % either side of mean (13,92-14,12) the following confidence limits apply *i.e.* the probability of this interval covering the mean	
2 samples	59 %
3 samples	69 %
5 samples	82 %
8 samples	91 %
Barley Screenings Mean = 2,04 Maximum = 2,8 Minimum = 1,7 using a range of 0,3 % either side of mean (1,74-2,34) the following confidence limits apply *i.e.* the probability of this interval covering the mean	
2 samples	67 %
3 samples	76 %
5 samples	87 %
8 samples	95 %
Barley Moisture Content Mean = 14,06 Maximum = 14,28 Minimum = 13,82 using a range of 0,1 % either side of mean (13,96-14,16) the following confidence limits apply *i.e.* the probability of this interval covering the mean	
2 samples	58 %
3 samples	70 %
5 samples	79 %
8 samples	89 %
Barley Nitrogen Mean = 1,77 Maximum = 1,78 Minimum = 1,72 using a range of 0,01 % either side of mean (1,76-1,78) the following confidence limits apply *i.e.* the probability of this interval covering the mean	

Table 8 *(continued)*

2 samples	52 %
3 samples	61 %
5 samples	71 %
8 samples	84 %

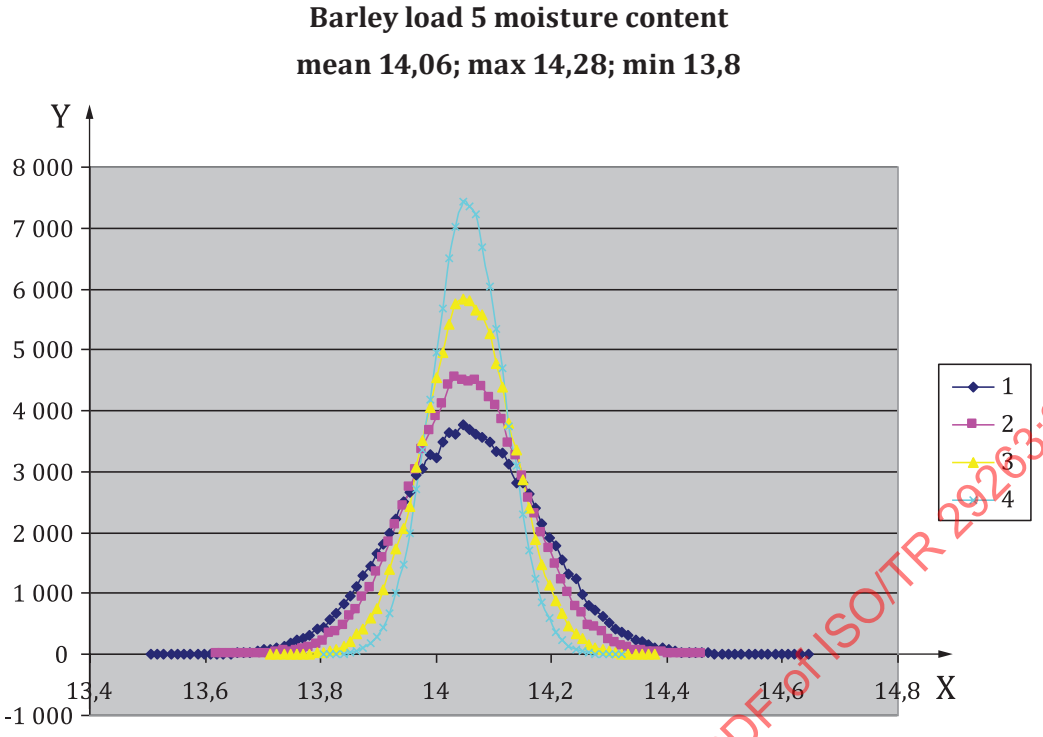
Figures 4 and 5 show two graphical representations of the results of the Monte Carlo simulations on reliability of results from using differing numbers of samples.



Key

X hagberg falling number
Y frequency
1 2 samples
2 3 samples
3 5 samples
4 8 samples

Figure 4 — Graphical representation of the impact of sampling intensity on reliability of result for Hagberg falling number in a lorry load of wheat



Key

X moisture content
Y frequency
1 2 samples
2 3 samples
3 5 samples
4 8 samples

Figure 5 — Graphical representation of the impact of sampling intensity on reliability of result for moisture content in a lorry load of barley

When the differences between the results of using a mean of individual sample measurements, a mean from a series of samples from a composite or the mean from a series of samples taken from a single large bulk sample were compared there were no statistically significant difference between them. On some occasions, the difference was positive whilst in others the difference was negative. The types of samples that showed the least difference (analysed by eye) were the mean of the eight individual samples and the mean of the 5 samples from the composite sample.

5.4.2.4 Discussion and conclusions

The collection of information on the approaches to sampling lorries under practical conditions suggested that there were wide differences in approach used by different stores. The Samplex CS90 was the equipment most commonly used although the exact method of use varied greatly. The next most common method was a manual spear but once again, the number and size of sample collected varied. The information collected supports the targeting of CS90 samplers and manual spearing during this project.

Guidelines for lorry sampling are specified in some contracts. These are all based directly on the ISO and BSI standards and specify the number and positions of sampling points for loads of certain weights. However, during the course of this work it became apparent that, in practice, with manual sampling it was impossible to observe these guidelines and to also observe HSE safety constraints and food safety regulation advise against an operator walking on the grain. Similar limitations applied to the

use of the CS90. When using this machine, even if ideally placed, it is impossible to reach up 40 % of the load in modern bulk trailers without moving the lorries. It is not common practice to move vehicles to overcome this limitation and therefore the sampling is limited to a central portion of the trailer in most cases. The manufactures of the CS90 are aware of this limitation but point out that, whilst it is possible to re-design the machine to extend its reach to cover the entire load, this would require a much larger and far more expensive unit. These are important issues and need to be addressed by the British Standards Institute and those responsible for drawing up contracts.

5.4.2.4.1 Inherent variation in grain

All grain sampled during this work at stores 1, 2 and 3 had been in store for at least 8 months. The grain had originally come from a number of sources but would have been mixed to some extent when entering storage. It is also reasonable to expect that extremes in moisture content would have been much reduced during storage. This is confirmed by the reduction in the difference between the maximum and minimum values for moisture shown by comparing the intake sampling at Store 3 with the values obtained during the project when assessing the grain at out-loading. However, the results obtained indicate that some inherent variation in the grain is still present after bulks of grain from farms are mixed and stored at commercial stores. The variation in the quality of grain within in a single lorry load was sufficient to make the difference between the grain being rejected or accepted. For example, the moisture content of load 10 at store 2 varied sufficiently that a single sample could have permitted the end user to accept or reject given a contract level of 15 %. Similar important variation was apparent in the protein content and falling number values of samples from Store 3. The origins of the grain at store 4 are unknown but at least some loads are likely to have come directly from farm stores.

In the case of the two stores where the grain was sampled with a CS90, the variability between individual samples may have been enhanced by the large differences in sample weight; up to two-fold. However, large differences in sample weight did not occur at Store 2 where the grain was sampled manually yet variation in quality was still present between individual samples.

Another factor that could influence the apparent variation between samples is any change to the method of measurement. The assessment of this factor was not a direct part of this project. However, some observations on the effects of changes in the method of measurement on the results were obtained. Large difference in screenings were found in the same samples of barley (Store 1) depending on whether they were tested by the store staff or project staff (see Table 2). The mechanical shaker used at the store always produced a higher assessment of fine material (+ about 30 %) than the manual sieving used by the project staff. However, this is not an indication of variability in the grain because the difference between the 2 methods remained about the same. These findings are not surprising as the model and setting of the shaker and the age of the sieves can influence the results (C. Finch, Pneumac, pers. com.). In addition the results from all samples of both wheat and barley collected from lorries and those from other parts of the Grain Sampling and Analysis (GSA) project, indicate that the distribution of fine material or screenings within a bulk or lorry-load of grain is variable and, as far as can be judged from results, random. As a result, the measurement of screenings is very likely to be subject to larger errors of prediction than other quality parameters. This may be worthy of more detailed research and perhaps should be born in mind when considering contractual obligations.

Tests done on other quality parameters throughout the GSA project on the Foss Infratec machines suggest that this instrument is relatively consistent. Where the same sample was put through the machine multiple times there was almost no variation in the results. The results obtained at Store 4 suggest that the values obtained by the project analysis were in general lower than those obtained by the mill analysis although the differences were mostly relatively small. It is possible that this trend was more a factor of analytical methods than sampling error. The largest difference between the two assessments was always in the estimation of screenings.

In general, the variation between samples taken from the same lorry-load was not of statistical significance. However, in many cases these differences were of great commercial importance. For example, with barley from Store 1 the screenings could vary by 1,5 % and the moisture by 1,4 % between samples from the same load. With milling wheat from store 3 the protein could vary by 0,5 % and the screenings by 1 % within a load. These data clearly demonstrate the dangers of basing a quality assessment of a lorry-load of grain on a single discrete sample irrespective of the method of collection.

Statistical analysis of the data provided useful information. The amount of variability for each quality parameter within a load or a group of loads was calculated and the ability to predict this variability was assessed. Obviously, each quality parameter behaved differently but most was broadly similar except screenings where the variation was much greater. Once the variability was calculated, limits could be set for the accuracy of prediction (for example: ± 0,05 % for protein in wheat). The numbers of samples used has a fundamental influence on the ability to approach these limits. A single sample may only offer a 50 % chance that the result will be close to such limits. In practical terms, this means that when lorries are sampled with a single sample, in the event of a dispute, there is a 50/50 chance that re-sampling will give a different result.

The confidence of prediction rises as the number of samples used in the assessment rises and 8 samples/load would appear to give a good compromise between confidence in the results and workload. There was no relationship between quality prediction and the position from which a sample was collected: the variability appeared random. Therefore, the exact siting of the sample points is of much less importance than the number of sample points, although spreading the points evenly over the entire load should be preferred. Collecting multiple samples (preferably 8/load), mixing these thoroughly and then using a sub-sample for quality assessment would appear to be an effective approach to sampling lorry-loads of grain.

The results from this work confirm the earlier limited work on sampling lorries (HGCA Project Report 79) but it does not appear possible to implement the use of the methodology suggested in ISO or BSI Standards because of limitations in the current equipment. However, they provide the basis for a new approach to standards with a sound technical base and may allow some general codes of practice to be constructed.

5.4.2.4.2 Recommendations and protocol for lorry sampling

5.4.2.4.2.1 Recommendations

a) **Screenings**

Throughout the sampling component of the GSA Project, the error of prediction of screenings has been greater than other quality parameters. This difficulty does not seem to be reflected in commercial contracts. It is recommended that more work be done to establish better limits of assessment that could be used that would provide a fairer approach for both buyers and sellers of grain.

b) **National and International Standards**

The results of this work should be discussed with the appropriate Standards organisations with a view to producing revised standards that correctly reflect the limitations of equipment and safety concerns.

Code of Practice: consideration should be given, in cooperation with appropriate organisations, to the drawing up of guidelines for the effective sampling of loads of grain.

c) **Influence of method of measurement**

Consider the interchange of samples between the project and commercial laboratories to investigate the effects of different methods of measuring quality parameters of grain.

5.4.2.4.2.2 Protocol for collecting samples from lorry-loads of grain

These guidelines are intended to offer advice to any organisation that sample grain in lorries. They are not intended as dogmatic instructions that can be applied at every site and there may be a need for some flexibility in approach. However, if used, the guidelines will help to minimise sampling error and provide a consistent method of collecting samples from lorry-loads of grain.

a) **General**

— All equipment should be clean and in good working condition;

- Operators should be familiar with the equipment and have an understanding of the problems of sampling grain;
- Safety should always be the first consideration in any sampling operation.

b) **Taking manual samples:**

- Use a manual spear that is at least 1,5 m long;
- Use a spear that will collect multiple sub-samples from different depths;
- Collect about 500 g/sample point. This may require 2 insertions/sample point;
- Collect samples from at least 5 (preferably 8) sample points/load. These points should be spread as widely as possible across the load without risk to the operator. In some case, it may be appropriate to move the lorry to give better access;
- Add the samples together to form a composite sample representing the load;
- Mix the composite sample thoroughly;
- Divide the composite sample with a sample divider. If this is not available the sample should be divided by coning and quartering to give a working sample. Failure to use a sample divider or to cone and quarter the sample is likely to increase the error in the measurements (particularly screenings);
- It may be appropriate to retain part of the composite sample in case of any dispute over the grain quality.

c) **Taking samples with a remote sampling probe**

- Where the amount of grain collected per insertion can be adjusted, it may be appropriate to set this to maximum. This will help to overcome the collection of small samples when the spear is inserted into troughs in the load;
- Try to ensure that the lorry is positioned with the sampler about half way along the trailer so that it can access as much of the load as possible;
- If the sampler has an automatic programme, choose the option that collects 8 samples from different points;
- If the probe is controlled manually, collect 8 samples/load from different points spread as far apart as possible over the load;
- Mix the composite sample thoroughly and then divide using a sample divider or by coning and quartering. Failure to use a sample divider or to cone and quarter the sample is likely to increase the error in the measurements (particularly screening);
- It may be appropriate to retain part of the composite sample in case of any dispute over the grain quality.

6 Study n°2: extract from "Sampling grain in static and flowing condition – alternatives to the regulatory protocol"

6.1 General

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This is the report of a 36-month project. The work was managed in IRTAC and funded by IRTAC members, FranceAgriMer, ANMAC, ARVALIS, GNIS

6.2 Context

6.2.1 Regulatory aspect

Regulation has been published at European level concerning sampling and methods for analyzing cereals and cereal products, within the framework of assessing average batch mycotoxin content: regulation n° 401/2006 of 23/2/2006 (Official Journal of 9/3/06).

The regulation concerns official controls. Nevertheless, if there is a too large gap between a protocol applied by the official bodies in charge of regulatory checking and those implemented by the cereal industry operators, this might result in different evaluations of the average content of the batches of cereals. This situation is a potential source of disputes. Commercial operators are directly concerned with these regulatory protocols insofar as they are entrusted with some official analyses (*e.g.* within the framework of Community intervention).

Furthermore, attempts to take the “contaminants” issue into account in the "Intervention" procedure, in particular for the application of **(EC) 856/2005** regulation, are confronted by certain concrete limits in implementing methods specified for sampling, due to the characteristics specific to big cereal batches concerned and in particular their size.

6.2.2 Normative aspect

Three main international sampling standards were available at the time of the studies:

— ISO 24333;

— ISO 542:1999;

— ISO 6497:2005.

These standards only deal with the evaluation of “uniformly” distributed characteristics ("homogeneous") such as protein or moisture.

ISO 24333 is the only one proposing sampling protocols suited to contaminants spread in a heterogeneous manner, and in particular in the field of mycotoxins such as *Fusarium* toxins.

NOTE The two standards ISO 6644:2002 and ISO 13690:1999 were withdrawn at the publication of ISO 24333.

6.3 Studies conducted and objectives

Two studies were conducted: the first in 2004 to 2005 (“study A”) and the second in 2006 to 2007 (“study B”).

6.3.1 Study A

The purpose of this study was respectively:

a) to compare 3 protocols with the aim of estimating the possible differences between:

   — a "regulatory" protocol whose sampling method corresponds to the one set out in EU regulation n° 401/2006[2];

   — a "normative" protocol whose sampling method is adapted to certain situations experienced by cereal operators (e.g. intervention scheme);

— a “routine” protocol whose sampling method is adapted to “day to day” control by cereal operators (e.g. intervention scheme).

For the 3 protocols, an aggregate sample was made from the individual samples and then analysed to determine an average content of the batch and to assess the related accuracy.

b) to evaluate the heterogeneity of 3 silos for the analysis of DON; of 2 silos for the analysis of nitrogen and falling number; of 1 silo for the analysis of moisture. For each of these analysis/silo, the 100 samples were individually analysed.

6.3.2 Study B

This study was conducted on large storage silos, on flowing of grains of corn, contaminated by Fusarium toxins, mainly fumonisins, but also deoxynivalenol and zearalenone.

The aim of this study B is to determine relationships between the average mycotoxin content and the variance of silos in order to estimate, for a given number of samples and for a given level of contamination, the accuracy that can be expected.

A part of this study was also devoted to assessing the impact of reducing the size of the laboratory sample.

6.4 Study A: silos and lorries of wheat and corn – Fusariotoxins and quality assessment

6.4.1 Organising field tests

6.4.1.1 Conducting tests

6.4.1.1.1 Setting up the tests

The tests were conducted on volunteer sites. A questionnaire was prepared and circulated in order to identify the interesting sites.

Furthermore, in order to ensure a prompt reaction when a batch was identified, and to ensure that the tests conducted in a reliable and rigorous manner, a single person was entrusted with conducting all of the samplings carried out in the field.

The same method was applied to similar situations, as far as possible, with the same equipment being used for all the trials: manual probes, divider and grinders. The automatic probes varied from a site to another.

6.4.1.1.2 Methods corresponding to the protocols tested

2 series of tests were conducted:

— the 1st series consisted of testing 3 sampling plans: the protocol corresponding to regulation n° 401/2006 for fusarium toxins, the "normative" protocol drafted by the "standardisation” working group (ISO 24333) and the “routine” protocol with less incremental samples per unit than the 2 others.

The grain sampling situations were as follows:

— flowing from silos (transferring the contents of one silo to another one, discharge and Redler samplings, or train discharge);

— static by sampling from lorries;

— static by sampling from flat or vertical silos.

The aim was to see whether the alternatives protocol correctly estimates the average content of a batch in the same way as the regulatory protocol, taking into account some experimental errors. The regulatory protocol is used here as a reference. The characteristics used for estimation are the DON content, the protein content and the Hagberg Falling number for wheat. For maize, the content values taken into account are: DON, protein content, promatest and zearalenone.

— The aim of the 2nd series was to evaluate silo heterogeneity in order to be able to estimate the relationship between the confidence interval of the average content of the silo and the number of incremental samples. Furthermore, resultant modelling enables to evaluate the risk taken if the batch is accepted on the basis of the number of incremental samples chosen.

The heterogeneity tests were conducted on silos of 500 t and for each, 100 elementary samples were taken and individually analysed: 3 silos for the analysis of DON; 2 silos for the analysis of nitrogen and Falling number; 1 silo for the analysis of moisture. The batches tested weighed approximately 500 t. Heterogeneity was only tested on wheat batches.

Table 9 — Characteristics of the tests conducted

Tests of sampling plans : regulation / standard / routine						
				regulatory method	normative method	routine method
flowing grains (by 500 t)	Type of grains	nb essais				
	wheat	1	sampling equipment	scoop	scoop	scoop
			number of increments	100/500T	25/500T	10/500T
	corn	4	mass of increment (kg)	0,3 à 1,6	0,3 à 1,8	0,3 à 1,9
			mass of aggregate sample (kg)	32 à 165	8 à 44	3 à 19
			mass of laboratory sample (kg)	9,5 à 9,8	8 à 10,6	3 à 10,4
			division of the aggregate sample	Boerner divider	Boerner divider	
Static lorries						
	wheat	6	sampling equipment	manual or automatic sampling equipment	automatic sampling equipment	automatic sampling equipment
			number of increments	100	10	3
	corn	8	mass of increment (kg)	0,4 à 1,5	0,4 à 1,5	0,3 à 1,9
			mass of aggregate sample (kg)	43 à 150	3,8 à 15	1,2 à 4,2
			mass of laboratory sample (kg)	9,3 à 10,9	3,8 à 10,9	1,2 à 4,2
			division of the aggregate sample	Boerner divider	Boerner divider	Boerner divider
Flat or vertical silos (by 500 t or by batch)						
	wheat	3	sampling equipment	manual sampling equipment or probes by aspiration	manual sampling equipment or probes by aspiration	manual sampling equipment or probes by aspiration
			number of increments	100	50	10
			mass of increment (kg)	1,9 à 2,7	1,9 à 2,7	2,2 à 3,0
			mass of aggregate sample (kg)	180 à 265	95 à 140	22 à 30
			mass of laboratory sample (kg)	11	10,3	10,7
			division of the aggregate sample	Boerner divider	Boerner divider	Boerner divider
Tests d'héréogénéité						
Test of Heterogeneity on 2 flat silos and 1 verical silo	type de produits	nb essais				

Table 9 *(continued)*

Tests of sampling plans : regulation / standard / routine						
				regulatory method	normative method	routine method
	wheat	3	sampling equipment	probes by aspiration		
			number of increments	100		
			mass of increment (kg)	1,8 à 2,7		
			mass of aggregate sample (kg)	180 à 265		
			mass of laboratory sample (kg)	1,8 à 2,7		
Tests realised 2005/09/20						

6.4.1.1.3 Description of the tests conducted

— 1st series → 25 tests were conducted on:

  — flowing grain: manual sampling equipment; 4 in silos - 3 maize, 1 wheat, from 500 t sections; 1 train –maize. The samples were taken at regular intervals in accordance with the flow and the number of samplings required;

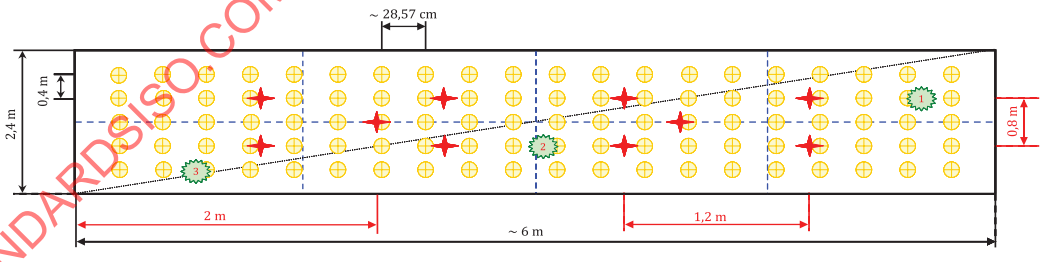
  — static grains: manual or automatic sampling equipment; 14 lorries (6 wheat, 8 maize), 3 types of probe. In 10 cases, a manual sampling was used; In 7 cases, a mechanical sampling was used; In 3 cases, the manual sampling was compared to the mechanical sampling;

  — 2 vertical silos and 1 horizontal silo: on wheat; the samples were taken from 500 t batches using mechanical probes (suction-operated probes).

The aggregate samples are obtained by grouping together the individual samples corresponding to each sampling situation and each method → for each situation; there are thus two aggregate samples (regulatory method – normative method). The aggregate samples obtained were homogenized and then divided in order to obtain a 10 kg laboratory sample. Aggregate samples of less than 10 kg were just homogenized and then ground (Labormill grinders).

The diagrams below show sampling examples taken from lorries.

Camion cas S/V.P) C1 (1)



Key

:100 points of sampling

:10 points of sampling

:3 points of sampling randomly distributed

Figure 6 — Example of sampling from lorries

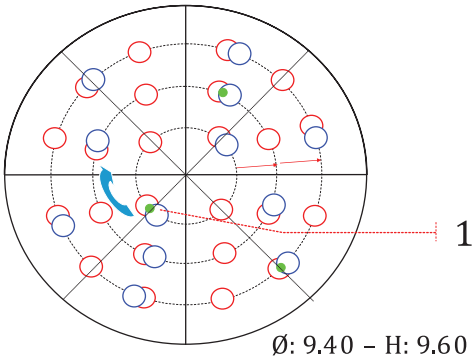


Figure 7 — Example of sampling from silo

Figure 8 — Boerner divider

Figure 9 — Example of sampling equipment

Key

○	24 places of sampling
○	12 places of sampling
●	3 places of sampling
1	1st sampling

— 2nd series: heterogeneity tests → 3 trials were conducted on 2 horizontal silos and 1 vertical silo. Grids were drawn on the top of the silos in order to ensure a consistent sample across the whole batch sampled. Each 100 elementary sample was individually homogenized and ground before being analysed.

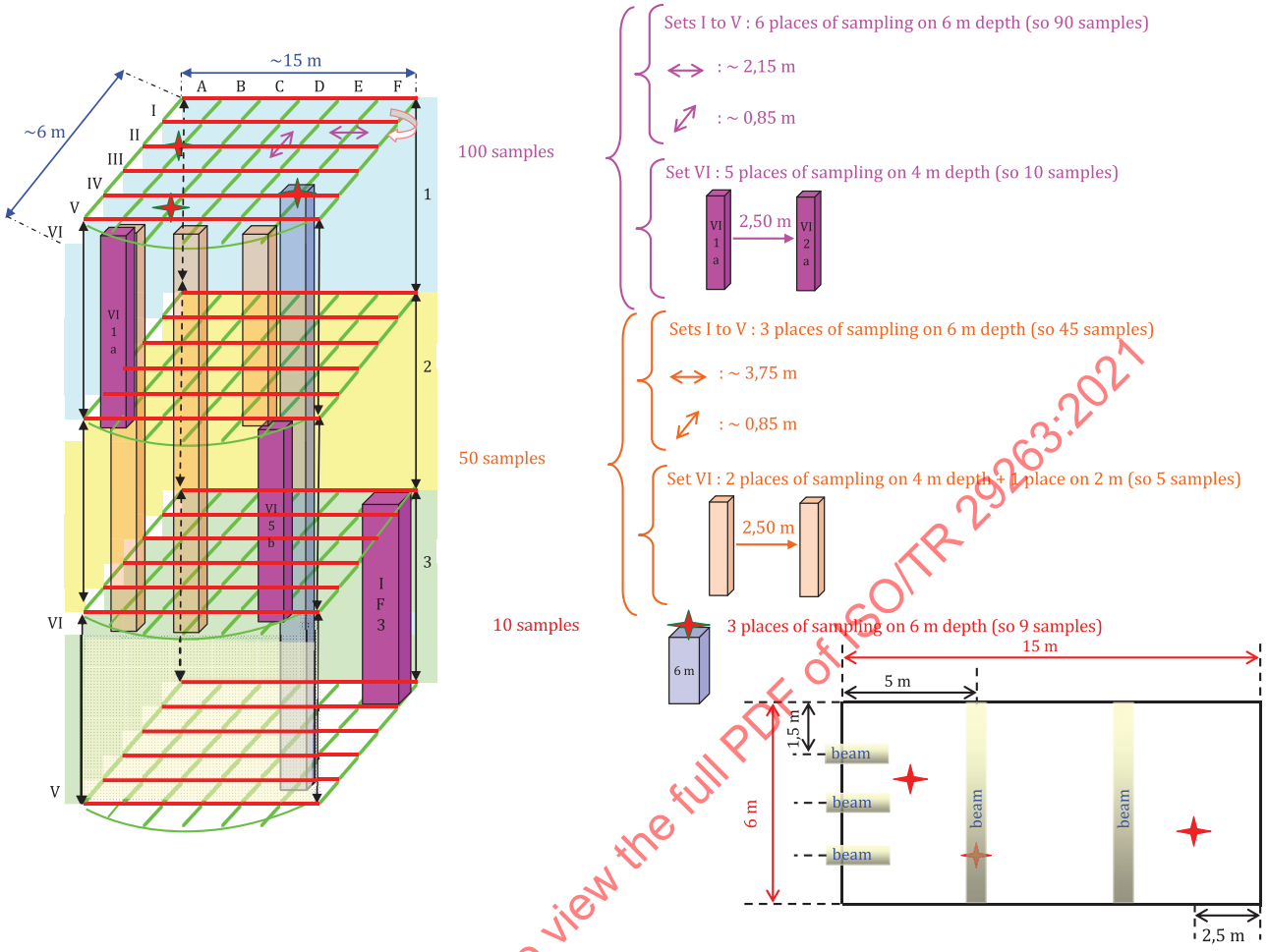


Figure 10 — Illustration of sampling from silos

— Table 10 summarises the different situations and sampling equipment.

— Tables 11 to 13 summarise the quantities sampled, homogenized, divided and ground, as well as the corresponding necessary times.

Table 10 — Situations and sampling equipment (1 of 2)

Reference	type of sampling	Product sampled	Traceability of the batch - size of the batch sampled	Screening by kit Elisa - DON (ppb)	Sampling equipment (Ma: manual - Me : mechanical)
flowing grain (by 500 t)					
(I.V / G.P) M (1) Ç A10	flowing grain - exit silo	wheat	during transilage from Cell A10 of silo 2 - batch of 380 t - Flow of grain : 100 t/h	2 197	scoop (manual)
(A.F / O) M (1)	flowing grain - exit silo	corn	Cell 307 - batch 500 t - flow of grain : 250 t/h	827	scoop (manual)
(CAVS / M) M1 (1)	flowing grain - exit silo	corn	Cell 10 of 11 000 t - on redler - batch sampled : 500 t - flow of grain : 75 t/h	DON: 2 000 (Zéa: 470 - Fumonisines: 62)	scoop (manual)
(CAVS / M) M2 (1)	flowing grain - exit silo	corn	Cell 10 of 11 000 t - on redler - batch sampled : 500 t - flow of grain : 100 t/h	DON: 2 000 (Zéa: 470 - Fumonisines: 62)	scoop (manual)
(R.F / L) T (1)	flowing grain - exit train	corn	train - batch 494 T (8 wagons / 2 cell by wagon - flow of grain : ~ 185 t/h by cell - sampling of 494 t		probe (manual)
Static - lorries					
(I.V / G.P) C1 (M) Ç A10	lorry 1/3	wheat	from cell A10 silo 2 (ensiled from private cell T1 silo 6) - lorry tow of 18 t	2 197	probe Tripette et Renaud (manual)
(I.V / G.P) C1 (A) Ç A10	lorry 1/3	wheat	from cell A10 silo 2 (ensiled from private cell T1 silo 6) - lorry tow of 18 t	2 197	probe SAMPLEX CS90 (mechanical)
(I.V / G.P) C2 (M) Ç A10	lorry 2/3	wheat	from cell A10 silo 2 (ensiled from private cell T1 silo 6) - lorry tow of 18 t	2 197	probe Tripette et Renaud (manual)
(I.V / G.P) C2 (A) Ç A10	lorry 2/3	wheat	from cell A10 silo 2 (ensiled from private cell T1 silo 6) - lorry tow of 18 t	2 197	probe Tripette & Renaud modèle Préleveur "Pick-up" (mechanical)
(I.V / G.P) C3 (M) Ç A10	lorry 3/3	wheat	from cell A10 silo 2 (ensiled from private cell T1 silo 6) - lorry tow of 18 t	2 197	probe Tripette et Renaud (manual)

Table 10 *(continued)*

Reference	type of sampling	Product sampled	Traceability of the batch - size of the batch sampled	Screening by kit Elisa - DON (ppb)	Sampling equipment (Ma: manual - Me : mechanical)
(I.V / G.P) C3 (A) Ç A10	lorry 3/3	wheat	from cell A10 silo 2 (ensiled from privated cell T1 silo 6) - lorry tow of 18 t	2 197	"Tout pour le Grain" (mechanical)
(C.C / T) C1 (1)	lorry 1/4	corn	batch 1 : loading of the lorry from batch 1 - lorry tow of 27,2 t	749	probe by aspiration Testermatic foss t14 (mechanical)
(C.C / T) C2 (1)	lorry 2/4	corn	batch 1 : loading of the lorry from batch 1 - lorry tow of 26,7 t	749	probe by aspiration Testermatic foss t14 (mechanical)
(C.C / T) C3 (1)	lorry 3/4	corn	batch 1 : loading of the lorry from batch 1 - lorry tow of 26,8 t	749	probe by aspiration Testermatic foss t14 (mechanical)
(C.C / T) C4 (1)	lorry 4/4	corn	batch 1 : loading of the lorry from batch 1 - lorry tow of 27,1 t	749	probe by aspiration Testermatic foss t14 (mechanical)
(R.D / M) C (1)	lorry 1/1	corn	5 cells - 3 500 t - lorry tow of 19 t	758	probe Tripette et Renaud (manual)
(S / V.P) C1 (1)	lorry 1/2	wheat	cell BC7 of 80 T - lorry tow of 25 t	800	probe Tripette et Renaud (manual)
(S / V.P) C2 (1)	lorry 2/2	wheat	cell BC7 of 80 T - lorry tow of 25 t	800	probe Tripette et Renaud (manual)
(CAVS / M) C1 (1)	lorry n° 1/3	corn	cell 10 of 11 000 t - bushel of loading - lorry tow of 25 t	DON: 2 000 (Zea: 470 - Fumonisines: 62)	probe Tripette et Renaud (manual)
(CAVS / M) C2 (1)	lorry n° 2/3	corn	cell 10 of 11 000 t - bushel of loading - lorry tow of 25 t	DON: 2 000 (Zea: 470 - Fumonisines: 62)	probe Tripette et Renaud (manual)
(CAVS / M) C3 (1)	lorry n° 3/3	corn	cell 10 of 11 000 t - bushel of loading - lorry tow of 12 t	DON: 2 000 (Zea: 470 - Fumonisines: 62)	probe Tripette et Renaud (manual)
(CER / M) H7 (C1) Ç 4	lorry 1/1	wheat	cell 4 of 15*6*- (L*l*H) - capacity of 12 000 t - lot of 2 726 tonnes - lorry tow of 25 t	1 138	probe Tripette et Renaud (manual)
Flat or vertical silos (by 500 t or by lot)					
(I.V / G.P) SV (1) Ç K23	vertical silo	wheat	cell K23 silo 2 - Ø: 9,40 - H: 9,60 - sampling on 650 t	1 403	probe by aspiration Agronet (mechanical)
(I.V / G.P) H1 Ç A10	vertical silo	wheat	cell A10 silo 2 (ensiled from private cell T1 silo 6) - - sampling on 500 t	2 197	probe by aspiration Agronet (mechanical)

Table 10 *(continued)*

Reference	type of sampling	Product sampled	Traceability of the batch - size of the batch sampled	Screening by kit Elisa - DON (ppb)	Sampling equipment (Ma: manual - Me : mechanical)
(CER / M) H7 Ç 4	flat silo	wheat	cell 4 of 15*6*- (L*l*H) - capacity of 12 000 t - batch of 2 726 tonnes - sampling on 500 t	1 138	probe by aspiration Agronet (mechanical)
Heterogeneity of silos					
(C.C / T) H8 C A2	Heterogeneity of flat silo	wheat	cell 18,4*18,4*10,3 (L*l*H) - 2 726 tonnes - sampling on 500 t	902	probe by aspiration Agronet (mechanical)
(I.V / G.P) H1 Ç A10	Heterogeneity of vertical silo	wheat	cell A10 silo 2 (ensiled from private cell T1 silo 6) - sampling on 500 t	2 197	probe by aspiration Agronet (mechanical)
(CER / M) H7 Ç 4	Heterogeneity of flat silo	wheat	cell 4 of 15*6*-(L*l*H)- capacity of 12 000 t - sampling on 500 t	1 138	probe by aspiration Agronet (mechanical)

Table 11 — Quantities sampled, homogenized, divided and ground – time required (1 of 3)

Reference	situation	products	according to the regulation						according to the draft standard						according to the routine method					
			mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)
flowing grain (par 500 t)																				
(I.V / G.P) M (1) Ç A10	flowing grain exit silo	wheat	122	228	9,74	175	55	458	32	(1 increment every 09 min 07 sec)	9,71	45	63	336	13	(1 increment every 22 min 48 sec)	9,38	25	48	301
(A.F / O) M (1)	flowing grain exit silo	corn		120	9,61	58	130	308	17	(1 increment every 04 min 48 sec)	10,47	10	54	184	7	(1 increment every 12 min)	only homogenisation	3	39	162
(CAVS / M) M1 (1)	flowing grain exit silo	corn	165	400	9,58	135	410	945	45	(1 increment every 16 min)	10,56	95	66	561	19	(1 increment every 40 min)	9,22	24	62	486
(CAVS / M) M2 (1)	flowing grain exit silo	corn	108,166	300	9,54	77	310	687	29	(1 increment every 12 min)	10,05	20	58	378	14	(1 increment every 30 min)	10,40	9	59	368
(R.F / L) T (1)	flowing grain vidange Train	corn	31,598	10	9,72	15	20	45	8		only homogenisation	3	55	68	3		only homogenisation	1	35	46
Static - lorries																				
(I.V / G.P) C 1 (M) Ç A10	lorry 1/3	wheat	63,5	95	9,71		66	161									only homogenisation		41	41
(I.V / G.P) C 1 (A) Ç A10	lorry 1/3	wheat	126	155	10,39		41	196	12	20	9,79		66	86	3	10	only homogenisation		36	46
(I.V / G.P) C 2 (M) Ç A10	lorry 2/3	wheat	54	135	9,32		46	181									only homogenisation		30	30
(I.V / G.P) C 2 (A) Ç A10	lorry 2/3	wheat	37,5	95	10,87	90	42	227	4	85 min for Normative and Routine method	only homogenisation		21	64	1,2	85 min for Normative and Routine method				42

Table 11 *(continued)*

Reference	situation	products	according to the regulation						according to the draft standard						according to the routine method					
			mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)
(I.V / G.P) C 3 (M) Ç A10	lorry 3/3	wheat	54	150	9,27		45	195												
(I.V / G.P) C 3 (A) Ç A10	lorry 3/3	wheat	74	135	9,99		48	183	7	90 min for Normative and Routine method	only homogenisation		27	72	2,4	90 min for Normative and Routine method				45
(C.C / T) C1 (1)	lorry 1/4	corn	151	68	10,12	155	53	276	15	7	10,13	30	49	86	4	4	only homogenisation	5	40	49
(C.C / T) C2 (1)	lorry 2/4	corn	149	46	10,15	160	62	268	15	4	9,89	20	57	81	4	2	only homogenisation	5	46	53
(C.C / T) C3 (1)	lorry 3/4	corn	136	45	10,19	140	67	252	14	7	10,88	15	50	72	4	2	only homogenisation	5	35	42
(C.C / T) C4 (1)	lorry 4/4	corn	140	46	9,59	160	71	277	14	2	10,36	20	56	78	4	1	only homogenisation	5	36	42
(R.D / M) C (1)	lorry 1/1	corn	46	84	9,99	27	54	165	5	12	only Homogenisation	2	36	50	1,5	21	only homogenisation	1	24	46
(S / V.P) C1 (1)	lorry 1/2	wheat	56	145	10,28	73	37	255	6	42 min for Normative and Routine method	only homogenisation	3	26	50	1,6	42 min for Normative and Routine method	only homogenisation	1	24	46
(S / V.P) C2 (1)	lorry 2/2	wheat	55	174 for the 3 method	10,22	58	43	159	5	174 for the 3 method	only homogenisation	2	26	86	1,7	174 for the 3 method	only homogenisation	1	20	79
(CAVS / M) C1 (1)	lorry n° 1/3	corn	47	117	10,54	43	72	232	5	15	only homogenisation	2 min 17 sec	42	59	1,5	11	only homogenisation	1 min 35 sec	28	40

Table 11 *(continued)*

Reference	situation	products	according to the regulation						according to the draft standard						according to the routine method					
			mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)
(CAVS / M) C2 (1)	lorry n° 2/3	corn	47	99 for the 3 method	10,22	33	64	130	5	99 for the 3 method	only homogenisation	2	43	78	1,5	99 for the 3 method	only homogenisation	1	27	61
(CAVS / M) C3 (1)	lorry n° 3/3	corn	43	100	10,78	31	67	198	4	15	only homogenisation	2	45	62	1,4	8	only Homogenisation	1 min 20 sec	28	37
(CER / M) H7 (C1) Ç 4	lorry 1/1	wheat	52	125	9,73	72	94	291	5	55 min for Normative and Routine method	only homogenisation	5	46	78	1,7	55 min for Normative and Routine method	only Homogenisation	2	27	56
Flat or vertical silos (by 500Tou by lot)																				
(I.V / G.P) SV (1) ÇK23	vertical silo	wheat	190	1 080	11,23	330	57	1 467	105	420	10,34	130	64	614	22	45	10,68	40	64	149
(I.V / G.P) H1 Ç A10	vertical silo	wheat	265						141	240	8,83	180	81	501	30	90	9,05	40	84	214
(CER / M) H7 Ç 4	flat silo	wheat	234	740					96	360	10,64	57	57	474	22		10,81	9	55	64
Heterogeneity of silos - 100 increments by silo																				
(C.C / T) H8 C A2	Heterogeneity - flat silo	wheat	180	960			26 min * 100 samples = 2 600 min	26 min * 100 samples = 2 600 min												
(I.V / G.P) H1 Ç A10	Heterogeneity - vertical silo	wheat	265	1 500			26 min * 100 samples = 2 600 min	26 min * 100 samples = 2 600 min												

Table 11 *(continued)*

Reference	situation	products	according to the regulation						according to the draft standard						according to the routine method					
			mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)	mass of sample taken (kg)	time of sampling (min)	mass of sample after division (kg)	time of homogenisation + division (min)	time of grinding + cleaning (min)	total time (min)
(CER / M) H7 Ç 4	Heterogeneity - flat silo	wheat	234	1 260			26 min * 100 samples = 2 600 min	26 min * 100 samples = 2 600 min												

6.4.2 Results and conclusions

6.4.2.1 Sample analysis results

6.4.2.1.1 Results of the comparison tests involving 2 protocols

For each of the situations, the samples representing the regulatory and normative sampling methods were subjected to Deoxynivalenol analyses (the content of other trichothecenes, TCT A and other TCT B, were lower than methods' detection limits).

— Table 12 overview of the DON analysis results of the aggregate samples obtained from the different batches analysed

These results are also presented in graphical form (see Figure 11) for each type of sampling: for each test conducted, the average DON values covered by the analytical measuring uncertainties are presented applicable to the two protocols.

It can be noted that the thresholds of the European regulation are adequately included in the values of the average contaminations of silos: the average values observed are within 477 and 3 461 ppb DON.

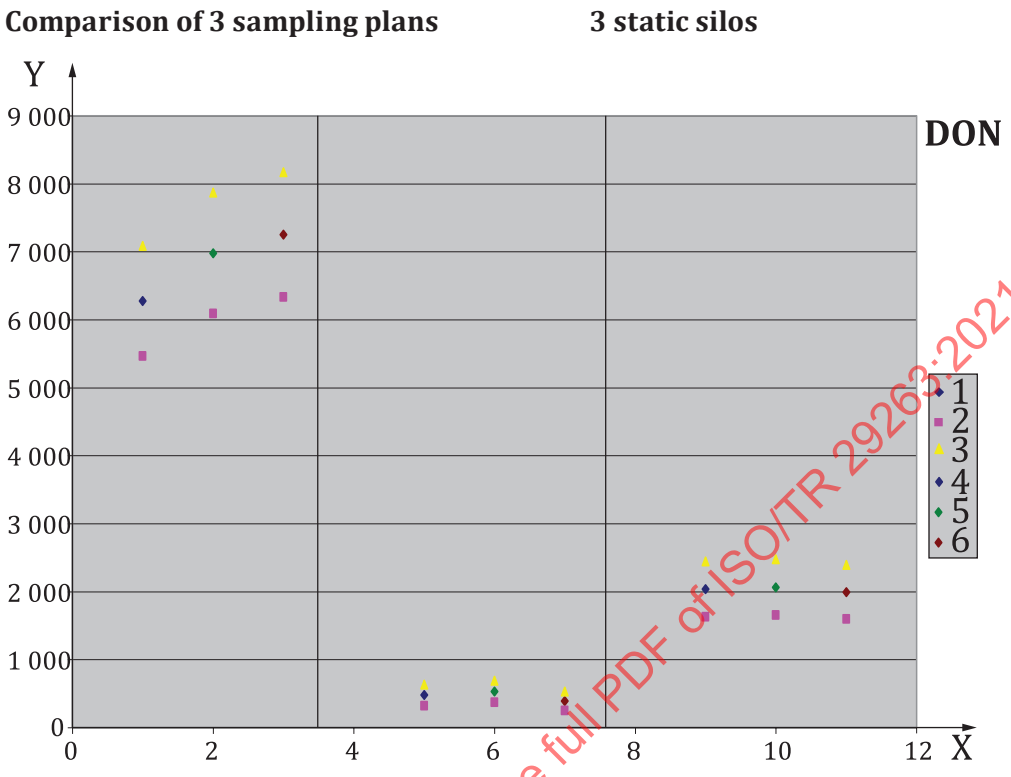
Tables 15 and 16 give the analytical results of the aggregate samples obtained from the different batches analysed for protein and Hagberg of wheat; protein, promatest and zearalenone of corn.

Table 12 — Overview of the DON content measured on the aggregate samples obtained from the different batches analysed (1 on 2)

Reference	type of sampling	products	Sampling equipment (Ma : manual - Me : mechanical)	Results of DON analysis (ppb)					
				regulatory method		normative method		routine method	
				average (ppb)	Range (average +/- uncertainty in ppb)	average (ppb)	Range (average +/- uncertainty in ppb)	average (ppb)	Range (average +/- uncertainty in ppb)
flowing grain (by 500 t)									
(I.V / G.P) M (1) Ç A10	flowing grain - exit silo	wheat	scoop (manual)	3 461	2 967 - 3 955	3 569	3 063 - 4 075	3 218	2 751 - 3 684
(A.F / O) M (1)	flowing grain - exit silo	corn	scoop (manual)	1 154	920 - 1 387	1 104	876 - 1 331	1 197	959 - 1 435
(CAVS / M) M1 (1)	flowing grain - exit silo	corn	scoop (manual)	1 023	804 - 1 242	1 055	833 - 1 277	1 007	791 - 1 224
(CAVS / M) M2 (1)	flowing grain - exit silo	corn	scoop (manual)	1 001	785 - 1 217	1 083	858 - 1 308	1 080	855 - 1 305
(R.F / L) T (1)	flowing grain - exit train	corn	probe (manual)	1 135	904 - 1 367	1 154	921 - 1 388	1 274	1 027 - 1 521
Static - lorries									
(I.V / G.P) C1 (M) Ç A10	lorry 1/3	wheat	probe Tripette et Renaud (manual)	3 167	2 707 - 3 628				
(I.V / G.P) C1 (A) Ç A10	lorry 1/3	wheat	probe SAMPLEX CS90 (mechanical)	2 983	2 543 - 3 423	2 802	2 382 - 3 221	2 142	1797 - 2487
(I.V / G.P) C2 (M) Ç A10	lorry 2/3	wheat	probe Tripette et Renaud (manual)	1 775	1 471 - 2 078				
(I.V / G.P) C2 (A) Ç A10	lorry 2/3	wheat	probe Tripette & Renaud modèle Préleveur "Pick-up" (mechanical)	1 582	1 300 - 1 864	1 753	1 452 - 2 054	1 267	1021 - 1513

Table 12 *(continued)*

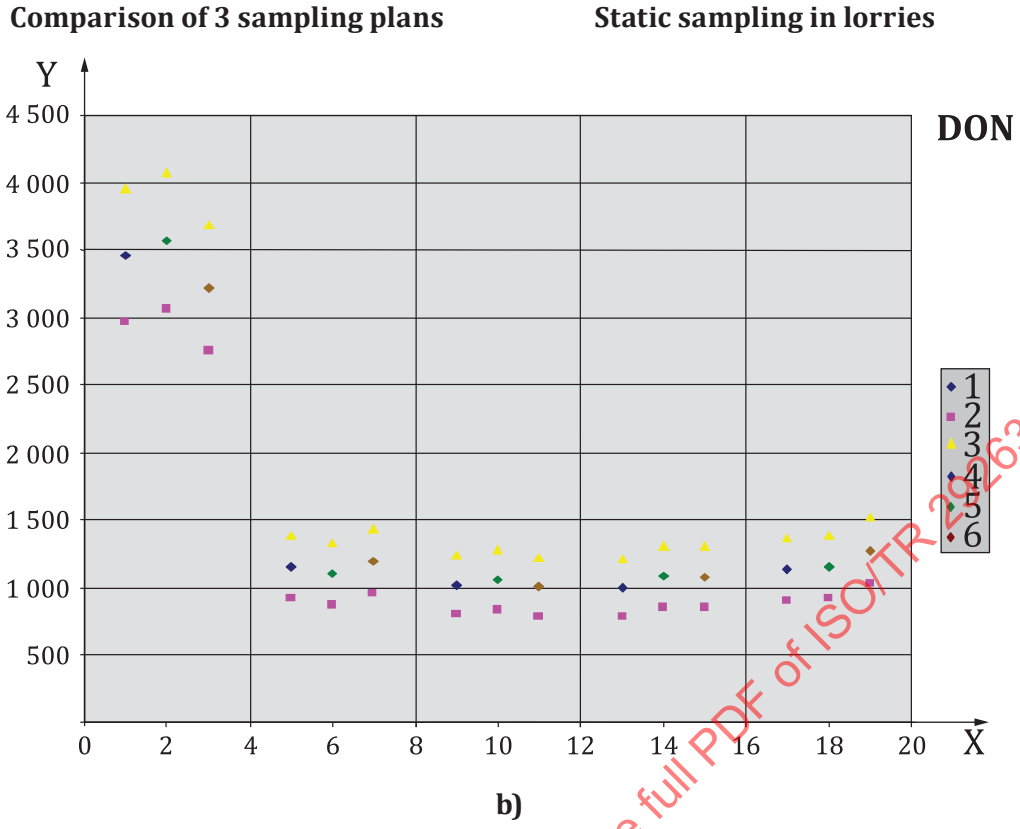
Reference	type of sampling	products	Sampling equipment (Ma : manual - Me : mechanical)	Results of DON analysis (ppb)					
				regulatory method		normative method		routine method	
				average (ppb)	Range (average +/- uncertainty in ppb)	average (ppb)	Range (average +/- uncertainty in ppb)	average (ppb)	Range (average +/- uncertainty in ppb)
(I.V / G.P) C3 (M) Ç A10	lorry 3/3	wheat	probe Tripette et Renaud (manual)	1 801	1 495 - 2 108				
(I.V / G.P) C3 (A) Ç A10	lorry 3/3	wheat	"Tout pour le Grain" (mechanical)	1 759	1 458 - 2 061	1 884	1 568 - 2 200	1 509	1235 - 1 782
(C.C / T) C1 (1)	lorry 1/4	corn	probe by aspiration Testermatic foss t14 (mechanical)	876	674 - 1 077	880	678 - 1 083	864	663 - 1 064
(C.C / T) C2 (1)	lorry 2/4	corn	probe by aspiration Testermatic foss t14 (mechanical)	828	632 - 1 025	841	643 - 1 039	827	630 - 1 023
(C.C / T) C3 (1)	lorry 3/4	corn	probe by aspiration Testermatic foss t14 (mechanical)	864	663 - 1 065	828	632 - 1 025	838	641 - 1 036
(C.C / T) C4 (1)	lorry 4/4	corn	probe by aspiration Testermatic foss t14 (mechanical)	848	649 - 1 046	854	654 - 1 053	836	638 - 1 033
(R.D / M) C (1)	lorry 1/1	corn	probe Tripette et Renaud (manual)	1 062	839 - 1 285	1 035	815 - 1 255	987	773 - 1 202
(S / V.P) C1 (1)	lorry 1/2	wheat	probe Tripette et Renaud (manual)	717	533 - 901	744	557 - 931	inf LQ (285)	
(S / V.P) C2 (1)	lorry 2/2	wheat	probe Tripette et Renaud (manual)	730	545 - 915	808	614 - 1 003	1 693	1 399 - 1 987
(CAVS / M) C1 (1)	lorry n° 1/3	corn	probe Tripette et Renaud (manual)	1 143	911 - 1 376	1 255	1 010 - 1 500	1 398	1 137 - 1 659
(CAVS / M) C2 (1)	lorry n° 2/3	corn	probe Tripette et Renaud (manual)	1 162	928 - 1 396	1 428	1 164 - 1 692	1 309	1058 - 1 560
(CAVS / M) C3 (1)	lorry n° 3/3	corn	probe Tripette et Renaud (manual)	1 188	951 - 1 425	1 255	1 011 - 1 500	1 151	918 - 1 384
(CER / M) H7 (C1) Ç 4	lorry 1/1	wheat	probe Tripette et Renaud (manual)	1 371	1 113 - 1 628	1 412	1 150 - 1 675	1 132	901 - 1 363
Flat or vertical silos (by 500Tor by lot)									
(I.V / G.P) SV (1) Ç K23	vertical silo	wheat	probe by aspiration Agronet (mechanical)	6 275	5 463 - 7 087	6 978	6 087 - 7 870	7251	6 329 - 8 174
(I.V / G.P) H1 Ç A10	vertical silo	wheat	probe by aspiration Agronet (mechanical)	2 038	1 630 - 2 446	2 064	1 651 - 2 477	1994	1 595 - 2 393
(CER / M) H7 Ç 4	flat silo	wheat	probe by aspiration Agronet (mechanical)	477 (average) - min: 163 - max: 1480	420 - 534	532	475 - 589	389	332 - 446
Heterogeneity of silos - 100 increments by silo									
(C.C / T) H8 C A2	Heterogeneity test in flat silo	wheat	probe by aspiration Agronet (mechanical)	see paragraph A.2.1.2.					
(I.V / G.P) H1 Ç A10	Heterogeneity test in vertical silo	wheat	probe by aspiration Agronet (mechanical)	see paragraph A.2.1.2.					
(CER / M) H7 Ç 4	Heterogeneity test in flat silo	wheat	probe by aspiration Agronet (mechanical)	see paragraph A.2.1.2.					



a)

Key

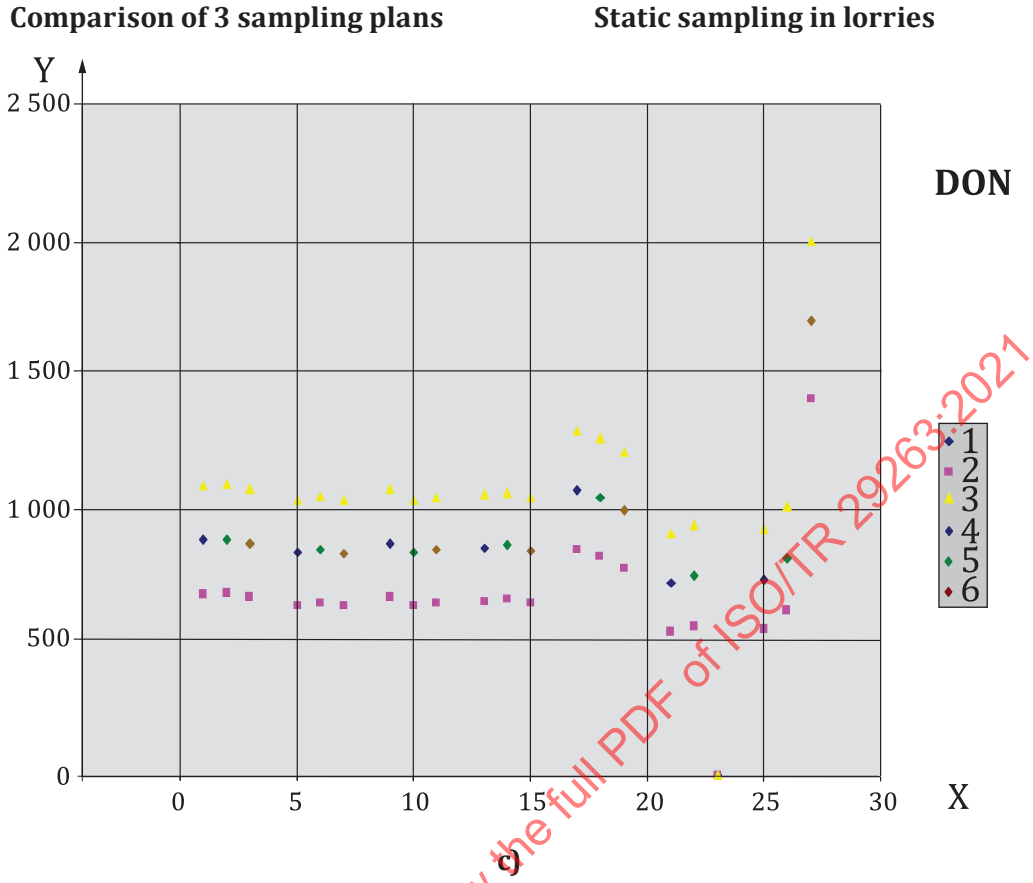
X sampling plans on 3 static lorries : regulatory – normative - routine
Y concentration DON (ppb)
1 average
2 minimum
3 maximum
4 regulatory
5 normative
6 routine



b)

Key

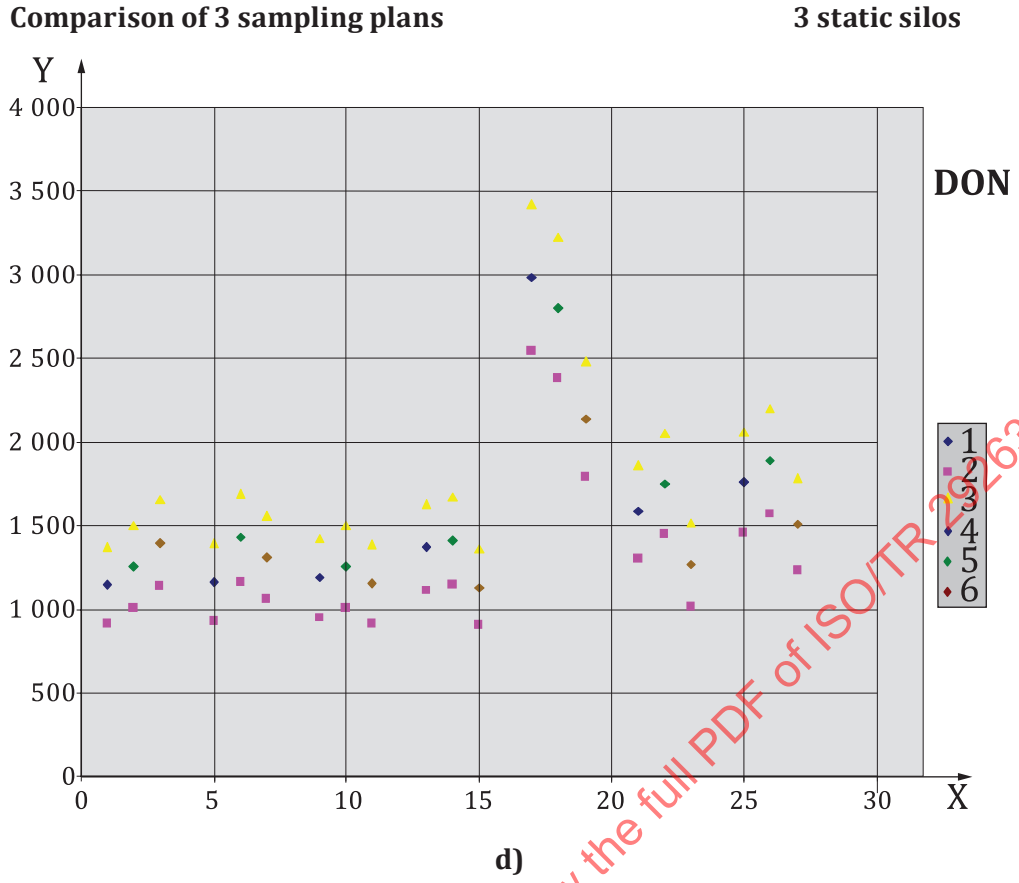
X sampling plans on 14 static lorries : regulatory – normative - routine
Y concentration DON (ppb)
1 average
2 minimum
3 maximum
4 regulatory
5 normative
6 routine



c)

Key

X sampling plans on 14 static lorries : regulatory – normative - routine
Y concentration DON (ppb)
1 average
2 minimum
3 maximum
4 regulatory
5 normative
6 routine



d)

Key

X sampling plans on 14 static lorries : regulatory – normative - routine
Y concentration DON (ppb)
1 average
2 minimum
3 maximum
4 regulatory
5 normative
6 routine

Figure 11 — Sampling results for 3 protocols (DON analysis)

Table 13 — Overview of the other analyses measured on the aggregate samples obtained from the different batches analyzed (1 of 2)

Refer-en-ce	Type of sampling	prod-ucts	Sam-pling equip-ment	analysis results Protéines (%MS - N*6,25)			analysis results Hagberg (on wheat)			analysis results Promatest (on corn)			analysis results zéaralénone (on corn)		
				accord-ing regu-la-tion	accord-ing stand-ard	accord-ing routine method	accord-ing regu-la-tion	accord-ing stand-ard	accord-ing routine method	accord-ing regu-la-tion	ac-cor-ding stand-ard	accord-ing routine method	ac-cor-ding regu-la-tion	Ac-cor-ding stan-dard	Ac-cor-ding routine me-thod
				aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	ave-ra-ge	ave-ra-ge	ave-ra-ge
flowing grain (by 500 t)															
(I.V / G.P) M (1) Ç A10	f l o w i n g grain - exit silo	wheat	manual	11,28	11,25	11,28	352	339	356						
(A.F / O) M (1)	f l o w i n g grain - exit silo	corn	manual	8,82	8,81	8,85				17,7	24,0	18,3	207	116	144
(CAVS / M) M1 (1)	f l o w i n g grain - exit silo	corn	manual	9,01	9,03	8,96				14,6	15,6	16,3	238	302	335
(CAVS / M) M2 (1)	f l o w i n g grain - exit silo	corn	manual	8,87	8,86	8,83				15,1	15,6	16,3	112	209	154
(R.F / L) T (1)	f l o w i n g grain - exit train	corn	manual	8,56	8,54	8,57				26,3	25,0	26,3	< 40	52	< 40
Static - lorries															
(I.V / G.P) C1 (M) Ç A10	lorry 1/3	wheat	manual												
(I.V / G.P) C1 (A) Ç A10	lorry 1/3	wheat	me-chani-cal	11,39	11,37	11,34	357	347	334						
(I.V / G.P) C2 (M) Ç A10	lorry 2/3	wheat	manual												
(I.V / G.P) C2 (A) Ç A10	lorry 2/3	wheat	me-chani-cal	11,42	11,38	11,40	348	367	356						
(I.V / G.P) C3 (M) Ç A10	lorry 3/3	wheat	manual												
(I.V / G.P) C3 (A) Ç A10	lorry 3/3	wheat	me-chani-cal	11,38	11,34	11,39	337	354	335						
(C.C / T) C1 (1)	lorry 1/4	corn	me-chani-cal	9,08	9,24	9,08				14,0	15,3	14,6	< 40	< 40	< 40
(C.C / T) C2 (1)	lorry 2/4	corn	me-chani-cal	9,23	9,19	9,15				14,6	14,4	13,5	< 40	< 40	< 40
(C.C / T) C3 (1)	lorry 3/4	corn	me-chani-cal	9,17	9,18	9,14				13,5	14,0	14,9	< 40	< 40	< 40
(C.C / T) C4 (1)	lorry 4/4	corn	me-chani-cal	9,16	9,08	9,13				14,0	13,2	13,2	< 40	< 40	< 40
(R.D / M) C (1)	lorry 1/1	corn	manual	9,35	9,45	9,51				11,4	< 10	11,8	< 40	107	89

Table 13 *(continued)*

Refer-en-ce	Type of sampling	prod-ucts	Sam-pling equip-ment	analysis results Protéines (%MS - N*6,25)			analysis results Hagberg (on wheat)			analysis results Promatest (on corn)			analysis results zéaralénone (on corn)		
				accord-ing regu-la-tion	accord-ing stand-ard	accord-ing routine method	accord-ing regu-la-tion	accord-ing stand-ard	accord-ing routine method	accord-ing regu-la-tion	ac-cor-ding stand-ard	accord-ing routine method	ac-cor-ding regu-la-tion	Ac-cor-ding stan-dard	Ac-cor-ding routine me-thod
				aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	aver-age	ave-ra-ge	ave-ra-ge	ave-ra-ge
(S / V.P) C1 (1)	lorry 1/2	wheat	manual	11,10	11,02	11,04	376	384	375						
(S / V.P) C2 (1)	lorry 2/2	wheat	manual	11,02	11,05	10,98	391	377	398						
(CAVS / M) C1 (1)	lorry n° 1/3	corn	manual	8,94	8,91	8,92				14,9	15,9	16,8	216	217	206
(CAVS / M) C2 (1)	lorry n° 2/3	corn	manual	8,88	8,92	8,96				14,2	14,4	15,1	199	173	177
(CAVS / M) C3 (1)	lorry n° 3/3	corn	manual	9,03	8,94	9,01				14,9	16,8	14,9	215	76	337
(CER / M) H7 (C1) Ç 4	lorry 1/1	wheat	manual	10,11	10,17	10,25	249	254	247						
Flat or vertical silos (by 500Tons by lot)															
(I.V / G.P) SV (1) Ç K23	vertical silo	wheat	me-chani-cal	11,05	11,09	11,06	356	350	344						
(I.V / G.P) H1 Ç A10	vertical silo	wheat	me-chani-cal												
(CER / M) H7 Ç 4	flat silo	wheat	me-chani-cal												

6.4.2.1.2 Results of the heterogeneity analyses

For the 3 silos studied, the 100 individual samples were analysed separately.

For 2 of the 3 silos, the DON analyses were completed with protein (nitrogen) and Hagberg analyses. The Table A6 gives the averages and characteristics statistics obtained by averaging the 100 individual samples of each batch.

Table 14 — Overview of the average analyses conducted on different batches

	silo (C.C/T) H8 CA2				Silo (I.V/G.P) H1 CA10)			Silo CER/M) H7 C4
	DON (Kit)	Moisture	Hagberg	Nitro-gen	DON (kit)	Hagberg	Nitrogen	DON (kit)
Average	1 613	11,25	317,02	11,61	1 998	349,82	11,61	477
min	211	10,43	276	11,21	1 006	321	11,3	163
max	5 355	12,39	364	12,13	3 376	376	12,0	1 480
Standard deviation	1 223	0,36	15,35	0,21	650	11,76	0,15	263
confidence interval	240	0,07	3,01	0,04	127	2,31	0,03	51

Table 14 *(continued)*

	silo (C.C/T) H8 CA2				Silo (I.V/G.P) H1 CA10)			Silo CER/M) H7 C4
	DON (Kit)	Moisture	Hagberg	Nitro-gen	DON (kit)	Hagberg	Nitrogen	DON (kit)
relative standard de-viation	75,8	3,17	4,84	1,84	32,5	3,36	1,27	55,1

6.4.2.2 Statistical validation

6.4.2.2.1 Results of comparison tests involving 3 sampling plans

6.4.2.2.1.1 Results of comparison tests involving 3 protocols for DON

The protocol for comparing results underwent the student's t-test for comparing averages (paired observations) between regulatory protocol and normative protocol and between regulatory protocol and routine protocol.

Table 15 — Comparison for the 3 protocols for DON

	Flowing grain		Static - lorries		Static - silos		Combined all situa-tions	
	Regulato-ry versus normative	Regulato-ry versus routine	Regulato-ry versus normative	Regulato-ry versus routine	Regulato-ry versus normative	Regulato-ry versus routine	Regulato-ry versus normative	Regulato-ry versus routine
Num. repe-titions	5	5	14	13	3	3	22	21
Average difference [a]	-38	0	-48	34	-261	-281	75	-19
Probability associat-ed with student's t-test	0,23 (NS)	0,99 (NS)	0,12 (NS)	0,76 (NS)	0,36 (NS)	0,50 (NS)	0,05 (NS)	0,82 (NS)

[a] Average difference: average of the differences of average DON content obtained by applying two protocols.

6.4.2.2.1.2 Analysis of the statistical test results

In this statistical analysis of the results, we are interested in determining whether there was a meaningful difference between the result groups taken in pairs, obtained by the application of the regulatory protocol / normative protocol and regulatory protocol / routine protocol: in other words, the hypothesis according to which the different sampling methods do not significantly modify the evaluation of the average mycotoxin content of the sampled batch. The Student's t-test can be used to measure the gap between 2 groups: this statistic is essentially the difference of the averages, divided by a value that takes into account the variance of the 2 groups. The calculated probabilities are compared with the chosen signification threshold, namely 0,05 (or 5 %).

— None of the tests applied highlighted a statistically significant difference between the protocols taken in pairs.

— We can draw the conclusion that the alternative protocols (normative and routine) results in the same estimation of average mycotoxin content of a batch as the regulatory protocol.

6.4.2.2.1.3 Results of the sampling method comparison tests: manual and automatic

Table 16 — Results of the comparison between methods (student's t-test, paired observations)

	manual vs automatic sampling
Num of repetitions	3
Average difference	140
probability	0,10 (NS)

Here again, there is no statistically significant difference.

6.4.2.2.1.4 Results of comparison tests involving 2 protocols for the other analyses

The application of the statistical tests for wheat (protein and Hagberg) and for maize (protein and promatest) concluded that there was no significant difference between the regulatory protocol and the normative protocol when estimating:

— the nitrogen content of a batch of wheat;

— Hagberg falling number of a batch of wheat;

— the nitrogen content of a batch of maize;

— promatest of a batch of maize.

6.4.2.2.2 Heterogeneity test results

6.4.2.2.2.1 Descriptive statistics

The DON content of the silos presents very different variability characteristics depending on the silo (see table 20).

Table 17 — Variability between silos — Heterogeneity tests

	Silo CC/T H8 CA2	Silo IV/GP H1 CA10	Silo CER/M H7 C4
n	100	100	100
average	1 613	1 998	477
Min	211	1 006	163
Max	5 355	3 376	1 480
Standard deviation	1 223	650	263
Confidence interval	240	127	51
relative standard deviation	75,8	32,5	55,1

The 3 silos have different average contents and variability. The higher the silo's average content, the greater its variability. However, the low number of silos observed precludes this relationship from being modelled.

6.4.2.2.2.2 Intra-silo variability study

In the estimation of a silo average, the variability percentage linked to technical errors (under-sampling + analytical error) cannot be reduced, i.e. it will not decrease even if the number of samples is increased.

The variation coefficient of this error is estimated at 20 % for DON. However, the variability connected with the sampling will decrease with the number of samples taken.

By means of simulation it is thus possible to calculate, for each silo, the change in terms of variability linked to the sampling, depending on the sample size.

For the three silos sampled, the relationship between the confidence interval of the silo's average content and the number of samples is as follows.

NOTE This confidence interval takes into account the total variability which is the variability due to the sampling and the analysis of the samples.

Table 18 — Relationship between confidence interval of the silo's average content and the number of individual samples taken in each silo

Number of increments or individual samples	Silo (C.C/T) H8 CA2	Silo (I.V/G.P) H1 CA10	Silo (CER/M) H7 C4
10	758	403	163
25	479	255	103
50	339	180	73
100	240	127	52

For a similar number of samples, the confidence intervals are different depending on the characteristics of the silos studied. However, it can be observed that when the number of samples exceeds 25, the gain in accuracy becomes prohibitive with reference to the sampling effort (technical, human and financial) that this would require.

It is important to observe that the variability due solely to sampling is lower than or equal to the variability of the actual analysis, for a number of samplings varying between 10 and 25, depending on the silo studied.

Table 19 — Relationship between analytical and sampling variability and the number of individual samples taken in each silo

	Silo (C.C/T) H8 CA2	Silo (I.V/G.P) H1 CA10	Silo (CER/M) H7 C4
Analytical variability	323	400	95
Sampling variability >= Analytical variability for (number of increments)	25	10	25

6.4.2.2.2.3 Analysis of the statistical test results

The simulation of the calculation of a confidence interval depending on a theoretical number of samples leads to an estimation of the variability due to sampling being lower or equal to that of the analytical variability for, on average, a sampling plan of 10 to 25 samples depending on the average and heterogeneity of the silo.

6.4.2.2.2.4 Error risk study

The objective of this part is to attempt to evaluate the estimated error of mycotoxin contamination depending on the number of samples taken per 500 t silo, with reference to the results obtained by the regulatory protocol (100 samples by 500t).

For this, we have conducted a simulation of 10,000 samples taken at random from the silo "population" of the 100 individual samples (for the 3 silos).

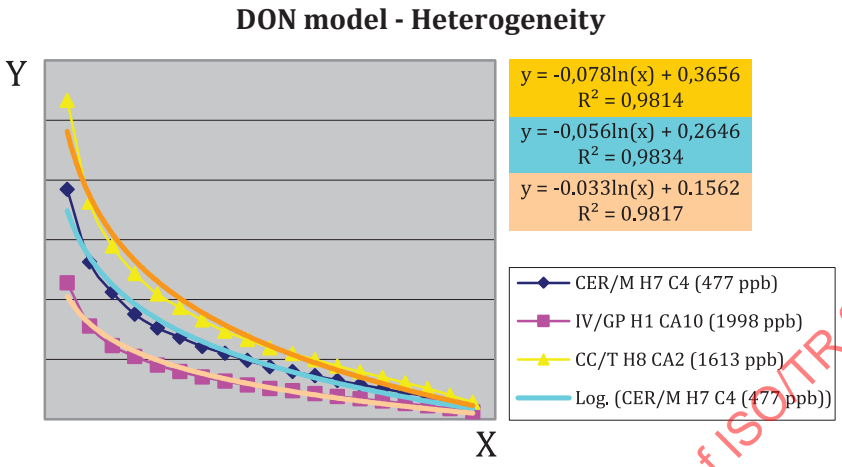
The method was the following one: we randomly extract 25 samples from the 100; we calculate the average. We compare the average of these 25 samples with that of 100 samples. We run 10,000 trials. We thus obtain 10,000 differences. Then, we calculate the average of the 10, 000 differences to have an idea of the average distance between 25 and 100.

Finally, we return this average error of sampling in % with respect to the mean content of the 100 samples. This "simulation" was run for 5, 10, 15, 20 .....95, 100 samples.

Curves thus represent a risk of supplementary error with regard to 100 (because we do not know the true mean content of the silo).

6.3.2.2.2.3.1 Error risk study on the estimation of average content of silos

— for DON



Key

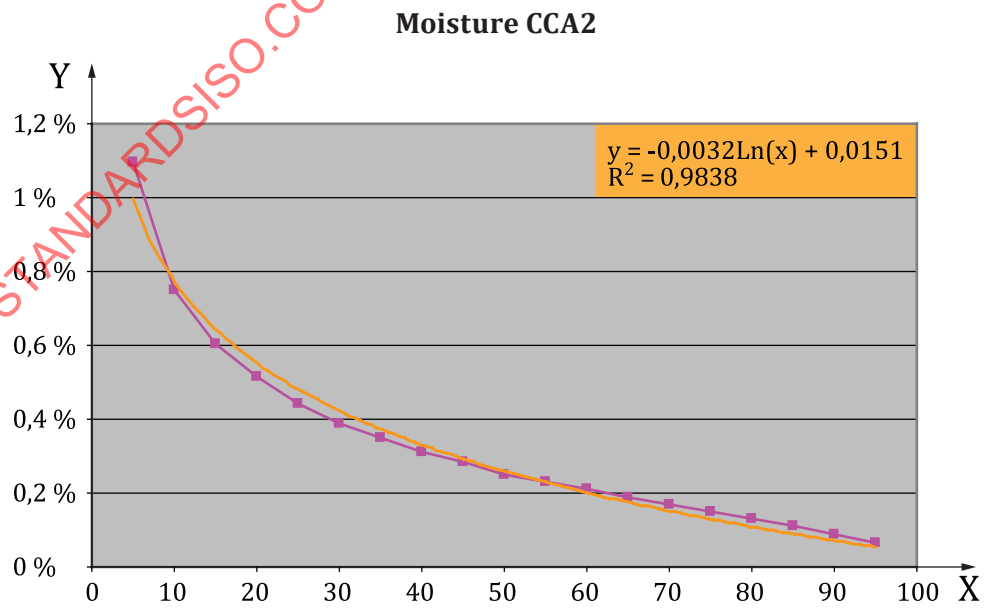
X number of samples / 500 t

Y risk to have an average different from that of 100 samples

Figure 12 — Error risk on the estimation of average content of DON in 3 silos

The results show that for 20 individual samples taken in a silo of 500 t, the risk of having an estimation of the mean content of the silo different from the one that we would have with 100 samples is included between 5 and 12 % according to the level of contamination of the silo; for 25 individual samples, the risk is included between 5 and 10 %.

— for moisture (on 1 silo : CC/T H8 CA2)



Key

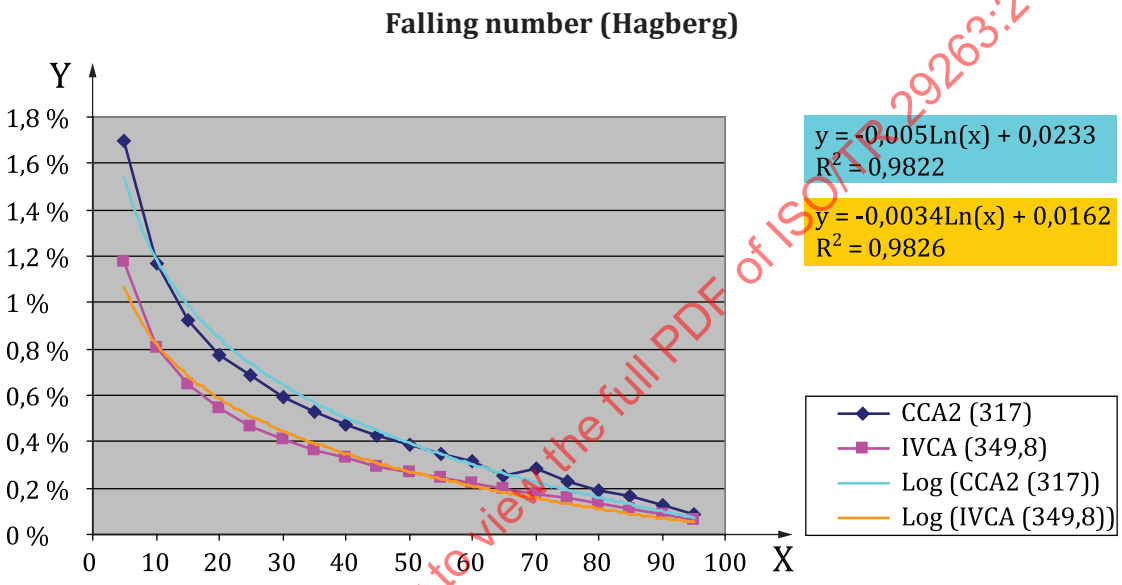
X number of samples per 500 t

Y % of risk to have an average different from that of 100 samples

Figure 13 — Error risk on the estimation of average content of moisture in 1 silo

The results show that for 5 individual samples taken in a silo of 500 t, the risk of having an estimation of the mean content of the silo different from the one that we would have with 100 samples is lower than 1,1 %; for 25 elementary samples, the risk is about 0,4 %.

— for falling number (Hagberg) (on 2 silos : CC/T H8 CA2 and IV/GP H1 CA10)



Key

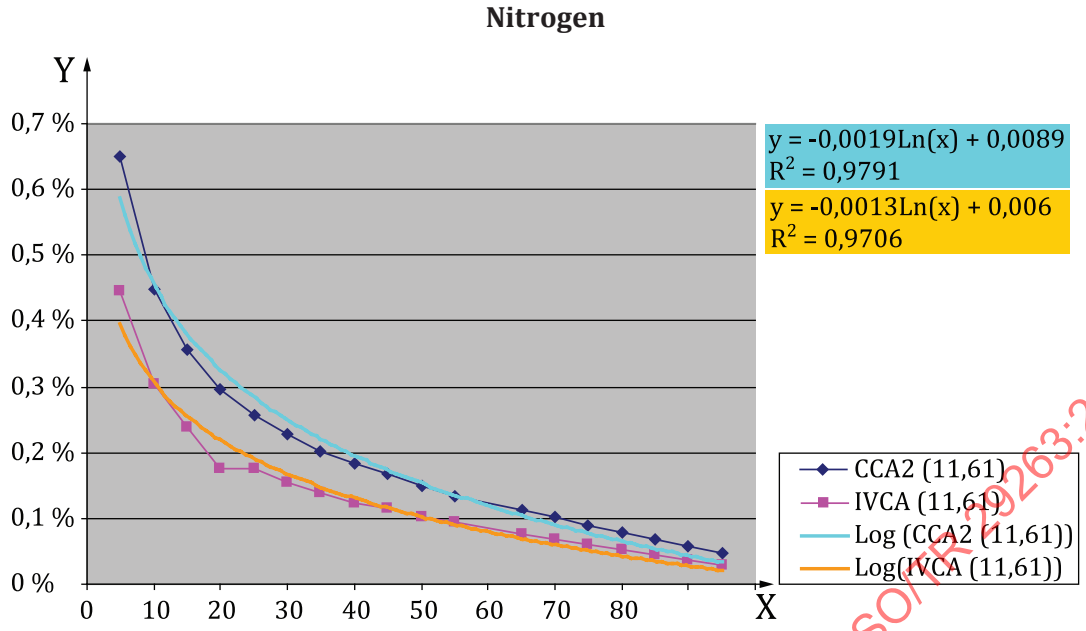
X number of samples / 500 t

Y % of risk to have an average different from that of 100 samples

Figure 14 — Error risk on the estimation of average content of falling number in 2 silos

The results show that for 5 individual samples taken in a silo of 500 t, the risk of having an estimation of the mean content of the silo different from the one that we would have with 100 samples is lower than 1,8 %; for 25 elementary samples, the risk is lower than 0,7 %.

— for nitrogen (on 2 silos : CC/T H8 CA2 and IV/GP H1 CA10)



Key

X number of samples / 500 t

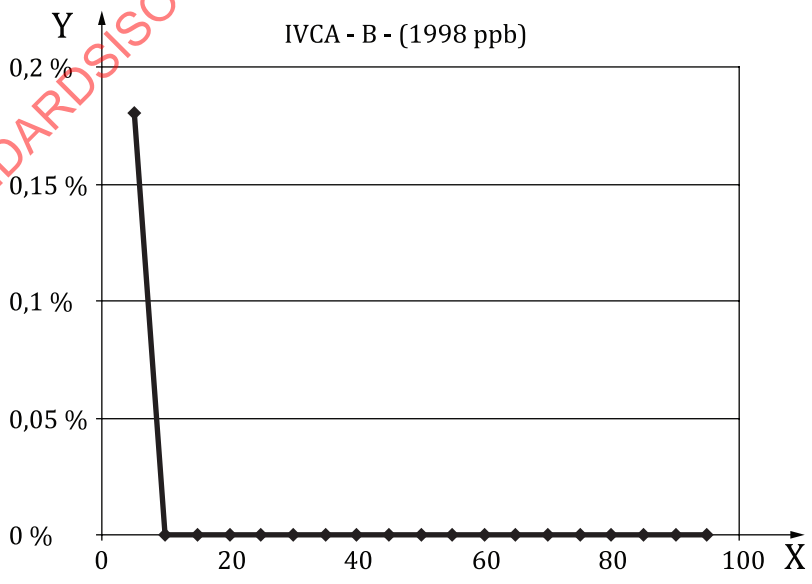
Y % of risk to have an average different from that of 100 samples

Figure 15 — Error risk on the estimation of average content of nitrogen in 2 silos

The results show that for 5 elementary samples taken in a silo of 500 t, the risk of having an estimation of the mean content of the silo different from the one that we would have with 100 samples is lower than 0,7 %; for 25 elementary samples, the risk is lower than 0,3 %.

6.3.2.2.2.3.2 Modelling of the risk of error to accept wrongly a contaminated lot (in reference to the legal threshold of 1 250 ppb for wheat)

The same operation was performed but with the aim of evaluating the risk to accept a lot within the real average of the silo is above 1 250 ppb (for DON).

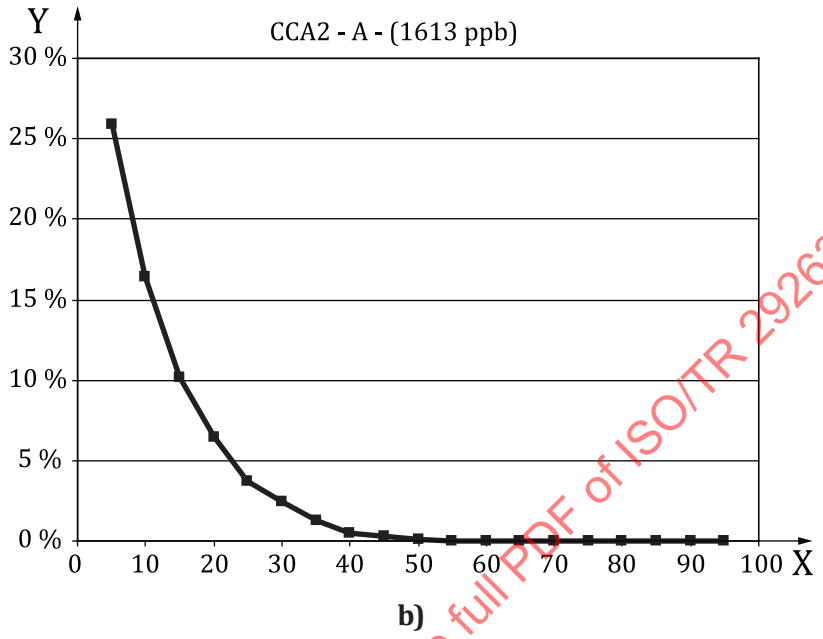


a)

Key

X number of samples

Y error with regard to the threshold of 1250 ppb (number of cases below the threshold)



b)

Key

X number of samples

Y error with regard to the threshold of 1250 ppb (number of cases below the threshold)

Figure 16 — Modelling of the error risk to accept wrongly a contaminated lot for 2 silos

In the case of silo CC/T H8 CA2 ("CCA2" in graph), with 5 individual samples taken in the 500 t batch, the simulation of 10 000 samples shows that we would have a risk of 25 % of accepting the lot while the real average of the silo is above 1 250 ppb. For 20 samples, the risk will be about 6 % and for 25 samples, the risk will be 4 %.

For IV/GP H1 CA10 ("IVCA" in graph), the risk is very low due to the fact that the level of contamination is far from 1 250 ppb.

6.4.2.3 Study A conclusions

Applying the two protocols showed an equivalent estimation of the average content of DON, nitrogen, moisture and falling number contamination of a batch of cereals given the analytical uncertainties.

As a result, implementing a sampling protocol based on a lower but sufficient number of samplings from a batch does not lead to an underestimation of the average mycotoxin content of this batch.

Consequently, using the regulatory protocols incurs unjustified costs.

To illustrate this point, applying the 2 protocols for a horizontal 10 000 t silo (per 500 t section) enabled the cost of the following sampling to be estimated:

— regulatory protocol: € 10,300 euros representing 470 h sampling, in other words 67 d;

— standard protocol: € 4,000 euros representing 185 h sampling, in other words 26,5 d.

6.5 Study B : silos of corn – Fusariotoxins; flowing grains

6.5.1 Organising field tests

6.5.1.1 Conducting tests

6.5.1.1.1 Setting up the tests

— Identification of silos with contamination levels very different;

— Selection of 10 vertical silos of corn; sampling of moving grain; same sampling plan;

— 4 silos sampled on 500 t; 1 silo for 625 t; 3 silos for 1 000 t; 1 silo for 1 250 t; 1 silo for 1 500 t.

Table 20 — Characteristics of the tests conducted

	Silo 06-01	Silo 06-02	Silo 06-03	Silo 06-04	Silo 06-05	Silo 06-06	Silo 07-01	Silo 07-02	Silo 07-03	Silo 07-04
Type of corn	Waxy	Maïs Roux	Waxy	Maïs Roux Classe A	Maïs Classe A	Maïs Roux	Maïs Roux		Starch.	Maize mills
Initial Quantity of the silo (t)	10 500	8 000	3 400	4 500	6 000	1 3200	5 000	4 000	4 500	1 092
Cell diamèter (m)	26 (conical)	18 (flat silo)	17 (conical)	17,5 (conical)	17,8 (conical)	33 (flat silo)	12 (flat silo)			
Cleaning before drying	Purifier	Purifier	No	Cleaner CESBRON	Cleaner MAROT	Cleaner - separator	CleanerMAROT			
Cooling before storage	Dry	Dry	No	No	No	Dry	No	No	No	No
Cell ventilation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Grain handling	Yes	No	No	No	No	Yes	Yes	No	No	No
Type of filling	Continuous	Continuous	Continuous	Intermittent	Intermittent	Intermittent	Conti-nuous	Continuous	Continuous	Continuous
Nomber of extractions	12	-	2	-	450	-	-	1	1	-
Remaining stock (t)	3 830	2 900	2 400	1 800	650	3 000	5 000	2 800	2 800	1 092
Quantity studied (t)	500	1 000	1 000	500	625	1 250	1 500	1 000	500	500
Remarks						Sampling after transilage	Sampling after transilage			

6.5.1.1.2 Method applied

— The samples were taken on a single lot in a continuous discharge, every 15 t to 20 t (in response to situations of filling trucks).

— For the silo 07-01, a first set of samples was done every 20 t and a 2nd series every 5 t and finally a 3rd series again every 20 t.

— 25 samples per 500 t were made on samples leaving the silo, (after passage of 4 t to 5 t) and after stabilization of flow. In the case of silo 07-01, the 1st series consists of 25 samples, the 2nd 100 and 3rd in 25.

— Each individual sample had a mass of 750 to 1 000 g of grain.

Table 21 — Sampling organisation

	Silo 06-01	Silo 06-02	Silo 06-03	Silo 06-04	Silo 06-05	Silo 06-06	Silo 07-01	Silo 07-02	Silo 07-03	Silo 07-04
Type of handling	reddler under cell	reddler under cell	reddler under cell	reddler under cell	reddler under cell	reddler under cell	reddler under cell	reddler under cell	Rubber carrier belt	reddler under cell
Type of pickup	bucket elevator	bucket eleva-tor	bucket eleva-tor	bucket eleva-tor	bucket eleva-tor	bucket eleva-tor	bucket eleva-tor	bucket eleva-tor	bucket elevator	bucket elevator
Transfer rate(t/h)	220	320	320	80	150	50	40-80	300	300	100
Weighing	Yes	No	No	Yes	Yes	Yes	No			
Quantity studied (t)	500	1 000	1 000	625	525	1 250	1 500	1 000	500	500
Nomber of individual samples	25	50	50	25	21	50	150	50	25	25
Interval between individual samples	20 t	20 t	20 t	25 t	25 t	25 t	5 t 20 t	20 t	20 t	20 t

6.5.1.2 Sample analyses and study of reducing the size of samples

— All individual samples were stored in cold storage before being analysed.

— An average sample was made up for each lot and analysed to ensure the level of contamination. To do this, an aliquot of the individual sample was taken from the divider Boerner, the other part was retained for subsequent analysis.

— The analysis of fumonisins, zearalenone, deoxynivalenol were performed by a laboratory according to the following standards:

  — determination of fumonisins B1 and B2 in corn. Purification by HPLC with solid phase extraction - EN 13585;

  — determination of zearalenone by chromatography with immunoaffinity column and HPLC - Draft ISO 17372;

  — determination of deoxynivalenol in wheat and maize - HPLC assay method followed by UV detection and purification on immunoaffinity column - Draft CEN/TC 275/ WG 5 "Biotoxins"

— Individual samples submitted to analysis were pre-crushed to 1 mm and then ground to 0,5 mm.

— A study of reducing the mass of the sample for the laboratory have been conducted on 2 samples of corn for fumonisin test and 2 samples of wheat for DON test. The 20 kg of the initial samples have been splitted to obtain final samples of grain of 500 g.

6.5.2 Results and conclusions

6.5.2.1 Sample analysis results

6.5.2.1.1 Results of the average samples

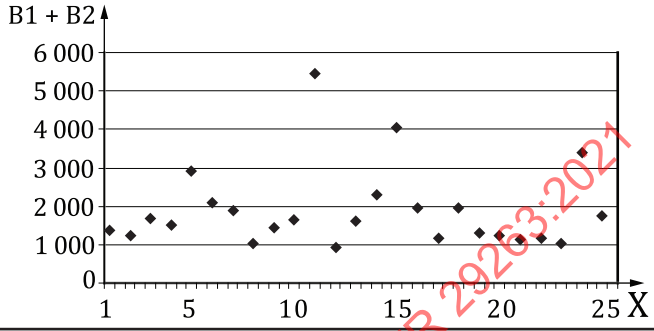
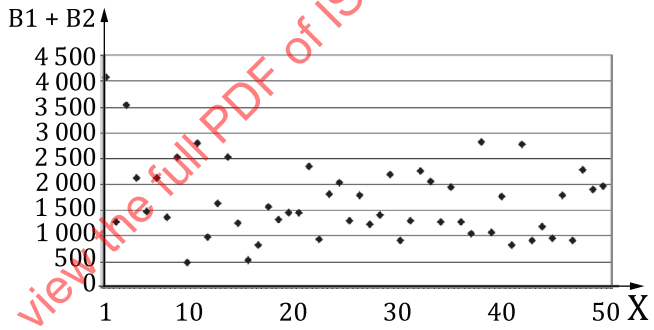
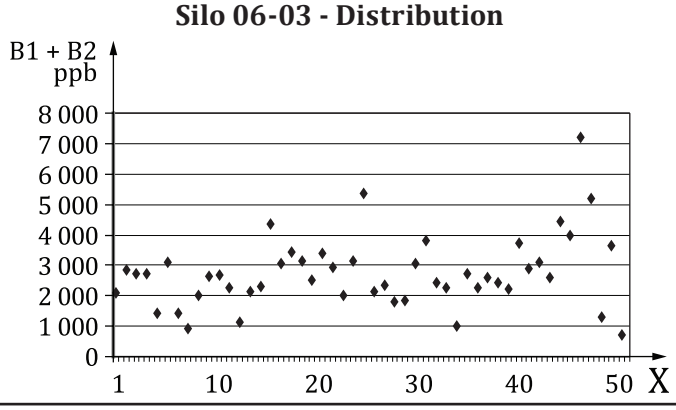
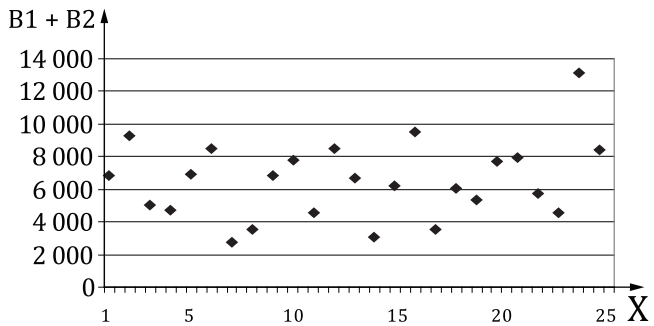
Table 22 — Results of average content of silos in fumonisins, zearalenone and deoxynivalenol

	Silo 06-01	Silo 06-02		Silo 06-03		Silo 06-04	Silo 06-05	Silo 06-06	
Quantity studied (t)	500	1 000		1 000		500	525	1 250	
Number of series	1	2		2		1	1	2	
Quantity of each serie (t)	500	500	500	500	500	500	525	625	625
Number of individual samples	25	25	25	25	25	25	21	21	25
Frequency of sampling (t)	20	20	20	20	20	20	25	25	25
Average sample in Fumonisins (ppb)	1 311	2 154	1 709	2 237	3 124	4 236	699	3 265	1 942
Calculated average content of the silo (from individual samples) in Fumonisins (ppb)	1 889	1 753	1 598	2 638	2 892	6 533	Not keep	1 722	1 887

	Silo 07-01				Silo 07-02		Silo 07-03	Silo 07-04
Quantity studied (t)	1 500				1 000		500	500
Number of series	4				2		1	1
Quantity of each serie (t)	500	250	250	500	500	500	500	500
Number of individual samples	25	50	50	25	25	25	25	25
Frequency of sampling rate (t)t	20	5	5	20	20	20	20	20
Average sample in Fumonisins (ppb)	5 698	5 258	4 581	5 180	384	477	-	-
Calculated average content of the silo (from individual samples) in Fumonisins (ppb)	7 422	7 185	6 637	7 724	502	-	-	-
Average sample in Zéaralénone (ppb)	-	-	-	-	338	484	non	113
Calculated average content of the silo (from individual samples) in Zéaralénone (ppb)					371	428	683	139
Average sample in Déoxynivalénol (ppb)	-	-	-	-	2 492	2 324	-	-
Calculated average content of the silo (from individual samples) in Déoxynivalénol (ppb)	-	-	-	-	2 779	2 486	-	-

6.5.2.1.2 Evolution of mycotoxin content during flowing of grains

6.5.2.1.2.1 Results for Fumonisins

a) Silo : 500 t; 25 samples Average content in Fumonisins (ppb): B1 : 1 289; B2 : 600; B1+B2 : 1 889	
b) Silo : 1 000 t; 50 samples Average content in Fumonisins (ppb): 1st serie (1 to 25 samples) B1 : 1 272; B2 : 481; B1+B2 : 1 753 2sd serie (26 to 50 samples) B1 :1 170; B2 : 429; B1+B2 : 1 598	
c) Silo : 1 000 t; 50 samples Average content in Fumonisins (ppb): 1st serie (1 to 25 samples) B1 : 1 828; B2 : 810; B1+B2 : 2 638 2sd serie (26 to 50 samples) B1 :2 002 ; B2 : 890; B1+B2 : 2 892	
d) Silo : 500 t; 25 samples Average content in Fumonisins (ppb): B1 : 4 504; B2 : 2 029; B1+B2 : 6 533	

e)

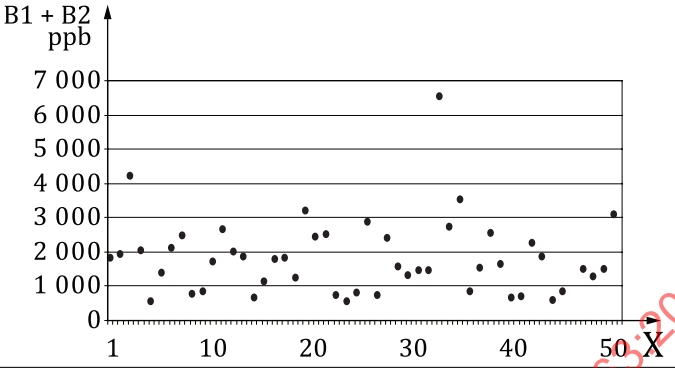
Silo : 1 000 t; 50 samples

Average content in Fumonisins (ppb):

1st serie (1 to 25 samples) B1 : 1 228; B2 : 494; B1+B2 : 1 722

2sd serie (26 to 50 samples) B1 :1 313; B2 :; B1+B2 : 1 887

Silo 06-06 - Distribution



f)

Silo 07-01 : 1 500 t; 150 samples

Average content in Fumonisins (ppb):

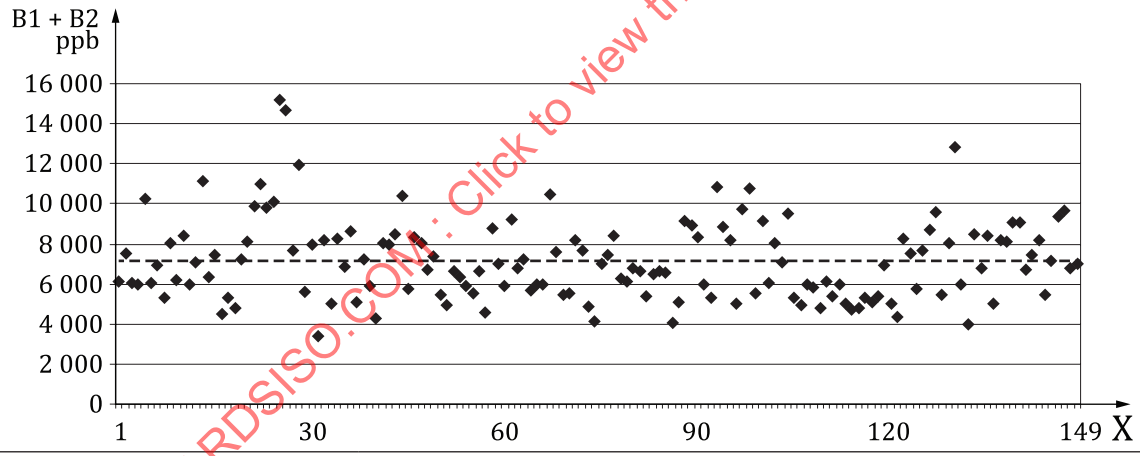
1st serie (1 to 25 samples) B1+B2 : 7 422

2sd serie (26 to 50 samples) B1+B2 : 7 875 (51 to 75 samples) B1+B2 : 6 496

3sd serie (76 to 100 samples) B1+B2 : 7 183 (101 to 125 samples) B1+B2 : 6 092

4th serie (126 to 150 samples) B1+B2 : 7 724

Silo 07-01 - Distribution



g)

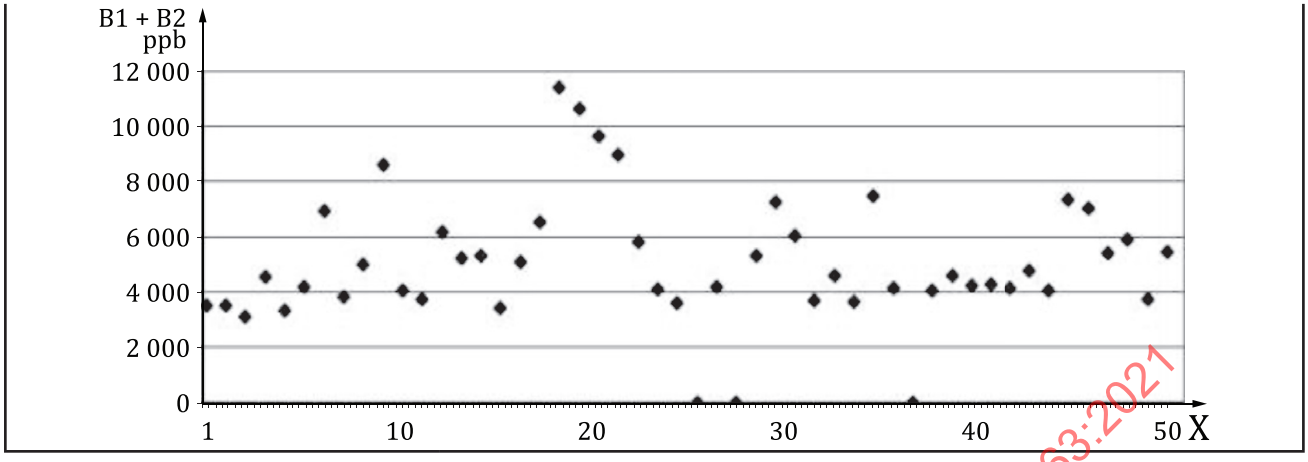
Silo : 1 000 t; 50 samples

Average content in Fumonisins (ppb):

1st serie (1 to 25 samples) B1 : 381; B2 : 179; B1+B2 : 560

2sd serie (26 to 50 samples) B1 :297 ; B2 : 147; B1+B2 : 444

Silo 07-02- Distribution

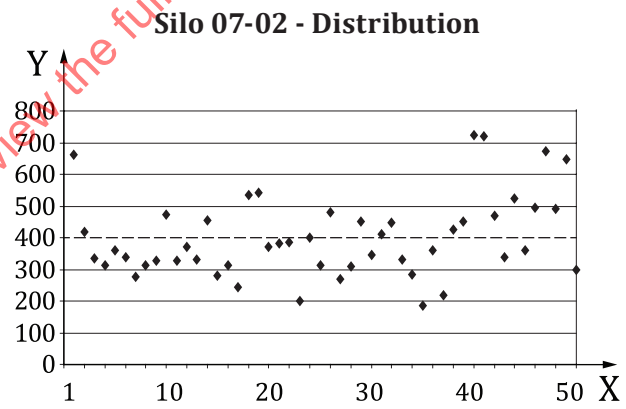
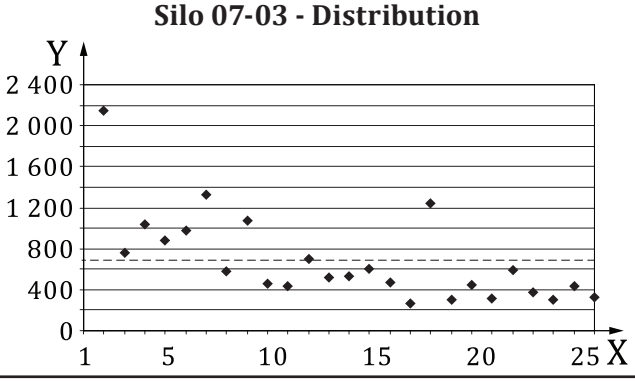


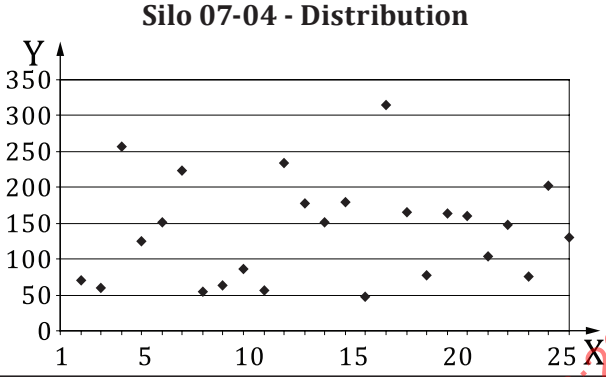
Key

X N° echantillon

Figure 17 — Evolution of fumonisins during flowing of grains

6.5.2.1.2.2 Results for Zearalenone (ZEA)

a) Silo : 1 000 t; 50 samples Average content in Zearalenone (ppb): 1st serie (1 to 25 samples) : 371 2sd serie (26 to 50 samples) : 428	
b) Silo : 500 t; 25 samples Average content in Zearalenone (ppb): 1 serie (1 to 25 samples) : 683	

c) Silo : 500 t; 25 samples Average content in Zearalenone (ppb): 1 serie (1 to 25 samples) : 139	

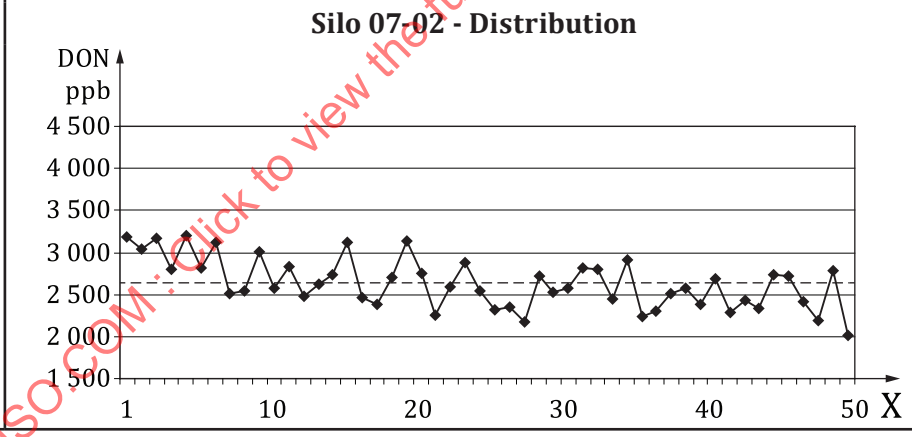
Key

X N° sample

Y zearalenone ppb

Figure 18 — Evolution of zearalenone during flowing of grains

6.5.2.1.2.3 Results for deoxynivalenol (DON)

Silo : 1 000 t; 50 samples Average content in DON (ppb): 1st serie (1 to 25 samples) : 2 779 2sd serie (26 to 50 samples) : 2 486	

Key

X N° sample

Y DON ppb

Figure 19 — Evolution of DON during flowing of grains

6.5.2.2 Statistical validation

6.5.2.2.1 Fumonisins

6.5.2.2.1.1 Descriptive statistics

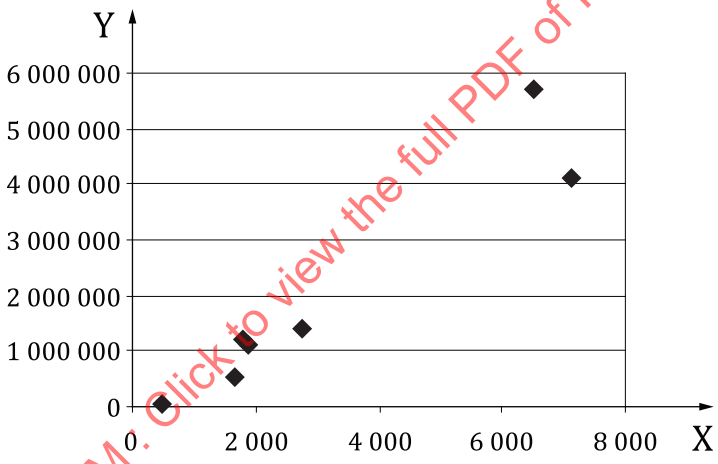
The 7 silos have average grades and variability of very different (see Table 26). The mean levels vary from 502 ppb to 7 132 ppb. The standard deviations ranged from 230 ppb to 2 387 ppb.

Table 23 — Descriptive statistics for fumonisins

	06-03	06-01	06-04	06-06	06-02	07-01	07-02
Number of individual samples	50	25	25	49	50	150	50
average	2 765	1 889	6 533	1 803	1 675	7 132	502
min	723	928	2 780	539	495	3 378	0
max	7 178	5 439	13 120	6 530	4 064	15 199	1 140
variance	1,403,892	1,121,961	5,696,655	1,201,553	546,862	4,096,068	53,077
standard deviation	1 185	1 059	2 387	1 096	740	2 024	230
relative standard deviation	42,9	56,1	36,5	60,8	44,1	28,4	45,9

There is a link between the variability of a silo and its average content: the higher the average level of the silo, the greater its variability is large (see Figure 20).

This type of relationship has been observed with other mycotoxins and other crops in the silo but also in the field (see review on DON and libraries).

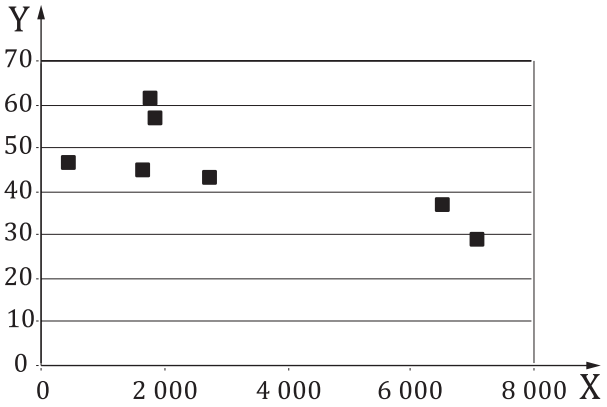


Key

X average level

Y variance

Figure 20 — Relationship variance — Mean



Key

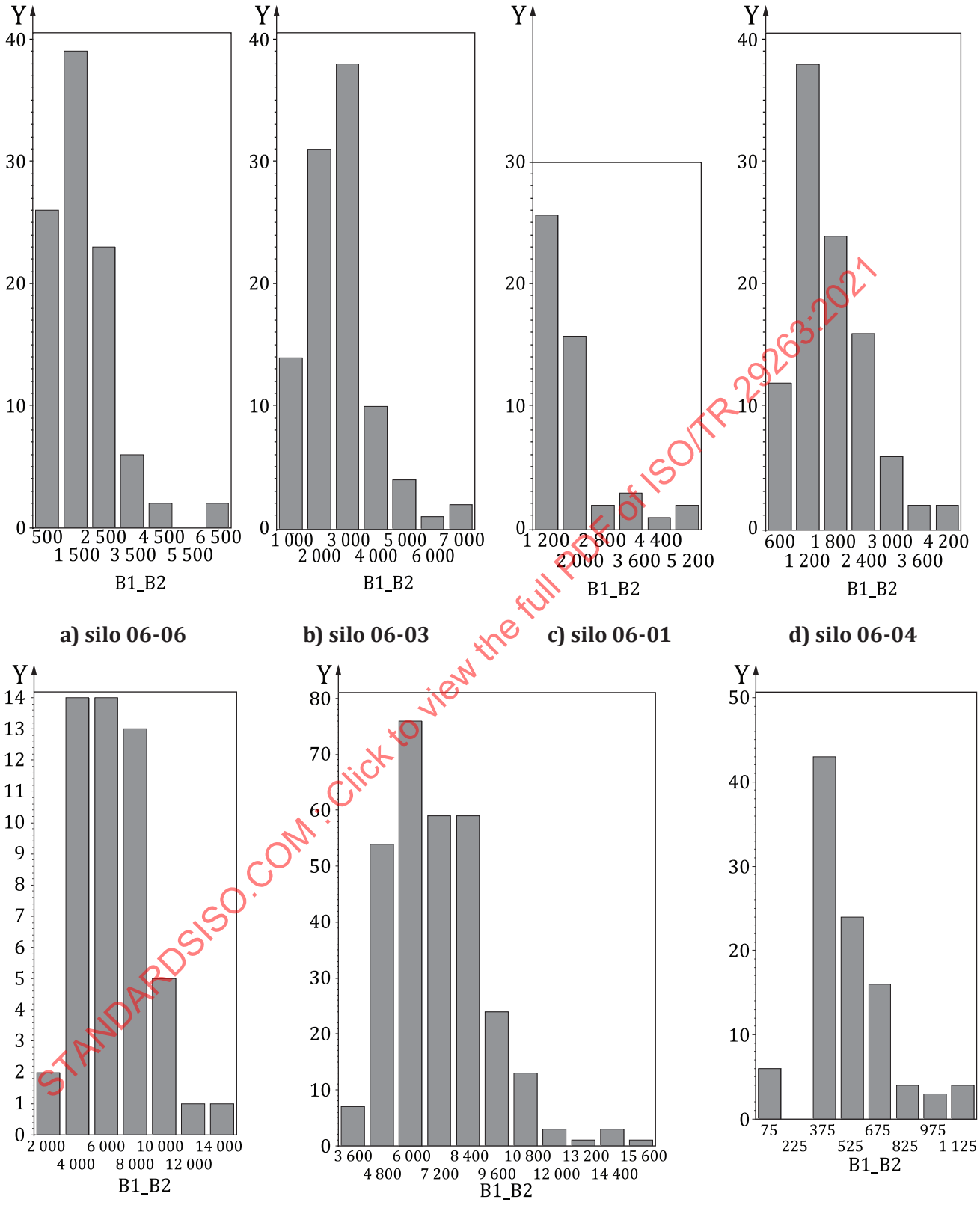
X average level

Y variation coefficient

Figure 21 — Relationship C_V - average

Conversely, the relative variability (C_V) appears to be relatively independent of the average, with average C_V of around 45 % (see Figure 21).

6.4.2.2.1.1.1 Distribution histograms



a) silo 06-06 **b) silo 06-03** **c) silo 06-01** **d) silo 06-04**

e) silo 06-02 **f) silo 06-04** **g) silo 07-01**

Key

Y frequency

Figure 22 — Distribution histograms for fumonisins

Histograms of the levels of fumonisin (Figure 22 g)) showed asymmetric distributions, with sometimes very strong values.

6.4.2.2.1.1.2 Temporal distribution and variograms

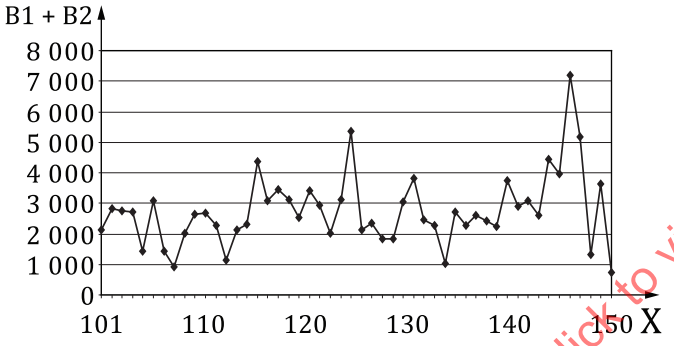
Figures 20 to 26 show the temporal distribution and variograms of fumonisin levels for each silo (fumonisin levels in relation with the number of individual samples to determine the existing structure.

We can see that the fumonisin content is structured temporally if 2 close samples are more similar than 2 distant samples. Conversely we can see that the structure is erratic (irregular) if the observed values, when one moves along the axis of time, are the result of random.

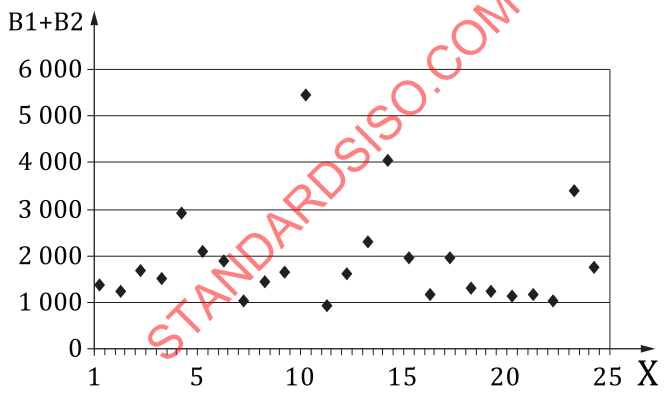
The variogram is a measure of variability between two observations based on the distance h between them. If the content is structured temporally fumonisins, 2 points closer are more alike than 2 points away, the variability between these observations is low. Therefore the value of the variogram is low for low values of h, it increases when the distance between observations increases.

Conventionally, a variogram is bounded, that is to say that from a certain distance (= the scope), the value of the variogram stabilizes to reach a limit. The scope is interpreted as the zone of influence of an observation: beyond the scope, two observations are not correlated.

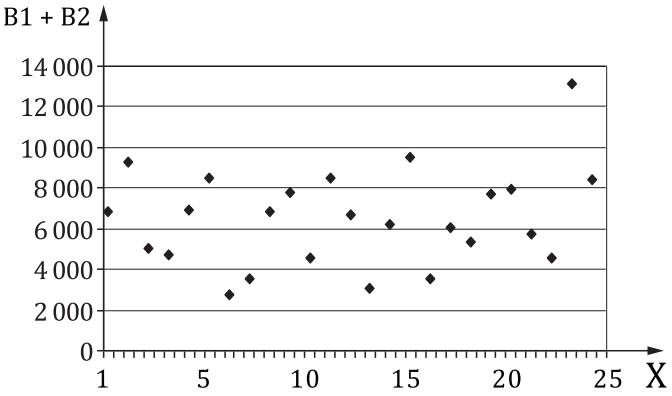
a) Silo 06-03



b) Silo 06-01

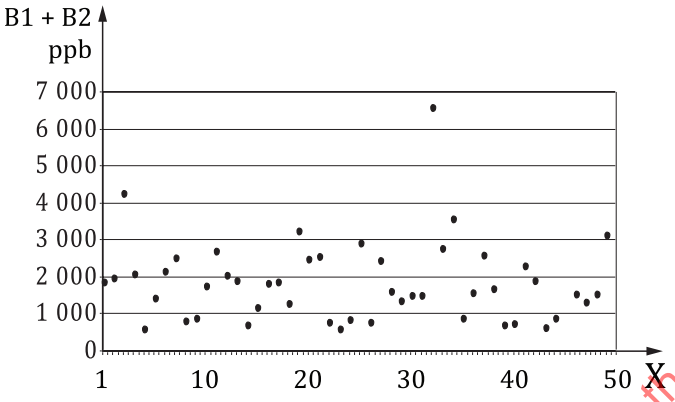


c) Silo 06-04



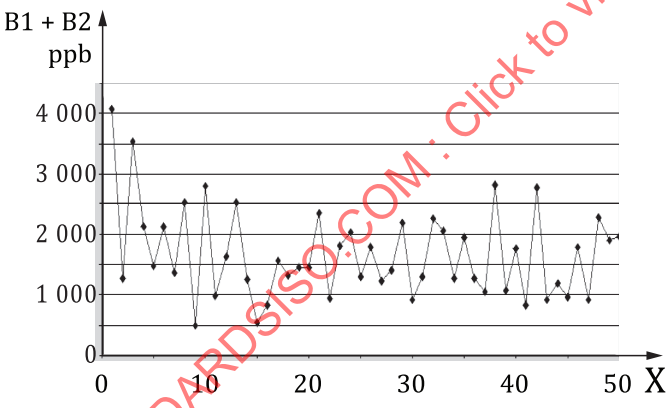
Distance (tons)

d) Silo 06-06



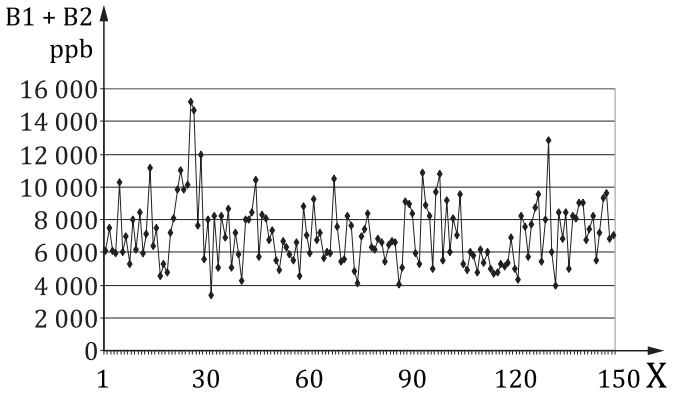
Distance (tons)

e) Silo 06-02



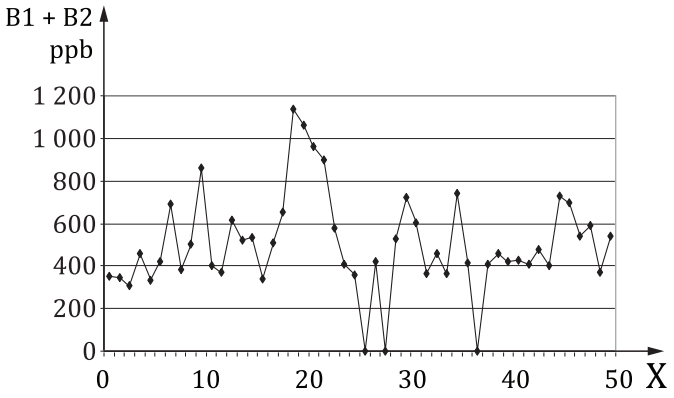
Distance (tons)

f) Silo 07-01



Distance (tons)

g) Silo 07-02



Distance (tons)

Key

X N° of sample

Figure 23 — Temporal distribution and variogram

The observation of variograms shows that the temporal distributions of the fumonisin content is not clearly structured, under the scale of the study, for the first 5 bins (the shape of the variogram is flat).

The values are randomly distributed (as opposed to what was observed on DON in wheat).

— The silo 07-01 has a slight temporal structure. It is probably partly due to the fact that the 26 to 125 samples were collected every 5 t instead of every 20 t as in the other silos. In this case, a weak correlation appears between samples.

— The silo 07-02 presents a clearer temporal structure, but this is mainly due to samples 19 to 22.

In conclusion, fumonisin content shows no or very little temporal structure, at the scale studied.

6.5.2.2.1.2 Error estimation of the average of a lot

6.4.2.2.1.2.1 Decomposition of variability

The variability of values observed in a silo is the result of two sources: sampling variability and analysis variability (technical error or repeatability). Since we had 2 tests per sample, we were able to estimate these two sources of variability. The results are given in Table 27.

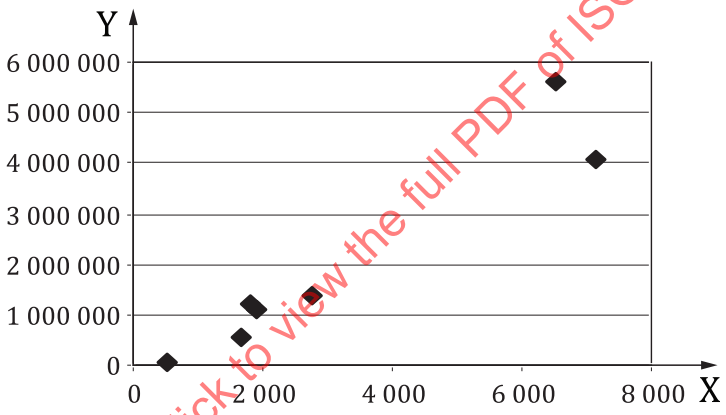
Table 24 — Estimation of sampling variances and repeatability

	06-03	06-01	06-04	06-06	06-02	07-01	07-02
Number of individual samples	50	25	25	49	50	150	50

Table 24 *(continued)*

	06-03	06-01	06-04	06-06	06-02	07-01	07-02
average	2 765	1 889	6 533	1 803	1 675	7 132	502
variance (repetability)	12 045	10 242	166 474	4 143	6 559	46 732	418
Standard deviation (repetability)	110	101	408	64	81	216	20
relative standard deviation (repetability)	4,0	5,4	6,2	3,6	4,8	3,0	4,1
variance (sampling error)	1 393 382	1 116 839	5 613 418	1 199 481	543 582	4 072 702	52 868
Standard deviation (sampling error)	1 180	1 057	2 369	1 095	737	2 018	230
relative standard deviation (sampling error)	42,7	55,9	36,3	60,7	44,0	28,3	45,8

Again the 7 bins have very different variability, both for the sampling variance and for the variance of repeatability. We can deduce from the results that as greater is the average content of a silo, as greater is the associated variability (see Figure 24 for the sampling variance and Figure 25 for the variance of repeatability).

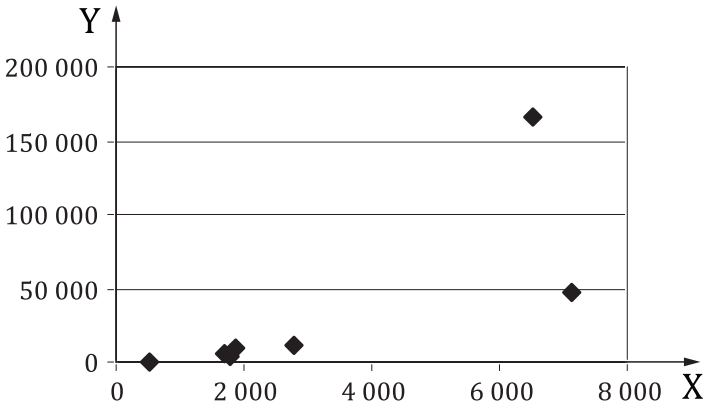


Key

X average level

Y sampling variance

Figure 24 — Relationship between sampling variance and average



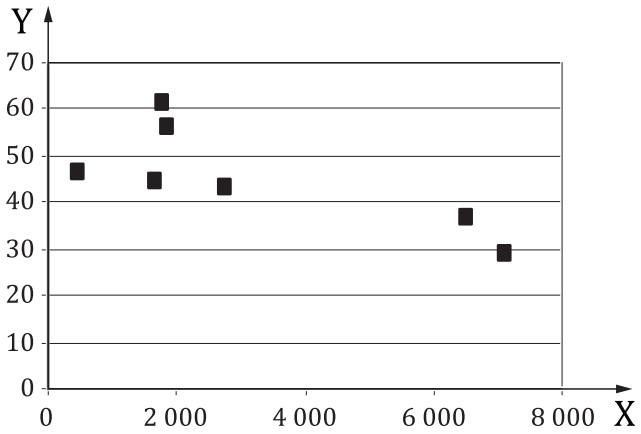
Key

X average level

Y repeatability variance

Figure 25 — Relationship between variance of repeatability and average

For cons, the relative variability (C_V) appears to be relatively independent of the average. The average C_V for sampling is around 45 % (see Figure 26), and the average C_V for repeatability is around 4,5 % (see Figure 27).

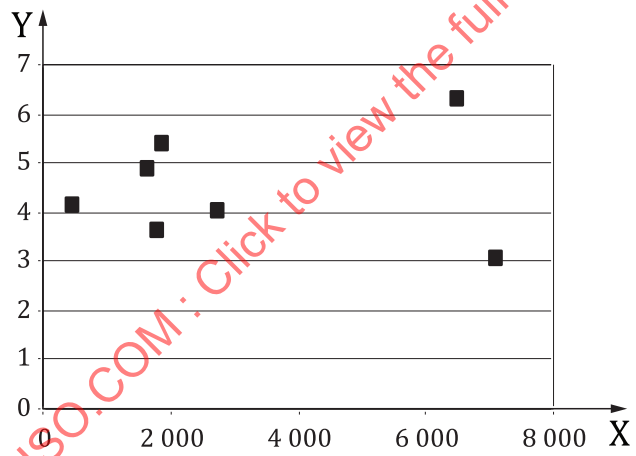


Key

X average level

Y sampling variation coefficient

Figure 26 — Relationship between C_V of sampling and average



Key

X average level

Y repeatability variation coefficient

Figure 27 — Relationship between C_V of repeatability and average

6.4.2.2.1.2.2 Variance of the average

The fact of working on a composite sample, whose result from the mixing of all individual samples, is equivalent to the average of all individual values. However, in estimating the mean of a silo, a single analysis is performed and therefore the part of variability due to technical errors is incompressible, that is to say that this technical errors will not diminish even if we increase the number of samples. By cons, the variability related to sampling will decrease with the number of samples taken.

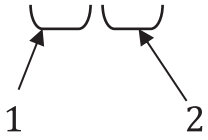
If σ_e^2 is the variance of the sampling error, and σ_t^2 is the variance of the analysis error (= variance of repeatability), then the variance of one observation is:

$$\sigma_x^2 = \sigma_e^2 + \sigma_t^2 \quad (1)$$

The Formula (1) shows that the observed error on one value is the sum of 2 sources of error: sampling and analysis errors.

When we work on a composite sample obtained from n individual samples, the variance is given by the variance of an average:

$$\sigma_{\bar{x}}^2 = \frac{\sigma_e^2}{n} + \sigma_t^2 \quad (2)$$



Key

1 part 1

2 part 2

Figure 28 — Decomposition of the average of the observed error

The Formula (2) shows that the error observed on an average can be decomposed into two parts: one part due to sampling errors (part 1) and one part due to analysis error (part 2).

The part due to sampling errors can be reduced if we increase the number of individual samples (= n). By cons, the part due to analysis error is incompressible. It is interesting to calculate the ratio between the variance of technical errors and the variance of sampling errors:

$$\sigma_t^2 \Big/ \frac{\sigma_e^2}{n} \quad (3)$$

But what value does it take for σ_t^2 ?

The calculation of the technical variance on the 7 silos led to a repeatability C_V of about 5 %. This value underestimates, almost certainly, the variability to be observed in practice because it takes into account neither the errors related to sub-sampling of a composite sample, or the inter-laboratory variability (C_V of reproducibility).

The variability due to errors of sub-sampling and analysis is probably closer to 20 % than 5 %.

We therefore consider the following 3 hypotheses for the variability of technical errors :

— an optimistic scenario with a CVT of 10 %;

— a realistic scenario with a CVT of 20 %;

— a pessimistic scenario with a CVT of 30 %.

From the variance of sampling calculated on each silo, we can calculate the variability due to sampling errors in relation with the number of individual samples (part 1 of Formula 2):

Table 25 — Variance of sampling (part 1 of Formula 2) in relation with the number of individual samples

Number of individual samples (n)	silos						
	06-03	06-01	06-04	06-06	06-02	07-01	07-02
10	139 338	111 684	561 342	119 948	54 358	407 270	5 287
15	92 892	74 456	374 228	79965	36 239	271 513	3 525
20	69 669	55 842	280 671	59 974	27 179	203 635	2 643
25	55 735	44 674	224 537	47 979	21 743	162 908	2 115

If we assume a CVT from 10 % for the technical errors, we obtain for the 7 silos, the following values for the variance due to technical errors:

Table 26 — Variance of the technical errors in the case of a $C_{V,T}$ of 10 %

	silos						
	06-03	06-01	06-04	06-06	06-02	07-01	07-02
Average of silo	2 765	1 889	6 533	1 803	1 675	7 132	502
C_V (laboratory or technical errors)	10	10	10	10	10	10	10
Variance (laboratory or technical errors)	76 459	35 701	426 780	32 508	28 056	508 654	2 520

We can then evaluate the ratio between the variance of technical errors and variance of sampling errors, in relation with the number of individual samples:

Table 27 — Ratio between the variance of technical errors and variance of sampling errors in the case of a $C_{V,T}$ of 10 %

	silos							
Number of individual samples	06-03	06-01	06-04	06-06	06-02	07-01	07-02	average
10	0,5	0,3	0,8	0,3	0,5	1,2	0,5	0,6
15	0,8	0,5	1,1	0,4	0,8	1,9	0,7	0,9
20	1,1	0,6	1,5	0,5	1,0	2,5	1,0	1,2
25	1,4	0,8	1,9	0,7	1,3	3,1	1,2	1,5

Thus, for example, in the case of 20 individual samples, the technical errors are 1,1 times higher than the sampling error, for the silo 06-03.

Generally, we observe a relationship between the variance of technical errors and the variance of sampling error of the order of 1. This means that the part of sampling errors in the total error is around 50 %. A decrease of the sampling error will have a significant impact on the total error. In these conditions, it seems interesting to increase the number of individual samples.

Similar calculations were made for $C_{V,T}$ of 20 and 30 % (Tables 31 and 32).

Table 28 — Ratio between variance of technical errors and variance of sampling errors in the case of a $C_{V,T}$ of 20 %

n	06-03	06-01	06-04	06-06	06-02	07-01	07-02	average
10	2,2	1,3	3,0	1,1	2,1	5,0	1,9	2,4
15	3,3	1,9	4,6	1,6	3,1	7,5	2,9	3,5
20	4,4	2,6	6,1	2,2	4,1	10,0	3,8	4,7

Table 28 *(continued)*

n	06-03	06-01	06-04	06-06	06-02	07-01	07-02	average
25	5,5	3,2	7,6	2,7	5,2	12,5	4,8	5,9

Table 29 — Ratio between variance of technical errors and variance of sampling errors in the case of a $C_{V,T}$ of 30 %

n	06-03	06-01	06-04	06-06	06-02	07-01	07-02	average
10	4,9	2,9	6,8	2,4	4,6	11,2	4,3	5,3
15	7,4	4,3	10,3	3,7	7,0	16,9	6,4	8,0
20	9,9	5,8	13,7	4,9	9,3	22,5	8,6	10,6
25	12,3	7,2	17,1	6,1	11,6	28,1	10,7	13,3

In the case of a $C_{V,T}$ of 20 %, there is, on average, a ratio between the variance of technical errors and variance of sampling error of about 4. This means that the part of sampling errors in the total error is around 20 %. A decrease in error due to sampling will have little impact on total error. In these conditions, it does not seem interesting to increase the number of individual samples.

In the case of a $C_{V,T}$ of 30 %, there is, on average, a ratio between the variance of technical errors and variance of sampling error of about 9. This means that the part of sampling errors in the total error is around 10 %. A decrease in error due to sampling will have very little effect on the total error. Under these conditions there is no interest in increasing the sampling effort.

6.4.2.2.1.2.3 Accuracy of the average

The accuracy of the mean is provided by calculating a confidence interval around this average. Under the assumption of normality, the confidence interval of an average is given, for a confidence level of 95 %, by the following Formula (4):

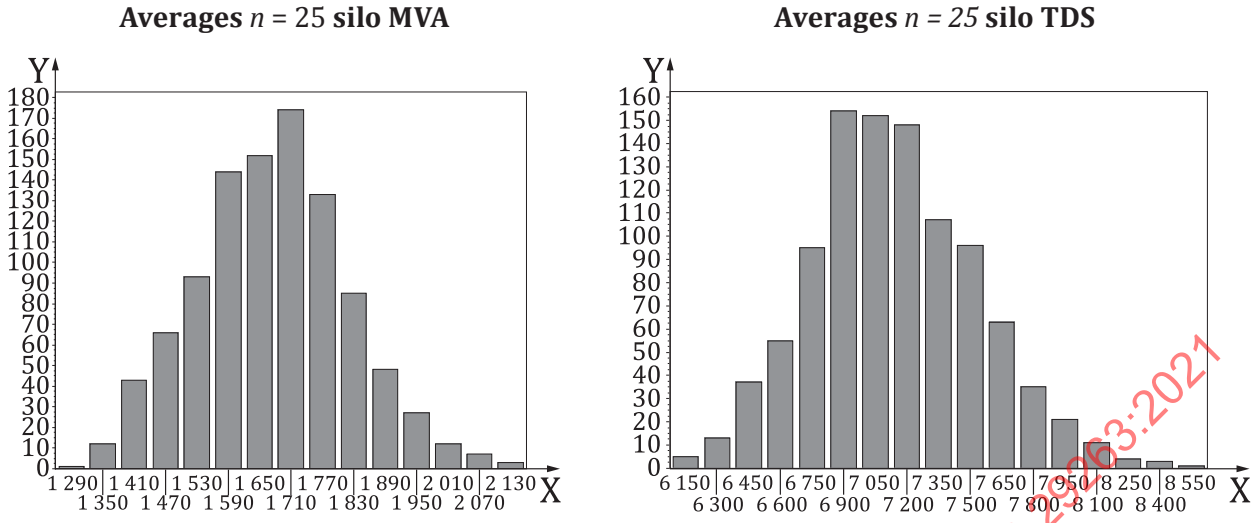
$$IC : \bar{x} \pm 2\sigma_{\bar{x}} \tag{4}$$

The standard deviation of the average, $\sigma_{\bar{x}}$, is calculated from the Formula (2).

The assumption that the data follow a normal distribution is implausible (see Figures 19 to 20 g)), but it is acceptable in the case of averages calculated with a sufficient number of observations. Simulations were performed on 2 silos.

A simulation was performed by randomly select 25 data in a silo and then calculates the correspondant average. 1 000 simulations were performed for each silo.

These simulations result in 1 000 averages per silo whose histograms are shown in Figure 26. In view of these histograms, we will consider that the assumption of normality of averages is acceptable.



Key

Y frequency

Figure 29 — Simulations of the calculated averages from 25 individual samples taken randomly in a silo

The relative accuracy is the confidence interval, expressed in % of the average:

$$100\frac{2\sigma_{\bar{x}}}{\bar{x}} \tag{5}$$

If we assume that the $C_{V,e}$ of sampling and the $C_{V,t}$ of technical errors is constant, the relative accuracy does not depend on the average.

Assuming a sampling $C_{V,e}$ equal to 45 % and a $C_{V,t}$ of technical errors equal to 10, 20 or 30 %, we obtain the following information regarding the number of individual samples:

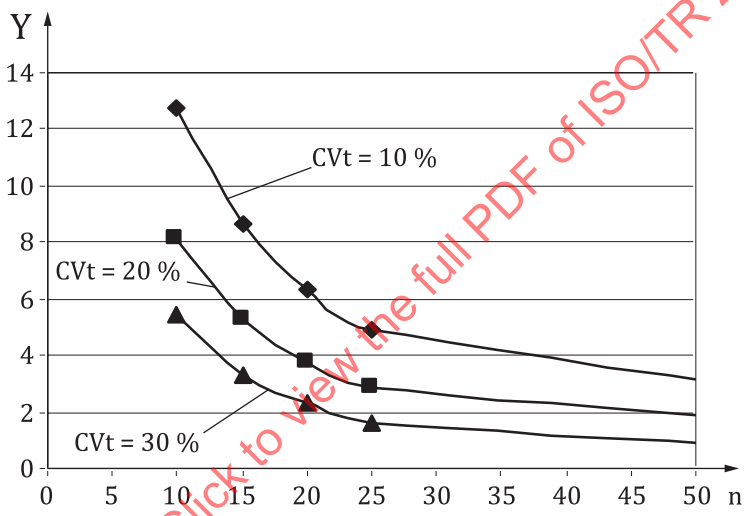
Table 30 — Relative accuracy for $C_{V,t}$ of technical errors equal to 10, 20 and 30 %, and $C_{V,e}$ of sampling error equal to 45 %

	Relative accuracy		
Number of individual samples	$C_{V,t}$ = 10 %	$C_{V,t}$ = 20 %	$C_{V,t}$ = 30 %
10	35	49	66
15	31	46	64
20	28	45	63
25	27	44	63
100	22	41	61
infinite	20	40	60

Table 34 and figure 29 reflects the loss of relative accuracy obtained in diminishing the number of individual samples compared to the precision observed with 100 samples.

Table 31 — Loss of relative accuracy from *n* to 100 samples

	loss of relative accuracy from *n* to 100 individual samples		
n	$C_{V,t}$ = 10 %	$C_{V,t}$ = 20 %	$C_{V,t}$ = 30 %
10	13	8	5
15	9	5	3
20	6	4	2
25	5	3	2
100	0	0	0



Key

Y lost of relative precision (%)

Figure 30 — Loss of relative accuracy from *n* to 100 samples

6.5.2.2.1.3 Buyer or seller risks

— Buyer risk = is the risk that a lot is considered good alors que then it is bad;

— Seller Risk = is the risk that a lot is considered bad alors que then it is good.

a) **Estimated decision rule:**

In the case of fumonisins on maize, the regulatory threshold is set to 4 000 ppb (NOTE: European threshold at the moment of the study). If a consignment is declared bad when the analysis shows a concentration above this threshold, then:

— buyer risk = risk that the result of analysis is <4 000 ppb since the real value of the batch is >4 000 ppb;

— seller risk = risk that the result of analysis is >4 000 ppb since the real value of the batch is <4 000 ppb.

The ability to correctly predict if a lot is below or above the regulatory threshold will depend on, one hand, the precision of the estimation of the concentration of the lot and, on the other hand, the real discrepancy between the level of the lot and the regulatory threshold.

The calculation of buyer and seller risk from the observed data is not possible because we do not know the real concentration of the silos sampled. It was therefore necessary to proceed by simulation. Whitaker uses the negative binomial distribution to describe the distribution of aflatoxin in peanuts, cotton and corn. Fitting a negative binomial distribution from fumonisins data on corn is also satisfactory. So, this law has been used for simulations of silos.

The following method was used:

— simulation of silos with different average levels (from 2 500 to 5 500 ppb) ~ negative binomial distribution;

— sampling randomly a number of samples within these silos (10, 15, 20, 25, 50 or 100);

— average of the n individual samples;

— simulation of a random technical error following a normal distribution with a mean of zero and a $C_{V,t}$ of 20 %;

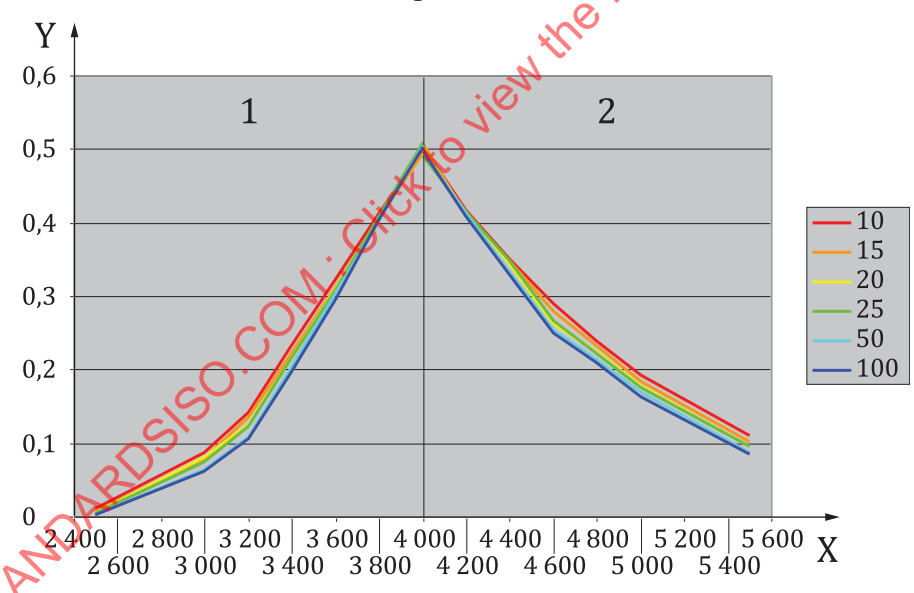
— final value = sample average + technical error;

— comparison of the final average to the regulatory threshold (4 000 ppb);

— iterate 2 000 times;

— calculation of % of error of classification (= seller or buyer risk).

The Figure 30 shows the simulation results: for each value of number of individual samples, the figure shows the risk of error based on the final average value of the silo.



Key

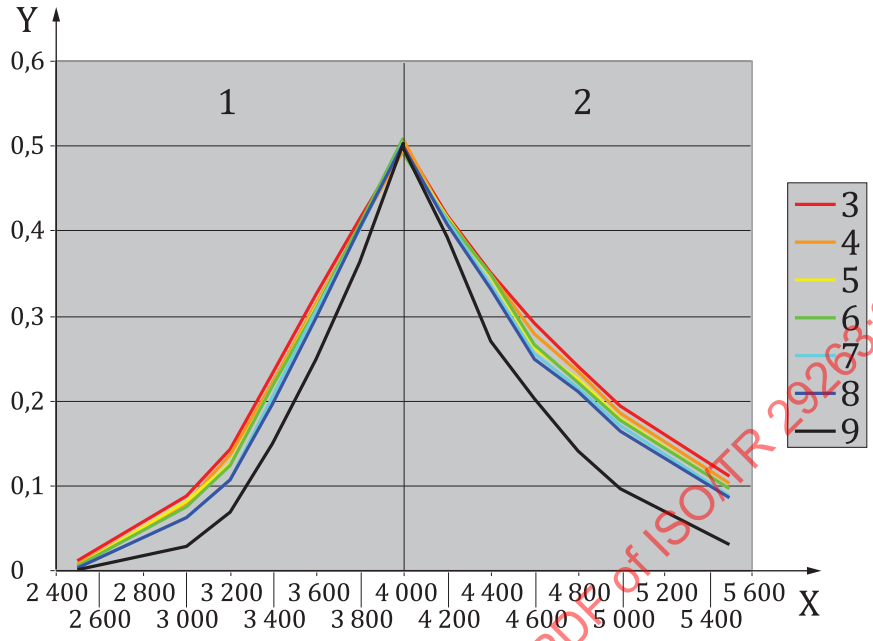
X average of the batch
Y risk (%)
1 seller risk
2 buyer risk

Figure 31 — Simulation of seller and buyer risks in relation with the final average value of the silo for a $C_{V,t}$ of 20 %

To assess the effect of analytical error on the risk of errors, simulations were performed with a $C_{V,t}$ of technical error of 10 %, so half value as before.

These new simulations have been performed only for a number of individual samples equal to 10.

Figure 31 shows the results of simulations and the previous simulation results with a $C_{V,t}$ = 20 %.



Key

X average of the batch
Y risk
1 seller risk
2 buyer risk
3 10 sample - $C_{V,t}$ =20%
4 15 sample - $C_{V,t}$ =20%
5 20 sample - $C_{V,t}$ =20%
6 25 sample - $C_{V,t}$ =20%
7 50 sample - $C_{V,t}$ =20%
8 100 sample - $C_{V,t}$ =20%
9 10 sample - $C_{V,t}$ =10%

Figure 32 — Simulation of seller and buyer risks in relation with the final average value of the silo for a $C_{V,t}$ of 20 % and 10 %

The figures show the week influence of the number of individual samples on the risk of error.

6.5.2.2.2 Zearalenone

6.5.2.2.2.1 Descriptive statistics

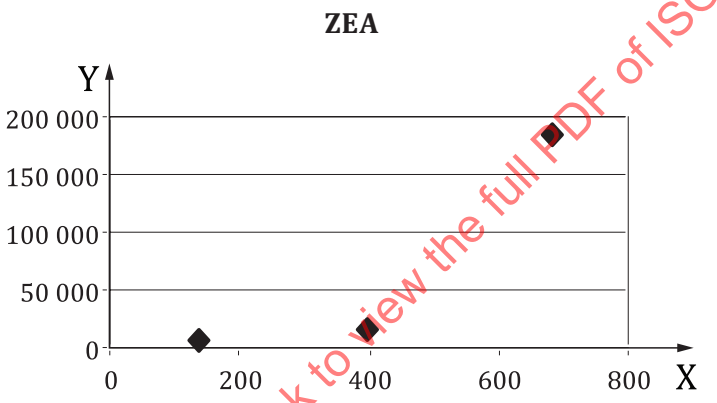
Table B.32 summarizes the main descriptive statistics for the 3 silos sampled. The 3 silos have very different average values and variability. The mean levels vary from 139 ppb to 683 ppb. The standard deviations vary from 71 ppb to 431 ppb.

Table 32 — Descriptive statistics for zearalenone

	silos		
	07-03	**07-02**	**07-04**
Number of individual samples	25	50	25
Average of the silo	683	399	139
min	266	185	48
max	2 151	724	315
variance	185 444	16 434	5 053
Standard deviation	431	128	71
C_V (relative standard deviation or variability)	63,0	32,1	51,1

As for fumonisins, there is a relation between the variability of a silo and its average content: the higher the average level of the silo, the greater is its variability (see Figure 32).

For cons, the relative variability (C_V) appears to be relatively independent of the average content of the silo, with an average C_V of around 50 % (see Figure 33).

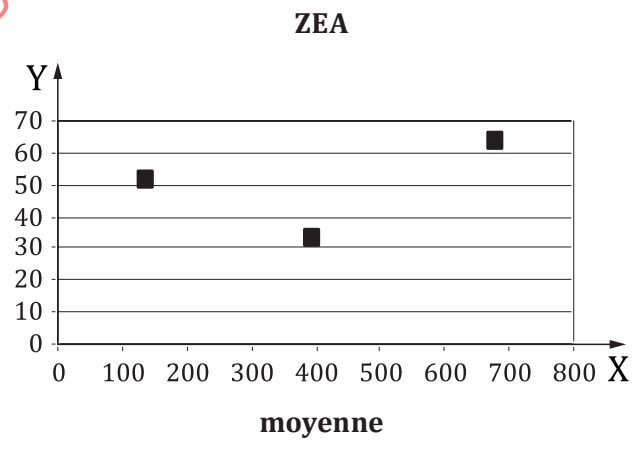


Key

X average of the lot (ppb)

Y total variance

Figure 33 — Relationship between variance and mean



Key

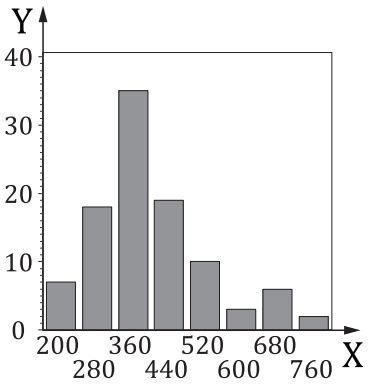
X average of the lot (ppb)

Y relative variability, C_V

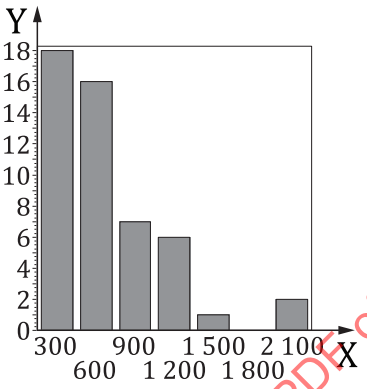
Figure 34 — Relationship between C_V and average

6.4.2.2.2.1.1 Distribution histograms of the content of zearalenone

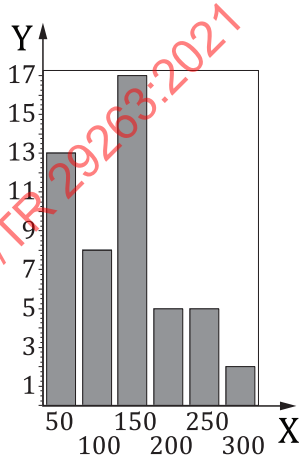
silo=AP



silo=OTT



silo=PR



Key

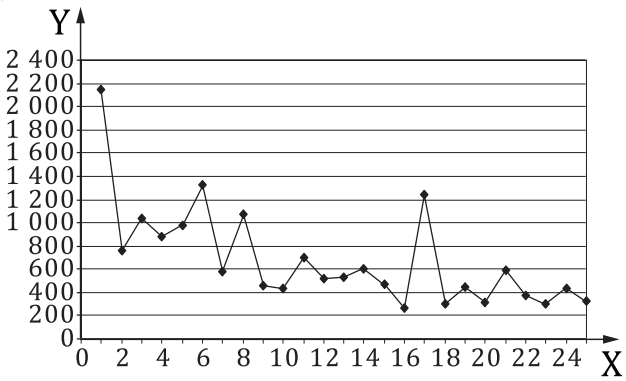
X zea

Y frequency

Figure 35 — Silo 07-02 **Figure 36 — Silo 07-03** **Figure 37 — silo 07-04**

As for fumonisins, the histograms of the levels of ZEA (Figures 34 to 36) show asymmetric distributions, with very high values relatively frequent.

6.4.2.2.2.1.2 Temporal distribution and variograms

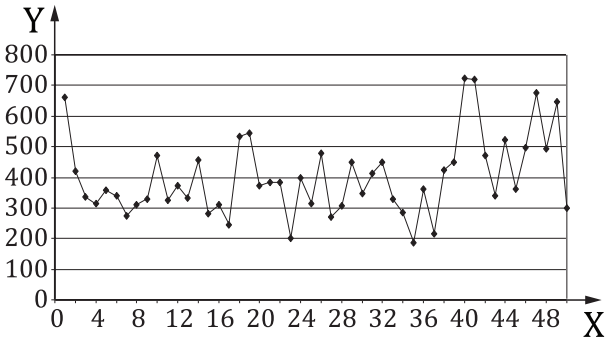


Key

X N° echantillon

Y zéaralénone ppb

Figure 38 — Temporal distribution and variogram - silo 07-03

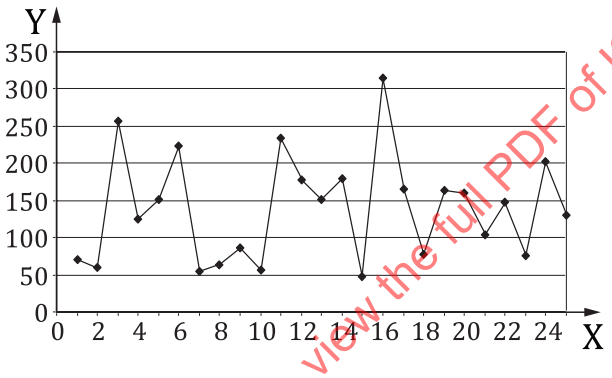


Key

X échantillon

Y zéaralénone ppb

Figure 39 — Temporal distribution and variogram - silo 07-02



Key

X N° echantillon

Y zéaralénone ppb

Figure 40 — Temporal distribution and variogram - silo 07-04

The 3 silos show quite different temporal structures. Silo 07-03 has a clear and important structure. The silo 07-02 has also a temporal structure but lesser pronounced. The silo 07-04 shows no temporal structure.

6.4.2.2.2.1.3 Decomposition of variability

The variability of values observed in a silo is due to two sources: variability related to sampling (sampling variability) and variability related to the analysis (technical error or repeatability). Given that there are two analysis per individual sample, we are able to estimate these two sources of variability. The results are given in Table 33.

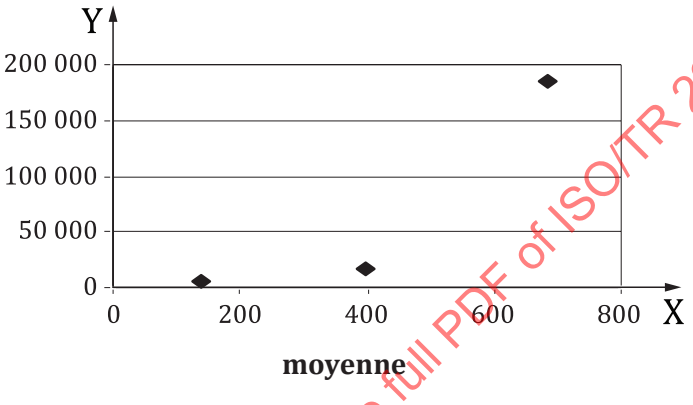
Table 33 — Estimated sampling variances and repeatability

	silos		
	07-03	**07-02**	**07-04**
variance of repetability (analysis)	1 527	468	85
Standard deviation of repetability	39	22	9
C_V (relative standard deviation or variability)	5,7	5,4	6,6

Table 33 *(continued)*

	silos		
	07-03	**07-02**	**07-04**
Variance of sampling (sampling error)	184 681	16 259	5 010
Standard deviation (sampling error)	430	128	71
C_V (relative standard deviation on sampling)	62,9	31,9	50,9

Again, the 3 silos have very different variability, both for the sampling variance and for the variance of repeatability (or analysis). We can see also that higher is the average content of the silo, greater is its variability (see Figure 40 for the sampling variance and Figure 41 for the variance of repeatability).

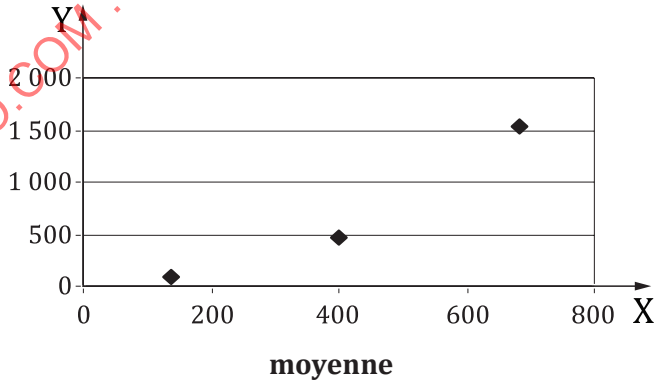


Key

X average of the lot (ppb)

Y sampling variance

Figure 41 — Relationship between sampling variance and mean



Key

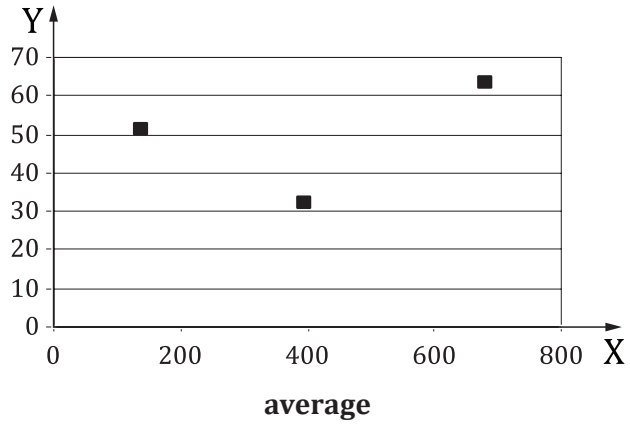
X average of the lot (ppb)

Y relative variability CV

Figure 42 — Relationship between C_V and average

By cons, as for fumonisins, the relative variability (C_V) appears to be relatively independent of the average, with a mean C_V (moyen) of around 50 % for the C_V sampling (see Figure 42), and of 5 % for the C_V of repeatability or analysis (see Figure 43).

Also we can note that the relative variability observed on zearalenone is on the same order of magnitude as those observed on fumonisins.

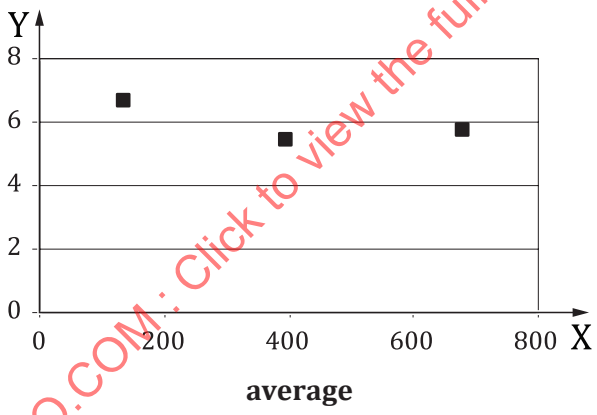


Key

X average of the lot (ppb)

Y relative variability of sampling variance

Figure 43 — Relationship between relative variability of sampling and mean



Key

X average of the lot (ppb)

Y relative variability of repetability CV

Figure 44 — Relationship between C_V of repetability and average

6.5.2.2.2 Conclusions

Despite a temporal structure which appears in some cases more pronounced than for fumonisins, the same conclusions than for fumonisins can be applied to zearalenone.

6.5.2.3 Deoxynivalenol

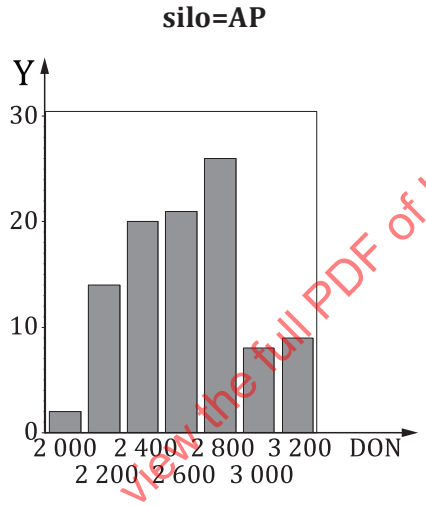
6.5.2.3.1 Descriptive statistics

Table 34 shows the main descriptive statistics for the silo 07-02 sampled.

Table 34 — Descriptive statistics

	Silo 07-02
Number of individual samples	50
Average content (ppb)	2 633
min	2 010
max	3 207
variance	88 379
standard deviation	297
CV (relative standard deviation or variability)	11,3

5.4.2.2.3.1.1 Distribution histograms of the content of DON

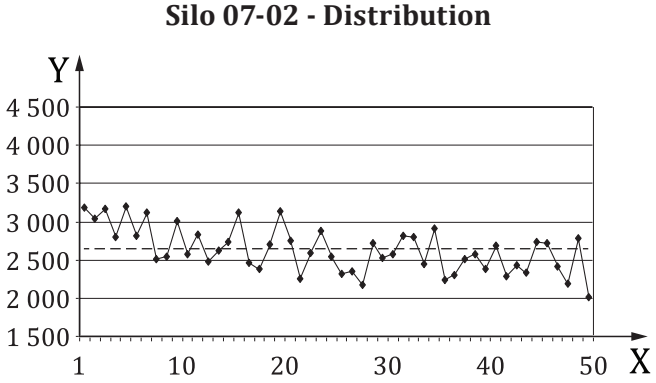


Key

Y frequency

Figure 45 — Silo 07-02

5.4.2.2.3.1.2 Temporal distribution and variograms



Key

X N° sample

Y DON ppb

Figure 46 — Temporal distribution and variogram - silo 07-02

The silo 07-02 has a clear and relatively important temporal structure.

5.4.2.2.3.1.3 Decomposition of variability

The variability of values observed in a silo is due to two sources: variability related to sampling (sampling variability) and variability related to the analysis (technical error or repeatability). Given that there are two tests per individual sample, we are able to estimate these two sources of variability. The results are given in Table 35.

Table 35 — Estimation of sampling variance and repeatability

	07-03
variance of repetability (analysis)	4 244
Standard deviation of repetability	65
C_V (relative standard deviation or variability)	2,5

Variance of sampling (sampling error)	86 257
Standard deviation (sampling error)	294
C_V (relative standard deviation on sampling)	11,2

6.5.2.2.3.2 Conclusions

The behavior of DON (in terms of variability and temporal distribution) is significantly different from those observed on fumonisins. It will be necessary, in this case, to have additional information in order to draw sound conclusions.

We can however argue (advancer) that, in the case of the one silo sampled, the application of conclusions obtained on fumonisins lead to results as accurate.

6.5.2.3 Reducing the pressure of sampling

Regulation 401-2006 fixed to 100 the number of samples required to properly determine the average level of mycotoxin contamination of a silo of 500 t.

The purpose of this section is to assess the risk of error incurred when we reduce the number of samples collected at least 100. In other words, what is the loss of accuracy due to lower pressure sampling?

6.5.2.3.1 Method of calculation

Let "x" be the number of samples taken in a cell of 500 t (with x <100). We take as example x = 25.

We randomly choose 25 samples among the 100 taken, without repetition, and we average the results obtained. We then calculate the difference (absolute value) between the average of 25 samples and that of 100.

$$\begin{aligned} R_{100} &= M + |e_{100}| + |a_{100}| \\ R_{25} &= M + |e_{25}| + |a_{25}| \\ |R_{100} - R_{25}| &= |e_{100} - e_{25}| \end{aligned} \quad (6)$$

where

R_{100} is the average results of analysis of 100 samples;

R_{25} is the average results of analysis of 25 samples;

M is the true average of the silo (unknown);

e_{100} or e_{25} are the error due to sampling;

a_{100} or a_2 are the analytical error.

The total error on an analytical result can be decomposed into two types of errors: a sampling error + an analytical error (due to sub-sampling, extraction process, precision of the instruments ...). The analytical error here concerns the analysis on each of the samples. We assume a constant analytical error regardless of the number of tests. Thus, when subtracting the average of 100 samples to the average of 25, the analytical error, constant vanishes and there remains only the error due to sampling plan.

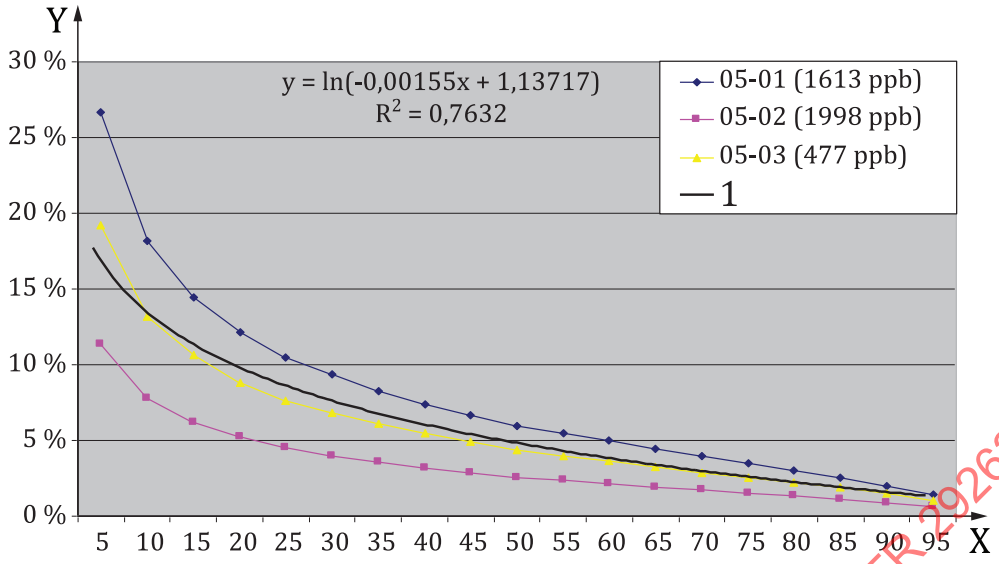
We repeat 10 000 times the operation and we calculate the average from the 10 000 differences to define an average of sampling error for 25 samples (ppb). This average of sampling error is reduced by% from the average of 100 samples.

For the rest, we think in relative error in fixing arbitrarily "| e100 |" to 0, even if for 100 samples by 500 t, there is already an error related to sampling. We therefore calculate an additional risk compared to a result obtained 100 samples because we do not know the true mean of each silo or sampling error of 100 samples.

6.5.2.3.2 Results of the contamination of wheat by deoxynivalenol

For the 5 silos selected for DON on wheat, the pressure sampling varies from 25 to 100 samples per cell of 500 t.

The methodology described allows to construct the curves in Figure 47 for the silos 05-01, 05-02 and 05-03. In some cases, we see that with 25 samples, we add an average error of + / -8 %. The risk increases significantly when further reduces the pressure sampling: ex with 10 samples, the average error is + / -13 %.



Key

X samples number per 500 t

Y additional risk of error compared to the average 100 samples

1 logarithmic (average)

Figure 47 — Risk of additional error in decreasing the number of samples (DON on wheat, silos 05-01 to 03)

For the silos 06-07 and 06-08, Figure 48 represents also the risk involved in more than one due to the initial sampling (25 here, not 100 as in the previous case). For these two silos, measurements were performed before and after cleaning.