**SAMPLING PLANS AND PERFORMANCE CRITERIA FOR DEOXYNIVALENOL (DON) IN
CEREAL-BASED FOODS FOR INFANTS AND YOUNG CHILDREN;
IN FLOUR, MEAL, SEMOLINA AND FLAKES DERIVED FROM WHEAT, MAIZE OR BARLEY; AND IN
CEREAL GRAINS (WHEAT, MAIZE AND BARLEY) DESTINED FOR FURTHER PROCESSING**

Cereal grains (wheat, maize and barley) destined for further processing

Maximum level	2000 µg/kg DON
Increments	increments of 100 g, depending on the lot weight (≥ 0.5 tonnes)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm - 20 mesh)
Laboratory sample weight	≥ 1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the DON-sample test result for the laboratory samples is equal or less than 2000 µg/kg, accept the lot. Otherwise, reject the lot.

Cereal-based foods for infants and young children

Maximum level	200 µg/kg DON
Increments	10 x 100 g
Sample preparation	None
Laboratory sample weight	1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the DON sample test result is equal or less than 200 µg/kg, accept the lot. Otherwise, reject the lot.

Flour, semolina, meal and flakes derived from wheat, maize or barley

Maximum level	1000 µg/kg DON
Increments	10 x 100 g
Sample preparation	None
Laboratory sample weight	1 kg
Number of laboratory samples	1
Test portion	25 g test portion
Method	HPLC
Decision rule	If the DON sample test result is equal or less than 1000 µg/kg, accept the lot. Otherwise, reject the lot.

DEFINITIONS

Lot	An identifiable quantity of a food commodity delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor, or markings.
Sublot	Designated part of a larger lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.
Sampling plan	It is defined by a DON test procedure and an accept/reject level. A DON test procedure consists of three steps: sample selection, sample preparation and analysis or DON quantification. The accept/reject level is a tolerance usually equal to the Codex maximum level (ML).

Incremental sample	The quantity of material taken from a single random place in the lot or sublot.
Aggregate sample	The combined total of all the incremental samples that is taken from the lot or sublot. The aggregate sample has to be at least as large as the laboratory sample or samples combined.
Laboratory sample	The smallest quantity of shelled cereal comminuted in a mill. The laboratory sample may be a portion of or the entire aggregate sample. If the aggregate sample is larger than the laboratory sample(s), the laboratory sample(s) should be removed in a random manner from the aggregate sample in such a way to ensure that the laboratory sample is still representative of the sublot sampled.
Test portion	A portion of the comminuted laboratory sample. The entire laboratory sample should be comminuted in a mill. A portion of the comminuted laboratory sample is randomly removed for the extraction of the DON for chemical analysis.

SAMPLING PLAN DESIGN CONSIDERATIONS

MATERIAL TO BE SAMPLED

1.   Each lot of cereal, which is to be examined for DON, must be sampled separately. Lots larger than 50 tonnes should be subdivided into sublots to be sampled separately. If a lot is greater than 50 tonnes, the lot should be subdivided into sublots according to Table 1.

Table 1. Subdivision of cereal sublots according to lot weight

Lot weight (t)	Maximum Weight or minimum number of sub lots	Number of incremental sample	Minimum laboratory Sample Weight (kg)
≥ 1500	500 tonnes	100	1
> 300 and < 1500	3 sublots	100	1
≥ 100 and ≤ 300	100 tonnes	100	1
≥ 50 and < 100	2 sublots	100	1
< 50	-	3-100*	1

 * see table 2

2.   Taking into account that the weight of the lot is not always an exact multiple of the weight of sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 20%.

INCREMENTAL SAMPLE

3.   The suggested minimum weight of the incremental sample should be 100 grams for lots ≥ 0.5 tonnes.

4.   For lots less than 50 tonnes, the sampling plan must be used with 3 to 100 incremental samples, depending on the lot weight. For very small lots (≤ 0.5 tonnes) a lower number of incremental samples may be taken, but the aggregate sample uniting all incremental samples shall be also in that case at least 1 kg. Table 2 may be used to determine the number of incremental samples to be taken.

Table 2. Number of incremental samples to be taken depending on the weight of the lot of

Lot weight (t)	Number of incremental sample	Minimum Laboratory Sample Weight (kg)
≤ 0.05	3	1
> 0.05 - ≤ 0.5	5	1
> 0.5 - ≤ 1	10	1
> 1 - ≤ 3	20	1
> 3 - ≤ 10	40	1
> 10 - ≤ 20	60	1
> 20 - < 50	100	1

STATIC LOTS

5. A static lot can be defined as a large mass of shelled cereal contained either in a large single container such as a wagon, truck or railcar or in many small containers such as sacks or boxes and the cereal is stationary at the time a sample is selected. Selecting a truly random sample from a static lot can be difficult because all containers in the lot or sublot may not be accessible.

6. Taking incremental samples from a static lot usually requires the use of probing devices to select product from the lot. The probing devices should be specifically designed for the commodity and type of container. The probe should (1) be long enough to reach all products, (2) not restrict any item in the lot from being selected, and (3) not alter the items in the lot. As mentioned above, the aggregate sample should be a composite from many small incremental samples of product taken from many different locations throughout the lot.

7. For lots traded in individual packages, the sampling frequency (SF), or number of packages that incremental samples are taken from, is a function of the lot weight (LT), incremental sample weight (IS), aggregate sample weight (AS) and the individual packing weight (IP), as follows:

    $SF = (LT \times IS)/(AS \times IP)$.

8. The sampling frequency (SF) is the number of packages sampled. All weights should be in the same mass units such as kg.

DYNAMIC LOTS

9. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of shelled cereal as the lot is transferred from one location to another. When sampling from a moving stream, take small incremental samples of product from the entire length of the moving stream; composite the incremental samples to obtain an aggregate sample; if the aggregate sample is larger than the required laboratory sample(s), then blend and subdivide the aggregate sample to obtain the desired size laboratory sample(s).

10. Automatic sampling equipment such as a cross-cut sampler is commercially available with timers that automatically pass a diverter cup through the moving stream at predetermined and uniform intervals. When automatic sampling equipment is not available, a person can be assigned to manually pass a cup through the stream at periodic intervals to collect incremental samples. Whether using automatic or manual methods, incremental samples should be collected and composited at frequent and uniform intervals throughout the entire time the cereal flow past the sampling point.

11. Cross-cut samplers should be installed in the following manner: (1) the plane of the opening of the diverter cup should be perpendicular to the direction of the flow; (2) the diverter cup should pass through the entire cross sectional area of the stream; and (3) the opening of the diverter cup should be wide enough to accept all items of interest in the lot. As a general rule, the width of the diverter cup opening should be about two to three times the largest dimensions of items in the lot.

12. The size of the aggregate sample (S) in kg, taken from a lot by a cross cut sampler is:

    $S=(D \times LT) / (T \times V)$,

    where D is the width of the diverter cup opening (cm), LT is the lot size (kg), T is interval or time between cup movement through the stream (seconds), and V is cup velocity (cm/sec).

13. If the mass flow rate of the moving stream, MR (kg/sec), is known, then the sampling frequency (SF), or number of cuts made by the automatic sampler cup can be computed as a function of S, V, D, and MR.

    $SF = (S \times V) / (D \times MR)$.

PACKAGING AND TRANSPORTATION OF SAMPLES

14. Each laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination, sunlight, and against damage in transit. All necessary precautions shall be taken to avoid any change in composition of the laboratory sample, which might arise during transportation or storage. Samples should be stored in a cool dark place.

15. Each laboratory sample taken for official use shall be sealed at the place of sampling and identified. A record must be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

SAMPLE PREPARATION

16. Sunlight should be excluded as much as possible during sample preparation, since DON may gradually break down under the influence of ultra-violet light. Also, environmental temperature and relative humidity should be controlled and not favour mould growth and DON formation.

17. As the distribution of DON is extremely non-homogeneous, laboratory samples should be homogenised by grinding the entire laboratory sample received by the laboratory. Homogenisation is a procedure that reduces particle size and disperses the contaminated particles evenly throughout the comminuted laboratory sample.

18. The laboratory sample should be finely ground and mixed thoroughly using a process that approaches as complete homogenisation as possible. Complete homogenisation implies that particle size is extremely small and the variability associated with sample preparation approaches zero. After grinding, the grinder should be cleaned to prevent DON cross-contamination.

TEST PORTION

19. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 25 g

20. Procedures for selecting the test portion from the comminuted laboratory sample should be a random process. If mixing occurred during or after the comminuting process, the test portion can be selected from any location throughout the comminuted laboratory sample. Otherwise, the test portion should be the accumulation of several small portions selected throughout the laboratory sample.

21. It is suggested that three test portions be selected from each comminuted laboratory sample. The three test portions will be used for enforcement, appeal, and confirmation if needed.

ANALYTICAL METHODS

22. A criteria-based approach, whereby a set of performance criteria is established with which the analytical method used should comply, is appropriate. The criteria-based approach has the advantage that, by avoiding setting down specific details of the method used, developments in methodology can be exploited without having to reconsider or modify the specific method. A list of possible criteria and performance levels are shown in Table 3). Utilising this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 3. Proposed method criteria for DON in cereals.

Commodity	ML (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Precision on HorRat	Minimum applicable range (mg/kg)	Recovery
Cereal grains (wheat, maize and barley) destined for further processing	2.0	≤ 0.2	≤ 0.4	≤ 2	1-3	80 - 110%
Cereal-based foods for infants and young children	0.2	≤ 0.02	≤ 0.04	≤ 2	0.1 – 0.3	80 – 110%
Flour, semolina, meal and flakes derived from wheat, maize or barley	1.0	≤ 0.1	≤ 0.2	≤ 2	0.5 – 1.5	80 – 110%