

**L.N. 488 of 2004**

**FOOD SAFETY ACT  
(CAP. 449)**

**Contaminants in Food (Sampling and Analysis Methods)  
Regulations, 2004**

IN exercise of the powers conferred by article 10 of the Food Safety Act, the Minister of Health, the Elderly and Community Care has made the following regulations:

1.1 The title of these regulations is the Contaminants in Food Title  
(Sampling and Analysis Methods) Regulations, 2004.

1.2 These regulations implement the provisions of:

(a) Commission Directive 98/53/EC of the 16<sup>th</sup> July, 1998 laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs;

(b) Commission Directive 2002/26/EC of the 13<sup>th</sup> March, 2002 amending Directive 98/53/EC laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs;

(c) Commission Directive 2002/27/EC of the 13<sup>th</sup> March, 2002 amending Directive 98/53/EC laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs;

(d) Commission Directive 2003/121/EC of 15<sup>th</sup> December, 2003 amending Directive 98/53/EC laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs;

(e) Commission Directive 2004/43/EC of 13<sup>th</sup> April, 2004 amending Directive 98/53/EC and Directive 2002/26/EC as regards sampling methods and methods of analysis for the official control of the levels of aflatoxin and ochratoxin A in food for infants and young children;

(f) Commission Directive 2001/22/EC of 8<sup>th</sup> March, 2001 laying down the sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs;

(g) Commission Directive 2002/69/EC of 26<sup>th</sup> July, 2002 laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs;

(h) Commission Directive 2004/44/EC of 13<sup>th</sup> April, 2004 amending Directive 2002/69/EC laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs

(i) Commission Directive 2002/26/EC of 13<sup>th</sup> March, 2002 laying down the sampling methods and the methods of analysis for the official control of the levels of ochratoxin A in foodstuffs;

(j) Commission Directive 2003/78/EC of 11<sup>th</sup> August, 2003 laying down the sampling methods and the methods of analysis for the official control of the levels of patulin in foodstuffs.

Sampling and analytical methods for aflatoxins in foodstuffs

2.1 The sampling for the official control of the levels of aflatoxins in foodstuffs shall be carried out in accordance with the methods described in the First Schedule to these regulations.

2.2 The sample preparation and methods of analyses used for the official control of the levels of aflatoxins in foodstuffs shall comply with the criteria described in the Second Schedule to these regulations.

Sampling and analytical methods for lead, cadmium, mercury and 3-MCPD in foodstuffs

3.1 The sampling for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs shall be carried out in accordance with the methods described in the Third Schedule to these regulations.

3.2 The sample preparation and methods of analyses used for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs shall comply with the criteria described in the Fourth Schedule to these regulations.

Sampling and analytical methods for ochratoxin A in foodstuffs

4.1 The sampling for the official control of the levels of ochratoxin A in foodstuffs shall be carried out in accordance with the methods described in the Fifth Schedule to these regulations.

4.2 The sample preparation and methods of analyses used for the official control of the levels of ochratoxin A in foodstuffs shall comply with the criteria described in the Sixth Schedule to these regulations.

5.1 The sampling for the official control of the levels of dioxins and furans and the determination of the levels of dioxin-like PCBs in foodstuffs shall be carried out in accordance with the methods described in the Seventh Schedule to these regulations.

Sampling and analytical methods for dioxins and dioxin-like PCBs in foodstuffs.

5.2 The sample preparation and methods of analyses used for the official control of the levels of dioxins and furans and the determination of the levels of dioxin-like PCBs in foodstuffs shall comply with the criteria described in the Eighth Schedule to these regulations.

6.1 The sampling for the official control of the levels of patulin in foodstuffs shall be carried out in accordance with the methods described in the Ninth Schedule to these regulations.

Sampling and analytical methods for patulin in foodstuffs.

6.2 The sample preparation and methods of analyses used for the official control of the levels of patulin in foodstuffs shall comply with the criteria described in the Tenth Schedule to these regulations.

7.1 The Contaminants (Sampling and Analysis Methods) Regulations, 2003 are hereby repealed.

Repeal of L.N. 240 of 2003.

FIRST SCHEDULE  
(Equivalent to Annex I of Commission Directive 98/53/EC)

**Methods of sampling for official checking control of the levels of aflatoxins in  
certain foodstuffs**

1. *Purpose and scope*

Samples intended for official checking of the levels of aflatoxin content in foodstuffs shall be taken according to the methods described below. Aggregate samples thus obtained shall be considered as representative of the lots. Compliance with maximum limits laid down in Commission Regulation (EC) No 1525/98 shall be established on the basis of the levels determined in the laboratory samples.

2. *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 large lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.

Incremental sample: a quantity of material taken from a single place in the lot or sublot.

Aggregate sample: the combined total of all the incremental samples taken from the lot or sublot.

Laboratory sample: sample intended for the laboratory (=subsample).

3. *General provisions*

3.1. Personnel

Sampling shall be performed by an official analyst as specified by the Act.

3.2. Material to be sampled

Each lot which is to be examined must be sampled separately. In accordance with the specific provisions in point 5 of this Schedule, large lots should be subdivided into sublots to be sampled separately.

3.3. Precautions to be taken

In the course of sampling and preparation of the laboratory samples precautions must be taken to avoid any changes which would affect the aflatoxin content, adversely affect the analytical determination or make the aggregate samples unrepresentative.

3.4. Incremental samples

As far as possible incremental samples should be taken at various places distributed throughout the lot or subplot. Departure from this procedure must be recorded in the record provided for in 3.8.

3.5. Preparation of the aggregate sample and the laboratory samples (subsamples)

The aggregate sample is made up by uniting and sufficiently mixing the incremental samples. After mixing, the aggregate sample must be divided into equal subsamples in accordance with the specific provisions of point 5 of this Schedule. The mixing is necessary to ensure that each subsample contains portions of the whole lot or subplot.

3.6. Replicate samples

The replicate samples for enforcement, trade (defence) and referee purposes are to be taken from the homogenised laboratory sample.

3.7. Packaging and transmission of laboratory samples

Each laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination 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.

3.8. Sealing and labelling of laboratory samples

Each 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.

4. *Explanatory provisions*

4.1. Different types of lots

Food commodities may be traded in bulk, containers, or individual packings (sacks, bags, retail packings, etc.). The sampling procedure can be applied to all the different forms in which the commodities are put on the market. Without prejudice to the specific provisions as laid down in point 5 of this Schedule, the following formula can be used as a guide for the sampling of lots traded in individual packings (sacks, bags, retail packings, etc.):

Sampling frequency (SF) = {Weight of the lot} x {Weight of the incremental sample} / {Weight of the aggregate sample} x {Weight of individual packing}

- Weight: in kg
- Sampling frequency (SF): every nth sack or bag from which an incremental sample must be taken (decimal figures should be rounded to the nearest whole number).

4.2. Weight of the incremental sample

The weight of the incremental sample should be about 300 grams unless otherwise defined in point 5 of this Schedule and with the exception of spices in which case the weight of the incremental sample is about 100 grams. In the case of retail packings, the weight of the incremental sample depends on the weight of the retail packing.

4.3. Number of incremental samples for lots of less than 15 tonnes

The number of incremental samples to be taken depends on the weight of the lot, with a minimum of 10 and a maximum of 100, unless otherwise defined in point 5 of this Schedule. The figures in the following table may be used to determine the number of incremental samples to be taken.

*Table 1: Number of incremental samples to be taken depending on the weight of the lot*

Lot weight (tonnes)	No of incremental samples
≤ 0.1	10
> 0.1 - ≤ 0.2	15
> 0.2 - ≤ 0.5	20
> 0.5 - ≤ 1.0	30
> 1.0 - ≤ 2.0	40
> 2.0 - ≤ 5.0	60
> 5.0 - ≤ 10.0	80
> 10.0 - ≤ 15.0	100

5. *Specific provisions*

5.1. General survey of the sampling procedure for groundnuts, nuts, dried fruit, spices and cereals

*Table 2: Subdivision of lots into sublots depending on product and lot weight*

Commodity	Lot weight (tonnes)	Weight or number of sublots	Number of incremental samples	Aggregate sample weight (kg)
Dried figs and other dried fruit	≥ 15	15-30 tonnes	100	30
	< 15	-	10-100 (*)	≤ 30
Groundnuts, pistachios, Brazil nuts and other nuts	≥ 500	100 tonnes	100	30
	> 125 & < 500	5 sublots	100	30
	≥ 15 & ≤ 125	25 tonnes	100	30
	< 15	-	10-100 (*)	≤ 30
Cereals	≥ 1500	500 tonnes	100	30
	> 300 & < 1500	3 sublots	100	30
	≥ 50 & ≤ 300	100 tonnes	100	30
	< 50	-	10-100 (*)	1-10

\* Depending on the lot weight – see point 4.3 or 5.3 of this Schedule

Commodity	Lot weight (tonnes)	Weight or number of sublots	Number of incremental samples	Aggregate sample weight (kg)
Spices	$\geq 15$	25 tonnes	100	10
	$< 15$	-	10-100 (*)	1-10

## 5.2. Groundnuts, pistachios and Brazil nuts

### Dried figs

### Cereals (lots $\geq 50$ tonnes)

### Spices

#### 5.2.1. Sampling procedure

- On condition that the subplot can be separated physically, each lot must be subdivided into sublots following Table 2 at point 5.1. Taking into account that the weight of the lot is not always an exact multiple of the weight of the sublots, the weight of the subplot may exceed the mentioned weight by a maximum of 20 %,
- each subplot must be sampled separately,
- number of incremental samples: 100. In the case of lots under 15 tonnes, the number of incremental samples to be taken depends on the weight of the lot, with a minimum of 10 and a maximum of 100 (see point 4.3),
- weight of the aggregate sample = 30 kg which has to be mixed and to be divided into three equal subsamples of 10 kg before grinding (this division into three subsamples is not necessary in the case of groundnuts, nuts, dried fruit and maize intended for further sorting or other physical treatment, however, this will depend upon the availability of equipment which is able to homogenise a 30 kg sample). In cases where the aggregate sample weights are under 10 kg, the aggregate sample must not be divided into three subsamples. In the case of spices the aggregate sample weighs not more than 10 kg and therefore no division in subsamples is necessary.
- laboratory sample: a subsample of 10 kg (each subsample must be separately ground finely and mixed thoroughly to achieve complete homogenisation, in accordance with the provisions laid down in the Second Schedule),
- if it is not possible to carry out the method of sampling described above because of the commercial consequences resulting from damage to the lot (because of packaging forms, means of transport, etc.) an alternative method of sampling may be applied provided that it is as representative as possible and is fully described and documented.

#### 5.2.2. Acceptance of a lot or subplot

- For groundnuts, nuts, dried fruit and maize subjected to a sorting or other physical treatment and spices:

- acceptance if the aggregate sample or the average of the subsamples conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- rejection if the aggregate sample or the average of the subsamples exceeds the maximum limit beyond reasonable doubt, taking into account the measurement uncertainty and correction for recovery.
- for groundnuts, nuts, dried fruit and cereals intended for direct human consumption and cereals, with the exception of maize, to be subjected to a sorting or other physical treatment:
  - acceptance if none of the subsamples exceeds the maximum limit, taking into account the measurement uncertainty and correction for recovery,
  - rejection if one or more of the subsamples exceeds the maximum limit beyond reasonable doubt, taking into account the measurement uncertainty and correction for recovery.
- where the aggregate sample is under 10 kg:
  - acceptance if the aggregate sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
  - rejection if the aggregate sample exceeds the maximum limit beyond reasonable doubt, taking into account the measurement uncertainty and correction for recovery.

5.3. Nuts other than groundnuts, pistachios and Brazil nuts  
Dried fruit other than figs  
Cereals (lots under 50 tonnes)

5.3.1. Sampling procedure

For these products, the sampling procedure laid down in point 5.2.1 may be applied. However, taking into account the low incidence of contamination for these products and/or the newer forms of packaging in which products can be traded, simpler sampling methods may be applied. For cereal lots under 50 tonnes, a sampling plan consisting of, depending on the lot weight, 10 to 100 incremental samples each of 100 grams, resulting in an aggregate sample of 1 to 10 kg may be used. The figures in the following table can be used to determine the number of incremental samples to be taken.

*Table 3: Number of incremental samples to be taken depending on the weight of the lot of cereals*

Lot weight (tonnes)	Number of incremental samples
≤ 1	10
> 1 - ≤ 3	20
> 3 - ≤ 10	40
> 10 - ≤ 20	60
> 20 - ≤ 50	100



5.3.2. Acceptance of a lot or subplot

See point 5.2.2.

5.4. Milk

5.4.1. Sampling procedure

Sampling in accordance with Commission Decision 91/180/EEC of 14 February 1991 laying down certain methods of analysis and testing of raw milk and heat-treated milk <sup>1</sup> :

- number of incremental samples: minimum 5,
- weight of aggregate sample: minimum 0,5 kg or litres.

5.4.2. Acceptance of a lot or subplot

- Acceptance if the aggregate sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- rejection if the aggregate sample exceeds the maximum limit beyond reasonable doubt taking into account the measurement uncertainty and correction for recovery.

5.5. Derived products and compound foods

5.5.1. Milk products

5.5.1.1. Sampling procedure

Sampling in accordance with Commission Directive 87/524/EEC of 6 October 1987 laying down Community methods of sampling for chemical analysis for the monitoring of preserved milk products <sup>2</sup>.

Number of incremental samples: minimum 5.

For the other milk products an equivalent method of sampling is used.

5.5.1.2. Acceptance of a lot or subplot

- Acceptance if the aggregate sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- rejection if the aggregate sample exceeds the maximum limit beyond reasonable doubt taking into account the measurement uncertainty and correction for recovery.

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<sup>1</sup> OJ L 93, 13. 4. 1991, p. 1.

<sup>2</sup> OJ L 306, 28. 10. 1987, p. 24.

5.5.2. Other derived products with very small particle weight, i.e. flour, fig paste, peanut butter (homogeneous distribution of aflatoxin contamination)

5.5.2.1. Sampling procedure

- Number of incremental samples: 100. For lots of under 50 tonnes the number of incremental samples should be 10 to 100, depending on the lot weight (see Table 3 at point 5.3.1 of this Schedule),
- the weight of the incremental sample should be about 100 grams. In the case of lots in retail packing, the weight of the incremental sample depends on the weight of the retail packing,
- weight of aggregate sample = 1-10 kg sufficiently mixed.

5.5.2.2. Number of samples to be taken

- The number of aggregate samples to be taken depends on the lot weight. The division of large lots into sublots must be done as defined for cereals in Table 2 under point 5.1,
- each subplot must be sampled separately.

5.5.2.3 Acceptance of a lot or subplot

- Acceptance if the aggregate sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- rejection if the aggregate sample exceeds the maximum limit beyond reasonable doubt taking into account the measurement uncertainty and correction for recovery.

5.6. Other derived products with a relatively large particle size (heterogeneous distribution of aflatoxin contamination)

Sampling procedure and acceptance as defined at points 5.2 and 5.3 of this Schedule for the raw agricultural product.

5.7. Foods intended for infants and young children

5.7.1 Sampling procedure

The sampling procedure as mentioned for milk and derived products as well as for compounded food in points 5.4, 5.5 and 5.6 applies.

5.7.2 Acceptance of a lot

- Acceptance if the aggregate sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- Rejection if the aggregate sample exceeds the maximum limit beyond reasonable doubt, taking into account the measurement uncertainty and correction for recovery.

6. Sampling at retail stage

Sampling of foodstuffs at the retail stage should be done where possible in accordance with the above sampling provisions. Where this is not possible, other effective sampling procedures at retail stage can be used provided that they ensure sufficient representativeness for the sampled lot.

SECOND SCHEDULE  
(Equivalent to Annex II of Commission Directive 98/53/EC)

**Sample preparation and criteria for methods of analysis used in official checking of  
the levels of aflatoxins in certain foodstuffs**

*1. Introduction*

*1.1. Precautions*

Daylight should be excluded as much as possible during the procedure, since aflatoxin gradually breaks down under the influence of ultra-violet light. As the distribution of aflatoxin is extremely non-homogeneous, samples should be prepared – and especially homogenised – with extreme care. All the material received by the laboratory is to be used for the preparation of test material.

*1.2. Calculation of proportion of shell/kernel of whole nuts*

The limits fixed for aflatoxins in the Contaminants in Food Regulations apply to the edible part. The level of aflatoxins in the edible part can be determined by:

- shelling samples of nuts 'in shell' and the level of aflatoxins is directly determined in the edible part,
- homogenise the nuts 'in shell' by taking them through the sample preparation procedure. The sampling and analytical procedure must estimate the weight of nut kernel in the aggregate sample. The weight of nut kernel in the aggregate sample is estimated after establishing a suitable factor for the proportion of nut shell to nut kernel in whole nuts. This proportion is used to ascertain the amount of kernel in the bulk sample taken through the sample preparation and analysis procedure. Approximately 100 whole nuts are taken at random separately from the lot or are to be put aside from each aggregate sample. The ratio may, for each laboratory sample, be obtained by weighing the whole nuts, shelling and re-weighing the shell and kernel portions. However, the proportion of shell to kernel may be established by the laboratory from a number of samples and so can be assumed for future analytical work. But if a particular laboratory sample is found to be in contravention of any limit, the proportion should be determined for that sample using the approximately 100 nuts that have been set aside.

*2. Treatment of the sample as received in the laboratory*

Finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenisation.

In case the maximum level applies to the dry matter, the dry matter content shall be determined on a part of the homogenised sample, using a procedure that has been demonstrated to determine accurately the dry matter content.

### 3. *Subdivision of samples for enforcement and defence purposes*

The replicate samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenized material.

### 4. *Method of analysis to be used by the laboratory and laboratory control requirements*

#### 4.1. Definitions

A number of the most commonly used definitions that the laboratory will be required to use are given below:

The most commonly quoted precision parameters are repeatability and reproducibility.

$r$  = repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions (i. e. same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95 %) and hence  $r = 2.8 \cdot s_r$

$s_r$  = Standard deviation, calculated from results generated under repeatability conditions

$RSD_r$  = relative standard deviation, calculated from results generated under repeatability conditions  $[(S_r/x) \cdot 100]$ , where  $x$  is the average of results over all laboratories and samples

$R$  = reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e. on identical material obtained by operators in different laboratories, using the standardised test method) may be expected to lie within a certain probability (typically 95 %);  $R = 2.8 \cdot s_R$

$s_R$  = standard deviation, calculated from results under reproducibility conditions

$RSD_R$  = relative standard deviation calculated from results generated under reproducibility conditions  $[(S_R/x) \cdot 100]$

#### 4.2. General requirements

Methods of analysis used for food control purposes must comply whenever possible with the provisions of points 1 and 2 of the Annex to Council Directive 85/591/EEC.

#### 4.3. Specific requirements

Where no specific methods for the determination of aflatoxin levels in foodstuffs are prescribed at Community level, laboratories may select any method provided the selected method meets the following criteria:

Criterion	Concentration range	Recommended value	Maximum permitted value
Blanks	All	Negligible	
Recovery – Aflatoxin M1	0.01 – 0.05 µg/kg > 0.05 µg/kg	60 to 120 % 70 to 110 %	
Recovery – Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	< 1.0 µg/kg 1-10 µg/kg > 10 µg/kg	50 to 120 % 70 to 110 % 80 to 110 %	
Precision RSD <sub>R</sub>	All	As derived from Horwitz equation	2 x value derived from Horwitz equation

Precision RSD<sub>R</sub> may be calculated as 0.66 times precision RSD<sub>R</sub> at the concentration of interest.

#### *Notes:*

- Values to apply to both B<sub>1</sub> and sum of B<sub>1</sub>+B<sub>2</sub>+G<sub>1</sub>+G<sub>2</sub>,
- if sum of individual aflatoxins B<sub>1</sub>+B<sub>2</sub>+G<sub>1</sub>+G<sub>2</sub> are to be reported, then response of each to the analytical system must be either known or equivalent,
- the detection limits of the methods used are not stated as the precision values are given at the concentrations of interest,
- the precision values are calculated from the Horwitz equation, i. e.:  

$$RSD_R = 2^{(1-0.5 \log C)}$$
 where:
  - RSD<sub>R</sub> is the relative standard deviation calculated from results generated under reproducibility conditions [(S<sub>R</sub>/x) .100]
  - C is the concentration ratio (i. e. 1 = 100 g/100 g, 0,001 = 1 000 mg/kg).
 This is a generalised precision equation which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

#### 4.4. Recovery calculation

The analytical result is to be reported corrected or uncorrected for recovery. The manner of reporting and the level of recovery must be reported. The analytical

result corrected for recovery is used for checking compliance (see First Schedule, points 5.2.2, 5.3.2, 5.4.2, 5.5.1.2 and 5.5.2.3).

The analytical result has to be reported as  $x \pm U$  whereby  $x$  is the analytical result and  $U$  is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level confidence of approximately 95 %.

#### 4.5. Laboratory quality standards

Laboratories must comply with Council Directive 93/99/EEC.

THIRD SCHEDULE  
(Equivalent to Annex I of Commission Directive 2001/22/EC)

**Methods Of Sampling For Official Control Of The Levels Of Lead, Cadmium,  
Mercury And 3-MCPD In Certain Foodstuffs**

1. PURPOSE AND SCOPE

Samples intended for the official control of the levels of lead, cadmium, mercury and 3-MCPD contents in foodstuffs shall be taken according to the methods described below. Aggregate samples thus obtained shall be considered as representative of the lots or sublots from which they are taken. Compliance with maximum levels laid down in the Contaminants in Food Regulations shall be established on the basis of the levels determined in the laboratory samples.

2. DEFINITIONS

*Lot*: an identifiable quantity of food delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings. In the case of fish, also the size of fish shall be comparable.

*Sublot*: designated part of a large lot in order to apply the sampling method on that designated part. Each sublot must be physically separated and identifiable.

*Incremental sample*: a quantity of material taken from a single place in the lot or sublot.

*Aggregate sample*: the combined total of all the incremental samples taken from the lot or sublot.

*Laboratory sample*: sample intended for the laboratory

3. GENERAL PROVISIONS

3.1. **Personnel**

Sampling shall be performed by an authorised analyst as specified by the Act.

3.2. **Material to be sampled**

Each lot which is to be examined must be sampled separately.

3.3. **Precautions to be taken**

In the course of sampling and preparation of laboratory samples precautions must be taken to avoid any changes which would affect the lead, cadmium, mercury and 3-MCPD contents, adversely affect the analytical determination or make the aggregate samples unrepresentative.



**3.4. Incremental samples**

As far as possible incremental samples shall be taken at various places distributed throughout the lot or subplot. Departure from this procedure must be recorded in the record provided for under 3.8.

**3.5. Preparation of the aggregate sample**

The aggregate sample is made up by uniting all incremental samples. It shall be at least 1 kg unless not practical, e.g. when a single package has been sampled.

**3.6. Subdivision of aggregate sample in laboratory samples for enforcement, defence and referee purposes**

The laboratory samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised aggregate sample. The size of the laboratory samples for enforcement shall be sufficient to allow at least for duplicate analyses.

**3.7. Packaging and transmission of aggregate and laboratory samples**

Each aggregate and laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination, from loss of analytes by adsorption to the internal wall of the container and against damage in transit. All necessary precautions shall be taken to avoid change of composition of the aggregate and laboratory samples which might arise during transportation or storage.

**3.8. Sealing and labelling of aggregate and laboratory samples**

Each 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.

**4. SAMPLING PLANS**

Sampling should ideally take place at the point where the commodity enters the food chain and a discrete lot becomes identifiable. The sampling method applied shall ensure that the aggregate sample is representative for the lot that is to be controlled.

**4.1. Number of incremental samples**

In the case of liquid products for which a homogeneous distribution of the contaminant in question can be assumed within a given lot, it is sufficient to take one incremental sample per lot which forms the aggregate sample. Reference to the lot number shall be given. Liquid products containing hydrolysed vegetable protein (HVP) or liquid soya sauce shall be shaken well, or homogenised by other suitable means, before the incremental sample is taken. For other products, the minimum number of incremental samples to be taken from the lot shall be as

given in Table 1. The incremental samples shall be of similar weight. Departure from this procedure must be recorded in the record provided for under 3.8.

*Table 1: Minimum number of incremental samples to be taken from the lot*

Weight of lot (kg)	Minimum number of incremental samples to be taken
< 50	3
50 to 500	5
> 500	10

If the lot consists of individual packages, then the number of packages which shall be taken to form the aggregate sample is given in Table 2.

*Table 2: Number of packages (incremental samples) which shall be taken to form the aggregate sample if the lot consists of individual packages*

Number of packages or units in the lot	Number of packages or units to be taken
1 to 25	1 package or unit
26 to 100	About 5 %, at least 2 packages or units
> 100	About 5 % at maximum 10 packages or units

## 5. COMPLIANCE OF THE LOT OR SUBLOT WITH THE SPECIFICATION

The control laboratory shall analyse the laboratory sample for enforcement at least in two independent analyses, and calculate the mean of the results. The lot is accepted if the mean conforms to the respective maximum level as laid down in the Contaminants in Food Regulations. It is rejected if the mean exceeds the respective maximum level.

## FOURTH SCHEDULE

(Equivalent to Annex II of Commission Directive 2001/22/EC)

**Sample Preparation And Criteria For Methods Of Analysis Used In Official Control Of The Levels Of Lead, Cadmium, Mercury And 3-MCPD In Certain Foodstuffs**

## 1. INTRODUCTION

The basic requirement is to obtain a representative and homogeneous laboratory sample without introducing secondary contamination.

## 2. SPECIFIC SAMPLE PREPARATION PROCEDURES FOR LEAD, CADMIUM AND MERCURY

There are many satisfactory specific sample preparation procedures which may be used for the products under consideration. Those described in the draft CEN Standard 'Foodstuffs — Determination of trace elements — Performance criteria and general consideration' have been found to be satisfactory (<sup>3</sup>) but others may be equally valid.

The following points must be noted for any procedure used:

- bivalve molluscs, crustaceans and small fish: where these are normally eaten whole, the viscera are to be included in the material to be analysed,
- vegetables: only the edible portion of is to be tested, with note to be taken of the requirements of the Contaminants in Food Regulations.

## 3. METHOD OF ANALYSIS TO BE USED BY THE LABORATORY AND LABORATORY CONTROL REQUIREMENTS

## 3.1. Definitions

A number of the most commonly used definitions that the laboratory will be required to use are given below:

$r$  = repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions (i.e., same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95 %) and hence  $r = 2.8 \cdot s_r$ .

$s_r$  = standard deviation, calculated from results generated under repeatability conditions.

$RSD_r$  = relative standard deviation, calculated from results generated under repeatability conditions  $[(s_r / \bar{x}) \times 100]$ , where  $\bar{x}$  is the average of results over all laboratories and samples.

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<sup>3</sup> Draft Standard prEN 13804, 'Foodstuffs — Determination of Trace Elements — Performance Criteria and General Considerations', CEN, Rue de Stassart 36, B-1050 Brussels.

R = reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e., on identical material obtained by operators in different laboratories, using the standardised test method), may be expected to lie within a certain probability (typically 95 %);  $R = 2.8 \cdot s_R$ .

$s_R$  = standard deviation, calculated from results under reproducibility conditions.

$RSD_R$  = relative standard deviation calculated from results generated under reproducibility conditions  $[(s_R / \bar{x}) \times 100]$

$HORRAT_r$  = the observed  $RSD_r$  divided by the  $RSD_r$  value estimated from the Horwitz equation using the assumption  $r = 0,66R$

$HORRAT_R$  = the observed  $RSD_R$  value divided by the  $RSD_R$  value calculated from the Horwitz equation (<sup>4</sup>).

### 3.2. General requirements

Methods of analysis used for food control purposes must comply whenever possible with the provisions of paragraphs 1 and 2 of the Annex to Directive 85/591/EEC of the European Community. For the analysis of lead in wine, Commission Regulation (EEC) No 2676/90(<sup>5</sup>) determining Community methods for the analysis of wines lays down the method to be used in Chapter 35 of its Annex.

### 3.3. Specific requirements

#### 3.3.1. Lead, cadmium and mercury analyses

Specific methods for the determination of lead, cadmium and mercury contents are not prescribed. Laboratories shall use a validated method that fulfils the performance criteria indicated in Table 3. Where possible, the validation shall include a certified reference material in the collaborative trial test materials.

Table 3: Performance criteria of methods for lead, cadmium and mercury analyses

Parameter	Value/comment
Applicability	Foods specified in the Contaminants in Food Regulations
Detection limit	No more than one tenth of the value of the specification in the Contaminants in Food Regulations, except if the value of the specification

<sup>4</sup> W Horwitz, 'Evaluation of Analytical Methods for Regulation of Foods and Drugs', Anal. Chem., 1982, No 54, 67A-76A

<sup>5</sup> OJ L 272, 3.10.1990, p. 1.

Parameter	Value/comment
	for lead is less than 0.1 mg/kg. For the latter, no more than one fifth of the value of the specification
Limit of quantification	No more than one fifth of the value of the specification in the Contaminants in Food Regulations, except if the value of the specification for lead is less than 0.1 mg/kg. For the latter, no more than two fifths of the value of the specification
Precision	HORRAT <sub>I</sub> or HORRAT <sub>R</sub> values of less than 1.5 in the validation collaborative trial
Recovery	80-120 % (as indicated in the collaborative trial)
Specificity	Free from matrix or spectral interferences

### 3.3.2. 3-MCPD analysis

Specific methods for the determination of 3-MCPD contents are not prescribed. Laboratories shall use a validated method that fulfils the performance criteria indicated in Table 4. Where possible, the validation shall include a certified reference material in the collaborative trial test materials. A specific method has been validated by collaborative trial and has been shown to meet the requirements of Table 4 <sup>(6)</sup>.

*Table 4: Performance criteria of methods for 3-MCPD analysis*

Criterion	Recommended value	Concentration
Field blanks	Less than the detection limit	-
Recovery	75 – 110 %	All
Limit of quantification	10 (or less) µg on a dry matter basis	-
Standard deviation of the field blank signal	Less than 4 µg/kg	-
In-house precision estimates – standard deviation of replicate measurements at different concentrations	< 4 µg/kg < 6 µg/kg < 7 µg/kg < 8 µg/kg < 15 µg/kg	20 µg/kg 30 µg/kg 40 µg/kg 50 µg/kg 100 µg/kg

### 3.4. Estimation of the analytical trueness and recovery calculations

Wherever possible the trueness of the analysis shall be estimated by including suitable certified reference materials in the analytical run. The ‘Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement’ <sup>(7)</sup>

<sup>6</sup> Method of Analysis to determine 3-Monochloropropane-1,2-Diol in Food and Food Ingredients using Mass Spectrometric Detection, submitted to CEN TC 275 and AOAC International (also available as ‘Report of the Scientific Cooperation task 3.2.6: Provision of validated methods to support the Scientific Committee on Food’s recommendations regarding 3-MCPD in hydrolysed protein and other foods’).

<sup>7</sup> ISO/AOAC/IUPAC Harmonised Guidelines for the Use of Recovery Information in Analytical Measurement. *Edited* Michael Thompson, Steven L R Ellison, Ales Fajgelj, Paul Willetts and Roger Wood, Pure Appl. Chem., 1999, No 71, 337-348

developed under the auspices of IUPAC/ISO/AOAC shall be taken into account. The analytical result shall be reported corrected or uncorrected. The manner of reporting and the level of recovery must be reported.

**3.5. Laboratory quality standards**

Laboratories must comply with Directive 93/99/EEC.

**3.6. Expression of results**

The results shall be expressed in the same units as the maximum levels laid down in the Contaminants in Foods Regulations.

FIFTH SCHEDULE  
(Equivalent to Annex I of Commission Directive 2002/26/EC)

**Methods of Sampling for Official Control of the Levels of Ochratoxin A in certain foodstuffs**

**1. Purpose and scope**

Samples intended for official checking of the levels of ochratoxin A content in foodstuffs shall be taken according to the methods described below. Aggregate samples thus obtained shall be considered as representative of the lots. Compliance with maximum limits laid down in Regulation (EC) No 466/2001 shall be established on the basis of the levels determined in the laboratory samples.

**2. 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 lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.

**Incremental sample:** a quantity of material taken from a single place in the lot or sublot

**Aggregate sample:** the combined total of all the incremental samples taken from the lot or sublot.

**3. General provisions**

*3.1. Personnel*

Sampling shall be performed by an authorised person as specified by the Food Safety Commission.

*3.2. Material to be sampled*

Each lot which is to be examined must be sampled separately. In accordance with the specific provisions of this Schedule, large lots should be subdivided into sublots to be sampled separately.

*3.3. Precautions to be taken*

In the course of sampling and preparation of the samples precautions must be taken to avoid any changes which would affect the ochratoxin A content, adversely affect the analytical determination or make the aggregate samples unrepresentative.

### 3.4. *Incremental samples*

As far as possible incremental samples should be taken at various places distributed throughout the lot or subplot. Departure from this procedure must be recorded in the record.

### 3.5. *Preparation of the aggregate sample*

The aggregate sample is made up by uniting the incremental samples.

### 3.6. *Replicate samples*

The replicate samples for enforcement, trade (defence) and referee purposes are to be taken from the homogenised sample, unless this conflicts with other applicable rules on sampling.

### 3.7. *Packaging and transmission of samples*

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

### 3.8. *Sealing and labelling of samples*

Each sample taken for official use shall be sealed at the place of sampling and identified following appropriate procedures established by the Food Safety Commission. 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.

## 4. **Specific provisions**

### 4.1. *Different types of lots*

Food commodities may be traded in bulk, containers, or individual packings (sacks, bags, retail packings, etc.). The sampling procedure can be applied to all the different forms in which the commodities are put on the market.

Without prejudice to the specific provisions as laid down in points 4.3, 4.4 and 4.5 of this Schedule, the following formula can be used as a guide for the sampling of lots traded in individual packings (sacks, bags, retail packings, etc.):

$$\text{Sampling frequency (SF) } n = \frac{\text{Weight of the lot} \cdot \text{Weight of the incremental sample}}{\text{Weight of the aggregate sample} \cdot \text{Weight of individual packaging}}$$

— Weight: in kg

— Sampling Frequency (SF): every nth sack or bag from which an incremental sample must be taken (decimal figures should be rounded to the nearest whole number).

### 4.2. *Weight of the aggregate sample*



The weight of the incremental sample should be about 100 grams, unless otherwise defined in this Schedule. In the case of lots in retail packings, the weight of the incremental sample depends on the weight of the retail packing.

#### 4.3. General survey of the sampling procedure for cereals and dried vine fruit

*Table 1: Subdivision of lots into sublots depending on product and lot weight*

Commodity	Lot weight (tonnes)	Weight or number of sublots	Number of incremental samples	Aggregate sample Weight (kg)
Cereals and cereal products	$\geq 1500$	500 tonnes	100	10
	$> 300$ and $< 1500$	3 sublots	100	10
	$\geq 50$ and $\leq 300$	100 tonnes	100	10
	$< 50$	-	10-100 <sup>(8)</sup>	1-10
Dried vine fruit (currants, raisins and sultanas)	$\geq 15$	15–30 tonnes	100	10
	$< 15$	-	10-100 <sup>(9)</sup>	1-10

#### 4.4. Sampling procedure for cereals and cereal products (lots $\geq 50$ tonnes) and dried vine fruit (lots $\geq 15$ tonnes)

- On condition that the subplot can be separated physically, each lot must be subdivided into sublots following Table 1. Taking into account that the weight of the lot is not always an exact multiple of the weight of the sublots, the weight of the subplot may exceed the mentioned weight by a maximum of 20 %.
- Each subplot must be sampled separately.
- Number of incremental samples: 100. In the case of lots of cereals under 50 tonnes and lots of dried vine fruit under 15 tonnes, see point 4.5. Weight of the aggregate sample = 10 kg.
- If it is not possible to carry out the method of sampling described above because of the commercial consequences resulting from damage to the lot (because of packaging forms, means of transport, etc.) an alternative method of sampling may be applied provided that it is as representative as possible and is fully described and documented.

#### 4.5. Sampling provisions for cereals and cereal products (lots $< 50$ tonnes) and for dried vine fruit (lots $< 15$ tonnes)

<sup>8</sup> Depending on the lot weight – See Table 2 of this Schedule.

<sup>9</sup> Depending on the lot weight – See Table 3 of this Schedule.

For cereal lots under 50 tonnes and for dried vine fruit lots under 15 tonnes, the sampling plan has to be used with 10 to 100 incremental samples, depending on the lot weight, resulting in an aggregate sample of 1 to 10 kg.

The figures in the following table can 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 cereals*

Lot weight (tonnes)	Number of incremental samples
$\leq 1$	10
$> 1 - \leq 3$	20
$> 3 - \leq 10$	40
$> 10 - \leq 20$	60
$> 20 - \leq 50$	100

*Table 3: Number of incremental samples to be taken depending on the weight of the lot of dried vine fruit*

Lot weight (tonnes)	Number of incremental samples
$\leq 0.1$	10
$> 0.1 - \leq 0.2$	15
$> 0.2 - \leq 0.5$	20
$> 0.5 - \leq 1.0$	30
$> 1.0 - \leq 2.0$	40
$> 2.0 - \leq 5.0$	60
$> 5.0 - \leq 10.0$	80
$> 10.0 - \leq 15.0$	100

#### *4.6 Sampling procedure for foods intended for infants and young children*

The sampling procedure as mentioned for cereals and cereal products in point 4.5 of this Schedule applies. This means that the number of incremental samples to be taken depends on the weight of the lot, with a minimum of 10 and a maximum of 100, in accordance with Table 2 at point 4.5.

- The weight of the incremental sample should be about 100 grams. In case of lots in retail packing, the weight of the incremental sample depends on the weight of the retail packing.
- Weight of aggregate sampling = 1 to 10 kg sufficiently mixed.

#### 4.7. *Sampling at retail stage*

Sampling of foodstuffs at the retail stage should be done where possible in accordance with the above sampling provisions. Where this is not possible, other effective sampling procedures at retail stage can be used provided that they ensure sufficient representativeness for the sampled lot.

#### 5. **Acceptance of a lot or subplot**

- Acceptance if the aggregate sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- Rejection if the aggregate sample exceeds the maximum limit beyond reasonable doubt, taking into account the measurement uncertainty and correction for recovery.

SIXTH SCHEDULE  
(Equivalent to Annex II of Commission Directive 2002/26/EC)

**Sample Preparation and Criteria for Methods of Analysis used in Official Checking  
of the Levels of Ochratoxin A in certain foodstuffs**

**1. Precautions**

As the distribution of ochratoxin A is non-homogeneous, samples should be prepared — and especially homogenised — with extreme care. All the material received by the laboratory is to be used for the preparation of test material.

**2. Treatment of the sample as received in the laboratory**

Finely grind and mix thoroughly the complete aggregate sample using a process that has been demonstrated to achieve complete homogenisation.

In case the maximum level applies to the dry matter, the dry matter content shall be determined on a part of the homogenised sample, using a procedure that has been demonstrated to determine accurately the dry matter content.

**3. Subdivision of samples for enforcement and defence purposes**

The replicate samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised material unless this conflicts with other applicable rules on sampling.

**4. Method of analysis to be used by the laboratory and laboratory control requirements**

*4.1. Definitions*

A number of the most commonly used definitions that the laboratory will be required to use are given below:

The most commonly quoted precision parameters are repeatability and reproducibility.

$r =$  Repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions (i.e. same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95 %) and hence  $r = 2,8 \times sr$

$sr = 1$  Standard deviation, calculated from results generated under repeatability conditions

- $RSD_r$  = Relative standard deviation, calculated from results generated under repeatability conditions  $\left[ \frac{s_r}{\bar{x}} \cdot 100 \right]$  where  $\bar{x}$  is the average of results over all laboratories and samples
- $R$  = Reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e. on identical material obtained by operators in different laboratories, using the standardised test method) may be expected to lie within a certain probability (typically 95 %);  $R = 2,8 \times s_R$
- $s_R$  = Standard deviation, calculated from results under reproducibility conditions
- $RSD_R$  = Relative standard deviation calculated from results generated under reproducibility conditions  $\left[ \frac{s_R}{\bar{x}} \cdot 100 \right]$ .

#### 4.2. General requirements

Methods of analysis used for food control purposes must comply with the provisions of items 1 and 2 of the Annex to Directive 85/591/EEC concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption.

#### 4.3. Specific requirements

Where no specific methods for the determination of ochratoxin A levels in foodstuffs are prescribed at Community level, laboratories may select any method provided the selected method meets the following criteria:

##### *Performance characteristics for ochratoxin A*

Level (µg/kg)	Ochratoxin A		
	$RSD_r$ (%)	$RSD_R$ (%)	Recovery (%)
< 1	≤ 40	≤ 60	50 to 120
1-10	≤ 20	≤ 30	70 to 110

- The detection limits of the methods used are not stated as the precision values are given at the concentrations of interest.
- The precision values are calculated from the Horwitz equation:

$$RSD_R = 2^{(1-0.5 \log C)}$$

where:

- $RSD_R$  is the relative standard deviation calculated from results generated under reproducibility conditions  $[(S_R/\bar{x} \cdot 100)]$ ,
- C is the concentration ratio (i.e. 1 = 100 g/100 g, 0,001 = 1,000 mg/kg).  
This is a generalised precision equation, which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

#### 4.4. *Recovery calculation*

The analytical result is to be reported corrected or uncorrected for recovery. The manner of reporting and the level of recovery must be reported. The analytical result corrected for recovery shall be used for checking compliance (see Fifth Schedule, point 5).

The analytical result has to be reported as  $x \pm U$ , whereby x is the analytical result and U is the expanded measurement uncertainty.

U is the expanded uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 %.

#### 4.5. *Laboratory quality standards*

Laboratories must comply with Directive 93/99/EEC on the subject of additional measures concerning the official control of foodstuffs.

SEVENTH SCHEDULE  
(Equivalent to Annex I of Commission Directive 2002/69/EC)

**Methods of Sampling for Official Control of the Levels of Dioxins (PCDD/PCDF)  
and the Determination of Dioxin-like PCBs in certain foodstuffs**

**1. Purpose and scope**

Samples intended for the official control of the levels of dioxins (PCDD/PCDF) content, as well for the determination of the content of dioxin-like PCBs <sup>(10)</sup> in foodstuffs shall be taken according to the methods described below. Aggregate samples thus obtained shall be considered as representative of the lots or sublots from which they are taken. Compliance with maximum levels laid down in Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs shall be established on the basis of the levels determined in the laboratory samples.

**2. Definitions**

Lot: an identifiable quantity of food delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings. In the case of fish and fishery products, also the size of fish shall be comparable.

Sublot: designated part of a large lot in order to apply the sampling method on that designated part. Each sublot must be physically separated and identifiable.

Incremental sample: a quantity of material taken from a single place in the lot or sublot.

Aggregate sample: the combined total of all the incremental samples taken from the lot or sublot.

Laboratory sample: a representative part/quantity of the aggregate sample intended for the laboratory.

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<sup>10</sup> Table WHO TEFs for human risk assessment based on the conclusions of the World Health Organisation meeting in Stockholm, Sweden, 15-18 June 1997 (Van den Berg *et al.*, (1998) Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and for Wildlife. Environmental Health Perspectives, 106(12), 775).

Congener	TEF value
<b>Dibenzo-p-dioxins (PCDDs)</b>	
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.0001
<b>Dibenzofurans (PCDFs)</b>	
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OCDF	0.0001
<b>‘Dioxin-like’ PCBs</b>	
<b>Non-ortho PCBs + Mono-ortho PCBs</b>	
<b>Non-ortho PCBs</b>	
PCB 77	0.0001
PCB 81	0.0001
PCB 126	0.1
PCB 169	0.01
<b>Mono-ortho PCBs</b>	
PCB 105	0.0001
PCB 114	0.0005
PCB 118	0.0001
PCB 123	0.0001
PCB 156	0.0005
PCB 157	0.0005
PCB 167	0.00001
PCB 189	0.0001
Abbreviations used: T = tetra; Pe = penta; Hx = hexa; Hp = hepta; O = octa; CDD = chlorodibenzodioxin; CDF = chlorodibenzofuran; CB = chlorobiphenyl	



### 3. General provisions

#### 3.1. *Personnel*

Sampling shall be performed by an authorised qualified person as specified by the Food Safety Commission.

#### 3.2. *Material to be sampled*

Each lot, which is to be examined, must be sampled separately.

#### 3.3. *Precautions to be taken*

In the course of sampling and preparation of laboratory samples precautions must be taken to avoid any changes, which would affect the content of dioxins and dioxin-like PCBs, adversely affect the analytical determination or make the aggregate samples unrepresentative.

#### 3.4. *Incremental samples*

As far as practical incremental samples shall be taken at various places distributed throughout the lot or subplot. Departure from this procedure must be recorded in the record provided for under 3.8.

#### 3.5. *Preparation of the aggregate sample*

The aggregate sample is made up by uniting all incremental samples. It shall be at least 1 kg unless not practical, e.g. when a single package has been sampled.

#### 3.6. *Subdivision of aggregate sample in laboratory samples for enforcement, defence and referee purposes*

The laboratory samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised aggregate sample unless this conflicts with other provisions on sampling. The size of the laboratory samples for enforcement shall be sufficient to allow at least for duplicate analyses.

#### 3.7. *Packaging and transmission of aggregate and laboratory samples*

Each aggregate and laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination, from loss of analytes by adsorption to the internal wall of the container and against damage in transit. All necessary precautions shall be taken to avoid change of composition of the aggregate and laboratory samples, which might arise during transportation or storage.

#### 3.8. *Sealing and labelling of aggregate and laboratory samples*

Each sample taken for official use shall be sealed at the place of sampling and identified following the procedures established by the Food Safety Commission. 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.

### 4. Sampling plans

The sampling method applied shall ensure that the aggregate sample is representative for the lot that is to be controlled.

*Number of incremental samples*

In the case of milk and oils, for which a homogeneous distribution of the contaminants in question can be assumed within a given lot, it is sufficient to take three incremental samples per lot which forms the aggregate sample. Reference to the lot number shall be given. For other products, the minimum number of incremental samples to be taken from the lot shall be as given in Table 1. The aggregate sample uniting all incremental samples shall be at least 1 kg (see point 3.5). The incremental samples shall be of similar weight. The weight of an incremental sample should be at least 100 grams. The weight of the incremental sample is dependent on the size of the particles in the lot. Departure from this procedure must be recorded in the record provided for under 3.8. In accordance with the provisions of Commission Decision 97/747/EC of the 27<sup>th</sup> October, 1997 fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products <sup>(11)</sup>, the sample size for hen eggs is at least 12 eggs (for bulk lots as well for lots consisting of individual packages, Tables 1 and 2).

TABLE 1

**Minimum number of incremental samples to be taken from the lot**

Weight of the lot (in kg)	Minimum number of incremental samples to be taken
< 50	3
50 to 500	5
> 500	10

If the lot consists of individual packages, then the number of packages, which shall be taken to form the aggregate sample, is given in Table 2.

TABLE 2

**Number of packages (incremental samples) which shall be taken to form the aggregate sample if the lot consists of individual packages**

Number of packages or units in the lot	Number of packages or units to be taken
1 to 25	1 package or unit
26 to 100	about 5 %, at least 2 packages or units
> 100	about 5 %, at maximum 10 packages or units

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<sup>11</sup> OJ L 303, 6.11.1997, p. 12.

#### 4.1 *Specific provisions for the sampling of lots containing whole fishes*

The number of incremental samples to be taken from the lot is defined in Table 1. The aggregate sample uniting all incremental samples shall be at least 1 kg (see point 3.5).

- In case the lot to be sampled contains small fish (individual fish weighing < 1 kg), the whole fish is taken as incremental sample to form the aggregate sample. In case the resulting aggregate sample weighs more than 3 kg, the incremental samples can consist of the middle part, weighing each at least 100 grams, of the fish forming the aggregate sample. The whole part to which the maximum level is applicable is used for homogenisation of the sample.
- In case the lot to be sampled contains larger fish (individual fish weighing more than 1 kg), the incremental sample consists of the middle part of the fish. Each incremental sample weighs at least 100 grams. In case the lot to be sampled consist of very large fish (e.g. > 6 kg) and taking a piece of the middle part of the fish would result in significant economic damage, taking three incremental samples of at least 350 grams each can be considered sufficient, independently of the size of the lot.

### 5. **Compliance of the lot or subplot with the specification**

The lot is accepted if the analytical result of a single analysis does not exceed the respective maximum level as laid down in Regulation (EC) No 466/2001, taking into account the measurement uncertainty.

The lot is non-compliant with the maximum level as laid down in Regulation (EC) No 466/2001, if the analytical result confirmed by duplicate analysis and calculated as the mean of at least two separate determinations exceeds the maximum level beyond reasonable doubt, taking into account the measurement uncertainty.

The taking into account of the measurement uncertainty can be done according to one of the following approaches:

- by calculating the expanded uncertainty, using a coverage factor of 2, which gives a level of confidence of approximately 95 %,
- by establishing the decision limit ( $CC\alpha$ ) according to the provisions of Commission Decision 2002/657/EC of the 12<sup>th</sup> August, 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results <sup>(12)</sup> (point 3.1.2.5 of the Annex — the case of substances with established permitted levels).

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<sup>12</sup> OJ L 221, 17.8.2002, p. 8. Decision as last amended by Decision 2004/25/EC (OJ L 6, 10.1.2004, p. 38).

The present interpretation rules apply for the analytical result obtained on the sample for official control. In case of analysis for defence or referee purposes, the national rules apply.

## EIGHTH SCHEDULE

**Sample Preparation and Requirements for Methods of Analysis used in Official Control of the Levels of Dioxins (PCDD/PCDF) and the Determination of Dioxin-like PCBs in certain foodstuffs****1. Objective and field of application**

These requirements should be applied where foodstuffs are analysed for the official control of the levels of dioxins (polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF)) and the determination of dioxin-like PCBs.

Monitoring for the presence of dioxins in foodstuffs can be performed by a strategy involving a screening method in order to select those samples with levels of dioxins and dioxin-like PCBs that are less than 30-40 % below or exceed the level of interest. The concentration of dioxins in those samples with significant levels needs to be determined/confirmed by a confirmatory method.

Screening methods are methods that are used to detect the presence of dioxins and dioxin-like PCBs at the level of interest. These methods have a capacity for a high sample throughput and are used to sift large numbers of samples for potential positives. They are specifically designed to avoid false negatives.

Confirmatory methods are methods that provide full or complementary information enabling the dioxins and dioxin-like PCBs to be identified and quantified unequivocally at the level of interest.

**2. Background**

Because environmental and biological samples (including samples of foodstuffs) in general contain complex mixtures of different dioxin congeners, the concept of Toxic Equivalency Factors (TEFs) has been developed to facilitate risk assessment. These TEFs have been established to express concentrations of mixtures of 2,3,7,8-substituted PCDDs and PCDFs, and more recently, some non-ortho and mono-ortho chlorine substituted PCBs which possesses dioxin-like activity in toxic equivalents (TEQs) of 2,3,7,8-TCDD (see Seventh Schedule, footnote 1).

The concentrations of the individual substances in a given sample are multiplied by their respective TEF and subsequently summed to give the total concentration of dioxin-like compounds expressed as TEQs.

The concept of 'upperbound' requires using the limit of quantification for the contribution of each non-quantified congener to the TEQ.

The concept of 'lowerbound' requires using zero for the contribution of each non-quantified congener to the TEQ.

The concept of 'mediumbound' requires using half of the limit of quantification calculating the contribution of each non-quantified congener to the TEQ.

For the purposes of this Schedule only, the accepted specific limit of quantification of an individual congener is the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions, to be monitored with an S/N (signal/noise) ratio of 3:1 for the less sensitive signal and fulfilment of the basic requirements such as, e.g., retention time, isotope ratio according to the determination procedure as described in EPA method 1613 revision B.

### **3. Quality assurance requirements to be complied with for sample preparation**

- Measures must be taken to avoid cross-contamination at each stage of the sampling and analysis procedure.
- The samples must be stored and transported in glass, aluminium, polypropylene or polyethylene containers. Traces of paper dust must be removed from the sample container. Glassware should be rinsed with solvents previously controlled for the presence of dioxins.
- The sample storage and transportation has to be performed in a way that maintains the integrity of the foodstuff sample.
- Insofar as relevant, finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenisation (e.g. ground to pass a 1 mm sieve); samples have to be dried before grinding if moisture content is too high.
- Perform a blank analysis by carrying out the entire analytical procedure omitting only the sample.
- Sample weight used for the extraction must be sufficient to fulfil the requirements with respect to sensitivity.
- There are many satisfactory specific sample preparation procedures, which may be used for the products under consideration. The procedures have to be validated according to internationally accepted guidelines.

### **4. Requirements for laboratories**

- Laboratories shall demonstrate the performance of a method in the range of the level of interest, e.g.  $0,5 \times$ ,  $1 \times$  and  $2 \times$  the level of interest with an acceptable coefficient of variation for repeated analysis. For details of acceptance criteria, see point 5.

- Limit of quantification for a confirmatory method should be in the range of about one fifth of the level of interest, to make sure that acceptable coefficients of variations are met in the range of the level of interest.
- Regular blank controls and spiking experiments or analysis of control samples (preferably, if available, certified reference material) should be performed as internal quality control measures.
- Successful participation in interlaboratory studies that assess laboratory proficiency is the best way to prove the competence in specific analyses. However successful participation in interlaboratory studies for, e.g. soil or sewage samples, does not necessarily prove the competence also in the field of food or feedingstuff samples, which present lower contamination levels. Therefore, the continuous participation in interlaboratory studies for the determination of dioxins and dioxin-like PCBs in the relevant feed/food matrices is mandatory.
- In accordance with the provisions of Directive 93/99/EEC, laboratories should be accredited by a recognised body operating in accordance with ISO Guide 58 to ensure that they are applying analytical quality assurance. Laboratories should be accredited following the ISO/IEC/17025:1999 standard.

## 5. Requirements to be met by analytical procedure for dioxins and dioxin-like PCBs

*Basic requirements for acceptance of analytical procedures:*

- *High sensitivity and low limits of detection.* For PCDDs and PCDFs, detectable quantities have to be in the picogram TEQ (10<sup>-12</sup> g) range because of extreme toxicity of some of these compounds. PCBs are known to occur at higher levels than the PCDDs and PCDFs. For most PCB congeners sensitivity in the nanogram (10<sup>-9</sup> g) range is already sufficient. However, for the measurement of the more toxic dioxin-like PCB congeners (in particular non-ortho substituted congeners), the same sensitivity must be reached as for the PCDDs and PCDFs.
- *High selectivity (specificity).* A distinction is required for PCDDs, PCDFs and dioxin-like PCBs from a multitude of other, coextracted and possibly interfering compounds present at concentrations up to several orders of magnitude higher than those of the analytes of interest. For gas chromatography/mass spectrometry (GC/MS) methods a differentiation among various congeners is necessary, such as between toxic (e.g. the seventeen 2,3,7,8-substituted PCDDs and PCDFs and dioxin-like PCBs) and other congeners. Bioassays should be able to determine TEQ values selectively as the sum of PCDDs, PCDFs and dioxin-like PCBs.
- *High accuracy (trueness and precision).* The determination should provide a valid estimate of the true concentration in a sample. High accuracy (accuracy of the measurement: the closeness of the agreement between the result of a measurement with the true or assigned value of the measurement) is necessary to avoid the

rejection of a sample analysis result on the basis of poor reliability of the estimate of TEQ. Accuracy is expressed as trueness (difference between the mean value measured for an analyte in a certified material and its certified value, expressed as percentage of this value) and precision (precision is usually calculated as a standard deviation including repeatability and reproducibility, and indicates the closeness of agreement between the results obtained by applying the experimental procedure several times under prescribed conditions).

Screening methods can comprise bioassays and GC/MS methods; confirmatory methods are high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) methods. Following criteria have to be complied with on total TEQ value:

	Screening methods	Confirmatory methods
False negative rate	< 1 %	
Trueness		-20 % to + 20 %
CV	< 30 %	< 15 %

#### 6. Specific requirements for GC/MS methods to be complied with for screening or confirmatory purposes

- Addition of  $^{13}\text{C}$ -labelled 2,3,7,8-chlorine substituted internal PCDD/F standards (and of  $^{13}\text{C}$ -labelled internal dioxin-like PCB standards, if dioxin-like PCBs have to be determined) must be carried out at the very beginning or start of the analytical method e.g. prior to extraction in order to validate the analytical procedure. At least one congener for each of the tetra to octa-chlorinated homologous groups for PCDD/F (and at least one congener for each of the homologous groups for dioxin-like PCBs, if dioxin-like PCBs have to be determined) must be added (alternatively, at least one congener for each mass spectrometric selected ion recording function used for monitoring PCDD/F and dioxin-like PCBs). There is a clear preference, certainly in case of confirmatory methods, of using all 17  $^{13}\text{C}$ -labelled 2,3,7,8-substituted internal PCDD/F standards and all 12  $^{13}\text{C}$ -labelled internal dioxin-like PCB standard (if dioxin-like PCBs have to be determined).

Relative response factors should also be determined for those congeners for which no  $^{13}\text{C}$ -labelled analogue is added by using appropriate calibration solutions.

- For foodstuffs of plant origin and foodstuffs of animal origin containing less than 10 % fat, the addition of the internal standards is mandatory prior to extraction. For foodstuffs of animal origin containing more than 10 % fat, the internal standards can be added either before extraction or after fat extraction. An appropriate validation of the extraction efficiency should be carried out, depending on the stage at which internal standards are introduced and on whether results are reported on product or fat basis.



- Prior to GC/MS analysis, 1 or 2 recovery (surrogate) standard(s) must be added.
- Control of recovery is necessary. For confirmatory methods, the recoveries of the individual internal standards should be in the range of 60 % to 120 %. Lower or higher recoveries for individual congeners, in particular for some hepta- and octa-chlorinated dibenzodioxins and dibenzofurans, are acceptable on the condition that their contribution to the TEQ value does not exceed 10 % of the total TEQ value (based on PCDD/F only). For screening methods, the recoveries should be in the range of 30 % to 140 %.
- Separation of dioxins from interfering chlorinated compounds such as PCBs and chlorinated diphenyl ethers should be carried out by suitable chromatographic techniques (preferably with a florisil, alumina and/or carbon column).
- Gas chromatographic separation of isomers should be sufficient (< 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF).
- Determination should be performed according to EPA Method 1613 revision B: Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS or another with equivalent performance criteria.
- The difference between upperbound level and lower bound level should not exceed 20 % for foodstuffs with a dioxin contamination of about 1 pg WHO-TEQ/g fat (based on PCDD/PCDF only). For foodstuffs with a low fat content, the same requirements for contamination levels of about 1 pg WHO-TEQ/g product have to be applied. For lower contamination levels, for example 0,50 pg WHO-TEQ/g product, the difference between upperbound and lowerbound level may be in the range of 25 to 40 %.

## 7. Screening methods of analysis

### 7.1. Introduction

Different analytical approaches can be performed using a screening method: a pure screening approach and a quantitative approach.

#### Screening approach

The response of samples is compared to that of a reference sample at the level of interest. Samples with a response less than the reference are declared negative, those with a higher response are suspected positives. Requirements:

- A blank and a reference sample(s) have to be included in each test series, which is extracted and tested at the same time under identical conditions. The reference sample must show a clearly elevated response in comparison to a blank.

- Extra reference samples  $0,5 \times$  and  $2 \times$  the level of interest should be included to demonstrate the proper performance of the test in the range of interest for the control of the level of interest.
- When testing other matrices, the suitability of the reference sample(s) has to be demonstrated, preferentially by including samples shown by HRGC/HRMS to contain a TEQ level around that of the reference sample or else a blank spiked at this level.
- Since no internal standards can be used in bioassays, tests on repeatability are very important to obtain information on the standard deviation within one test series. The coefficient of variation should be below 30 %.
- For bioassays, the target compounds, possible interferences and maximum tolerable blank levels should be defined.

#### Quantitative approach

The quantitative approach requires standard dilution series, duplicate or triplicate clean up and measuring as well as blank and recovery controls. The result may be expressed as TEQ, thereby assuming that the compounds responsible for the signal correspond to the TEQ principle. This can be performed by using TCDD (or a dioxin/furan standard mixture) to produce a calibration curve to calculate the TEQ level in the extract and thus in the sample. This is subsequently corrected for the TEQ level calculated for a blank sample (to account for impurities from solvents and chemicals used), and a recovery (calculated from the TEQ level in a quality control sample around the level of interest). It is essential to note that part of the apparent recovery loss may be due to matrix effects and/or differences between the TEF values in the bioassays and the official TEF values set by WHO.

#### *7.2. Requirements for methods of analysis used for screening*

- GC/MS methods of analysis and bioassays may be used for screening. For GC/MS methods the requirements as laid down in point 6 are to be used. For cell based bioassays specific requirements are laid down in point 7.3 and for kit-based bioassays in point 7.4.
- Information on the number of false-positive and false-negative results of a large set of samples below and above the maximum level or action level is necessary, in comparison to the TEQ content as determined by a confirmatory method of analysis. Actual false negative rates should be under 1 %. The rate of false positive samples should be low enough to make the use of a screening tool advantageous.
- Positive results have always to be confirmed by a confirmatory method of analysis (HRGC/HRMS). In addition, samples from a wide TEQ-range should be

confirmed by HRGC/HRMS (approximately 2 % to 10 % of the negative samples). Information on correspondence between bioassay and HRGC/HRMS results should be made available.

### 7.3. *Specific requirements for cell-based bioassays*

- When performing a bioassay, every test run requires a series of reference concentrations of TCDD or a dioxin/furan mixture (full dose-response curve with a  $R^2 > 0,95$ ). However, for screening purposes an expanded low level curve for analysing low level samples could be used.
- A TCDD reference concentration (about  $3 \times$  limit of quantification) on a quality control sheet should be used for the outcome of the bioassay over a constant time period. An alternative could be the relative response of a reference sample in comparison to the TCDD calibration line since the response of the cells may depend on many factors.
- Quality control (QC) charts for each type of reference material should be recorded and checked to make sure the outcome is in accordance with the stated guidelines.
- In particular for quantitative calculations, the induction of the sample dilution used must be within the linear portion of the response curve. Samples above the linear portion of the response curve must be diluted and re-tested. Therefore, at least three or more dilutions at one time are recommended to be tested.
- The percent standard deviation should not be above 15 % in a triplicate determination for each sample dilution and not above 30 % between three independent experiments.
- The limit of detection may be set as  $3 \times$  the standard deviation of the solvent blank or of the background response. Another approach is to apply a response that is above the background (induction factor  $5 \times$  the solvent blank) calculated from the calibration curve of the day. The limit of quantification may be set as  $5 \times$  to  $6 \times$  the standard deviation of the solvent blank or of the background response or to apply a response that is above the background (induction factor  $10 \times$  the solvent blank) calculated from the calibration curve of the day.

### 7.4. *Specific requirements for kit-based bioassays* <sup>(13)</sup>

- Manufacturer's instructions for sample preparation and analyses have to be followed.

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<sup>13</sup> No evidence have yet been submitted of commercially available kit-based bioassays having sufficient sensitivity and reliability to be used for screening for the presence of dioxins at the required levels in samples of foodstuffs and feedingstuffs.

- Test kits should not be used after the expiration date.
- Materials or components designed for use with other kits should not be used.
- Test kits should be kept within the specified range of storage temperature and used at the specified operating temperature.
- The limit of detection for immunoassays is determined as  $3 \times$  the standard deviation, based on 10 replicate analysis of the blank, to be divided by the slope value of the linear regression equation.
- Reference standards should be used for tests at the laboratory to make sure that the responsiveness to the standard is within an acceptable range.

## **8. Reporting of the result**

Insofar as the used analytical procedure makes it possible, the analytical results should contain the levels of the individual PCDD/F and PCB congeners and be reported as lowerbound, upperbound and mediumbound in order to include a maximum of information in the reporting of the results and thereby enabling the interpretation of the results according to specific requirements.

The report should also include the lipid content of the sample as well the method used for lipid extraction.

The recoveries of the individual internal standards must be made available in case the recoveries are outside the range mentioned in point 6, in case the maximum level is exceeded and in other cases upon request.

NINTH SCHEDULE  
(Equivalent to Annex I of Commission Directive 2003/78/EC)

**Methods of Sampling for Official Control of the Levels of Patulin in certain  
foodstuffs**

**1. Purpose and scope**

Samples intended for official checking of the levels of patulin in foodstuffs shall be taken according to the methods described below. Aggregate samples thus obtained shall be considered as representative of the lots. Compliance with maximum levels laid down in Commission Regulation (EC) No 466/2001 shall be established on the basis of the levels determined in the laboratory samples.

**2. Definitions**

**Lot:** an identifiable quantity of a food commodity delivered at one time and having been determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings.

**Sublot:** designated part of a lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.

**Incremental sample:** a quantity of material taken from a single place in the lot or sublot.

**Aggregate sample:** the combined total of all the incremental samples taken from the lot or sublot.

**3. General provisions**

*3.1. Personnel*

Sampling shall be performed by an authorised person as specified by the Food Safety Commission.

*3.2. Material to be sampled*

Each lot which is to be examined must be sampled separately.

*3.3. Precautions to be taken*

In the course of sampling and preparation of the samples precautions must be taken to avoid any changes, which would affect the patulin content, adversely affect the analytical determination or make the aggregate samples unrepresentative.

### *3.4. Incremental samples*

As far as possible incremental samples should be taken at various places distributed throughout the lot or subplot. Departure from this procedure must be recorded in the record.

### *3.5. Preparation of the aggregate sample*

The aggregate sample is made up by uniting the incremental samples. It shall be at least 1 kg unless not practical e.g. when a single package has been sampled.

### *3.6. Replicate samples*

Replicate samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised aggregate sample unless this conflicts with other provisions on sampling.

### *3.7. Packaging and transmission of samples*

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

### *3.8. Sealing and labelling of samples*

Each sample taken for official use shall be sealed at the place of sampling and identified following the procedures established by the Food Safety Commission.

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.

## **4. Sampling plans**

The sampling method applied shall ensure that the aggregate sample is representative for the lot that is to be controlled.

Number of incremental samples

The aggregate sample shall be at least 1 kg (see point 3.5), except where it is not possible e.g. when sampling a single package.

The minimum number of incremental samples to be taken from the lot shall be as given in Table 1. In the case of liquid products the lot shall be thoroughly mixed insofar as possible by either manual or mechanical means immediately prior to sampling. In this

case, a homogeneous distribution of patulin can be assumed within a given lot. It is therefore sufficient to take three incremental samples from a lot to form the aggregate sample.

The incremental samples shall be of similar weight. The weight of an incremental sample should be at least 100 grams, resulting in an aggregate sample of at least 1 kg. Departure from this procedure must be recorded in the record provided for under 3.8.

**Table 1**

**Minimum number of incremental samples to be taken from the lot**

Weight of lot (in kg)	Minimum number of incremental samples to be taken
< 50	3
50 to 500	5
> 500	10

If the lot consists of individual packages, then the number of packages, which shall be taken to form the aggregate sample, is given in Table 2.

**Table 2**

**Number of packages (incremental samples) which shall be taken to form the aggregate sample if the lot consists of individual packages**

Number of packages or units in the lot	Number of packages or units to be taken
1 to 25	1 package or unit
26 to 100	about 5 %, at least 2 packages or units
> 100	about 5 %, at maximum 10 packages or units

**5. Compliance of the lot or subplot with the specification**

The control laboratory shall analyse the laboratory sample for enforcement in duplicate analysis in case the obtained result of the first analysis is less than 20 % below or above the maximum level, and calculate the mean of the results.

The lot is accepted if the result of the first analysis is more than 20 % below the maximum level or, where duplicate analysis is necessary, if the mean does not exceed the respective maximum level as laid down in Regulation (EC) No 466/2001 taking into account the measurement uncertainty and correction for recovery.

The lot is non-compliant with the maximum level as laid down in Regulation (EC) No 466/2001, if the mean, corrected for recovery exceeds the maximum level beyond reasonable doubt taking into account the measurement uncertainty.

## TENTH SCHEDULE

### **Sample Preparation and Criteria for Methods of Analysis used in Official Checking of the Levels of Patulin in certain foodstuffs**

#### **1. Precautions**

As the distribution of patulin in certain foodstuffs could be non-homogeneous, samples should be prepared — and especially homogenised — with extreme care.

All the material received by the laboratory is to be used for the preparation of test material.

#### **2. Treatment of the sample as received in the laboratory**

Finely grind (insofar relevant) and mix thoroughly the complete aggregate sample using a process that has been demonstrated to achieve complete homogenisation.

#### **3. Subdivision of samples for enforcement and defence purposes**

The replicate samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised material unless this conflicts with other provisions on sampling.

#### **4. Method of analysis to be used by the laboratory and laboratory control requirements**

##### *4.1. Definitions*

A number of the most commonly used definitions that the laboratory will be required to use are given below.

The most commonly quoted precision parameters are repeatability and reproducibility.

$r =$  Repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions (i.e. same sample, same operator, same apparatus, same laboratory, and short interval of time) may be expected to lie within a specific probability (typically 95 %) and hence  $r = 2,8 \times s_r$ .

$s_r =$  Standard deviation, calculated from results generated under repeatability conditions.



$RSD_r =$  Relative standard deviation, calculated from results generated under repeatability conditions  $\left[ \frac{s_r}{\bar{x}} \cdot 100 \right]$  where  $\bar{x}$  is the average of results over all laboratories and samples.

$R =$  Reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions (i.e. on identical material obtained by operators in different laboratories, using the standardised test method) may be expected to lie within a certain probability (typically 95 %);  $R = 2,8 \times s_R$ .

$s_R =$  Standard deviation, calculated from results under reproducibility conditions.

$RSD_R =$  Relative standard deviation calculated from results generated under reproducibility conditions  $\left[ \frac{s_R}{\bar{x}} \cdot 100 \right]$ .

#### 4.2. General requirements

Methods of analysis used for food control purposes must comply with the provisions of items 1 and 2 of the Annex to Council Directive 85/591/EEC of the 20<sup>th</sup> December, 1985 concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption (<sup>14</sup>).

#### 4.3. Specific requirements

Where no specific methods for the determination of patulin in foodstuffs are prescribed at Community level, laboratories may select any method provided the selected method meets the following criteria:

Level µg/kg	Patulin		
	$RSD_r$ %	$RSD_R$ %	Recovery %
< 20	$\leq 30$	$\leq 40$	50 to 120
20-50	$\leq 20$	$\leq 30$	70 to 105
> 50	$\leq 15$	$\leq 25$	75 to 105

The detection limits of the methods used are not stated as the precision values are given at the concentrations of interest.

The precision values are calculated from the Horwitz equation:

<sup>14</sup> OJ L 372, 31.12.1985, p. 50.

$$RSD_R = 2^{(1-0.5 \log C)}$$

where:

- $RSD_R$  is the relative standard deviation calculated from results generated under reproducibility conditions  $[(S_R/\bar{x} \cdot 100)]$ .
- $C$  is the concentration ratio (i.e. 1 = 100g/100g, 0,001 = 1,000 mg/kg)

This is a generalised precision equation, which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

#### *4.4. Recovery calculation and reporting of results*

The analytical result is to be reported corrected or uncorrected for recovery. The manner of reporting and the level of recovery must be reported. The analytical result corrected for recovery is used for checking compliance (see Annex I, point 5).

The analytical result has to be reported as  $x \pm U$  whereby  $x$  is the analytical result and  $U$  is the measurement uncertainty.

#### *4.5. Laboratory quality standards*

Laboratories must comply with Council Directive 93/99/EEC of 29<sup>th</sup> October, 1993 on the subject of additional measures concerning the official control of foodstuffs.