# Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs (Text with EEA relevance)

ANNEX

PART A

## DEFINITIONS

For the purposes of this Annex, the following definitions shall apply:

| | | |
|---|---|---|
| ‘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; aggregate samples shall be considered as representative of the lots or sublots from which they are taken; |
| ‘laboratory sample’ | : | a sample intended for the laboratory. |

PART B

## SAMPLING METHODS

B.1. GENERAL PROVISIONS

B.1.1. Personnel

Sampling shall be performed by an authorised person as designated by the Member State.

B.1.2. Material to be sampled

Each lot or sublot which is to be examined shall be sampled separately.

B.1.3. Precautions to be taken

In the course of sampling, precautions shall be taken to avoid any changes which would affect the levels of contaminants, adversely affect the analytical determination or make the aggregate samples unrepresentative.

B.1.4. Incremental samples

As far as possible, incremental samples shall be taken at various places distributed throughout the lot or sublot. Departure from such procedure shall be recorded in the record provided for under point B.1.8. of this Annex.

B.1.5. Preparation of the aggregate sample

The aggregate sample shall be made up by combining the incremental samples.

B.1.6. Samples for enforcement, defence and referee purposes

The samples for enforcement, defence and referee purposes shall be taken from the homogenised aggregate sample unless this conflicts with the rules of the Member States as regards the rights of the food business operator.

B.1.7. Packaging and transmission of samples

Each 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 any change in composition of the sample which might arise during transportation or storage.

B.1.8. Sealing and labelling of samples

Each sample taken for official use shall be sealed at the place of sampling and identified following the rules of the Member States.

A record shall be kept of each sampling, permitting each lot or sublot to be identified unambiguously (reference to the lot number shall be given) and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

B.2. SAMPLING PLANS

Large lots shall be divided into sublots on condition that the sublot may be separated physically. For products traded in bulk consignments (e.g. cereals), Table 1 shall apply. For other products Table 2 shall apply. 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 sublot may exceed the mentioned weight by a maximum of 20 %.

The aggregate sample shall be at least 1 kg or 1 litre except where it is not possible e.g. when the sample consists of 1 package or unit.

The minimum number of incremental samples to be taken from the lot or sublot shall be as given in Table 3.

In the case of bulk liquid products the lot or sublot shall be thoroughly mixed in so far as possible and in so far it does not affect the quality of the product, by either manual or mechanical means immediately prior to sampling. In this case, a homogeneous distribution of contaminants is assumed within a given lot or sublot. It is therefore sufficient to take three incremental samples from a lot or sublot to form the aggregate sample.

The incremental samples shall be of similar weight. The weight of an incremental sample shall be at least 100 grams or 100 millilitres, resulting in an aggregate sample of at least about 1 kg or 1 litre. Departure from this method shall be recorded in the record provided for under point B.1.8. of this Annex.

TABLE 1

Subdivision of lots into sublots for products traded in bulk consignments

| **Lot weight (ton)** | **Weight or number of sublots** |
|---|---|
| ≥ 1 500 | 500 tonnes |
| > 300 and < 1 500 | 3 sublots |
| ≥ 100 and ≤ 300 | 100 tonnes |
| < 100 | — |

TABLE 2

Subdivision of lots into sublots for other products

| **Lot weight (ton)** | **Weight or number of sublots** |
|---|---|
| ≥ 15 | 15 to 30 tonnes |
| < 15 | — |

TABLE 3

Minimum number of incremental samples to be taken from the lot or sublot

| **Weight or volume of lot/sublot (in kg or litre)** | **Minimum number of incremental samples to be taken** |
|---|---|
| < 50 | 3 |
| ≥ 50 and ≤ 500 | 5 |
| > 500 | 10 |

If the lot or sublot consists of individual packages or units, then the number of packages or units which shall be taken to form the aggregate sample is given in Table 4.

TABLE 4

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

| **Number of packages or units in the lot/ sublot** | **Number of packages or units to be taken** |
|---|---|
| ≤ 25 | at least one package or unit |
| 26 to 100 | about 5 %, at least two packages or units |
| > 100 | about 5 %, at maximum 10 packages or units |

The maximum levels for inorganic tin apply to the contents of each can, but for practical reasons it is necessary to use an aggregate sampling approach. If the result of the test for an aggregate sample of cans is less than, but close to, the maximum level of inorganic tin and if it is suspected that individual cans might exceed the maximum level, then it might be necessary to conduct further investigations.

B.3. SAMPLING AT RETAIL STAGE

Sampling of foodstuffs at retail stage shall be done where possible in accordance with the sampling provisions set out in points B.1. and B.2. of this Annex.

Where this is not possible, an alternative method of sampling at retail stage may be used provided that it ensures sufficient representativeness for the sampled lot or sublot.

## PART C

## SAMPLE PREPARATION AND ANALYSIS

C.1. LABORATORY QUALITY STANDARDS

Laboratories shall comply with the provisions of Article 12 of Regulation (EC) No 882/2004[(1)].

Laboratories shall participate in appropriate proficiency testing schemes which comply with the 'International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories'[(2)] developed under the auspices of IUPAC/ISO/AOAC.

Laboratories shall be able to demonstrate that they have internal quality control procedures in place. Examples of these are the 'ISO/AOAC/IUPAC Guidelines on Internal Quality Control in Analytical Chemistry Laboratories'[(3)].

Wherever possible the trueness of analysis shall be estimated by including suitable certified reference materials in the analysis.

C.2. SAMPLE PREPARATION

C.2.1. Precautions and general considerations

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

All of the sample material received by the laboratory shall be used for the preparation of the laboratory sample.

Compliance with maximum levels laid down in Regulation (EC) No 1881/2006 shall be established on the basis of the levels determined in the laboratory samples.

C.2.2. Specific sample preparation procedures

C.2.2.1. Specific procedures for lead, cadmium, mercury and inorganic tin

The analyst shall ensure that samples do not become contaminated during sample preparation. Wherever possible, apparatus and equipment coming into contact with the sample shall not contain those metals to be determined and be made of inert materials e.g. plastics such as polypropylene, polytetrafluoroethylene (PTFE) etc. These should be acid cleaned to minimise the risk of contamination. High quality stainless steel may be used for cutting edges.

There are many satisfactory specific sample preparation procedures which may be used for the products under consideration. Those described in the CEN Standard 'Foodstuffs — Determination of trace elements — Performance criteria, general considerations and sample preparation'[(4)] have been found to be satisfactory but others may be equally valid.

In the case of inorganic tin, care shall be taken to ensure that all the material is taken into solution as losses are known to occur readily, particularly because of hydrolysis to insoluble hydrated Sn(IV) oxide species.

C.2.2.2. Specific procedures for benzo(a)pyrene

The analyst shall ensure that samples do not become contaminated during sample preparation. Containers shall be rinsed with high purity acetone or hexane before use to minimise the risk of contamination. Wherever possible, apparatus and equipment coming into contact with the sample shall be made of inert materials such as aluminium, glass or polished stainless steel. Plastics such as polypropylene or PTFE shall be avoided because the analyte can adsorb onto these materials.

C.2.3. Treatment of the sample as received in the laboratory

The complete aggregate sample shall be finely ground (where relevant) and thoroughly mixed using a process that has been demonstrated to achieve complete homogenisation.

C.2.4. Samples for enforcement, defence and referee purposes

The samples for enforcement, defence and referee purposes shall be taken from the homogenised material unless this conflicts with the rules of the Member States on sampling as regards the rights of the food business operator.

C.3. METHODS OF ANALYSIS

C.3.1. Definitions

The following definitions shall apply:

| | | |
|---|---|---|
| 'r' | = | Repeatability the value below which the absolute difference between 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 $[(s_r/\bar{x}) \times 100]$. |
| '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 $[(s_R/\bar{x}) \times 100]$. |
| 'LOD' | = | Limit of detection, smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. The limit of detection is numerically equal to three times the standard deviation of the mean of blank determinations ($n > 20$). |
| 'LOQ' | = | Limit of quantification, lowest content of the analyte which can be measured with reasonable statistical certainty. If both accuracy and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to six or 10 times the standard deviation of the mean of blank determinations ($n > 20$). |
| '$HORRAT_r$' | = | The observed $RSD_r$ divided by the $RSD_r$ value estimated from the Horwitz equation[5] using the assumption $r = 0,66R$. |
| '$HORRAT_R$' | = | The observed $RSD_R$ value divided by the $RSD_R$ value calculated from the Horwitz equation. |
| 'u' | = | Standard measurement uncertainty. |
| 'U' | = | The expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 % ($U = 2u$). |
| 'Uf' | = | Maximum standard measurement uncertainty. |

C.3.2. General requirements

Methods of analysis used for food control purposes shall comply with the provisions of points 1 and 2 of Annex III to Regulation (EC) No 882/2004.

Methods of analysis for total tin are appropriate for official control on inorganic tin levels.

For the analysis of lead in wine, Commission Regulation (EEC) No 2676/90[(6)] lays down the method to be used in chapter 35 of its Annex.

C.3.3. Specific requirements

C.3.3.1. Performance criteria

Where no specific methods for the determination of contaminants in foodstuffs are prescribed at Community level, laboratories may select any validated method of analysis (where possible, the validation shall include a certified reference material) provided the selected method meets the specific performance criteria set out in Tables 5 to 7.

TABLE 5

Performance criteria for methods of analysis for lead, cadmium, mercury and inorganic tin

| **Parameter** | **Value/Comment** |
|---|---|
| Applicability | Foods specified in Regulation (EC) No 1881/2006 |
| LOD | For inorganic tin less than 5 mg/kg.<br>For other elements less than one tenth of the maximum level in Regulation (EC) No 1881/2006, except if the maximum level for lead is less than 100 µg/kg. For the latter, less than one fifth of the maximum level |
| LOQ | For inorganic tin less than 10 mg/kg.<br>For other elements less than one fifth of the maximum level in Regulation (EC) No 1881/2006, except if the maximum level for lead is less than 100 µg/kg. For the latter, less than two fifth of the maximum level |
| Precision | $HORRAT_r$ or $HORRAT_R$ values of less than 2 |
| Recovery | The provisions of point D.1.2. apply |
| Specificity | Free from matrix or spectral interferences |

TABLE 6

Performance criteria for methods of analysis for 3-MCPD

| **Criterion** | **Recommended Value** | **Concentration** |
|---|---|---|
| Field blanks | Less than the LOD | — |
| Recovery | 75 to 110 % | all |
| LOD | 5 µg/kg (or less) on a dry matter basis | |
| LOQ | 10 µg/kg (or less) on a dry matter basis | — |
| Precision | < 4 µg/kg | 20 µg/kg |

| | |
|---|---|
| < 6 µg/kg | 30 µg/kg |
| < 7 µg/kg | 40 µg/kg |
| < 8 µg/kg | 50 µg/kg |
| < 15 µg/kg | 100 µg/kg |

TABLE 7

Performance criteria for methods of analysis for benzo(a)pyrene

| **Parameter** | **Value/Comment** |
|---|---|
| Applicability | Foods specified in Regulation (EC) No 1881/2006 |
| LOD | Less than 0,3 µg/kg |
| LOQ | Less than 0,9 µg/kg |
| Precision | $HORRAT_r$ or $HORRAT_R$ values of less than 2 |
| Recovery | 50 to 120 % |
| Specificity | Free from matrix or spectral interferences, verification of positive detection |

C.3.3.2. 'Fitness-for-purpose' approach

Where a limited number of fully validated methods of analysis exist, alternatively, a 'fitness-for-purpose' approach may be used to assess the suitability of the method of analysis. Methods suitable for official control must produce results with standard measurement uncertainties less than the maximum standard measurement uncertainty calculated using the formula below:

$$Uf = \sqrt{(LOD/2)^2 + (\alpha C)^2}$$

where:

Uf is the maximum standard measurement uncertainty (µg/kg);

LOD is the limit of detection of the method (µg/kg);

C is the concentration of interest (µg/kg);

α is a numeric factor to be used depending on the value of C. The values to be used are given in Table 8.

TABLE 8

Numeric values to be used for α as constant in formula set out in this point, depending on the concentration of interest

| **C (µg/kg)** | **α** |
|---|---|
| ≤ 50 | 0,2 |
| 51 to 500 | 0,18 |
| 501 to 1 000 | 0,15 |
| 1 001 to 10 000 | 0,12 |

| | |
|---|---|
| > 10 000 | 0,1 |

PART D

## REPORTING AND INTERPRETATION OF RESULTS

D.1. REPORTING

D.1.1. Expression of results

The results shall be expressed in the same units and with the same number of significant figures as the maximum levels laid down in Regulation (EC) No 1881/2006.

D.1.2. Recovery calculations

If an extraction step is applied in the analytical method, the analytical result shall be corrected for recovery. In this case the level of recovery must be reported.

In case no extraction step is applied in the analytical method (e.g. in case of metals), the result may be reported uncorrected for recovery if evidence is provided by ideally making use of suitable certified reference material that the certified concentration allowing for the measurement uncertainty is achieved (i.e. high accuracy of the measurement). In case the result is reported uncorrected for recovery this shall be mentioned.

D.1.3. Measurement uncertainty

The analytical result shall be reported as x +/– U whereby x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 % (U = 2u).

The analyst shall note the ‘Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions in EU food and feed legislation’[7].

D.2. INTERPRETATION OF RESULTS

D.2.1. Acceptance of a lot/sublot

The lot or sublot is accepted if the analytical result of the laboratory sample does not exceed the respective maximum level as laid down in Regulation (EC) No 1881/2006 taking into account the expanded measurement uncertainty and correction of the result for recovery if an extraction step has been applied in the analytical method used.

D.2.2. Rejection of a lot/sublot

The lot or sublot is rejected if the analytical result of the laboratory sample exceeds beyond reasonable doubt the respective maximum level as laid down in Regulation (EC) No 1881/2006 taking into account the expanded measurement uncertainty and correction of the result for recovery if an extraction step has been applied in the analytical method used.

D.2.3. Applicability

The present interpretation rules shall apply for the analytical result obtained on the sample for enforcement. In case of analysis for defence or reference purposes, the national rules shall apply.

(**1**) As amended by Article 18 of Commission Regulation (EC) No 2076/2005 (OJ L 338, 22.12.2005, p. 83).

(**2**) 'The international harmonized protocol for the proficiency testing of analytical chemistry laboratories' by M. Thompson, S.L.R. Ellison and R. Wood, Pure Appl. Chem., 2006, 78, 145-96.

(**3**) Edited by M. Thompson and R. Wood, Pure Appl. Chem., 1995, 67, 649-666.

(**4**) Standard EN 13804:2002, 'Foodstuffs — Determination of trace elements — Performance criteria, general considerations and sample preparation', CEN, Rue de Stassart 36, B-1050 Brussels.

(**5**) M. Thompson, Analyst, 2000, 125, 385-386.

(**6**) OJ L 272, 3.10.1990, p. 1. Regulation as last amended by Regulation (EC) No 1293/2005 (OJ L 205, 6.8.2005, p. 12).

(**7**) http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/sampling_en.htm