CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 3 **CX/MAS 18/39/3 Add.1**

April 2018

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Thirty-ninth Session

Budapest, Hungary, 7 – 11 May 2018

ENDORSEMENT OF METHODS OF ANALYSIS AND SAMPLING PLANS FOR PROVISIONS IN CODEX STANDARDS

1. This document contains the methods of analysis and/or sampling (Appendices I and II) proposed by the following Committees:

- Committee on Cereals, Pulses and Legumes (methods of analysis for quinoa);
- Committee on Contaminants in Foods (Sampling plan for MLs for methylmercury in fish).

CODEX COMMITTEE ON CEREALS, PULSES AND LEGUMES (CCCPL)

Methods of analysis for quinoa[1]

Note: The draft standard for quiona is forwarded to the Commission for adoption at Step 8.

2. The Committee **is invited to endorse** the methods of analysis in Appendix I.

COMMITTEE ON CONTAMINANTS IN FOODS (CCCF)

Sampling plan for MLs for methylmercury in fish(CXS 193-1995)[2]

3. CCCF12 agreed to send the sampling plan to CCMAS for endorsement.

4. The Committee **is invited** to **endorse** the sampling plan in Appendix II .

Note: accompanying questions are provided in CX/MAS 18/39/2 Add.1

[1] CL 2018/25-CPL Annex II

[2] REP18/CF, para 91, /Appendix IV, Part B

APPENDIX I

CODEX COMMITTEE ON CEREALS, PULSES AND LEGUMES (CCCPL)

Methods of analysis for Quinoa

	Method	**Principle**	**Type**
Moisture content	ISO 712	Gravimetric	1
Protein Content (N x 6.25) Dry weight basis	ISO 1871	Titrimetry, Kjeldahl	1

Appendix II

COMMITTEE ON CONTAMINANTS IN FOODS (CCCF)

PROPOSED DRAFT SAMPLING PLAN FOR METHYLMERCURY CONTAMINATION IN FISH

DEFINITIONS

The following definitions should apply:

Lot	An identifiable quantity of a food commodity delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor, or markings.
Sublot	Designated part of a larger lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.
Incremental sample	The quantity of material taken from a single random place in the lot or sublot.
Aggregate sample	The combined total of all the incremental samples that is taken from the lot or sublot. The aggregate sample has to be at least as large as the laboratory sample or samples combined.
Laboratory sample	A sample intended for a laboratory.

SAMPLING METHODS

GENERAL PROVISIONS

Personnel

Sampling should be performed by an authorised person as designated by the national authority.

Material to be sampled

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

Precautions to be taken

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

Incremental samples

As far as possible, incremental samples should be taken at various places distributed throughout the lot or sublot.

Preparation of the aggregate sample

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

Samples for enforcement, defence and referee purposes

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

Packaging and transmission of samples

Each sample should 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 should be taken to avoid any change in composition of the sample which might arise during transportation or storage.

Sealing and labelling of samples

Each sample taken for official use should be sealed at the place of sampling and identified following the locally applicable rules.

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

SAMPLING PLAN

Division of lots into sublots

Large lots should be divided into sublots on condition that the sublot may be separated physically. For products traded in bulk consignments Table 1 should apply. For other products Table 2 should 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%.

Number of incremental samples

The aggregate sample should be at least 1 kg 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 should be as given in Table 3.

The incremental samples should be of similar weight/volume. The weight/ volume of an incremental sample should be at least 100 grams, resulting in an aggregate sample of at least about 1 kg. Departure from this method should be recorded.

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	15-30 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)	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 should be taken to form the aggregate sample is given in Table 4.

Table 4 Number of packages or units (incremental samples) which should 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 1 package or unit
26-100	about 5%, at least 2 packages or units
> 100	about 5%, at maximum 10 packages or units

Specific provisions for the sampling of large fish arriving in large lots

In case the lot or sublot to be sampled contains large fish (individual fish weighing more than about 1 kg) and the lot or sublot weighs more than 500 kg, the incremental sample should consist of the middle part of the fish. Each incremental sample should weigh at least 100 g.

SAMPLING AT RETAIL STAGE

Sampling of foodstuffs at retail stage should be done where possible in accordance with the sampling provisions set out in this sampling plan.

Where it is not possible to carry out the method of sampling set out above because of the unacceptable commercial consequences (e.g. because of packaging forms, damage to the lot, etc.) or where it is practically impossible to apply the abovementioned method of sampling, an alternative method of sampling may be applied provided that it is sufficiently representative for the sampled lot or sublot and is fully documented.

SAMPLE PREPARATION AND ANALYSIS

LABORATORY QUALITY STANDARDS

Laboratories should 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 should be estimated by including suitable certified reference materials in the analysis.

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 should be used for the preparation of the laboratory sample.

Compliance with maximum levels laid down in the General Standard for Contaminants and toxins in Food and Feed should be established on the basis of the levels determined in the laboratory samples.

Specific sample preparation procedures

The analyst should ensure that samples do not become contaminated during sample preparation. Wherever possible, apparatus and equipment coming into contact with the sample should not contain mercury 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. For those aspects not specifically covered by this sampling plan, the CEN Standard 'Foodstuffs. Determination of elements and their chemical species. General considerations and specific requirements'[4] has been found to be satisfactory but other sample preparation methods may be equally valid.

[3] Edited by M. Thompson and R. Wood, Pure Appl. Chem., 1995, 67, 649-666.

[4] Standard EN 13804:2013, 'Foodstuffs. Determination of elements and their chemical species. General considerations and specific requirements', CEN, Rue de Stassart 36, B-1050 Brussels.

Treatment of the sample as received in the laboratory

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

Samples for enforcement, defence and referee purposes

The samples for enforcement, defence and referee purposes should be taken from the homogenised material unless this conflicts with the applicable rules at the national level on sampling as regards the rights of the food business operator.

METHODS OF ANALYSIS

Definitions

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 × 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 /) × 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 × 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 /) × 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 10 times the standard deviation of the mean of blank matrix determinations (n ≥ 20).
HORRAT[5] r	The observed RSD r divided by the RSD r value estimated from the (modified) Horwitz equation (2) (cf. point C.3.3.1 ('Notes to the performance criteria')) using the assumption r = 0,66 R.
HORRAT[6] R	The observed RSD R divided by the RSD R value estimated from the (modified) Horwitz equation[7] (cf. point 'Notes to the performance criteria').
u	Combined standard measurement uncertainty obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model[8]
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.

5 Horwitz W. and Albert, R., 2006, The Horwitz Ratio (HorRat): A useful Index of Method Performance with respect to Precision, Journal of AOAC International, Vol. 89, 1095-1109. (2) M. Thompson, Analyst, 2000, p. 125 and 385-386.

6 Horwitz W. and Albert, R., 2006, The Horwitz Ratio (HorRat): A useful Index of Method Performance with respect to Precision, Journal of AOAC International, Vol. 89, 1095-1109.

8 International vocabulary of metrology – Basic and general concepts and associated terms (VIM), JCGM 200:2008.

General requirements

Methods for analysis for total mercury are appropriate for screening purpose for control on methylmercury levels. If the total mercury concentration is below or equal to the maximum level for methylmercury, no further testing is required and the sample is considered to be compliant with the maximum level for methylmercury. If the total mercury concentration is at or above the maximum level for methylmercury, follow-up testing should be conducted to determine if the methylmercury concentration is above the maximum level for methylmercury.

Specific requirements

Performance criteria

Where no specific methods for the determination of contaminants in foodstuffs are prescribed at the Codex level, laboratories may select any validated method of analysis for the respective matrix provided that the selected method meets the specific performance criteria set out in Table 5.

It is recommended that fully validated methods (i.e. methods validated by collaborative trial for the respective matrix) are used where appropriate and available. Other suitable validated methods (e.g. in-house validated methods for the respective matrix) may also be used provided that they fulfil the performance criteria set out in Tables 5.

Where possible, the validation of in-house validated methods should include a certified reference material.

Table 5 Performance criteria for methods of analysis of mercury and methylmercury

Parameter	Criterion		
Applicability	Fish specified in the General Standard for Contaminants and Toxins in Food and Feed (GSCTFF, CXS 193-1995)		
Specificity	Free from matrix or spectral interferences		
Repeatability (RSD_r)	$HORRAT_r$ less than 2		
Reproducibility (RSD_R)	$HORRAT_R$ less than 2		
Recovery	The provisions of ‘Recovery calculations’ apply		
LOD	= three tenths of LOQ		
LOQ	Methylmercury	ML is < 0,100mg/kg	ML is ≥ 0,100 mg/kg
		≤ two fifths of the ML	≤ one fifth of the ML

Notes to the performance criteria:

The Horwitz equation[9] (for concentrations $1,2 \times 10^{-7} \leq C \leq 0,138$) and the modified Horwitz equation[10]

(for concentrations $C < 1,2 \times 10^{-7}$) are generalised precision equations which are independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

Modified Horwitz equation for concentrations $C < 1,2 \times 10^{-7}$:

RSD R = 22%

where:

- RSD R is the relative standard deviation calculated from results generated under reproducibility conditions [(s R /) × 100]
- C is the concentration ratio (i.e. 1 = 100 g/100 g, 0,001 = 1 000 mg/kg). The modified Horwitz equation applies to concentrations $C < 1,2 \times 10^{-7}$.

9 W. Horwitz, L.R. Kamps, K.W. Boyer, J.Assoc.Off.Analy.Chem.,1980, 63, 1344.
10 M. Thompson, Analyst, 2000, p. 125 and 385-386.

Horwitz equation for concentrations $1,2 \times 10^{-7} \leq C \leq 0,138$:

$RSD\ R = 2C^{(-0,15)}$

where:

- RSD R is the relative standard deviation calculated from results generated under reproducibility conditions [(s R /) × 100]
- C is the concentration ratio (i.e. 1 = 100 g/100 g, 0,001 = 1 000 mg/kg). The Horwitz equation applies to concentrations $1,2 \times 10^{-7} \leq C \leq 0,138$.

Fitness-for-purpose' approach

For in-house validated methods, as an alternative a 'fitness-for-purpose' approach[11] may be used to assess their suitability for official control. Methods suitable for official control must produce results with a combined standard measurement uncertainty (u) 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). The LOD must meet the performance criteria set in point C.3.3.1 for the concentration of interest.
- 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 6.

Table 6 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
51-500	0,18
501-1 000	0,15
1 001-10 000	0,12
> 10 000	0,1

Table 7: Calculated performance criteria for ML ≥ 0.1 mg/kg

				Min. applicable range		
	ML mg/kg	LOD mg/kg	LOQ mg/kg	From mg/kg	To mg/kg	Precision RSDR (%)
All Tuna	1.2	0.12	0.24	0.64	1.76	31.1
Alfonsino	1.5	0.15	0.3	0.823	2.177	30.1
All Marlin	1.7	0.17	0.34	0.947	2.453	29.5
Shark	1.6	0.16	0.32	0.885	2.315	29.8

[11] M. Thompson and R. Wood, Accred. Qual. Assur., 2006, p. 10 and 471-478.

REPORTING AND INTERPRETATION OF RESULTS

Expression of results

The results should be expressed in the same units and with the same number of significant figures as the maximum levels laid down in the *General Standard for Contaminants and Toxins in Food and Feed* (GSCTFF) (CXS 193-1995).

Recovery calculations

If an extraction step is applied in the analytical method, the analytical result should 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, 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), and thus that the method is not biased. In case the result is reported uncorrected for recovery this should be mentioned.

Measurement uncertainty

The analytical result should 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).

INTERPRETATION OF RESULTS

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 the General Standard for Contaminants and Toxins in Food and Feed (GSCTFF, CXS 193-1995), 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.

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 the General Standard for Contaminants and Toxins in Food and Feed (GSCTFF, CXS 193-1995), 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.

Applicability

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