

CODEX ALIMENTARIUS COMMISSION



Food and Agriculture
Organization of the
United Nations



World Health
Organization

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Agenda Item 3

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

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ENDORSEMENT OF THE SAMPLING PLAN FOR METHYLMERCURY IN FISH

1. This document contains the sampling plan for methylmercury in fish (Appendix I) proposed by the Codex Committee on Contaminants (CCCF17) (REP24/CF)¹, which CCCF17 agreed to send to CCMAS for endorsement (submitted for adoption at Step 5/8 by CAC47).
2. CCMAS **is invited to consider and endorse** the sampling plan in Appendix I.

¹ REP24/CF para. 71 (still to be published)

APPENDIX I

SAMPLING PLAN FOR METHYLMERCURY CONTAMINATION IN FISH

(For endorsement by CCMAS)

GENERAL CONSIDERATIONS

DEFINITION

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. A lot of whole fish should consist of one species and the length and/or weight should be comparable. In case the length and/or weight of the fish is not comparable, the consignment may still be considered as a lot, but a specific sampling procedure has to be applied (as described in paragraph 8).
Sublot	Designated part of a larger lot in order to apply the sampling method on that designated part. Each sub-lot must be physically separate and identifiable.
Sampling plan	A procedure for sampling of food from a certain lot with a view of a specific chemical analysis of that lot, in order to ensure that the sample that is taken, is representative for the concentration of the concerned chemical within the lot.
Methylmercury test procedure	A methylmercury test procedure consists of three steps: sample selection, sample preparation and methylmercury quantification. It contains an accept/reject level.
Decision rule	The accept/reject level is a level usually equal to the Codex maximum level (ML).
Incremental sample	The quantity of material taken from a single random place in the lot or sub-lot.
Aggregate sample	The combined total of all the incremental samples that is taken from the lot or sub-lot. The aggregate sample has to be at least as large as the laboratory sample or samples combined. The entire aggregate sample should be comminuted in a mill.
Laboratory sample	A sample intended for the laboratory, which consists out of a comminuted quantity of fish muscle, or whole fish. The laboratory sample may be a portion of or the entire aggregate sample. If the aggregate sample is larger than the laboratory sample(s), the laboratory sample(s) should be removed in a random manner from the homogenised aggregate sample.
Test portion	A randomly removed portion of the comminuted laboratory sample for the extraction of the methylmercury for chemical analysis.

MATERIAL TO BE SAMPLED

1. Each lot or sub-lot which is to be examined must be sampled separately.
2. Fresh or frozen whole (in general after removing digestive tract) or dressed fish (eviscerated fish with head and tail removed) and other non-bulk fishery products of lots greater than or equal to 15 metric tons (MT) should be subdivided into sub-lots of 15-30 MT in accordance with Table 2.
3. Lots of fishery products traded as bulk commodities of greater than 100 MT should be subdivided into sub-lots in accordance with Table 1 to be sampled separately.

Table 1. Subdivision of sub-lots according to bulk consignment lot weight

Commodity	Lot weight (MT ^a)	Weight or number of sub-lots (MT)
Fishery products (traded as bulk consignments)	≥ 1500	500
	> 300 and <1500	3 sub-lots (minimum 100 MT)
	≥ 100 and ≤300	100
	< 100	-

^a1 metric tonne (MT) = 1000 kilograms

Table 2. Subdivision of sublots according to other products lot weight

Commodity	Lot weight (MT ^a)	Weight or number of sub-lots (MT)
Fish (traded as non-bulk consignments)	≥ 15	15-30
	< 15	-

^a1 metric tonne (MT) = 1000 kilograms

4. Taking into account that the weight of the lot is not always an exact multiple of the weight of the sub-lots, the weight of the sub-lot may exceed the mentioned weight by a maximum of 20 %.

INCREMENTAL SAMPLE

5. The recommended minimum number of incremental samples taken from the lot or sub-lot is dependent on the size of the lot or sub-lot as specified in Table 3.
6. The aggregate sample should contain a quantity of sample of at least 1 kilogram. The minimum weight of the incremental sample should be determined by dividing 1 kilogram by the required number of incremental samples as listed in Table 3. Incremental samples taken from a lot or sub-lot should be of comparable weight.

Table 3. Number of incremental samples to be taken depending on the weight of the lot or subplot

Lot weight (MT ^a)	Number of incremental samples	Minimum laboratory sample weight (kg)
≤ 0.05	3	1
> 0.05 - ≤ 0.5	5	1
> 0.5	10	1

^a1 metric tonne (MT) = 1000 kilograms

7. Whole fish are considered to be of comparable length and weight class where the differences in size and/or weight do not exceed about 50%.
8. For lots where fish are not of comparable length and/or weight the following approaches are to be applied to taking the incremental samples:
- Where a length or weight class/category is predominant (80% or more of the fish lot or sub-lot are within the same length and/or weight class), the aggregate sample is combined only from incremental samples of fish within the predominant category and outliers are excluded. This aggregate sample is to be considered as being representative for the whole lot/sub-lot.
 - Where there is no predominant weight or size class and where the overall length and/or weight of the fish present in the lot or sub-lot varies by more than 50% but less than 100%, the lot or sub-

lot is separated into two length or weight classes and separate aggregate samples are composited from incremental samples taken independently from each length and/or weight class.

- c. Where there is no predominant weight or size class and where the overall length and/or weight of the fishes present in the lot differ more than 100%, the lot or sub-lot is separated into three length or weight classes and separate aggregate samples are composited from incremental samples taken independently from each length or weight class.
9. For lots or sub-lots of whole fish the part of the fish where the incremental sample is taken is informed by the weight of the whole fish as specified in Table 3. Some examples on sampling of batches of fishes of different size and/or weight can be found in Annex II.

Table 4. Tissue area the incremental sample is taken from for whole fish based on weight classes

Weight class of an individual whole fish	Sampled part
< 1 kg	Whole fish (after removing the digestive tract) For lots of 0.05MT or greater where the aggregate sample would exceed 3 kg the midline (halfway between the gill opening and the anus) strip from backbone to belly can be sampled
1-6 kg	Midline (halfway between the gill opening and the anus) strip from backbone to belly
> 6 kg	Midline (halfway between the gill opening and the anus) strip from backbone to belly Alternatively, equal composite parts of muscle from behind the head and close to the tail can be sampled For tuna, incremental samples can instead be taken from the muscle from close to the tail.

PACKAGING AND TRANSPORTATION OF SAMPLES

10. Each laboratory sample should be placed in a clean, inert container offering adequate protection from contamination, loss of analytes by adsorption to the internal wall of the container and against damage in transit. All necessary precautions, for example temperature control and storage in airtight containers, should be taken to avoid any change in composition of the sample which might arise during transportation or storage (for example avoiding excess heat or the sample drying out).

SEALING AND LABELLING OF SAMPLES

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

SAMPLE PREPARATION PRECAUTIONS

12. In the course of sampling, precautions, such as correct-sampling technique and limitation of cross contamination, should be taken to avoid any changes which would affect the levels of methylmercury, adversely affect the analytical determination, or make the aggregate samples unrepresentative.
13. Wherever possible, apparatus and equipment coming into contact with the sample should not contain mercury and should 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 to take increment samples and make comminuted samples.

HOMOGENIZATION – GRINDING

14. The complete aggregate sample should be finely comminuted and thoroughly mixed using a process that has been demonstrated to achieve complete homogenization. Depending on the equipment available frozen samples may need to be thawed prior to homogenisation.

TEST PORTION

15. Procedures for selecting the test portion from the comminuted laboratory sample should be a random process. Following homogenization and thorough mixing, the test portion can be selected from any location throughout the comminuted laboratory sample.
16. It is suggested that three test portions be selected from each comminuted laboratory sample. The three test portions will be used for enforcement, appeal, and confirmation if needed.

ANALYTICAL METHODS

17. A criteria-based approach, whereby a set of performance criteria is established with which the analytical method used should comply, is appropriate. The performance criteria-based approach has the advantage that, by avoiding setting down specific details of the method used, developments in methodology can be exploited without having to reconsider or modify the specific method. Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.
18. Refer to The Procedural Manual of the Codex Alimentarius Commission for principles for the establishment of methods of analysis.
19. Method performance criteria for methylmercury and total mercury are detailed for the species of fish for which there are Codex MLs in Annex I.
20. Countries or importers may decide to use their own screening when applying the ML for methylmercury in fish by analysing total mercury in fish. If the total mercury concentration is below or equal to the ML for methylmercury, no further testing is required, and the sample is determined to be compliant with the ML. If the total mercury concentration is above the ML for methylmercury, follow-up testing shall be conducted to determine if the methylmercury concentration is above the ML.

RECONDITIONING LOTS/SUB-LOTS

21. A lot or sub-lot where fish are not of comparable length and/or weight that is separated in to 2 to 3 length and/or weight classes should be analysed sequentially from the largest class first.
22. A lot or sub-lot where fish are not of comparable length and/or weight can be considered in compliance with the ML if the methylmercury concentration of the aggregate sample taken from the highest length and/or weight class is below the ML. However, export or trade requirements (e.g. certificates of analysis) may require testing lots or sub-lots of smaller length and/or weight classes.
23. Where the methylmercury concentration in the aggregate sample taken from a length and/or weight class is above the ML then the next largest length/weight class should also be analysed. If the methylmercury concentration in this sample is below the ML the lot or sub-lot can be reconditioned to remove length and/or weight classes that exceed the ML to ensure the remaining fish are in compliance with the ML.
24. For a lot or sub-lot separated into three length or weight classes paragraph 23 should be repeated for the smallest length/weight classes if the methylmercury concentration in the aggregate sample taken from the middle length/weight class is also above the ML.

ANNEX I
Method performance criteria for methylmercury and total mercury in fish

Species	ML (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	Precision (%)	Recovery (%)	Minimum applicable range (mg/kg)
Alfonsino	1.5	≤ 0.15	≤ 0.30	≤ 30	80-110	0.82 - 2.2
Marlin (all species)	1.7	≤ 0.17	≤ 0.34	≤ 30	80-110	0.95 – 2.5
Orange roughy	0.8	≤ 0.08	≤ 0.16	≤ 33	80-110	0.35- 1.04
Pink cusk-eel	1.0	≤ 0.10	≤ 0.20	≤ 32	80-110	0.52 – 1.5
Shark (all species)	1.6	≤ 0.16	≤ 0.32	≤ 30	80-110	0.88 - 2.3
Tuna (all species)	1.2	≤ 0.12	≤ 0.24	≤ 31	80-110	0.64 – 1.8

ANNEX II

Examples on how to apply provisions in the Sampling Plan

EXAMPLE 1

In case the size and/or weight of the fishes present in the lot differs more than 50 % but less than 100 %: two separate representative samples are taken from each size or weight class/category within a lot.

Example: 5 MT lot of fishes with weights from 2 kg to 3.5 kg.

A first aggregate sample is taken of the smaller sized (lot relative) fishes, which weigh about 2-2.75 kg: 10 incremental samples (fishes) are taken. Each incremental sample is constituted from the muscle meat of the middle part of the fish (slice backbone to belly, symmetrically taken around line B in Figure 1) and weighs about 100 grams. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately.

A second aggregate sample is taken of the larger sized (lot relative) fishes, which weigh about 2.75 -3.5 kg: 10 incremental samples (fishes) are taken. Each incremental sample is constituted from the muscle meat of the middle part of the fish (slice backbone to belly, symmetrically taken around line B in Figure 1) and weighs about 100 grams. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately.

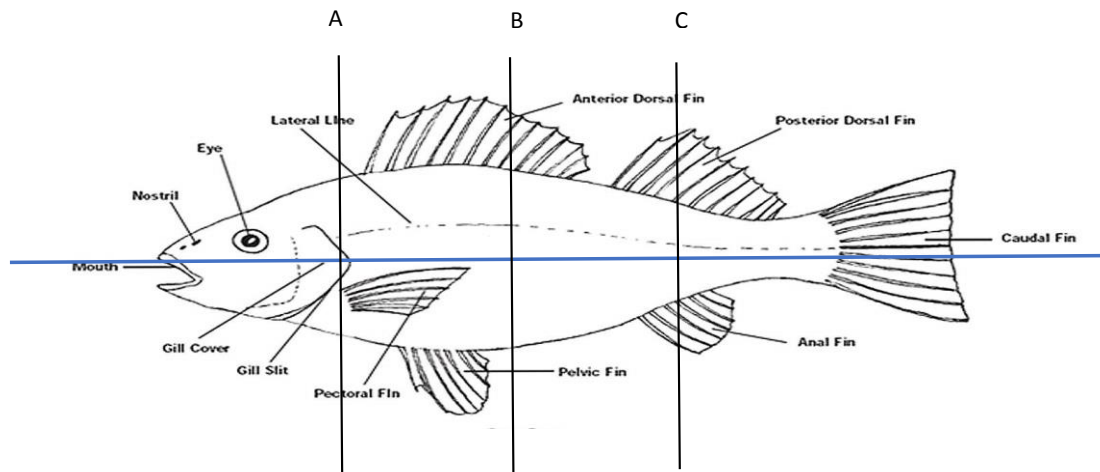


Figure 1: The different sections of a fish.

A) Laboratory performs a sequential analysis:

First the sample of the larger sized fishes is homogenised and analysed separately.

- In case the analytical result is compliant, the whole lot is compliant.
- In case the analytical result is non-compliant, as a second step the sample of the smaller sized fishes is homogenised and analysed separately.
- In case the analytical result of the sample of the smaller sized fishes is non-compliant, the whole lot is non-compliant.
- In case the analytical result of the sample of smaller sized fishes is compliant, then the smaller sized fishes (2-2.75 kg) have to be sorted out and these fishes are compliant. The remaining larger sized fishes (2.75-3.5 kg) are non-compliant.

B) Laboratory analyses both samples at the same time:

- In case both analytical results are compliant, the whole lot is compliant.
- In case both analytical results are non-compliant, the whole lot is non-compliant.
- In case the sample of the smaller sized fishes (2-2.75 kg) is compliant and the sample of the larger sized fishes (2.75-3.5 kg) not, then the smaller sized fishes (2-2.75 kg) have to be sorted out and these small sized fishes are compliant. The remaining larger sized fishes (2.75-3.5 kg) are non-compliant.

EXAMPLE 2

In case the size and/or weight of the fishes present in the lot differs more than 100%: three separate representative samples are taken from each size or weight class/category within a lot

Example: 10 MT lot of fishes with weights from 2 kg to 8 kg.

A first aggregate sample is taken of the smaller sized (lot relative) fishes, which weigh about 2-4 kg: 10 incremental samples (fishes) are taken, each incremental sample is constituted from the muscle meat of the middle part of the fish (slice backbone to belly, symmetrically taken around line B in Figure 1) and weighs about 100 grams. This results in one aggregate sample of about 1 kg, to be homogenised and analysed separately.

A second aggregate sample is taken of the fishes of medium size (lot relative) of about 4-6 kg: 10 incremental samples (fishes) are taken, each incremental sample is constituted from the muscle meat of the middle part of the fish (slice backbone to belly) and weighs about 100 grams. This results in one aggregate sample of about 1 kg, to be homogenised and analysed separately.

A third aggregate sample is taken of the larger sized (lot relative) fishes of about 6-8 kg: 10 incremental samples (fishes) are taken, each incremental sample is

- constituted of the right side dorso-lateral muscle meat in the middle part of the fish (symmetrically around line B in Figure 1 and above the horizontal line in Figure 1) and weighs about 100 grams. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately.

OR

- constituted of equal parts of 50 grams of the muscled meat close to the tail part (the region around line C in Figure 1) and the muscle meat close to the head part of one fish (the region of line A in Figure 1) which are combined to form an incremental sample of about 100 grams per fish. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately.