## JOINT FAO/WHO FOOD STANDARDS PROGRAMME CODEX COMMITTEE ON CONTAMINANTS IN FOODS

$17^{\text {th }}$ Session<br>15-19 April 2024<br>Panama City, Panama<br>SAMPLING PLANS FOR METHYLMERCURY IN FISH

## (At Step 4)

(Prepared by the Electronic Working Group chaired by New Zealand and co-chaired by Canada)

> Codex members and observers wishing to submit comments at Step 3 on sampling plans for methylmercury in fish should do so as instructed in CL 2024/03-CF available on the Codex webpage ${ }^{1}$

## BACKGROUND

1. The conclusions of the $11^{\text {th }}$ Session of the Codex Committee on Contaminants in Foods (CCCF11, 2017) in terms of progressing maximum levels (MLs) for methylmercury in fish identified that they should be accompanied by sampling plans ${ }^{2}$.
2. A general sampling plan for methylmercury in fish was developed using European Union (EU): Commission Regulation (EC) No 333/2007 as a basis. The draft sampling plan was discussed and presented to CCCF12 (2018) accompanying the proposed MLs for various fish species (CX/CF 18/12/7).
3. Following editorial amendments, CCCF12 agreed to send the sampling plans to the Codex Committee on Methods of Analysis and Sampling (CCMAS) for endorsement and to request advice on ${ }^{3}$ :
a. The necessary performance criteria for the MLs;
b. Whether there is evidence that methylmercury can vary widely between individual fish sampled at the same time. How this would apply to large fish sold as individual units and whether the sampling plan provides enough basis to deal with this; and
c. Whether the whole fish should be analysed or only specific fractions of edible portions. Currently only mention is made that the mid-section should be sampled for some large fish.
4. CCMAS39 (2018) was unable to respond to the questions raised in relation to the sampling plan as the questions were outside its remit (CX/CF 19/13/2). CCMAS endorsed the performance criteria for methods of analysis for methylmercury when amended to meet formatting requirements. However, the Committee did not endorse the sampling plan for MLs for methylmercury in fish and agreed to return the sampling plan to CCCF for further consideration.

[^0]5. At CCCF13 (2019) the Chair of the Electronic Working Group (EWG) informed the Committee that a revised sampling plan would not be presented for approval as there were areas of inconsistency with other sampling plans in the General Standard for Contaminants in Foods (CXS 193-1995) that needed to be addressed. In addition, the two remaining questions CCMAS was unable to respond to were not discussed as further consideration was necessary, these questions had also not been discussed by the EWG in advance of CCCF13. The Committee agreed to consider issues related to sampling plans for methylmercury in fish, through the consideration of contemporary scientific literature and national monitoring data, as part of the re-established EWG examining the feasibility of MLs for additional fish species. It was agreed that the EWG would present these findings for consideration at CCCF14. ${ }^{4}$
6. In preparation for CCCF14, the questions posed to CCMAS (para 3a-3c) were posed to the EWG and outcomes presented for consideration at CCCF14. The available body of evidence was presented addressing the two sample plan questions, for which it was confirmed that ongoing collection of information would be needed to develop the sampling plan.
7. At CCCF14 it was agreed to continue further work on the sampling plan following the approach proposed in Appendix III of CX/CF 21/14/11 to include provisions for different weight and values classes and that further work should ensure the practicality of the sampling plan. ${ }^{5}$
8. At CCCF15 (2022) it was agreed to progress further development of the sampling plan based on the approach referred to in CX/CF 22/15/8. This approach included provisions on weight and length, with confirmation of its practicality confirmed by member countries. The consideration of weights and values were proposed; however, it was agreed at that the monetary value of fish would not be included in the provisions of the sampling plan. It was also agreed to request information on national sampling plans for methylmercury or other contaminants in fish through a circular letter (CL) and that the work of CCMAS on the revision of the General Guidelines on Sampling be considered. Noting that sufficient time should be provided to gather information, CCCF agreed that the recommendations for the sampling plans be considered at CCCF17 (2024). ${ }^{6}$
9. The following information was sought from EWG and member countries via Circular Letter (CL 2022/47-CF) issued in September 2022 (deadline for comments December 2022):
a. National sampling plans available for mercury in fish, or other contaminants in fish, in particular: tuna, shark, alfonsino, and marlin, orange roughy and pink cusk-eel. Specific details requested include but were not limited to how and where the material has been sampled, typical ranges of commercial lot sizes and the feasibility of reconditioning sub-lots.
b. Data or studies from primary literature available on the distribution of mercury laterally and from top (dorsal) to bottom (ventral) for tuna, shark, alfonsino, marlin, orange roughy and pink cusk eel.
10. The proposed sampling plan is submitted for consideration by CCCF17 in Appendix I. Examples on how to apply to provisions in the sampling plan is presented in Appendix II to facilitate submission of comments. Appendix III presents a summary of key points of discussion in the EWG from 2023-2024 (paragraphs 26-37) including an assessment of the data/information provided in reply to CL 2022/47-CF (paragraphs 10-21) that supports the proposed sampling plans as presented to CCCF17 for consideration. Comments in reply to this circular letter are contained in Appendix IV.

## CONCLUSION[S]

11. Sampling plan for methylmercury in fish is being proposed based on the information available to date and information provided by certain member countries.
12. More information from members countries would be needed to address outstanding data gaps, especially on the methylmercury distribution in fish tissues for the species/groupings of fish which have established MLs , and data to confirm the practicality of the sampling plan. However, commitment from member countries to collect such data would be needed.
[^1]
## RECOMMENDATIONS

13. CCCF17 is invited to consider the sampling plan for methylmercury as presented in Appendix I and determine whether the sampling plan:
(i) can be recommended for final adoption to Step $5 / 8$ based on the data/information provided in the discussion paper (Appendix III) and the examples provided in Appendix II.
(ii) should be further developed in order to continue considering aspects raised in paragraphs 32,55 and 56 (Appendix III).

## APPENDIXI

PROPOSED SAMPLING PLAN FORMAT FOR METHYLMERCURY CONTAMINATION IN FISH

## (For comments at Step 3)

## GENERAL CONSIDERATIONS

DEFINITION
$\left.\begin{array}{|l|l|}\hline \text { Lot } & \begin{array}{l}\text { An identifiable quantity of a food commodity delivered at one time and } \\ \text { determined by the official to have common characteristics, such as origin, } \\ \text { variety, type of packing, packer, consignor, or markings. } \\ \text { A lot of whole fish should consist of one species and the length and/or } \\ \text { weight should be comparable. In case the length and/or weight of the fish is } \\ \text { not comparable, the consignment may still be considered as a lot, but a } \\ \text { specific sampling procedure has to be applied (as described in paragraph 8). }\end{array} \\ \hline \text { Sublot } & \begin{array}{l}\text { Designated part of a larger lot in order to apply the sampling method on that } \\ \text { designated part. Each sub-lot must be physically separate and identifiable. }\end{array} \\ \hline \text { Sampling plan } & \begin{array}{l}\text { It is defined by a methylmercury test procedure and an accept/reject level. A } \\ \text { methylmercury test procedure consists of three steps: sample selection, } \\ \text { sample preparation and methylmercury quantification. The accept/reject } \\ \text { level is a level usually equal to the Codex maximum level (ML). } \\ \text { Countries or importers may decide to use their own screening when } \\ \text { applying the ML for methylmercury in fish by analysing total mercury in fish. } \\ \text { If the total mercury concentration is below or equal to the ML for } \\ \text { methylmercury, no further testing is required, and the sample is determined } \\ \text { to be compliant with the ML. If the total mercury concentration is above the } \\ \text { ML for methylmercury, follow-up testing shall be conducted to determine if } \\ \text { the methylmercury concentration is above the ML. }\end{array} \\ \hline \text { Test portion } & \begin{array}{l}\text { The quantity of material taken from a single random place in the lot or sub- } \\ \text { lot. }\end{array} \\ \hline \text { Aggregate sample } & \begin{array}{l}\text { The combined total of all the incremental samples that is taken from the lot } \\ \text { or sub-lot. The aggregate sample has to be at least as large as the laboratory } \\ \text { sample or samples combined. }\end{array} \\ \hline \text { A portion of the comminuted laboratory sample. The entire laboratory } \\ \text { sample should be comminuted in a mill. A portion of the comminuted } \\ \text { laboratory sample is randomly removed for the extraction of the } \\ \text { methylmercury for chemical analysis. }\end{array}\right\}$

## 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 $15-30 \mathrm{MT}$ 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 $\left(\mathrm{MT}^{\text {a }}\right)$ | Weight or number of <br> sub-lots (MT) |
| :--- | :--- | :--- |
| Fishery products <br> (traded as bulk <br> consignments) | $\geq 1500$ | 500 |
|  | $>300$ and $<1500$ | 3 sub-lots <br> (minimum 100 MT$)$ |
|  | $\geq 100$ and $\leq 300$ | 100 |
|  | $<100$ | - |

${ }^{\text {a }} 1$ metric tonne $(\mathrm{MT})=1000$ kilograms
Table 2. Subdivision of sublots according to other products lot weight.

| Commodity | Lot weight (MTa) | Weight or number of <br> sub-lots (MT) |
| :---: | :--- | :--- |
| Fish (traded as non- <br> bulk consignments) | $\geq 15$ | $15-30$ |
|  | $<15$ | - |

${ }^{\text {a }} 1$ metric tonne $(\mathrm{MT})=1000$ 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 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 suggested minimum weight of the incremental sample should be an approximate division of the minimum aggregate sample based on the number of incremental samples taken from the lot as specified in Table 2 ( 100 g ) resulting in an aggregate sample of at least 1 kg . 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

| Lot weight (MTa) | Number of incremental <br> samples | Minimum laboratory <br> sample weight (kg) |
| :--- | :--- | :--- |
| $\leq 0.05$ | 3 | 1 |
| $>0.05-\leq 0.5$ | 5 | 1 |
| $>0.5-\leq 1$ | 10 | 1 |
| $>1-\leq 3$ | 20 | 1 |
| $>3-\leq 10$ | 40 | 1 |
| $>10-\leq 20$ | 60 | 1 |
| $>20$ | 100 | 1 |

[^2]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:
a. 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.
b. 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 Appendix 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 |
| :--- | :--- |
| $<1 \mathrm{~kg}$ | Whole fish (after removing the digestive tract) <br> For lots of 0.05MT or greater where the <br> aggregate sample would exceed 3 kg the midline <br> (halfway between the gill opening and the anus) <br> strip from backbone to belly should be sampled |
| $1-10 \mathrm{~kg}$ | Midline (halfway between the gill opening and <br> the anus) strip from backbone to belly <br> For lots of 0.05 MT or greater where the <br> aggregate sample would exceed 3kg, the muscle <br> close to the tail |
| $>10 \mathrm{~kg}$ | Equal composite of muscle from behind the head <br> and close to the tail |
| $>10 \mathrm{~kg}$ (significant commercial value) | 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.

## 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. Performance criteria are detailed for the species of fish for which there are Codex MLs in Annex 1 .
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 (CXS 193-1995; REP18/CF).

## 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 that are not of comparable length and/or weight and the aggregate sample is taken from the highest length and/or weight class can be considered in compliance if the methylmercury concentration 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.
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 criteria for methylmercury in fish with MLs.

| Species | ML (mg/kg) | LOD (mg/kg) | LOQ (mg/kg) | Precision (\%) | Recovery (\%)Minimum <br> applicable <br> range (mg/kg) |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Alfonsino | 1.5 | $\leq 0.15$ | $\leq 0.30$ | $\leq 30$ | $80-110$ | $0.82-2.2$ |
| Marlin (all <br> species) | 1.7 | $\leq 0.17$ | $\leq 0.34$ | $\leq 30$ | $80-110$ | $0.95-2.5$ |
| Orange roughy | 0.8 | $\leq 0.08$ | $\leq 0.16$ | $\leq 30$ | $80-110$ | $0.35-1.04$ |
| Pink cusk-eel | 1.0 | $\leq 0.1$ | $\leq 0.2$ | $\leq 32$ | $80-110$ | $0.52-1.5$ |
| Shark (all <br> species) | 1.6 | $\leq 0.16$ | $\leq 0.32$ | $\leq 30$ | $80-110$ | $0.88-2.3$ |
| Tuna (all <br> species) | 1.2 | $\leq 0.12$ | $\leq 0.24$ | $\leq 31$ | $80-110$ | $0.64-1.8$ |

## APENDIX II <br> (For information)

## EXAMPLE 1

In case the size and/or weight of the fishes present in the lot differs more than $\mathbf{5 0} \%$ but less than $\mathbf{1 0 0} \%$ : 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: 40 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 : 40 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 noncompliant.
- In case the analytical result of the sample of smaller sized fishes is compliant, then the smaller sized fishes (22.75 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 ( $2-2.75 \mathrm{~kg}$ ) is compliant and the sample of the larger sized fishes (2.75-3.5 kg) not, then the smaller sized fishes ( $2-2.75 \mathrm{~kg}$ ) have to be sorted out and these small sized fishes are compliant. The remaining larger sized fishes ( $2.75-3.5 \mathrm{~kg}$ ) 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: 40 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 $4-6 \mathrm{~kg}$ : 40 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 $6-8 \mathrm{~kg}$ : 3 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 350 grams. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately.

OR

- constituted of equal parts of 175 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 350 grams per fish. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately.


## APPENDIX III <br> SUMMARY REPORT <br> (For information)

## Advancements in the work 2022-2023

## Information available to determine appropriate size classes

1. It was recommended the proposed size classes could be further refined through the consideration of commercial weights for those species with MLs and national sampling plans.
2. EWG members were requested to provide information to determine appropriate size classes. No information was available, one member commented that information on the weight range of captured specimens/individual fish would be needed if sampling information is going to be provided for different weight classes of fish for which there are Codex MLs.
3. One member provided a link to seafood handling guidelines which included a size grading schedule for all major species (Sydney Fish Market Pty Ltd, 2015). In this grading schedule, the following species have size ranges allocated for extra small, small, medium, large extra-large and extra extra-large; measured at total fish length, centimetres (cm):
a. Alfonsino (small $<35 \mathrm{~cm}$, medium $35-40 \mathrm{~cm}$, large $>40 \mathrm{~cm}$ )
b. Pink cusk-eel (extra small $<40 \mathrm{~cm}$, small $40-50 \mathrm{~cm}$, medium $50-68 \mathrm{~cm}$, large $68-90 \mathrm{~cm}$, extra large $>90 \mathrm{~cm}$ )
c. Orange roughy (small $27-30 \mathrm{~cm}$, medium $30-40 \mathrm{~cm}$, large $>40 \mathrm{~cm}$ )
d. Bigeye tuna \& Yellowfin tuna (small <20kg, medium $20-40 \mathrm{~kg}$, large $>40 \mathrm{~kg}$ )
e. Sharks (Angel, Eastern fiddler; small $<50 \mathrm{~cm}$, medium $50-70 \mathrm{~cm}$, large $>70 \mathrm{~cm}$ )
f. Sharks (Whaler; small $>60 \mathrm{~cm}$, medium $60-80 \mathrm{~cm}$, large $>80 \mathrm{~cm}$ )
g. Sharks (Greeneye dogfish; small $<50 \mathrm{~cm}$, medium $50-60 \mathrm{~cm}$, large $60-70 \mathrm{~cm}$, extra-large $>70 \mathrm{~cm}$ )
4. Fish that are graded by length and weight prior to export as whole or further processed fish would be expected to show smaller variations in methylmercury. Where grading does not occur, further processed fishery products that are drawn from a broad range of fish sizes and catches from different regions may have larger variation in the methylmercury concentration in the lot. Where such products are sold by portions it may not be possible to address methylmercury variation through sampling of different weight or length classes given this information in unlikely to be unavailable.

## Mercury concentrations in fish tissue samples from different locations on the fish

5. A request for information was issued for any studies identifying the distribution of total mercury of methylmercury in muscle sampled from different areas of fish. Only one additional study on bluefin tuna was provided for EWG consideration.
6. A study by Piras et al. (2020) found that for bluefin tuna (Thunnus thynnus), the site 'anterior extremity of the upper loin' is representative of the mercurial content average of whole fish white muscular tissues. This finding is comparable to studies previously commented on by CCCF14 for data on bluefin tuna, which showed minimal variation between different sections of farmed fish, although between different muscle tissues that have varying lipid contents, there was notable variation (CX/CF 21/14/11).
7. As with previous studies reviewed by CCCF14, it was acknowledged that the production of a composite sample of a large size tuna would be expensive and time consuming.
8. The Piras et al. (2020) paper is generally consistent with respect to large fish with indications given in the Commission Regulation (EC) 333/2007 ${ }^{1}$ (B.2.3) and EC 2017/644 ${ }^{2}$ (3) upon which the sampling plan proposed is based upon. The sampling requirements advise to stratify sampling location based upon the size of the fish:

[^3]a) For fish sizes below 1 kg the whole fish is taken, unless this causes the aggregate sample to be too large ( $>3 \mathrm{~kg}$ ) in which case the middle part is used.
b) For fish of around 1 kg the middle part of the fish is taken for the incremental sample (of at least 100 g).
c) For fish of 1-6 kg the incremental sample is taken from a midline strip from backbone to belly (line B in Figure 1)
d) For fish > 6 kg the sample is taken from the right side dorso-lateral muscle meat in the middle of the fish (symmetrically around line B in Figure 1 and above the horizontal line in Figure 1), except if this will result in significant damage whereby the aggregate sample can be made up of three incremental samples of 350 g taken equally from the muscle close to the head, and close to the tail.

Figure 1. Sampling locations and instructions for determination of lateral variation of total and methylmercury in fish (obtained from CX/CF 21/14/11)


Measuring from the mouth to the start of the caudal fin (tail), divide fish lengthwise into four equal parts as depicted by the solid lines $A, B$ and $C$. Cut $\sim 2 \mathrm{~cm}$ either side of the lines $A, B$ and $C$ to obtain sufficient tissue for the analytical method.
9. No further studies were available for consideration on species/groupings of fish for which there are MLs for methylmercury established.

## Call for data outcomes

10. Seven member countries or organizations (Canada, Egypt, European Union, Japan, Peru, Saudi Arabia and Thailand) responded to the Circular Letter (2022/47-CF) which called for information on national sampling plans. Responses are summarised and key themes presented below (CX/CF 24/17/6).

## How lots of fish that are not of comparable length/weight are sub-divided into sub-lots for sampling

11. One member indicated 5 fish or fish products for a lot of fish is sampled, and the size distribution proportionate to the sizes of the fish in the lot. Tissue from the 5 sub-samples are composited to produce one result. A sample unit can be on animal, one package or one 'grab sample' with a minimum of 100 g of product. For fish that are consumed whole (e.g., capelin) or if once filleted and skin removed, there is insufficient tissue to make up the sample, the whole fish is sampled. For larger fish, three steaks in total are taken, each a transverse section of the fish at nape, mid-fish and tail regions.
12. The European Union sampling plan for mercury in fish (EC No. 333/2007) account for variation in weight/sizes of fish by separating a lot into either two weight or size classes, where overall variation across the lot in weight/size is $50-100 \%$; or three weight or size classes, where overall variation in weight/size is $>100 \%$. Separate aggregate samples are composited from incremental sampling of each weight or size class. The guidance also refers to sequential analysis of the aggregate samples from the largest size class first before descending to smaller size classes to establish conformity of the whole or parts of the lot. Should a size class be compliant with the ML then smaller size classes are also considered compliant.
13. The Department of Fisheries (DoF) Ministry of Agriculture and Cooperatives of Thailand developed their sampling plan according to the International Standard ISO 2859-1: 1999 Sampling procedures for inspection by attributes - Part 1: Sampling plans by acceptance quality level (AQL) for lot-by-lot inspection and work instruction for sampling fish and fishery products of the Fish Inspection and Quality Control Division (FIQD) of DoF methylmercury. Sampling plans are provided for fish as raw material depending on weight of the fish and for fishery products depending on the types of fishery products (chilled and frozen products; Table 1). The sampling procedure involves taking random samples, each sample consisting of least 5 parts of the fish or fishery product such as the upper, bottom, middle, front and tail.
Table 1. Sampling plans for fish as raw material and fishery products

| Weight of fish $\leq 5 \mathbf{k g}$ |  | Weight of fish $\geq 5 \mathrm{~kg}$ |  | Chilled and frozen fish |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Lot size (t) | Number of <br> samples <br> (fish) | Lot size (t) | Number of <br> samples <br> (fish) | Lot sizes <br> (packages) | Number of <br> samples <br> (packages) |
| 15 or less | 2 | 25 or less | 2 | 25 or less | 2 |
| $16-50$ | 3 | $26-150$ | 3 | $26-150$ | 3 |
| $51-150$ | 5 | $151-1200$ | 5 | $151-1200$ | 5 |
| $151-500$ | 8 | $1200-35,000$ | 8 | $1200-35,000$ | 8 |
| $501-3200$ | 13 | 35,0001 and <br> over | 13 | 35,0001 and <br> over | 13 |
| 3201 and <br> over | 20 |  | 20 |  |  |

14. Another member provided information on their procedure for sampling fish and crustaceans from aquaculture ${ }^{3}$ which are conducted annually. The annual sample size is based on the annual harvest volumes of fish and crustaceans from aquaculture at the national level. The minimum samples collected each year must be at least equal to 1 per 100 tons of annual production.
15. In consideration of the implications of taking samples from a location i.e. middle part of the fish which would result in significant economic damage, two member countries provided responses. One member country suggested that for large and expensive fish such as tuna, that sampling could be conducted from the muscle close to the tail in view of the economic implications as well as health protection of consumers. Their study findings in 2020 and 2021 of bluefin and bigeye tuna indicated that the average mercury concentrations between the seven parts (dorsal front, middle and rear; ventral front, middle and rear, and tail)) of the tuna analysed were significantly different, however, there was no statistically significant difference in methylmercury concentrations between the muscle of the tail part and the mean of the muscle of the other six parts.
16. Another member country proposed an alternative option for sampling fish that would result in significant damage if the sampling fraction was taken from the right side dorso-lateral muscle meat in the middle part of the fish. This meant three incremental samples of at least 350 grams may be sufficient independent of the lot or alternatively three incremental samples of at least 350 grams from an equal part ( 175 grams) of the muscle meat close to the tail part and the muscle meat close to the head part of each fish.
[^4]
## Information requested where there was limited/no information provided

17. No estimates of commercial size lots were provided.
18. No information on the typical size ranges of commercially harvested fish for which Codex MLs exist were provided.
19. Reconditioning (sorting of fish) information was included in one member country's sampling plan, for which it means removing the length/weight of class(es) that would exceed an ML so that the remaining fish would be in compliance. This approach was considered in previous iterations of the sampling plan and has been retained in Appendix I.
20. No further information was provided on the feasibility of reconditioning lots.
21. No further information was provided on the distribution of mercury laterally and from top (dorsal) to bottom (ventral) for the species of interest.

## Revised General Guidelines on Sampling

22. The Codex Committee for Methods and Analysis (CCMAS) agreed to forward the revised General Guidelines on Sampling (CXG 50-2004) to CAC 46 for adoption as per REP23/MAS. The revised guidelines reflect current scientific and statistical approaches for the development and evaluation of sampling plans.
23. It was agreed to inform relevant Codex committees to request that they review their sampling plans in light of the revised guidelines, and that sampling plans should be developed as needed in compliance with the General Guidelines on Sampling.
24. In order to facilitate the understanding and implementation of the revised guidelines, a supporting information document, namely, an e-book with the sampling plan applications, will be completed in 2023-2024. The information document will address more detailed examples on measurement uncertainty (MU) and some practical examples of sampling plans.
25. CCMAS as per the General Guidelines on Sampling (3.2.2) recommend that when commodity committees have included sampling plans in a Codex commodity standard, that these should be referred to CCMAS for endorsement along with relevant information to the sampling plan.

## 2024 Discussions and conclusions

26. The EWG commented on the updates added in this paper, four members (Brazil, Canada, Japan and the United States) provided comments on the sampling plan.
27. Two options were presented for the EWG to consider in light of the information available to date, they were to indicate their preference for or against.
28. The first option was to accept the current iteration of the sampling plan to put forward to plenary whilst noting; and acknowledgement that minor amendments are required for progressing the sampling plan further.
29. The basis for the proposal of the first option was the current data gap on methylmercury distribution in different fish tissues for the species/groupings of fish which have established MLs, with existing information available only for certain tuna species. The opportunities were noted for progressing acceptance of sampling plan, this included the opportunity for member countries to utilise the sampling plan and in turn provide recommendations for its refinement, and most importantly to standardise a fish sampling location for fish which varies in consideration of current available national protocols.
30. A note to further revise the sampling plan in 4-5 years as new data becomes available was considered. However, with no possible indications that member countries will collect data on mercury distribution in fish for which there are MLs, the intent would be void.
31. An alternative option put forward was to postpone development of sampling plan for 4-5 years, given the issues on practicality, size and weight provisions for which data to refine further are not available. This option was not supported by members as the Codex MLs for methylmercury in fish have been in place for a number of years and a corresponding plan should be in place. One member reiterated that additional information on the practicality of implementing the approaches discussed in Appendix I, such as sorting lots by weight or size to obtain representative samples.
32. All four EWG members considered the sampling plan acceptable, on the provision the following are considered. A further note is made on how EWG members feedback have been addressed and outstanding issues and data gaps are referred to in paragraphs 44 and 45.
a. That an option for sampling from the muscle close to the tail for fish weighing over >10kg in the sampling plan be included, as it is supported by evidence and from a practical standpoint achievable for inspectors to prepare samples. This has been included in Table 4 of the sampling plan.
b. That the execution of the sampling plan could be difficult for lots of large fishes, especially when the lot has a huge individual length variation. This has been addressed in the sampling plan (Appendix I) which targets sampling to a length/weight class that is either representative of the lot/sub-lot and or in the case of notable variation separate the lot to provide representative samples of each weight and size range. However, it is noted by one member that the execution and practicality of the approach might not be feasible.
c. The suitability of the tissue sampling locations for the 3 weight classes presented in the sampling plan for all fish species. This has not been addressed for all species for which MLs have been established due to lack of data on methylmercury distribution in some species.
d. That additional information is needed on implementing the approaches discussed including sorting lots by weight/size to obtain representative samples and obtaining a representative mercury concentration based on the location of the sampling site relative to the length/weight of the fish. It is critical that the approaches adopted are practical reflecting the CCMAS General Guidelines for Sampling that note "that sampling should be practical in terms of cost of sampling and testing and ease of use." No information is available on implementing the approaches discussed in the sampling plan, however the current sampling plan is based on a national sampling plan (European Union: Commission Regulation (EC) No 333/2007) with minor edits that has been in place for some time.
33. Issues with the practicality of sampling plan were also raised, mainly around the approach for reconditioning lots of fish and the sampling locations for obtaining a sample for the whole fish based on a weight class. One member noted that it would likely be expensive or impractical to recondition lots on the approach proposed, instead it was suggested that for fish for no predominant weight or size class, that incremental samples should be collected from individuals of a size class in a proportion corresponding to sizes variation observed.
34. A difficulty in establishing the sampling plan is that the datasets from which species MLs have been set upon is not standardised in terms of sampling location, with this differing on the national sampling protocols and practices. Consequently, the datasets from species were MLs have been established will encompass different forms of sampling which may have resulted in a variation of methylmercury concentrations.
35. The differences in sizes amongst the species/groupings for which MLs have been established is considerable (paragraph 3 ; alfonsino $<50 \mathrm{~cm}$, Atlantic blue marlin up to 500 cm ) and within groupings the variability in size may also be large (bullet tuna: $\sim 50 \mathrm{~cm}$; bluefin tuna: $\sim 200 \mathrm{~cm}$ ). As a result of these differences, using a general sampling plan to encompass the four species/groupings of fish would therefore be difficult. An approach to develop species specific annexes was suggested but not favoured (REP21/CF).
36. To address suggestions from EWG members, subdivision of sub-lots for fishery products traded as non-bulk consignments have been added back into the sampling plan taken from the EWG discussion paper in preparation for CCCF14, including additional information on the minimum number of incremental samples (Tables 1-3, Appendix I)
37. General amendments and clarifications were suggested by members were incorporated where possible.

## Sampling plan

38. A general set of provisions around length/weight classes for sampling methylmercury in fish is proposed in Appendix I.
39. Fish are considered of comparable size and weight where either parameter does not vary more than $50 \%$ across the lot. Where fish size varies more than this but $80 \%$ or more of the fish in lot are within the same size or weight class then this weight class is considered representative and thus the incremental samples are taken only from fish in this class. Where there is no predominant weight or size class then the lot is separated into either two weight or size classes, where overall variation across the lot in weight/size is $50-100 \%$; or three weight or size classes, where overall variation in weight/size is $>100 \%$. Separate aggregate samples are composited from incremental sampling of each weight or size class.
40. This approach accounts for the variation as lots/sub-lots of whole fish or dressed fish may show considerable length/weight variation and consequently methylmercury variation across the lot.
41. The sampling scheme differentiates sampling location based on the size of the fish, this ensures that the sampling is targeted to tissues most representative of the whole fish except for species (typically the largest) whether this would cause significant economic loss to the whole carcass and/or impractical to conduct.
42. The commercial catch weight of species for which MLs are set identifies stratification in the weight classes of $1-10 \mathrm{~kg}$ (alfonsino, small tuna species and dogfish) and $>10 \mathrm{~kg}$ (marlin, large tuna species, large shark species) (CX/CF 21/14/11). The sampling scheme separates weight classes into three categories (10kg) and provides a simple graduated approach to balancing intent to derive a representative sample against limiting economic loss from sampling damaging high value marketable cuts or carcasses.
43. In regard to the practicality, the General Guidelines on Sampling note that is important to ensure that any sampling plan chosen will be practical in terms of cost of sampling and testing and ease of use.

## Data gaps and outstanding issues

44. Following data gaps remain;

- Data on the methylmercury distribution in different fish tissues for the species/groupings of fish which have established MLs, as the only information available is for tuna.
- Data to confirm the practicality of the sampling plan., i.e. the feasibility of reconditioning lots, and the estimates of commercial size lots.

45. Following outstanding issues raised by EWG members that could not be addressed due to lack of information;

- That the execution of the sampling plan could be difficult for lots of large fishes, especially when the lot has a huge individual length variation. That additional information is needed on implementing the approaches discussed including sorting lots by weight/size to obtain representative samples and obtaining a representative mercury concentration based on the location of the sampling site relative to the length/weight of the fish. It is critical that the approaches adopted are practical reflecting the CCMAS General Guidelines for Sampling that note "that sampling should be practical in terms of cost of sampling and testing and ease of use."
- The suitability of the tissue sampling locations for the 3 weight classes presented in the sampling plan for all fish species.


## References

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## APPENDIXIV

## Comments received in reply to CL 2023/47-CF <br> (For information) <br> ORIGINAL LANGUAGE ONLY

## COMMENT

a) The information provided is used in Canada, by the Canadian Food Inspection Agency (CFIA), to sample all types of fish for the analysis of all types of contaminants.
The sampling parameters provided apply to the CFIA's sampling of all imported and domestic fish species, both fresh and frozen, as well as processed fish products (e.g. canned fish), that are sold for human consumption in Canada.
i. Canadian fish inspectors are directed to sample 5 fish or fish products; for a lot of fish, the size distribution should be proportionate to the sizes of fish in the lot. Tissue from the 5 sub-samples is composited and analysed to produce one result.
The sampling guidance provided is: "Each sample should consist of five sample units. A unit can be one animal, one package or one "grab sample" with a minimum of 100 g of product or animals (ex.: one can of lobster meat, one lobster, one eel or 100 g of frozen shrimp in a sample bag). The lab will provide one analytical result, based on the composite of the five sample units.
ii. To date, samples have been collected by competent authority, which is, in Canada, inspectors from the Canadian Food Inspection Agency (CFIA); samples have been collected from commercial fish shipments. Sampling in the future may include contracted sampling (third party) collecting samples from retail outlets.
iii. For small fish that are consumed whole (e.g. capelin), or if, once filleted and the skin removed, there is insufficient tissue remaining to comprise the sample to be homogenized, the whole fish is sampled.
For larger fish not typically eaten whole and from which sufficient tissue can be sampled, three steaks (total) are taken, one from each the nape, mid-fish and tail regions.
Each steak is a transverse section through the fish and therefore includes both dorsal and ventral parts of the fish's body.
iv. Fish and fish products are sampled as presented in commercial shipment.

Small fish that are typically consumed whole (e.g. capelin) or cannot be filleted due to their small size are homogenized whole, including bones and skin. Large fish are filleted and the tissue is sampled; bones and skin are removed as much as possible.
v. Commercial lot sizes are variable. In general, shipments of fresh fish are of smaller fish but are shipped in higher frequency and shipments of frozen fish consist of relatively larger fish and are shipped less frequently.
vi. Information on the typical size ranges of commercially harvested fish for which Codex MLs exist is not available.
vii. Reconditioning (sorting) of fish is not part of the Canadian sampling plan itself, but would be considered as an option to bring a portion of a lot into compliance.
viii. The Canadian Food Inspection Agency is in the process of compiling and publishing the results for mercury and other metals collected by the Agency since 2000. Making these results publicly available will provide industry data on mercury, and other metal levels, by species, to inform their processing and distribution decisions.

Once these data are publicly available, Canada could provide a link to the database where it is housed.
Data on the distribution of mercury in fish, either laterally or vertically, is not available from Canada's sampling results.

## MEMBER/OBSERVER

| COMMENT |
| :--- |
| Egypt's Data for the CCCF17: CL 2022/47-CF - Request for information on national sampling plans for methylmercury in fish or other contaminants in fish, <br> is already sent via email. |

Following the request to submit information on the Codex Members' sampling plans to further develop the sampling plan for methylmercury in fish, the European Union would like to submit the following information on the existing European Union sampling plans for mercury in fish, which can also be consulted in Regulation (EC) No 333/2007 (https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1664547253349\&uri=CELEX\%3A32007R0333).
i. How lots of fish that are not of comparable length or weight are sub-divided into sub-lots for sampling

Large lots shall be divided into sublots on the condition that the sublot may be separated physically in accordance with 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 sublot may exceed the mentioned weight by a maximum of $20 \%$.
Table 1
Subdivision of lots into sublots for products not traded in bulk consignments
Lot weight (ton) Weight or number of sublots
$\geq 15 \quad 15-30$ tonnes
< 15 -
ii. How samples are taken

* Number of incremental samples

For fish the aggregate sample shall be at least 1 kilogram. For fish, the minimum number of incremental samples to be taken from the lot or sublot shall be in accordance with Table 2.
Table 2
Minimum number of incremental samples to be taken from the lot or sublot of food, other than food supplements
Weight or volume of lot/sublot (in kilogram) Minimum number of incremental samples to be taken
< 503
$\geq 50$ and $\leq 500 \quad 5$
$>500 \quad 10$
The incremental samples shall be of similar weight/volume.
For fish an incremental sample shall be at least 100 grams, resulting in an aggregate sample of at least about 1 kilogram.

* Specific provisions for sampling of lots of fish containing whole fish of comparable size and/or weight

The number of incremental samples to be taken from the lot is set out in Table 2. The aggregate sample uniting all incremental samples shall be at least 1 kilogram.

## MEMBER/OBSERVER

Egypt

European Union

## COMMENT

- Where the lot to be sampled contains small fish (individual fish weighing < 1 kilogram), the whole fish is taken as incremental sample to form the aggregate sample. Where the resulting aggregate sample weighs more than 3 kilogram, the incremental samples may consist of the middle parts of the fish, weighing each at least 100 grams, forming the aggregate sample. The whole part to which the maximum level is applicable, is used for homogenisation of the sample.
The middle part of the fish is where the centre of gravity is. This is located in most cases at the dorsal fin (in case the fish has a dorsal fin) or halfway between the gill opening and the anus.
- Where the lot to be sampled contains larger fish (individual fish weighing $\geq 1$ kilogram), the incremental sample consists of the middle part of the fish. Each incremental sample weighs at least 100 grams.
For fish of intermediate size ( $\geq 1$ kilogram and < 6 kilogram) the incremental sample is taken as a slice of the fish from backbone to belly in the middle part of the fish.
For very large fish ( $\geq 6$ kilogram), the incremental sample is taken from the right side (frontal view) dorso-lateral muscle meat in the middle part of the fish. Where the taking of such a piece of the middle part of the fish would result in a significant economic damage, the taking of three incremental samples of at least 350 grams each may be considered as being sufficient independent of the size of the lot or alternatively three incremental samples of at least 350 grams each from an equal part ( 175 grams) of the muscle meat close to the tail part and the muscle meat close to the head part of each fish may be considered as being sufficient independent of the size of the lot.';
*Specific provisions for sampling of lots of fish containing whole fish of different size and/or weight
The provisions for sampling of lots of fish containing whole fish of comparable size and/or weight shall apply.
Where a size or weight class/category is predominant (about $80 \%$ or more of the lot), the sample is taken from fish with the predominant size or weight. This sample is to be considered as being representative for the whole lot.
Where no particular size or weight class/category predominates, then it shall be ensured that the fish selected for the sample are representative for the lot. Specific guidance for such cases and examples are provided in 'Guidance document on sampling of whole fish of different size and/or weight' (https://food.ec.europa.eu/system/files/2022-05/cs contaminants sampling guid-samp-fishes.pdf).
For batches of fishes of different size and/or weight, in case no particular size or weight class/category predominates, the following sample procedure is proposed:

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.
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.
The laboratory may perform a sequential analysis on the samples of the different size/weight classes/categories of one lot, whereby the sample representing the largest fishes is analysed first.

- In case the analytical result of this sample is compliant with the maximum level, the whole lot is considered to be compliant.
- In case the analytical result of this sample is exceeding the EU maximum level, then the sample taken from the medium size fishes is analysed.
o In case this analytical result is compliant then no analysis is necessary of the sample taken from the smallest size fishes (in case the lot is divided into three size classes).


## COMMENT

o In case the analytical result of the sample of the medium size fishes is non-compliant with the EU maximum level, in case of three separate samples, then the sample from the smallest size fishes is analysed.
Based on the analytical results of one or more samples, the whole or parts of the lot can be accepted or rejected.

## EXAMPLES

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 ton 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 $2.75-3.5 \mathrm{~kg}: 10$ 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 ( $2.75-3.5 \mathrm{~kg}$ ) 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 ( $2-2.75 \mathrm{~kg}$ ) is compliant and the sample of the larger sized fishes ( $2.75-3.5 \mathrm{~kg}$ ) not, then the smaller sized fishes ( $2-2.75 \mathrm{~kg}$ ) have to be sorted out and these small sized fishes are compliant. The remaining larger sized fishes ( $2.75-3.5 \mathrm{~kg}$ ) are non-compliant. 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 ton 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.

## COMMENT

A second aggregate sample is taken of the fishes of medium size (lot relative) of about $4-6 \mathrm{~kg}$ : 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: 3 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 350 grams. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately. OR
- constituted of equal parts of 175 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 350 grams per fish. This results in one aggregate sample of about 1 kg to be homogenised and analysed separately.
A) The laboratory performs a sequential analysis:

First the sample of the larger sized fishes ( $6-8 \mathrm{~kg}$ ) 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 medium sized fishes ( $4-6 \mathrm{~kg}$ ) is homogenised and analysed separately.
-- In case the analytical result of the sample of medium sized fishes ( $4-6 \mathrm{~kg}$ ) is compliant, then the larger sized fishes ( $6-8 \mathrm{~kg}$ ) have to be sorted out and these fishes ( $6-8 \mathrm{~kg}$ ) are non-compliant. The remaining smaller ( $2-4 \mathrm{~kg}$ ) and medium sized ( $4-6 \mathrm{~kg}$ ) fishes are compliant.
-- In case the analytical result of the sample of medium sized fishes ( $4-6 \mathrm{~kg}$ ) is non-compliant, as a third step the sample of the smaller sized fishes (2-4 kg) is homogenised and analysed.
-- -- In case the analytical result of the sample of smaller sized fishes ( $2-4 \mathrm{~kg}$ ) is non-compliant, then the whole lot of fish is non-compliant
-- -- In case the analytical result of the sample of smaller sized fishes ( $2-4 \mathrm{~kg}$ ) is compliant, then the smaller fishes ( $2-4 \mathrm{~kg}$ ) have to be sorted out and these fishes ( $2-4 \mathrm{~kg}$ ) are compliant. The remaining medium ( $4-6 \mathrm{~kg}$ ) and larger sized fishes ( $6-8 \mathrm{~kg}$ ) are not compliant.
B) The laboratory analyses all three samples at the same time
- In case all three analytical results are compliant, the whole lot is compliant.
- In case all three analytical results are non-compliant, the whole lot is non-compliant.
- In case the sample of the smaller fishes ( $2-4 \mathrm{~kg}$ ) is compliant and the sample of the medium sized ( $4-6 \mathrm{~kg}$ ) and larger fishes ( $6-8 \mathrm{~kg}$ ) not, then the smaller fishes ( $2-4 \mathrm{~kg}$ ) have to be sorted out and these fishes are compliant. The remaining medium sized ( $4-6 \mathrm{~kg}$ ) and larger sized fishes ( $6-8 \mathrm{~kg}$ ) are noncompliant.
- In case the sample of the smaller ( $2-4 \mathrm{~kg}$ ) and medium sized fishes ( $4-6 \mathrm{~kg}$ ) is compliant and the sample of the larger sized fishes ( $6-8 \mathrm{~kg}$ ) not, then the larger sized fishes ( $6-8 \mathrm{~kg}$ ) have to be sorted out and these fishes ( $6-8 \mathrm{~kg}$ ) are non-compliant. The remaining smaller ( $2-4 \mathrm{~kg}$ ) and medium sized fishes ( $4-6$ kg ) are compliant.
iii. where on the fish the sample is taken, both laterally and top (dorsal) to bottom (ventral)


## COMMENT

- Where the lot to be sampled contains small fish (individual fish weighing < 1 kilogram), the whole fish is taken as incremental sample to form the aggregate sample. Where the resulting aggregate sample weighs more than 3 kilogram, the incremental samples may consist of the middle parts of the fish, weighing each at least 100 grams, forming the aggregate sample. The whole part to which the maximum level is applicable, is used for homogenisation of the sample.
The middle part of the fish is where the centre of gravity is. This is located in most cases at the dorsal fin (in case the fish has a dorsal fin) or halfway between the gill opening and the anus.
- Where the lot to be sampled contains larger fish (individual fish weighing $\geq 1$ kilogram), the incremental sample consists of the middle part of the fish. For fish of intermediate size ( $\geq 1$ kilogram and < 6 kilogram) the incremental sample is taken as a slice of the fish from backbone to belly in the middle part of the fish.
For very large fish ( $\geq 6$ kilogram), the incremental sample is taken from the right side (frontal view) dorso-lateral muscle meat in the middle part of the fish. Where the taking of such a piece of the middle part of the fish would result in a significant economic damage, the taking of three incremental samples of at least 350 grams each may be considered as being sufficient independent of the size of the lot or alternatively three incremental samples of at least 350 grams each from an equal part ( 175 grams) of the muscle meat close to the tail part and the muscle meat close to the head part of each fish may be considered as being sufficient independent of the size of the lot.';
For further details see examples under point ii and figure 1.
iv. the tissues included in the sample (e.g., skin is removed, red muscle tissue should not be sampled, deboned);

The whole part to which the maximum level is applicable is used for homogenisation of the sample. In the EU this means that the fish meat is sampled without the skin, bones or viscera.
vi. typical size ranges of commercially harvested fish for which Codex MLs are established;

- tuna
- shark,
- alfonsino
- marlin
- orange roughy
- pink cusk-eel
vii. if reconditioning sub-lots is practical and feasible; reconditioning involves removing the length/weight class(es) that exceed the ML, so that the remainder of the lot of smaller fish are in compliance; and
For batches of fishes of different size and/or weight, in case no particular size or weight class/category predominates, the following sample procedure is proposed:

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.
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.

## COMMENT

MEMBER/OBSERVER
The laboratory may perform a sequential analysis on the samples of the different size/weight classes/categories of one lot, whereby the sample representing the largest fishes is analysed first.

- In case the analytical result of this sample is compliant with the maximum level, the whole lot is considered to be compliant.
- In case the analytical result of this sample is exceeding the EU maximum level, then the sample taken from the medium size fishes is analysed.
o In case this analytical result is compliant then no analysis is necessary of the sample taken from the smallest size fishes (in case the lot is divided into three size classes).
o In case the analytical result of the sample of the medium size fishes is non-compliant with the EU maximum level, in case of three separate samples, then the sample from the smallest size fishes is analysed.
Based on the analytical results of one or more samples, the whole or parts of the lot can be accepted or rejected. For further details see example under point ii.
viii. information on relevant risk management measures (e.g., catch, sorting) that could be incorporated in into the sampling plan.

Based on the analytical results of one or more samples of different size classes, the whole or parts of the lot can be accepted or rejected.
Following the request for data or studies on the distribution of mercury laterally and from top (dorsal) to bottom (ventral) for tuna, shark, alfonsino, marlin, orange roughy and pink cusk eel, the European Union (EU) would like to inform that it has no such studies available.
From CCCF12, Japan has suggested that for large and expensive fish such as tuna, sampling should be conducted from the muscle close to tail (hereinafter referred to as "the muscle of tail part"), in view of economic implications as well as health protection of consumers (Please refer to CRD07, CCCF14: https://www.fao.org/fao-who-codexalimentarius/sh-
proxy/en/?lnk=1\&url=https\%253A\%252F\%252Fworkspace.fao.org\%252Fsites\%252Fcodex\%252FMeetings\%252FCX-735-
$14 \% 252$ FCRDs $\% 252$ Fcf14_crd07x.pdf). In 2020 and 2021, the Ministry of Agriculture, Forestry and Fisheries of Japan investigated the distribution of concentrations of methylmercury in muscle of different parts of tuna. The data obtained indicate that sampling from the muscle of tail part is appropriate not only from feasibility but also from a scientific standpoint for consistent conformity judgment of the maximum level in each country.
Samples were obtained from the muscle of each of seven parts (dorsal front, middle and rear; ventral front, middle and rear, and tail) of each of five bluefin tuna and five bigeye tuna, and the concentration of methylmercury in sample was determined in accredited laboratories. The measured values were analyzed to determine (1) whether there was a difference in the concentrations among the muscle of seven parts and (2) whether there was a difference in the concentrations between the muscle of tail part and the mean of the muscle of the other six parts. The results are as follows:
(1) Difference in concentration among seven parts

There was a statistically significant difference in methylmercury concentrations among the seven parts.
(2) Difference in concentration between the tail and the mean of the other six parts

There was no statistically significant difference in methylmercury concentrations between the muscle of tail part and the mean of the muscle of the other six parts.

## COMMENT

MEMBER/OBSERVER
These results indicate that although there was a significant difference among the parts, the concentration in the muscle of tail part can be considered representative of the methylmercury concentration in the whole fish for tuna. For large and expensive fish like tuna, sampling from the muscle of tail part is most appropriate because it save the time for the collection of samples from multiple parts and preparing composite samples, thus making sampling faster and simpler. It also reduces economic losses for the food business operators by avoiding the collection of samples from expensive parts. Detailed information on sampling, analytical methods and results of analysis are shown in the Appendix.

Respecto a la información sobre los planes nacionales de muestreo para seguir desarrollando el plan de muestreo para el metilmercurio en el pescado u otros contaminantes en el pescado, el Perú desea expresar que la Autoridad Sanitaria cuenta con planes de control oficial para peces y moluscos, donde se monitorean contaminantes como Metales pesados (cadmio, plomo, mercurio). Los cuales pueden revisarse en los siguientes enlaces:
https://www.sanipes.gob.pe/web/index.php/es/acuicultura/tus-programas/control-de-moluscos-bivalvos
https://www.sanipes.gob.pe/web/index.php/es/pesca/tus-programas/control-oficial-de-productos-hidrobiologicos-nacionales-y-de-exportacion Y para mayor información acerca de los planes de control oficial que se manejan, pueden revisar el Informe de inocuidad, en este enlace:
https://www.sanipes.gob.pe/Informe-de-inocuidad-2017-2019/archivos/INFORME-DE-INOCUIDAD-201-2019.pdf
Usually, the concentration of all forms of mercury correlated with fish age and weight. Most of available studies in literature reports levels of this mercury mainly for muscles. Fewer data are available on the concentrations of Mercury ( Hg ) and its species in other tissues (skin, gills) and organs (liver, kidney, heart, spleen, digestive tract) of marine fish.
Some data distribution of mercury for selected products by the committee found in literature is attached and sent by email.
Sampling plans for contaminants including methylmercury in fish and fishery products of Thailand
The Department of Fisheries (DoF), Ministry of Agriculture and Cooperatives of Thailand, is as a Competent Authority (CA) for regulating and controlling aquaculture production and fishery products. DoF develops the sampling plans for contaminants including methylmercury in fish and fishery products to provide guidance information for sampling. This document is developed according to International Standard ISO 2859-1: 1999 Sampling procedures for inspection by attributes - Part 1: Sampling plans by acceptance quality level (AQL) for lot-by-lot inspection and work instruction for sampling fish and fishery products of the Fish Inspection and Quality Control Division (FIQD) of DoF. Detail of sampling plans is as follows:
Sampling plans for fish as raw material
Each examined lot must be sampled depending on the weight of fish. The numbers of samples may be taken as following provisions laid down in Tables 1 and 2.
Table 1: The numbers of samples to be taken depending on the weight of fish less than or equal to 5 kg
Lot size Inspection Level Number of samples
( t )
(S-3)
(fish)
15 or less A $\quad 2$
16-50 B 3
51-150 C 5
151-500 D 8
501-3,200 E 13
3,201 and over F 20

Peru

## Saudi Arabia

## Thailand



APPENDIX IV<br>List of Participants<br>\section*{Chair}<br>Jeane Nicolas<br>Specialist Adviser Toxicology<br>New Zealand Food Safety<br>Ministry for Primary Industries<br>\section*{Co-chair}<br>Dr Rosalie Awad<br>Head, Food Contaminants Section<br>Bureau of Chemical Safety<br>Health Canada

## Australia

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Larissa Bertollo Gomes Porto
Health Regulation Specialist
Brazil Health Regulatory Agency

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Food Directorate, Health Canada
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Bureau of Chemical Safety, Health Products and Food Branch, Health Canada

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## European Union

Veerle Vanheusden
European Commission

## France

Celine Schmidt
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Karine Bertholon
Ministry of Agriculture

## India

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Plant Protection \& Biosafety
N. Palanikumar

Assistant Director
Export Inspection Council
C.N. Ravishankar

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ICAR-IARI

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## Mexico

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Secretaría de Economía

## Morroco

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Head of Toxicology Hydrolgy \& Forensic Toxicology
Departement
National Institut of Hygiene -Rabat
Varsha Misra
Dy. Director
National Accreditation board for certification Bod

## New Zealand

Fiapaipai Auapaau
Adviser Risk Assessment
Ministry for Primary Industries

## Nigeria

Hassan Modu Shettima
Principal Fishery Officer

## Spain

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Advance Technician of the Contaminants Management
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Spanish Agency for Food Safety and Nutrition

## Türkiye

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The Ministry of Agriculture and Forestry
Ms. Bengi AKBULUT PINAR
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Quynh-Anh Nguyen
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U.S. Food and Drug Administration


[^0]:    1 Codex webpage/Circular Letters:
    http://www.fao.org/fao-who-codexalimentarius/resources/circular-letters/en/.
    Codex webpage/CCCF/Circular Letters:
    http://www.fao.org/fao-who-codexalimentarius/committees/committee/related-circular-letters/en/?committee=CCCF
    REP 17/CF11 para. 140
    REP18/CF12 para. 87

[^1]:    4 REP19/CF13, paras. 124-127

[^2]:    ${ }^{\text {a }} 1$ metric tonne (MT) $=1000$ kilograms

[^3]:    ${ }^{1}$ 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
    https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex\%3A32007R0333
    ${ }^{2}$ https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX\%3A32017R0644

[^4]:    ${ }^{3}$ https://www.gob.pe/institucion/sanipes/normas-legales/2652692-001-2022-sanipes-pe

