



CD/K/526-4:2010  
ICS 67.120.30

## **EAST AFRICAN STANDARD**

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**Test methods for fish and fishery products — Part 4: Determination of total inorganic and organic mercury**

**EAST AFRICAN COMMUNITY**

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## Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that "Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose".

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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## Introduction

This method is applicable to the determination of total, inorganic and organic mercury in fish and fishery products as well as other biological tissues. Inorganic and organic mercury naturally occurs in the environment where fish lives. Over time the organisms can accumulate it in their tissues to a level where if consumed by human being can lead to mercury poisoning. The method will offer the cheapest and reliable means of ascertaining the levels of mercury. The detection limit of this method is 0.002 ppm.

In the preparation of this East African Standard, the following sources were consulted extensively:

KS 1754-4:2003, *Test methods for fish and fishery products — Part 4: Determination of total, inorganic and organic mercury*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

IS 4303-1:1975, *Code of hygienic conditions for fish industry — Part 1: Pre-processing stage*

IS 4303-2:1975, *Code of hygienic conditions for fish industry — Part 2: Canning stage*

Codex Alimentarius website: [http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd\\_q-e.jsp](http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp)

USDA Foreign Agricultural Service website: <http://www.mrldatabase.com>

USDA Agricultural Marketing Service website: <http://www.ams.usda.gov/AMSV1.0/Standards>

European Union: [http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index\\_en.htm](http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm)

Assistance derived from these sources is hereby acknowledged.

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## Test methods for fish and fishery products — Part 4: Determination of total inorganic and organic mercury

### 1 Scope

This method is applicable to fish and fishery products as well as other biological tissues.

### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CAC/GL 21, *Principles for the establishment and application of microbiological criteria for foods*

CAC/RCP 1, *Recommended international code of practice — General principles of food hygiene*

CAC/GL 30, *Principles and guidelines for the conduct of microbiological risk assessment*

CAC/GL 31, *Guidelines for the sensory evaluation of fish and shellfish in laboratories*

CAC/GL 48, *Model certificate for fish and fishery products*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

CAC/GL 53, *Guidelines on the judgement of equivalence of sanitary measures associated with food inspection and certification systems*

EAS 38, *Labelling of prepackaged foods — Specification*

### 3 Principle

**3.1** The tissue sample is digested at 10 °C using a 45 % NaOH solution containing cysteine as a mercury binder.

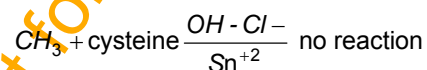
#### 3.2 Inorganic mercury analysis

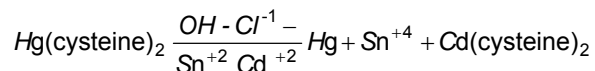
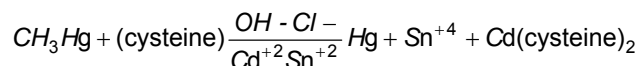
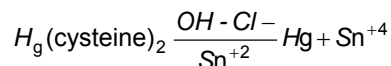
After binding to cysteine in solution, inorganic mercury ( $\text{Hg}^{+2}$ ) is reduced to elemental mercury by stannous ions in a strongly basic solution. The organic mercury-cysteine complexes are not reduced in this process.

#### 3.3 Total mercury analysis

In another subsample, a large excess of cadmium ion ( $\text{Cd}^{+2}$ ) is added with the stannous ions ( $\text{Sn}^{+2}$ ) to displace all type of mercury from cysteine in a strongly basic solution, and all forms of mercury are reduced to elemental mercury by stannous ions to give a total mercury determination. The result thus obtained is subtracted from inorganic mercury result, as determined in section 3.2, to give organic mercury by difference.

**3.4** Equations:





**3.5** The elemental mercury is portioned into air and determined either by flameless atomic absorption at 253.7 nm or by a mercury monitor.

## **4 Interferences**

There are no known significant interferences.

## **5 Sampling procedure and storage**

### **5.1 Commercial shipment**

Take a representative sample from the product lot and store as to maintain sample integrity.

### **5.2 Survey samples**

Fish may be either pooled or individual. For species normally greater than 30 cm in length, an individual fish may be used as a sample. For species less than 30 cm in length, a pooled sample is required. Store as to maintain integrity.

## **6 Sample preparation**

### **6.1 Commercial shipment**

Sample preparation should take into account the type of product and how it is used and prepared by the consumer.

**6.1.1** For fish and fish products that contains no free liquid, comminute the sample until homogeneous.

**6.1.2** For products that are packed in water, brine or similar medium that is normally discarded by the consumer; open the package and drain the product on an appropriate size sieve for 1 to 1½ minutes. Comminute the part of the sample retained by the screen until a homogeneous blend is obtained.

**6.1.3** For products that are packed in a medium that may be or is normally used by the consumer, e.g. fish canned in its own juice or oil; transfer the entire contents of the package into a homogenizer and blend for one minute or until a homogeneous mix is obtained.

### **6.2 Survey samples**

**6.2.1** For individual fish, weigh and measure the fork-length, i.e. from the nose to the fork of the tail, for size correlation.

**6.2.2** For a pooled sample, determine the average values for length and weight of the fish.

**6.2.3** Pass the skinned fillets through a commercial meat grinder a sufficient number of times to obtain a homogeneous blend (e.g. three times).

Collect the homogenized sample into a thoroughly cleaned, sealable plastic pot or glass bottle. Store

the sample in a refrigerator or freezer until required. Ensure that the prepared sample is still homogeneous prior to weighing. If liquid separates from the sample, thoroughly reblend before use.

## 7 Apparatus

7.1 **Flameless atomic absorption spectrophotometer**, with a mercury hollow cathode vapour lamp or a mercury monitor.

7.2 **Multimeter**, set on mv scale

7.3 **Chart recorder (optional)**

7.4 **Heating stirring hot plate**

7.5 **Taylor graduated digestion tubes**

7.6 **Aluminium block**, with bored holes to fit the Taylor digestion tubes

7.7 **Automatic pipettes 1000  $\mu$ l**

7.8 **Vacuum pump**

7.9 **Gas-air manometer**

7.10 **Tygon tubing  $\frac{1}{2}$ " diameter (ID)**

7.11 **Hot plate thermometer**

7.12 **Universal bottle top dispenser**

2 of 1-10 ml

1 of 1-50 ml

7.13 **Reagent containers**, 1 L or 2 L in plastic or glass.

7.14 **Reaction vessel** (see Figure 1) with dreshsel head having inlet converted to a glass funnel. Alternatively, a rubber stopper can be used.

7.15 **Midget impinger**

7.16 **Charcoal filter trap**

## 8 Reagents

8.1 **Sodium chloride (NaCl)**

**Sodium chloride solution (1%)**

8.2 **Sulfuric acid ( $H_2SO_4$ )**

8.3 **4.5N  $NH_4SO_4$  – 1% NaCl**, To 35g of NaCl add 3000 mL of  $H_2O$  and 441 mL of  $H_2SO_4$ . Dilute 3500 mL.

8.4 **L-cysteine** — L-cysteine solution (1 %)

8.5 **Stannous chloride ( $SnCl_2$ )**

8.6 **Stannous chloride solution**

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Add 10g of SnCl<sub>2</sub> and 1g of L-cysteine. Dilute to 500 mL with 4.5 N H<sub>2</sub>SO<sub>4</sub> – 1 % NaCl.

### 8.7 Cadmium chloride (CdCl<sub>2</sub>)

### 8.8 Stannous chlorid-cadmium chloride solution

Heat just to boil a solution of 10g of SnCl<sub>2</sub>, 2.5g of CdCl<sub>2</sub>, and 1g of L-Cysteine. Cool and dilute to 500 mL with 4.5N H<sub>2</sub>SO<sub>4</sub> – 1 % NaCl hydroxide.

### 8.9 Sodium hydroxide (NaOH)

#### 8.9.1 Sodium Hydroxide solution (35 % and 45 %)

### 8.10 n-octanol

### 8.11 Ethanol (95 %)

### 8.12 Antifoam solution

Add 1 ml of n-octanol to 9ml of ethanol (95 %).

### 8.13 Mercury chloride (HgCl<sub>2</sub>)

**8.13.1 Mercury primary standard (1000 µg/ml)** — Dissolve 0.134 5 g Hg Cl<sub>2</sub> in about 75ml distilled water, add 5 drops conc H<sub>2</sub>SO<sub>4</sub>, and dilute to 100 ml. Solution is stable for about 1 month at room temperature. Alternatively a commercially prepared primary standard may be used. If stored in a polyethylene container in a refrigerator, the primary standard is stable for up to a year.

**8.13.2 Mercury working standard (10 µg/ml)** — Dissolve 62.58 mg of CH<sub>3</sub>HgCl in 100ml of water.

**8.14.2 Methyl mercury working standard (5 µg/ml)** — Dilute 1ml of the primary standard in 100ml of H<sub>2</sub>O.

## 9 Procedure

**9.1** Accurately weigh between 0.1 and 0.5g of homogenized sample in a 50 ml graduated Taylor digestion tube. (Allow the sample to partially thaw prior to weighing). Sample weights will depend on the difficulty of digestion; e.g., fish meals, fish protein concentrate and fish oils will necessitate smaller sample weights.

**9.2** Prepare daily standards containing 0.1 to 0.4 µg Hg (10, 20, 30 and 40 µl of working mercury chloride solution) or 0.2 µg Hg (40 µl of methyl mercury working standard) to graduated Taylor digestion tube, and take both standard and blank through the entire procedure.

**9.3** Set up the flameless atomic absorption spectrophotometer or mercury monitor according to the manufacturer's instructions with the mercury lamp. Adjust the absorption cell in place, if necessary. Start vacuum pump. Adjust vacuum for 1 litre/min. Adjust the "zero" on the spectrophotometer of mercury Monitor and the recorder.

### 9.4 Total mercury analysis

**9.4.1** To the Taylor graduated digestion tubes containing the samples, standards (0.1. to 0.4 µg Hg) and blanks add 1.0 ml of cysteine solution (1 % NaCl, and 3.0 mL 45 % NaOH.

**9.4.2** Digest 1 hr at 100 °C while continuously shaking the tubes.

**9.4.3** Coll and dilute to 50 ml.

**9.4.4** Transfer 1.0ml to the reaction vessel with three drops of antifoam solution, 5.0ml SnCl<sub>2</sub>- CdCl<sub>2</sub> solution and 5.0 ml of 35 % NaOH. Immediately record the deflection.

**9.5 Inorganic mercury analysis**

Proceed as in 9.4 but use methyl mercury as standard (0.2 µg Hg).

Replace SnCl<sub>2</sub> – CdCl<sub>2</sub> solution with SnCl<sub>2</sub> solution in step 9.4.4. Record deflection.

**10 Calculations**

**10.1** Prepare a calibration curve of peak height versus µg Hg in the standards.

**10.2** Determine the mercury concentration in the intralaboratory check sample (if necessary), and in the sample by comparing the sample peak height to the calibration curve taking into account the sample weight and the dilution factor. Express result in terms of total mercury or inorganic mercury on a wet weight basis (ppm). Subtract inorganic mercury from total mercury to obtain organic mercury content in samples.

$$\text{ppm } (\mu\text{g/g}) = \frac{\text{height sample (cm)}}{\text{height calibration } \left(\frac{\text{cm}}{\mu\text{g}}\right)} \times \frac{\text{dilution factor (if necessary)}}{\text{weight sample (g)}}$$

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