



**NATIONAL STANDARD OF THE  
PEOPLE'S REPUBLIC OF CHINA**

**GB 5009.274-2016**

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**National food safety standard - Determination of Ciguatoxin in  
Aquatic Products**

**食品安全国家标准 水产品中西加毒素的测定  
(English Translation)**

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### 1 Scope

This Standard specifies methods for the determination of ciguatoxin in aquatic products.

This Standard applies to the determination of ciguatoxin in edible parts of aquatic products.

Method 1 -- Mouse bioassay

### 2 Principle

Use acetone to extract ciguatoxin in the aquatic product; after the extract is evaporated to dryness under reduced pressure, use 1% Tween-60 physiological saline as the dispersion medium to prepare a ciguatoxin-1% Tween-60 physiological saline suspension; inject the suspension into the peritoneal cavity of the mouse; observe the survival of the mouse.

According to the correlation chart between the death time of the mouse and the mouse unit, find out the corresponding mouse unit; correct the mouse unit according to the mouse weight; calculate and confirm the content of ciguatoxin (mouse unit virulence).

### 3 Reagents and materials

Unless otherwise specified, all the reagents are analytical reagents, the water is grade-1 water which is specified by GB/T 6682.

#### 3.1 Reagents

##### 3.1.1

Tween-60 ( $C_{64}H_{126}O_{26}$ ).

##### 3.1.2

Picric acid [ $HO-C_6H_2(NO_2)_3$ ].

##### 3.1.3

Acetone ( $CH_3COCH_3$ ).

##### 3.1.4

Methanol ( $CH_3OH$ ).

4.5 Nitrogen concentrator.

#### 5.1 Sample preparation

Take the edible portion of the aquatic product (boning); cut it into small pieces to make meat emulsion. Store it below  $-18^{\circ}C$ .

#### 5.2 Sample extraction

Take 200 g of sample in a plastic bag; place it in a water bath at  $70^{\circ}C$  for 15 min. Take it out for cooling; add 400 mL of acetone; homogenize for 10 min; use a Buchner funnel to extract and collect the filtrate. Extract the meat residue for twice more. Combine the filtrate; place it in a rotary evaporator at  $55^{\circ}C$  to concentrate to near dryness. Add 100 mL of methanol solution (90%) to dissolve; transfer to 500 mL separatory funnel; add

100 mL of n-hexane; shake well to extract; let stand for about 30 min. After layering is complete, discard the upper layer of n-hexane and collect the lower layer of methanol solution. Add 100 mL of n-hexane to repeat the extraction once more. Rotary evaporate the lower layer of methanol solution to dryness at  $55^{\circ}C$ ; use 100 mL of ethanol solution (25%) to dissolve; transfer to a separatory funnel; add 100 mL of diethyl ether and shake well; let stand for 10 min or more. After the layering, collect the upper layer of ether. Use diethyl ether to extract the lower solution twice more.

Combine the ether extracts; rotary evaporate to dryness at  $40^{\circ}C$ . Use 5 mL of chloroform-methanol solution (3+97) to dissolve the concentrate; transfer to a graduated

test tube; use nitrogen to concentrate to dryness at 45°C; use 1% Tween

60-normal saline to fix-volume to 2 mL; shake well to make even ciguatoxin-1% Tween

60-normal saline suspension. 0.5 mL of this suspension is equal to 50 g of sample.

Use this suspension to perform the mouse experiment.

**Note:** In order to avoid the harm of toxins, gloves shall be used for operation. The used equipment shall be soaked in sodium hypochlorite solution (5%) for more than 1 hour to decompose the toxin.

### 5.3.1 Breeding of experimental animals

The mouse shall be raised in a ventilated, light-transparent, clean indoor environment; and standard rodent food and water shall be normally supplied during the experiment.

### 5.3.2 Mouse test

The mice are randomly divided into a test group, a solvent-control group and a blank control group, of each group there are 3 mice and each mouse is weighed (accurate to 0.5 g); and the body weight is recorded. Use the staining method (such as 3% ~ 5%

Where:

X -- the virulence of ciguatoxin in the sample, in MU per 50 grams (MU/50g);

MU -- the mouse unit of ciguatoxin that is contained in 0.5 mL of mouse intraperitoneal injection, in MU per 50 grams (MU/50g);

C -- the correction coefficient of mouse weight;

f -- the dilution factor of sample extract.

1MU is defined as the virulence of death in 400 min of a specific pathogen-free (SPF)

Kunming male mouse of 20.0 g; it is equivalent to 9.10 ng of ciguatoxin.

## 6.2 Result judgment

If the death time of all mice in the test group is greater than 400 min, the virulence of ciguatoxin in the sample of the report is less than 0.98 MU/50g.

If the median death time of the test group is 50 min ~ 400 min, calculate according to Formula (1); and report the virulence of ciguatoxin in the sample as: xxx MU/50g.

If the median death time of the test group is less than 50 min, dilute the sample solution; and choose 3 mice for the experiment until the median death time is 50 min ~ 400 min; calculate according to Formula (1). Report the virulence of the ciguatoxin in the sample as:

xxxMU/50g.

## 7 Others

The detection-limit of the method is 0.98 MU/50g.

Method 2 -- Liquid chromatography - tandem mass spectrometry

## 8 Principle

The ciguatoxin in the sample is extracted by methanol, purified by C18 solid phase extraction column, determined by liquid chromatography-tandem mass spectrometry, and quantified by external standard method.

### 9.5 Materials

#### 9.5.1

##### **C18 solid phase extraction column**

200 mg/3mL. Before use, use 6 mL of methanol and 6 mL of water to activate it and to keep the column moist.

#### 9.5.2

##### **Microporous membrane**

0.22  $\mu\text{m}$ , organic phase.

### **10.1 Liquid chromatography - tandem mass spectrometer: equipped with electrospray**

ion source (ESI).

10.2 Analytical balance: sensitivity of 0.1mg and 0.01g.

10.3 Nitrogen concentrator.

10.4 Freeze-dryer.

10.5 Pulverizer.

10.6 Centrifuge: speed  $\geq 5\,000$  r/min.

10.7 Homogenizer: 3 400 r/min ~ 24 000 r/min.

10.8 Vortex oscillator.

10.9 Ultrasonic generator.