



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

GB 5009.261-2016

**National food safety standard - Determination of neurotoxic
shellfish poisoning in shellfish**

食品安全国家标准 贝类中神经性贝类毒素的测定

(English Translation)

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Foreword

This Standard replaces SN/T 1573-2013 "Inspection of neurotoxin shellfish poison in shellfish - Method of mouse biology".

Compared with SN/T 1573-2013, the main deviation in this Standard is as follow.

- changed the standard name as "National Food Safety Standard -

Determination of neurotoxic shellfish poisoning in shellfish".

National Food Safety Standard - Determination of

neurotoxic shellfish poisoning in shellfish

1 Scope

This Standard specifies the method of mouse biology for the determination of neurotoxic shellfish poisoning (NSP) in shellfish.

This Standard is applicable to the determination of neurotoxic shellfish poisoning (NSP) in shellfish.

2 Principle

Use ether to extract the neurotoxic shellfish poisoning in shellfish. After the extract is pressure-reduced and evaporated to dryness, use 1%Tween-60 saline as dispersion medium to prepare NSP-1%Tween-60 saline suspension.

Inject this suspension into abdominal cavity. Observe the survival of the mouse.

Calculate its virulence.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytically pure; the water is grade one water specified in GB/T 6682.

3.2.1 Sodium chloride solution (0.85%). weigh 0.85 g of NaCl, add water to dissolve and set volume to 100 mL.

3.2.2 1%Tween-60. weigh 1.0 g of Tween-60, use 0.85% sodium chloride solution to dissolve and set volume to 100 mL.

3.2.3 Sodium hypochlorite solution (5%). weigh 50 g of sodium hypochlorite, use water to dissolve and dilute to 1000 mL.

3.3 Standard product

Short naked toxin analog (brevetoxin-2), purity $\geq 90\%$.

3.4 Materials

Mouse. healthy ICR strain male mouse weighing 19g ~ 21g.

5 Analysis steps

NOTE: The used equipment such as rubber gloves, glass products, and discarded extracts shall be soaked in sodium hypochlorite solution (5%) for more than 1h so as to decompose toxins.

5.1.1 Sample collection

At least 10 shellfish shall be taken for each analysis sample to make the shellfish more than 200g.

The frozen sample is placed in an insulation box sent for inspection. Or ensure it is at a low temperature state ($0^{\circ}\text{C} \sim 10^{\circ}\text{C}$) sent for inspection. If it is a shell sample, it shall be opened to remove moisture and then sent for inspection.

5.1.2.1 Fresh shell sample

Use clean water to wash the shell surface thoroughly. Cut off the muscle. Open shell. Use water to rinse the interior so as to remove sediment and other foreign objects. Separate the occipital muscle from the tissue attached to the gluing. Take out the shellfish. Never cut the flesh. Do not heat or use anesthetic before opening the shell. Collect 200g of shellfish and drain it on a metal sieve with a pore size of about 2mm for 5min. Pick up broken shells and other debris. Homogenize the shellfish, for use.

5.1.2.2 Frozen sample

At room temperature, make the frozen sample semi-frozen. For shell frozen sample, according to the method in 5.1.2.1, clean, open shell, rinse and take the meat. Remove the borneol attached to the outside of the shellfish, wipe off the water, and then relax at room temperature. Collect 200g of shellfish and drain it on a metal sieve with a pore size of about 2mm for 5min. Homogenize the shellfish, for use.

5.1.2.3 Canned shellfish

Drain the contents of the can. Pour into the homogenizer for full homogenization, for use.

5.1.2.4 Dried shellfish product

Weigh 100g of dried product into sufficient water, soaking 24h ~ 48h (refrigerated at 4°C). Drain, homogenize, for use.

5.1.2.5 Salted product

Use clean water to rinse. Use running water to desalt. Drain, homogenize, for use.

5.2 Specimen extraction

Take 100g of specimen into a 500mL beaker. Add 5g of sodium chloride and

1mL of concentrated hydrochloric acid. Mix them well. While stirring, heat the mixture to it is boiling. Boil it on a slow fire for 5min. Cool to room temperature. Transfer the mixture to a 500mL centrifuge tube. Use 50mL of ether to rinse the beaker. Move the rinse together into the centrifuge tube. Add 100mL of ether into the centrifuge tube. Cap a plug. Shake thoroughly. Centrifuge at 6000 r/min for 15min.

After centrifugation, carefully pour off the ether layer (upper layer) solution into a 1000mL separatory funnel. Use ether to repeat the extraction of the precipitate in the centrifuge tube three times. The total amount of extraction for three times is 250 mL of ether. Transfer the ether layer liquid to the separatory funnel. Gently oscillate (cannot produce emulsion). Remove the water layer (lower layer) and shellfish fragments after static layering and stratification. Transfer the ether layer to a 500mL round bottom flask. Conduct concentration under reduced pressure (rotary evaporator, $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$) to remove ether. Use 1%Tween-60 saline to dilute the concentrate to 10 mL in a graduated tube. Make a full shake. Prepare it to a uniform NSP-1%Tween-60 saline suspension. At this time, the amount of 1 mL of liquid corresponds to the weight of 10 g of shelled shellfish measured in advance. Take this suspension as test stock solution for animal experiment.

5.3 Mouse test

Choose 6 healthy ICR strain male mice with a body weight of 19g ~ 21g. Divide them into 2 groups randomly. testing sample group and blank control group (1%Tween-60 saline), 3 mice/group.

Separately take 1 mL of testing solution or 1%Tween-60 saline for intraperitoneal injection. If the extract overflows during the injection, the mouse must be discarded and another mouse shall be re-injected. Record the time of completion of injection and the time of death when the mouse stops breathing, and observe 930min continuously. If the median death time of the mice is within