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

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**Water quality--Determination of the acute toxicity--Zebrafish
(Danio rerio) eggs method
(English Translation)**

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Foreword

In order to implement the "Environmental Protection Law of the People's Republic of China" and the "Law of the People's Republic of China on Water Pollution Control", protect the ecology the environment, safeguard human health, standardize the zebrafish egg method for the determination of acute toxicity of water quality, and formulate this standard.

This standard specifies the zebrafish egg method for the determination of acute toxicity in surface water, groundwater, domestic sewage and industrial wastewater.

Appendices A to G of this standard are informative appendices.

This standard is issued for the first time.

This standard is formulated by the Department of Eco-Environmental Monitoring, Laws and Standards Department of the Ministry of Ecology and Environment.

This standard was drafted. Changzhou Environmental Monitoring Center of Jiangsu Province, Environmental Benchmark and Risk Assessment of Chinese Academy of Environmental Sciences State Key Laboratory.

This standard is verified by. Environmental Analysis and Testing Technology Center of Chinese Academy of Environmental Sciences, China Environmental Monitoring Station, Health Research on Quality Standards of Agricultural Products of Nanjing Institute of Environmental Sciences, Zhejiang Province Environmental Monitoring Center, Zhejiang Academy of Agricultural Sciences Institute and Jiangsu Guochuang Environmental Technology Co., Ltd.

This standard was approved by the Ministry of Ecology and Environment on December 31, 2019.

This standard will be implemented from June 30, 2020.

This standard is explained by the Ministry of Ecology and Environment.

Water quality-Determination of acute toxicity-Zebrafish egg method

1 Scope

This standard specifies the zebrafish egg method for the determination of acute toxicity to water quality.

This standard applies to the determination of acute toxicity of surface water, groundwater, domestic sewage and industrial wastewater.

2 Normative references

The content of this standard refers to the clauses in the following documents. For undated references, the valid version applies to this standard.

GB/T 6920 Determination of pH value of water quality Glass electrode method GB/T 13267 Determination of acute toxicity of water quality substances to freshwater fish (zebrafish) HJ 91.1 Technical Specifications for Sewage Monitoring HJ 506 Determination of dissolved oxygen in water HJ/T 91 Technical specifications for surface water and sewage monitoring HJ/T 164 Technical Specifications for Groundwater Environmental Monitoring

3 Terms and definitions

The following terms and definitions apply to this standard.

Fish egg

Refers to egg cells that are in the entire developmental stage before rupture of the egg membrane. See Appendix A for zebrafish egg-related developmental stages.

Dilution level

The original water sample accounts for the reciprocal of the total number of points of the diluted water sample, which is generally expressed by D. For example, if the water sample is not diluted, Multiple D = 1; take 250 ml water sample and dilute to 1000 ml (that is, the volume fraction is 25%), then the dilution multiple D = 4.

Lowest ineffective dilution the minimum dilution factor that does not produce a test effect during the test. This standard refers to the lowest water sample when not less than 90% of the eggs are alive.

Dilution multiple, expressed as LID.

EC50

It refers to the percentage of the original water sample that caused 50% of zebrafish eggs to have an effect on the total volume of the diluted water sample after 48 hours of exposure. EC50 Means. EC50 in this standard is LC50.

Reference substance

In tests, known positive control substances used to verify the sensitivity of the method, such as 3,4-dichloroaniline in this standard.

4 Method principle

Multiwell cell culture plates were used to control 4-cells under conditions controlled by microplate controls, negative controls, and positive controls.

Stage to 128-cell stage zebrafish fertilized eggs are placed in water samples with different dilutions and cultured at $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 hours.

h. Calculate the LID or EC50 value based on the statistics of survival and death of fish eggs to characterize the acute toxicity of water samples.

5 Interference and cancellation

The turbidity or chromaticity of the water sample interferes with the end point of the test. A plastic dropper (7.11) can be used to slowly remove the porous cell culture plate (7.8) After a certain amount of water sample has been sucked out, observe it to avoid touching the eggs. After the observation, if you need to continue to expose, use plastic drops.

Tube (7.11) returns the original water sample to restore the original exposed volume.

6.1.1 Fish species

Use robust, disease-free, deformity-free, wild-type zebrafish with high fertility and fertilization rates as breeding fish for spawning. to make the fish body is about 3.5 cm long

and the fish age is between June and 24 months. The belly of healthy females is bright and plump. Healthy male body the figure is slender, the abdomen is flat, and the body has a golden yellow sheen. Breeders should not be given any medication for 6 months before spawning. Fish breeding See Appendix B for specific conditions for maintenance and maintenance.

6.1.2 Fish eggs

Ensure that the fertilization rate of the test fish eggs is $\geq 70\%$, and it is in the 4-cell phase to 128-cell phase (see Appendix A).

6.2 Reagents

Unless otherwise specified, analytical reagents that meet national standards are used. Test water using fresh distilled water or Ionized water meets the requirements of pH 6.5 ~ 8.5 and conductivity 7 instruments and equipment

7.1 Refrigerated sampling box. 2 °C ~ 8 °C;

7.2 pH meter. The measuring range is 0-14, and the minimum division is 0.1 pH unit;

7.3 Dissolved oxygen analyzer. The measurement range is 0 mg/L ~ 20 mg/L, and the minimum division is 0.1 mg/L;

7.4 Inverted microscope or stereo microscope. the minimum magnification is 30 x;

7.5 Refrigerator. 2 °C ~ 8 °C in refrigerating room; ≤ -18 °C in freezer;

7.6 Constant temperature incubator or constant temperature room. the temperature can be adjusted to $26\text{ °C} \pm 1\text{ °C}$;

7.7 Zebrafish breeding system. including systems for water purification, storage, water supply, water quality control and recycling;

7.8 Multiwell cell culture plate. 2.5ml ~ 5ml per well or other cell culture plates with the same function.

Commercially available

7.9 Spawning box. inert material, recommended size. 20 cm x 10 cm x 11 cm (see Appendix C), can be selected for sale;

7.10 Thermometer. 0 °C ~ 50 °C;

7.11 Plastic dropper. 5 ml, caliber greater than 3 mm;

7.12 Utensils and equipment commonly used in general laboratories.