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

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**Water quality - Identification of mutagenicity - Vicia faba root-tip
micronucleus test**

水质 致突变性的鉴别 蚕豆根尖微核试验法

(English Translation)

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Foreword

Protect the ecology for the implementation of the Environmental Protection Law of the People's Republic of China and the Law of the People's Republic of China on Water Pollution Prevention and Control this standard is formulated for the environment, the protection of human health, and the identification of the mutagenicity of water samples.

This standard specifies the micronucleus test of the broad bean root tip for the mutagenicity in surface water, groundwater, domestic sewage and industrial wastewater.

Test.

Appendix D of this standard is a normative appendix, Appendix A ~ Appendix C, Appendix E, Appendix F is an informative appendix.

This standard is the first release.

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

This standard was drafted. Jiangsu Changzhou Environmental Monitoring Center (Jiangsu Province Environmental Protection Water Environment Biological Monitoring Key Experiment Room), State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Academy of Environmental Sciences.

The standard verification unit. Shanghai Environmental Monitoring Center, Zhejiang Environmental Monitoring Center, Jiangsu Environmental Monitoring Center, Nanjing Environmental Monitoring Center of Jiangsu Province, Suzhou Environmental Monitoring Center of Jiangsu Province and Taizhou Environmental Monitoring Center of Jiangsu Province.

This standard is approved by the Ministry of Ecology and Environment on April 13, 2019.

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

Identification of water quality mutagenicity

1 Scope of application

This standard specifies the *Vicia faba* root tip micronucleus test method for identifying mutagenicity of water samples.

This standard applies to the mutagenic identification of surface water, groundwater, domestic sewage and industrial wastewater.

2 Normative references

This standard refers to the following documents or their terms. For undated references, the valid version applies to this standard.

GB/T 6920 Determination of pH of water - Glass electrode method HJ/T 91 Surface Water and Wastewater Monitoring Technical Specifications HJ/T 164 Technical Specifications for Groundwater Environmental Monitoring

3 Terms and definitions

The following terms and definitions apply to this standard.

Micronucleus micronuclei in eukaryotic cells, small nuclei that are free from the main nucleus are micronuclei, whose material composition and characteristics are consistent with the main nucleus.

Below 1/3 of the main nucleus, it is a manifestation of chromosomal aberrations.

Micronucleus rate

A certain sample is used to detect several root tips, and each root tip observes 1 000 mitotic interphase cells.

The ratio of the number of cells, in twips.

Mitotic index

Observation of 1 000 cells at the same root tip, in the mitosis phase (including pre-, mid-, post-, and end-stage, see Appendix) A Figure A.1) The ratio of the number of cells to the total number of cells, in twips.

Reference substance

A known positive control substance that verifies the sensitivity and effectiveness of the test method for determining the biological characteristics and test conditions of the test no significant changes have occurred, ensuring reliable and effective testing.

4 Principle of the method

The primary roots of broad bean grown after soaking and rooting are exposed to the sample for a certain period of time, and are restored, fixed, and dyed.

After the microscopic examination, the micronucleus rate of the primary meristem of the *Vicia faba* root tip (see Appendix A, Figure A.2) was counted. Mutagens it can act on nuclear material, causing chromosome breaks during mitosis to form fragments, the entire chromosome is detached from the spindle, and the spindle the function of silk traction chromosome movement is impaired. These movements are affected by chromosome

fragments or the entire chromosome cannot be stained with normal the color body moves toward the two poles of the cell to form a daughter cell nucleus, while the retained cytoplasm forms a daughter cell micronucleus and causes an increase in its number. Comparison Whether the micronucleus rate of the root tip cells of *Vicia faba* L. and the blank sample is significantly increased can determine whether the sample is mutagenic.

5 Reagents and materials

Unless otherwise stated, analytically pure reagents that meet national standards are used for the analysis, and experimental water is newly prepared deionized Water or distilled water, conductivity (25 ° C) ≤ 0.50 mS/m, pH (25 ° C) between 5.5 ~ 7.5.

5.1

Temperature-controlled water. The experimental water used for soaking seeds (8.2), rooting (8.3), and root tip treatment (10.1) must be placed in advance.

In a constant temperature incubator (6.3), equilibrate to a constant temperature reserve at $25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

5.2

Hydrochloric acid. $\rho(\text{HCl}) = 1.18$ g/ml.

5.3

sodium hydroxide (NaOH).

5.4

Acetic acid. $\rho(\text{C}_2\text{H}_4\text{O}_2) = 1.05$ g/ml.

5.5

Anhydrous ethanol. $\rho(\text{C}_2\text{H}_6\text{O}) = 0.79$ g/ml.

5.6

Basic fuchsin ($\text{C}_{20}\text{H}_{20}\text{ClN}_3$).

5.7

Sodium sulfite ($\text{Na}_2\text{S}_2\text{O}_5$) or potassium sulfite ($\text{K}_2\text{S}_2\text{O}_5$).

5.8

Activated carbon powder.

5.9

cyclophosphamide ($\text{C}_7\text{H}_{15}\text{Cl}_2\text{N}_2\text{O}_2\text{P} \cdot \text{H}_2\text{O}$), biological reagent (BR), purity $\geq 99.0\%$, reference material of this standard.