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

GB/T 44482-2024

**Chemicals - Tetrahymena thermophila multi-generational
reproductive test**

化学品 嗜热四膜虫多代繁殖毒性试验

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Foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for standardization work Part

1. Structure and drafting rules for standardization documents" Drafting is required. Please note that some of the contents of this document may involve patents. The issuing organization of this document does not assume the responsibility for identifying patents. This document is proposed and coordinated by the National Technical Committee on Hazardous Chemicals Management Standardization (SAC/TC 251). This document was drafted by: China University of Geosciences (Wuhan), Institute of Microbiology, Guangdong Academy of Sciences (Guangdong Microbiological Analysis and Detection Center Center),

China University of Mining and Technology, China Inspection and Testing (Tianjin) Co., Ltd., Ecological and Environmental Research Center of the Chinese Academy of Sciences, Ecological South China Institute of Environmental Sciences, Ministry of Environment (Institute of Ecological and Environmental Emergency, Ministry of Ecology and Environment), Hubei Academy of Ecological and Environmental Sciences (Provincial Ecological and Environmental Science Research Institute) Environmental Engineering Assessment Center), Linyi Inspection and Testing Center. The main drafters of this document are. Liu Chunsheng, Zhang Yongkang, Mei Chengfang, Cheng Shiyang, Gao Liang, Xu Shengnan, Kong Ren, Li Haishan, Zhou Lili, Qin Zhanfen, Dang Yao, Xiang Mingdeng, Yi Chuan, Liu Chaowu, Huo Jian, Zhang Yuling, Feng Lianna. Multi-generation reproduction toxicity test of chemicals on *Tetrahymena thermophila*

1 Scope

GB/T 44482-2024 is the Chinese multi-generation reproduction test on the ciliate *Tetrahymena thermophila* for chemical hazard assessment. It belongs to the family of alternative methods that regulators are encouraging: a protozoan test is quick and cheap, uses no vertebrates, and because the organism divides several times a day it can be carried through many generations in days rather than the months a fish or invertebrate study needs, which makes it a practical screen for effects on reproduction. The standard sets the test principle, the test organism, its strain and culture maintenance, the reagents, media and apparatus, the preparation of the test substance and its concentrations, the exposure procedure across the successive generations, the measurement of the population growth and reproduction endpoints at each, the controls and validity criteria, the calculation of the effect concentrations, and the interpretation and the test report. It takes effect on 1 April 2025.

This document describes the test principle, test substance information, reference substances, test system, and test results of the multi-generation toxicity test of *Tetrahymena thermophila*. systems, limit testing, quality control, data and reporting. This document applies to the multi-generation reproduction toxicity test of chemicals on *Tetrahymena thermophila*.

2 Normative references

This document has no normative references.

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 *tetrahymena thermophila* density; TD The number of *Tetrahymena thermophila* individuals contained in a unit volume [usually milliliters (mL)] of culture medium.

3.2 effect concentration; EC The concentration of a test substance that results in a x% decrease in the density of *Tetrahymena thermophila* compared to the control group during a given test period. Note

1.The English abbreviation for effect concentration is EC. ECx represents the concentration of the test substance when the inhibition rate is x%, such as EC50 represents the half-maximal effect concentration. Note

2.The effect concentration is expressed as the amount of test substance contained in each unit volume of culture medium.

3.3 lowest observed effect concentration; LOEC In a given assay period, the presence of a statistically significant effect ($p < 0.05$) on the density of *Tetrahymena thermophila* compared to the control group Lowest test substance concentration.

Note. The p-value is a measure of the statistical significance of the observed results. When performing hypothesis testing, the significance level is usually set to 0.05, and $p < 0.05$ were considered to be statistically significant.

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3.4 No observed effect concentration (NOEC) No statistically significant effect ($p \geq 0.05$) was observed on *Tetrahymena thermophila* density compared to the control group within a given assay period. The highest test substance concentration.

0.05 was considered to be statistically insignificant.

4 Test Principle

Thermophilic *Tetrahymena* can complete multiple generations of reproduction in a short test period. The addition of exogenous chemicals will affect its growth state and reproduction. The hazard assessment of chemicals can be completed in a short time. The density of *Tetrahymena thermophila* was measured in the culture medium, and the effect of exposure to the tested chemicals on the multi-generation reproduction of *Tetrahymena thermophila* was evaluated, and the The effect concentration (ECx), the lowest observable effect concentration (LOEC) and the no observable effect concentration (NOEC) of the test substance.