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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 46249-2025

Chemicals - Rapid androgen disruption activity reporter (RADAR) assay

化学品 快速雄激素干扰活性报告试验

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1.Structure and Drafting Rules of Standardization Documents". Drafting. Please note that some content in this document may involve patents. The issuing organization of this document assumes no responsibility for identifying patents. This document was proposed and is under the jurisdiction of the National Technical Committee on Standardization of Hazardous Chemicals Management (SAC/TC251). This document was drafted by: Shanghai Jiao Tong University, Beijing Normal University, Kezhihua Inspection and Testing (Fujian) Co., Ltd., and Jiangsu Provincial Product Quality Supervision and Inspection Center. Supervision and Inspection Institute, Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment, South China Institute of Environmental Sciences, Ministry of Ecology and Environment, Solid Waste Management Institute Waste and Chemicals Management Technology Center, Nanjing University of Finance and Economics, Guangzhou Institute of Energy Research, Chinese Academy of Sciences, CCIC (Tianjin) Testing & Inspection Co., Ltd. Limited Liability Company, Jiangsu Yaxin Kuncheng Testing Technology Co., Ltd. The main drafters of this document are. Yu Yunjiang, Liu Jining, Peng Ying, Wang Lei, Liu Hongying, Gu Aiguo, Liu Xiaojian, Fan Deling, Xiang Mingdeng, and Yang Qian. Zhou Lili, Ding Jie, Liu Jianmei, Zhang Lu, Ding Feng, Huang Jianmei, Zhou Tong, Yang Chang, Wang Xuefen, Yang Guangchao, Wang Liuhong, Li Guochen, Zhang

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This document utilizes the transgenic strain of Japanese medaka (Oryziaslatipes) carrying the spg1-gfp genetic construct. Because the estrogen receptor (ER) and androgen receptor (AR) of the Japanese medaka exhibit conformational conservation compared to their human counterparts, this paper... The test organism used was the transgenic Japanese medaka (Oryziaslatipes) carrying the spg1-gfp gene construct. The construct contains a promoter for the spiggin1 gene, which is coupled to a reporter gene of green fluorescent protein (GFP). The spiggin gene is a... The gene encoding a glycoprotein (a mucous-like gelatinous protein) produced by sexually mature male Gasterosteus culea. The promoter of the 4.159kb spiny stickleback (spiky stickleback) gene is present. The spiny stickleback gene in both Japanese killifish and spiny stickleback possesses a promoter structure. Tissue specificity. The spg1-gfp transgenic line completely replicates the characteristics of spiggin1, therefore GFP is specifically limited to the kidney (mesonephrium). Express. The purpose of this document is to identify the potential activity of the test substance along the androgen axis, not to obtain information for risk assessment. Toxicity data (e.g., concentrations with no observed effect or effective concentrations). This document can be used to guide OECD (Organisation for Economic Co-operation and Development) on internal... Level 3 of the secretion disruptor test framework, see reference [33], OECD Guideline No. 150, on the intergroup interpretation of test results. Explanation and extrapolation. This document can only use the spg1-gfp transgenic strain, Japanese medaka. If you require GFP expression driven by the spiggin1 promoter, please use a different strain. For other transgenic lines or reporter genes, complete methodological validation is required to adjust relevant validity criteria, statistical analysis, and fluorescence. Threshold. The endpoint of this experiment was inducing fluorescence in a single embryo. Compared to the control group, embryos exposed to the test substance either activated or inhibited heredity. During transcription of the construct, the embryo expresses more or less GFP, resulting in correspondingly stronger or weaker fluorescence. Another condition... The test substance was tested with or without 17alpha-methyl-5alpha-dihydrotestosterone (17MT, 3 μg/L, as an AR agonist). The level of androgens produced during the embryonic stage is low, therefore 17MT can be added to the exposure solution to detect its effect on 17MT availability. Test substances that have an antagonistic effect on AR, or test substances that show an antagonistic effect on AR. 17MT concentration screening tests determine the appropriate concentration of 17MT for combined exposure. The concentrations were 3 μg/L and 5 μg/L, with the selected concentration (3 μg/L) showing the highest sensitivity to the reference test substances for androgen-stimulating hormone and antiandrogen. Rapid androgen interference activity report test for chemicals

1 Scope

GB/T 46249-2025 is the Chinese national standard covering screening a chemical for

androgen disruption - the reporter cell line and its response, the agonist and antagonist modes run in parallel, the cytotoxicity control that stops a dead culture reading as a negative, and the validity and interpretation criteria. First edition, in force from 1 February 2026. Issued on 5 October 2025, it has been in force since 1 February 2026.

This document describes the test methods for reporting rapid androgen interference activity of chemicals. This document is applicable to the testing and evaluation of androgen-interfering activity of test substances, and is used to determine the test substance in transgenic lines containing spg1-gfp. Fluorescence intensity in Japanese medaka (Oryziaslatipes) embryos; also applicable to all non-volatile or low-volatile substances that can be accurately measured. Test sample. This document does not apply to volatile test substances.

Note. If used for testing mixtures, unidentified complex substances (UVCB), or multi-component substances, their components should be characterized, for example, their chemical properties and quantitative characteristics. Conditions and material properties.

2 Normative references

The contents of the following documents, through normative references within the text, constitute essential provisions of this document. Dated citations are not included. For references to documents, only the version corresponding to that date applies to this document; for undated references, the latest version (including all amendments) applies. This document.

GB/T 21801 Rapid biodegradability test of chemicals - breathability measurement method

GB/T 21802 Improved MITI test for rapid biodegradability of chemicals (I)

GB/T 21803 Rapid biodegradability test for DOC reduction of chemicals

GB/T 21831 Test method for rapid biodegradability of chemicals - Closed bottle method

GB/T 21856 Test for rapid biodegradability of chemicals and carbon dioxide generation

GB/T 21857 Improved OECD screening test for rapid biodegradability of chemicals

GB/T 27850 General Rules for Rapid Biodegradability of Chemicals

3 Terms, Definitions and Abbreviations

3.1 Terms and Definitions The following terms and definitions apply to this document.

3.1.1 Free embryo eleutheroembryo The stage of embryonic development after hatching, but before it can independently consume provided exogenous food.

Note. In this experiment, the developmental stage of the Japanese medaka embryo was defined as stage 39 (hatching stage) to stage 42 (when the structures required for

capturing prey have formed, including...). The shape of the upper jaw teeth, otoliths, and all fins.

3.1.2 Spiked mode Rapid androgen interference activity assay performed with the addition of an AR agonist.

3.1.3 unspiked mode Rapid androgen interference activity assay performed without the addition of AR agonists.