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

GB/T 45238-2025

**Chemicals - Determination of in vitro intrinsic clearance using
rainbow trout liver S9 sub-cellular fraction (RT-S9)**

化学品 用虹鳟肝S9亚细胞组分测定体外固有清除率试验

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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: Institute of Microbiology, Guangdong Academy of Sciences (Guangdong Microbiological Analysis and Testing Center), Solid Waste Management Department of the Ministry of Ecology and Environment Chemicals and Waste Management Technology Center, China Inspection and Quarantine (Tianjin) Inspection and Testing Co., Ltd., Pearl River Fisheries Research Institute of Chinese Academy of Fishery Sciences Research Institute. The main drafters of this document are: Mei Chengfang, Xu Meiyong, Chen Guilan, Wang Qingbai, Chen Huiming, Dou Congcong, Lin Jianhui, Zeng Guoqu, Zhou Lili, Liu Xiaojian, Liang Qiu, Liu Yanzhen, Liu Chunxin, Feng Baoxin, Tan Wenwei, Au Laihan, Liang Pinghuan, Wang Yingying.

In order to improve the ability to predict the bioaccumulation of chemicals in fish, it is necessary to establish an assessment method for biotransformation in the liver of fish and to The information is incorporated into established computer models. Currently, the intrinsic clearance of chemicals is mainly determined using in vitro metabolic systems of liver tissue. This document describes a substrate clearance assay based on the rainbow trout

(*Oncorhynchus mykiss*) liver S9 subcellular fraction (RT-S9). Test method for in vitro intrinsic clearance of test objects (CLIN VITRO, INT). OECD Chemical Testing Guideline No. 319A and OECD Chemical Testing Guidelines No. The Product Testing Guidance Document No. 280 describes the detailed steps for conducting the test and how to use CLIN VITRO, INT for bioaccumulation in fish. Computer model predictions. Briefly, intrinsic hepatic clearance was estimated based on the determined CLIN VITRO, INT, and physiologically based toxicokinetics. The bioaccumulation in fish can be further evaluated using the PBTK model. This value can also be extrapolated to the Extrapolate to obtain systemic (in vivo) biotransformation rate constants. If the test substance is within the application range of the model, the measured CLIN VITRO, INT was used as input information for the bioconcentration computer prediction model based on biotransformation. In vivo biotransformation rates can be used in computer models to predict bioconcentration factors (BCFs). Integration into a fish one-compartment model significantly improved model performance, incorporating biotransformation data compared to predictions obtained when metabolism was missing. The predictions of the bioconcentration results by the later model were closer to the experimental values. The reproducibility of this document has been verified by comparative tests performed by 6 laboratories (on 5 test substances and 1 reference substance). Some chemicals have been tested in fish hepatocytes, liver S9 subcellular fractions or microsomes and have obtained CLIN VITRO, INT. The Joint Research Centre of the European Commission has developed a database on in vitro biotransformation in fish covering biotransformations using in vitro methods (liver cells, Intrinsic clearance data from assays using cytosolic, S9, microsomes (eg, mitochondrial, erythropoietin, pyogenes, sarcoma) were used to inform the applicability of this document. If this document is used to test mixtures, difficult-to-test substances (such as unstable substances), or test substances not specified in the scope of application, first Consider whether the trial results are scientifically significant. Determination of Subcellular Fractions of Rainbow Trout Liver S9 for Chemical Use In vitro intrinsic clearance assay

1 Scope

GB/T 45238-2025 is the Chinese adoption of the rainbow trout liver S9 assay for measuring the in vitro intrinsic clearance of a chemical. Its purpose is to replace animals: bioaccumulation in fish has traditionally been assessed by a flow-through test in which live fish are exposed for weeks, and this assay instead measures how fast a liver preparation metabolises the substance and feeds that rate into a model that predicts the bioconcentration factor, which is a regulatory endpoint under chemical control regimes. The standard sets the test principle, the information required about the test substance, the reference substances used to demonstrate that the preparation is metabolically competent, the experimental preparation, the S9 fraction, the incubation medium and the conditions, and then the test procedure, the sampling over time, the analysis of substrate depletion, the calculation of the intrinsic clearance and its extrapolation, the acceptance criteria and the

test report. It takes effect on 1 May 2025, and for a chemical registrant working in the Chinese system it is a route to a bioaccumulation assessment without a fish study.

This document describes a test method for determining the in vitro intrinsic clearance of chemicals using the rainbow trout liver S9 subcellular fraction. Test material information, reference materials, test preparation, test procedures, data processing, quality assurance and quality control, and result reporting. This document is applicable to the determination of the in vitro intrinsic clearance of chemicals. The lowest in vitro first-order clearance rate constant (K_e) that can be accurately quantified is approximately

0.05 h⁻¹~

0.14 h⁻¹

1.If there is a special need, it can also be used to identify metabolites.

2 Normative references

The contents of the following documents constitute the essential clauses of this document through normative references in this document. For referenced documents without a date, only the version corresponding to that date applies to this document; for referenced documents without a date, the latest version (including all amendments) applies to This document.

GB/T 21801 Respirometric test for rapid biodegradability of chemicals

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

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

GB/T 21831 Ready biodegradability of chemicals. Closed bottle test

GB/T 21845 Test for water solubility of chemicals

GB/T 21852 Chemical partition coefficient (octanol-water) High performance liquid chromatography test

GB/T 21853 Partition coefficient of chemicals (octanol-water) Shake flask test

GB/T 21855 Test for hydrolysis of chemicals in relation to pH

GB/T 21856 Carbon dioxide generation test for readily biodegradable chemicals

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

GB/T 22052 Method for determining the relationship between vapor pressure and temperature and initial decomposition temperature of liquids using a liquid vapor pressure gauge

GB/T 22228 Determination of the vapor pressure of solids and liquids of industrial

chemicals in the range of 10-

3 Terms and definitions, symbols and abbreviations

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

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