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Chemicals - EASZY assay - Detection of endocrine active substances, acting through estrogen receptors, using transgenic tg(cyp19a1b:GFP) zebrafish embryos

化学品 EASZY试验 利用转基因 tg（cyp19a1b：GFP）斑马鱼胚胎通过雌激素受体检测内分泌活性物质

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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.

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 for Hazardous Chemicals Management Standardization (SAC/TC251).

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Introduction

EASZY [Detection of endocrine active substances via estrogen receptors using transgenic tg(cyp19a1b.GFP) zebrafish embryos] trial This is a mechanism-based in vivo screening assay designed to induce expression of green fluorescent protein (GFP) driven by the cyp19a1b promoter.

EASZY test can be used to detect the effects of chemical substances on transgenic tg (cyp19a1b.GFP) zebrafish embryos were exposed to estrogen activity for 96 h. Appendix A describes the genes used to establish transgenic lines structure.

The cyp19a1b gene encodes brain aromatase, an enzyme responsible for synthesizing estrogens from androgens.

The expression of the gene is restricted to radial glial cells, which are progenitor cells that generate new neurons from the embryonic stage to adulthood.

The transcription of estrogen requires activated estrogen receptors (ERs), ligand-ER complexes bound to estrogen response elements (EREs), and Half of the EREs in the promoter region of the cyp19a1b gene are involved. Cyp19a1b regulation also requires the glial cell x response element The synergistic effect of the glial cell-specific factor (GxRE) binding to the ERE sequence. The cell-specific role of GxRE in the cyp19a1b gene It plays an important role in the regulation of estrogen.

In addition to estrogen, steroid androgens can also regulate the transcriptional activity of

the cyp19a1b gene.

This regulation indicates that they can be aromatized to estrogens, which then bind to ERs and induce the expression of cyp19a1b.

Among the androgens, 11-ketotestosterone cannot induce the expression of cyp19a1b, while 5alpha-dihydrotestosterone (DHT) has been shown to have estrogen-like activity.

It induces the expression of cyp19a1b through an ER-dependent pathway. This may be due to the action of DHT through 3beta-hydroxysteroid dehydrogenase.

It is converted to beta-diol, a known estrogenic steroid (see Appendix B).

The expression of ERbeta1 and ERbeta2 mRNA can be detected in the telencephalon, preoptic area and hypothalamus of zebrafish embryos.

In zebrafish embryos at this developmental stage, no ERalpha transcripts were detected in their brains. This suggests that ERbeta1 and/or ERbeta2 can drive aromatic The expression of ERbeta2 in zebrafish was higher than that in ERalpha for natural and synthetic estrogens, which is consistent with the reported human ER subtypes have different affinities for these substances.

In transgenic tg(cyp19a1b.GFP) zebrafish, GFP perfectly mimics the expression of endogenous cyp19a1b in the zebrafish brain.

Therefore, measuring the reporter gene GFP in tg(cyp19a1b.GFP) embryos can be used to evaluate the expression of the cyp19a1b gene by chemical induction. The ability to reach.

The EASZY test technology has been successfully applied to test a number of compounds, including natural and synthetic hormones, drugs, pesticides, industrial chemicals The products were used to evaluate their ability to induce GFP in tg(cyp19a1b.GFP) zebrafish embryos.

The revised Guideline Document No. 150 of the Organization for Economic Cooperation and Development (OECD) has identified the EASZY test as an OECD endocrine Evaluation of the Tier 3 fish screening test in the conceptual framework, an in vivo test that provides data on a specific endocrine disruption mechanism/pathway.

The EASZY assay is a level 3 screening assay that identifies ER-regulated cyp19a1b cells by inducing GFP driven by the ER-regulated cyp19a1b promoter.

Active and inactive test substances on signaling pathways. Test data should not be used for risk assessment of chemicals.

Chemical EASZY test using transgenic tg (cyp19a1b.GFP) zebrafish embryos Estrogen receptor detection of endocrine active substances

1 Scope

This paper describes the use of transgenic tg(cyp19a1b.GFP) zebrafish embryos to detect endocrine-active substances via the estrogen receptor EASZY test method, including test principle, test material information, proficiency testing chemicals, test preparation, test procedures, data analysis and results Evaluation, quality assurance and quality control, and reporting of results.

This document is applicable to the screening and evaluation of endocrine active substances in chemicals.

2 Normative references

GB/T 13266

3.1 Terms and Definitions

The following terms and definitions apply to this document.

3.1.1 Agonist

Chemical substances or ligands that can bind to and activate specific nuclear receptors and induce nuclear receptors to regulate gene transcription activity.

3.1.2 Antagonist

A chemical substance or ligand that can bind to a nuclear receptor and block or inhibit the response mediated by the agonist ligand.

3.1.3 Active test chemical

The test compound can significantly induce GFP expression and the minimum average induction level of GFP is more than twice that of the blank control group.

3.1.4 Brain aromatase

Encoded by the cyp19a1b gene, the enzyme responsible for the endogenous synthesis of estrogens.

3.1.5 effect concentration;ECx

The concentration of test substance that results in a x% decrease in embryo fluorescence intensity compared to the control group during a given test period.

3.1.6 estrogenic activity

The ability of the test substance to mimic the effects of 17beta-estradiol, activate endogenous cyp19a1b and/or