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

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**Chemicals -- Test method of uterotrophic bioassay in rodents --
A short-term screening test for oestrogenic properties**

化学品 啮齿类动物子宫增重试验 雌激素作用的短期筛选试验

(English Translation)

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Foreword

This standard was drafted in accordance with GB/T 1.1-2009 given rules.

This standard technical content and Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals No.440 (2007) "sub-rodents Palace weight gain short-term effects of estrogen test screening test "(in English) the same.

This standard configuration and do the following editorial changes.

--- The OECD440 original in the "Preface" and "initial considerations and limitations" As part of this standard "Introduction";

--- Increasing the range a chapter;

--- The OECD440 original part of the content "is defined in Appendix 1" present standard "Terms and Definitions";

--- The original part OECD440 "Appendix 2 OECD endocrine disruptors Measurement and Evaluation conceptual framework" as a the standard "Appendix A".

This standard is managed by the National Standardization Technical Committee chemicals dangerous (SAC/TC251) and focal points.

This standard was drafted. Chinese Center for Disease Prevention and Control of Occupational Health and Poison Control, Occupational Disease Prevention Hospital in Liaoning Province, China Chemical Industry Technology Development Center, Chinese Academy of Inspection and Quarantine.

Introduction

In 1998, OECD has initiated action a high priority, namely the revision of existing guidelines established for screening and detection of potential New guidelines endocrine disruptors [1]. One of the elements of action is to build rodent uterotrophic test guidelines for testing. Thereafter, Rodent uterotrophic test conducted extensive verification procedures, including editing detailed background document [2-3] as well as through the application of strong

activity Reference estrogen, receptor agonist activity is weak, strong estrogen receptor antagonist activity, and other negative chemical reference substance used as test carried out Within the extensive laboratory and inter-laboratory comparison test to illustrate the relevance and reproducibility [4-9]. The test guide 440 is drawn Verification test experience and with reference to the estrogen agonist class of the test substance formed after the test results.

Uterotrophic test is a screening test began in the 20th century, the short 30 years [27-28], in 1962 was the first time a committee of experts as a screening test [32, 35]. It is based on the quality of the uterus or uterine weight gain increases the reaction (see 5.3), used to evaluate a chemical primer from the natural estrogen (e.g. 17beta- estradiol, 17beta-estradiol) agonists or antagonists of the action consistent with the ability of biological effects.

However, this method for detecting estrogen antagonist ratio for detecting an estrogen agonist much less. Uterine estrogen response methods are Two kinds. The initial reaction is to absorb moisture and cause the uterus to increase quality. Since then, the growth of uterine tissue lead to further increase its quality [30].

Uterine response in rats and mice are comparable.

This test is a screening test in vivo, its application can be found in "the conceptual framework of OECD endocrine disruptors Measurement and Evaluation" (attached Record A). In this conceptual framework, uterine weight test as an in vivo test is located at level 3 framework, clarifying an endocrine single Mechanisms, namely estrogen-like effects.

Uterotrophic test want to be included to detect potential endocrine disrupting effects in vivo and in vitro test series, and ultimately for human Health and the environmental impacts of risk assessment. OECD in carrying out the method validation tests, the use of strong activity and weak estrogenic activity stimulated Dynamic test for detection of the agent to evaluate the effectiveness of estrogen-like action substance [4-8]. Thus, in addition to the laboratory and inter-laboratory reproducibility good in addition, the test for the detection of estrogenic substances sensitivity has also been well documented.

In the verification test of the method, using only a negative reference substance, there are reports of uterine weight gain of the test as well as in vitro receptor substances and receptor binding assay testing was negative. In addition, other than the test data for verification were evaluated, these data further demonstrates Uterine weight gain specific screening test for estrogen-like effects of the substance.

As estrogen agonists and antagonists of estrogen receptor alpha and beta receptor ligands, receptors, respectively activate or inhibit transcription. This Cause potential health hazards, including effects on reproduction and development. Thus, chemicals need to quickly evaluate the possibility of estrogen Agonists or antagonists. Although the test substance with affinity for the estrogen receptor, estrogen receptor gene transcriptional activation in

vitro tests and other aspects of funding Material can provide information, but that information is to determine whether there is only one of the factors that may have harmful effects. Other chemical determinants comprising Enter the body after metabolic activation and inactivation in the distribution of the organizations, and so removed from the body; at least in part, by these cases it depends on the exposure pathway, and a test of the test substance. This requires understanding of the test substance in vivo activity of the environment, unless there is information available indicates that Absorbing chemicals - Distribution - Metabolism - Excretion features. Uterine tissue after being estrogen stimulation undergoing rapid growth, particularly rodents Class experimental animals (their estrous cycle lasts about 4d). Rodents, especially rats, are widely used in toxicological hazard assessment test in. Therefore, the rodent uterus for in vivo screening of estrogen agonists and antagonists are a suitable target organs.

This standard is based on those test methods used in the test verification OECD, the results of these validation tests have shown that the method Between the laboratory and the laboratory is reliable and reproducible [5,7]. You can now use two methods, namely removal of the ovaries of adult female animals Methods (ovx-adultmethod) and immature ovariectomized no way (immaturemethod). OECD validation test results If the display of these approaches has comparable sensitivity and reproducibility. However, immature animals, because it has a complete lower Hypothalamic - pituitary - gonadal (HPG) axis, when used in the test-specific reaction to be worse, but higher than the removal of the animal ovarian Fan Wai larger, because it can be used in addition to acting on the estrogen receptor material can also be used to detect the effect on the substance HPG axis.

In about 15 days of age, HPG axis rat-functional. Prior to this, with a gonadotropin-releasing hormone treatment can not promote Puberty. Just before arriving puberty, vaginal opening, females will experience several similar period of dormancy, during which not Cause vaginal opening and ovulation, but hormone levels fluctuate. If the chemicals directly or indirectly stimulate HGP axis, can cause youth Period ahead, early ovulation and accelerate the vaginal opening. In addition to acting on the HPG axis substances, although if the feed material but does not contain estrogen it may contain higher levels of metabolic energy, but also can stimulate animal growth, accelerate the vaginal opening. These substances do not cause ovariectomized Adult animals uterotrophic response, because their HPG axis does not function.

Taking into account the animal welfare, which should give priority to the use of immature rats, the animals avoid surgery can be avoided by the Estrus cycles in animals into the possibility of animal waste (see 5.4.1).

Uterine weight gain is not entirely caused by the estrogen, that in addition to estrogen agonists and antagonists of other substances may also be cited from uterine weight gain. For example, a relatively high dose of progesterone (progesterone), testosterone or various synthetic progesterone (progestin) are can stimulate uterine weight gain [30]. Any change in

uterine weight gain can be obtained by histological examination of the vagina and keratosis to be determined [30]. Whether induced by What factors, if uterine weight gain test result is positive, the general should be clarified further their cause. Other information, such as the estrogen receptor Funding in vivo binding experiments in vitro assay tests and estrogen receptor transcriptional activation assay and other data as well as analysis of adolescent females Material can also be used to demonstrate the estrogen-like effects.

Due to uterotrophic in vivo test is used as a screening test, therefore, the method carried out verification tests necessary to consider animal welfare to Requirements, but also consider strategies to carry out the test phases. Verification tests mainly in order to fully demonstrate the detection of estrogen-like effects (many chemical Aspects were of concern) reproducibility and sensitivity, and its detection of anti-estrogen-like effects made only a small amount of validation. Using only A strong anti-estrogen active substance is verified, it is because there is a clear anti-estrogen-like effects (effects were not some estrogenic activity Material interference) the information is very limited. Accordingly, the present standard for estrogen-like effect, described detection of anti-estrogen-like effect of the test Prescription rule contained in a guidance document. Anti-estrogenic substances reproducibility and sensitivity of this method for detecting simple in the future (When the detection of anti-estrogen-like effect of the conventional method is used for some time and the use of this method to detect a more anti-estrogenic effect After the substance) will be more clearly described.

As we all know, all animal related test procedures should be consistent with local standards of animal management; following the relevant animal management and disposal Management requirements are minimum standards, may be replaced by local animal management requirements. OECD gives the kind treatment of animals Guidance Document [25].

All tests carried out with the use of live animals, like, should determine the need to carry out the test before the test. For example, in the following two cases the test conditions can be carried out. (1) potential exposure amount (see Appendix A, the conceptual framework of level 1) or its results suggest that estrogen-like effects Required by the body to carry out tests to prove that role. (2) in vivo test results Conceptual Framework level 4 or 5 Tips confirmed the need the effect is to produce estrogen mechanisms involved, but this can not be proved in vitro tests.

Chemicals rodent uterotrophic test Short-term screening test for the role of estrogen

1 Scope

This standard specifies the terms and definitions chemical rodent uterotrophic test estrogenic effect of short-term screening test methods, test Principle test, test methods describe, explain and recognition of test procedures, test data and reporting the results.