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

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**Chemicals -- Test method of mammalian cell micronucleus in
vitro**

化学品 体外哺乳动物细胞微核试验方法

(English Translation)

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Foreword

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

This standard with the Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals No.487 (2010) "in vitro mammalian cell micronucleus test Test "is consistent (in English) of technical content.

This standard configuration and do the following editorial changes.

--- The OECD487 original in the "Introduction" and "initial consideration" As part of this standard "Introduction";

--- The OECD487 description in "Appendix. Definitions" as part of the contents of this standard "2 Terms and Definitions";

--- Increasing the range chapter.

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

The purpose of this test is by detecting the frequency in the cytoplasm of interphase cells micronucleus (micronuclei, MN) formed to evaluate the test substance Genotoxicity. No micro-approved from the centromere of chromosome fragments or in cell mitosis anaphase cells can not be moved to the poles of the entire dyeing Color bodies. This test is used to detect the cell during and after exposure to the test substance or exposure to the test substance to induce cell division experienced breakage and aneuploidy Active role in the body [1-2]. This allows the use of standard practices in the development of mobile and

without cytoplasmic blockers --- cytochalasin Su B (cytochalasinB, cytoB) were tested in two ways. Cell mitosis before joining cytoB, can block cytokinesis, the cells were double nucleated cells [3-4], allowing the completion of the first cell division in cells to identify the incidence of micronuclei election Optional analysis. Mitosis evidence as to provide the analyzed cell population occurs, the test may not use cytoB operation.

Further, in addition to the use of this method can identify chemicals induce micronuclei, but can also be applied cytoplasm mobile blocker, centromere immunolabeling, and Centromeric/end centromeric probe hybridization technology (Fluorescence in situ hybridization, fluorescenceinsituhybridisation, FISH), Providing chromosome damage and micronucleus formation mechanism of information [5-16]. In micronucleus formation was observed to increase, and this increase is desirable to determine fault for with or induced aneuploidy effect, applicable labeling and hybridization techniques to operate.

Micronuclei damage is passed to daughter cells, whereas chromosome division metaphase cells appear distortion is not transmitted to the next generation of cells;

Because detected micronucleus interphase cells is more objective, and therefore need only test whether or not a determination of cell division, as well as containing micro How many cell nuclei. Therefore, sample preparation, and analysis of records is relatively fast, but also to automate the analysis; this can only make the original Analysis of each treatment group to count hundreds of thousands of cells in the cell, thereby increasing the effectiveness of the detection test method. Finally, if the micro Nuclear from lagging whole chromosomes, the present method can use a conventional chromosome aberration analysis (OECD473) non-induced difficulties Aneuploidy effect chemicals were detected and analyzed [17].

However, if you do not use specific FISH and other techniques, in vitro micronucleus test is not Identification of chemical agents induce polyploidy or cleaving agent.

In vitro micronucleus test is a test in vitro conditions in vitro cultured human or rodent cells in. As the test at the same time it can detect aneuploidy agent role and the role of breaking agent, thus providing a comprehensive action to investigate the potential damage to chromosomes under in vitro conditions basis.

In vitro tests typically require the use of exogenous metabolic activation system. Exogenous metabolic activation system does not entirely mimic in vivo metabolic conditions.

It should be taken to avoid the presence of chemical factors inherent mutagenic activity, such as pH value, or significant changes in osmotic pressure, or at high concentration Degree under conditions that cause severe cytotoxicity test, in order to avoid false positive results occur.

In order to analyze the role of induced micronuclei, the most critical is treated and control groups cultures essential nucleus has experienced or complete nuclear Split. Micronucleus count is the most favorable period during and after the test substance or treated cells have completed a mitotic stage.

Situation in vitro micronucleus test using a variety of cell types tested are very effective, either with or without treatment cytoB Next; as has been a lot of research data shows that in vitro micronucleus test application of various rodent cell lines (CHO, V79, CHL/IU and L5178Y) and human lymphocytes were confirmed to be very effective [8,19-31]. These include, in particular, by the Technical Scientific Committee genotoxicity (Société Française de Toxicologie Génétique, SFTG) the effectiveness of international cooperation in research [8,19-22] and chaired the International genotoxic Test report of the Working Group.

The EU has an alternative to the effectiveness of [4,16] European Centre (European Centre for the Validation of Alternative Methods, ECVAM) a weight of evidence for the effectiveness of the Institute of retrospective re-evaluation of the data [2,33-34], and experts in the organization Advisory Committee (ECVAM Scientific Advisory Committee, ESAC) that the in vitro micronucleus test is scientifically valid. already There use to lymphoblastoid cell lines [5], HepG2 cell lines [36-37] and the Syrian hamster embryo cells report TK6 class [38], although these cells not validity study.

Chemicals

In vitro mammalian cell micronucleus test methods

1 Scope

This standard specifies the terms and definitions chemicals micronucleus test methods in vitro mammalian cell test principle, test methods, test number and, according to the report.

This standard applies to chemicals in vitro mammalian cell micronucleus test.

2 Terms and definitions

The following terms and definitions apply to this document.

Aneuploidy inducing (agent) aneugen Through mitosis and meiosis cycle related component interactions, causing cell or organism chromosome aneuploidy formation any substance (aneuploidy-inducing agent) or process.

Aneuploidy Normal diploid chromosome number (or haploid) number of different chromosomes, their number or single or multiple chromosomes increase or decrease, the offset is not changing the whole set of chromosomes, entire chromosomes of polyploid.

Apoptosis

It is programmed cell death, which is characterized by a series of successive cell disintegration step into a capsule surrounded by fine particles and then be swallowed fine Phagocytic cells slough off or excluded.

Proliferation celproliferation as a result of cell mitosis, the number of cells increases.

Centromere DNA (Deoxyribonucleic acid, DNA) chromatids within a region of two chromatids connected together.

Fracture (agent) clastogen any substance can cause chromosome structure or process cells or organisms distortion.

Cytokinesis cytoplasm divide Nuclei in mitosis or meiosis after forming cytoplasmic separation of the two daughter cells.

Cytoplasm move to block proliferation index cytokinesis-block proliferation index, CBPI
Number stimulate cell division the cytoplasm of the cell population of mobile-blocker treatment group and a control group of untreated cells ratio.