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Water quality — Evaluation of genotoxicity by measurement of the induction of micronuclei —

Part 2: Mixed population method using the cell line V79

Qualité de l'eau — Évaluation de la génotoxicité par le mesurage de l'induction de micronoyaux —

Partie 2: Méthode de la population mélangée à l'aide de la lignée de cellules V79

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 21427-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

ISO 21427 consists of the following parts, under the general title *Water quality — Evaluation of genotoxicity by measurement of the induction of micronuclei*:

— *Part 1: Evaluation of genotoxicity using amphibian larvae*

— *Part 2: Mixed population method using the cell line V79*

Water quality — Evaluation of genotoxicity by measurement of the induction of micronuclei —

Part 2: Mixed population method using the cell line V79

WARNING — Persons using this part of ISO 21427 should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this part of ISO 21427 be carried out by suitably trained staff.

1 Scope

This part of ISO 21427 specifies a method for the determination of genotoxicity of water and waste water using a mammalian *in vitro* test which detects damage, induced by water-soluble substances, to the chromosomes or the mitotic apparatus of V79 cells from the Chinese hamster.

The micronucleus test allows the identification of substances that cause cytogenetic damage which results in the formation of micronuclei containing lagging chromosome fragments and/or whole chromosomes.

The assay is based on the increase in the frequency of micronucleated cells after incubation with and without metabolic activation.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply:

3.1
cell lines
distinct families of cells grown in culture originated from a single clone

3.2
cofactor solution
aqueous solution of chemicals (e.g. NADP, Glucose-6-phosphate and inorganic salts) needed for the activity of the enzymes in the S9 fraction

3.3
dilution level D
denominator of the dilution coefficient (using the numerator 1) of a mixture of water or waste water with dilution water as integral number

NOTE For undiluted water or waste water, this coefficient is per definition 1:1. The corresponding smallest possible D value is 1.

3.4
D value
smallest value of D at which, under the conditions of this part of ISO 21427, no increase in the number of micronuclei per culture is detected

NOTE In the case of more than one D value (at maximum two are possible, see 9.2), the highest D value is decisive.

3.5
karyotype
characteristic of the nucleus of a cell, including its size, form and chromosome number

3.6
micronuclei
small particles consisting of acentric fragments of chromosomes and/or entire chromosomes which lag behind at anaphase stage of cell division and form, after telophase, single or multiple micronuclei in the cytoplasm

3.7
mitotic index
percentage of cells of a cell population under division at a particular time of observation

3.8
plating efficiency
measure of the number of colonies originated from single cells

3.9
proliferation index
rate at which cells are dividing within the culture

3.10
proliferation rate
rate with which cells replicate, calculated by a formula which takes into account 1, 2, 4 and 8 cell stages of clones

3.11
S9 fraction
9 000 g supernatant of a tissue homogenate in 0,15 mol/l KCl, obtained from livers of male rats (200 g to 300 g) pretreated with an appropriate substance or substance combination for enzyme induction

3.12
S9 mix
mixture of the S9 fraction and the cofactor solution

3.13
stock culture
frozen culture for the preservation of the characteristics of V79 cells

3.14
survival index
percentage of surviving cells compared to all cells, used as index of toxicity

3.15
test culture
culture of cells used for the study

4 Principle

The possible clastogenic and/or aneugenic activity of the test sample is detected by comparing, for the respective activation condition, the number of micronucleated cells in cultures treated with the negative control and the number in cultures treated with undiluted and diluted test samples, respectively.

During cell division, chromatid fragments without centromers will not move to the nuclei of the daughter cells and will stay within the cytoplasm. Part of the chromosome aberrations induced by the test item will be chromatid fragments without centromers and will, therefore, not be incorporated in the nuclei of the daughter cells. In addition, spindle disorders may lead to chromosomes which are not incorporated into the nucleus. These particles will form micronuclei in the plasma.

V79 cells are exposed for 24 h (4 h with the S9 mix) to a range of concentrations of a test sample. Thereafter, slides are prepared, and cells are stained and evaluated for the presence of micronucleated cells. An increased incidence of these micronucleated cells in comparison to the negative control indicates that the test item may cause chromosome breaks or spindle disorders in V79 cells *in vitro*.

5 Interferences

Biologically relevant alterations of the culture conditions may induce chromosome aberration due to secondary mechanisms resulting in artificial positive and, therefore, irrelevant results [16]. Those factors are, e.g. stronger changes in osmolality or pH, precipitation of test sample and phagocytosis thereof, and strong cytotoxic effects of the test sample. Therefore, test samples should be monitored at least for changes in pH or osmolality of the cultures using the same proportion of test item per culture as will be used later under test conditions. If there is a shift in pH in the culture, the test item should be adjusted to pH 7,0 ± 0,2. If there is a change in osmolality, the highest concentration used in the test has to be reduced so that no relevant alteration of osmolality occurs in the cultures. To avoid artifacts based on phagocytosis or severe cytotoxicity, limitations are given for the highest concentration, which should be used for testing (see 9.1 and 9.2).

6 Reagents and media

As far as possible use “reagent grade” chemicals.

If chemicals with different amounts of water of crystallization are used, calculate the needed amounts accordingly.

Always perform autoclaving for 20 min at 121 °C ± 2 °C. Cover vessels loosely (e.g. with aluminium foil). Never seal air-tight.

6.1 **Water**.

Prepare all aqueous solutions with water of a conductivity of ⩽ 5 µS/cm.

6.2 **Reagents**.

6.2.1 Glucose-6-phosphate dihydrate, $C_6H_{11}O_9PNa_2 \cdot 2\ H_2O$.

6.2.2 Nicotinamide adenine dinucleotide phosphate disodium salt, NADP, $C_{21}H_{26}N_7Na_2O_{17}P_3$.

6.2.3 Magnesium chloride hexahydrate, $MgCl_2 \cdot 6\ H_2O$.

6.2.4 Potassium dihydrogenphosphate, KH_2PO_4.

6.2.5 di-Sodium hydrogenphosphate dihydrate, $Na_2HPO_4 \cdot 2\ H_2O$.

6.2.6 Ethanol (absolute), C_2H_5OH.