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Biological evaluation of medical devices —

Part 3: Evaluation of genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity

Évaluation biologique des dispositifs médicaux —

Partie 3: Évaluation de la génotoxicité, de la cancérogénicité, de la toxicité sur la reproduction et de la toxicité sur le développement

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 206, *Biological and clinical evaluation of medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This fourth edition of ISO 10993-3 cancels and replaces ISO 10993-3:2014 and ISO/TR 10993-33:2015.

The main changes are as follows:

— this document has been entirely revised to emphasize evaluation of genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity instead of testing;

— chemical characterization and toxicological risk assessment have been added as an approach to address genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity;

— the follow-up evaluation has been changed when an in vitro genotoxicity test is positive and the flowchart for follow-up evaluation has been removed;

— reproductive toxicity and developmental toxicity have been described separately;

— the annex on cellular transformation has been deleted;

— ISO/TR 10993-33 has been merged into this document as Annex B on genotoxicity test methods.

A list of all parts in the ISO 10993 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The basis for biological evaluation of medical devices is often empirical and driven by relevant concerns for human safety. The risk of serious and irreversible effects, such as cancer or second-generation abnormalities, is of particular public concern. It is inherent in the provision of safe medical devices that such risks be minimized to the greatest extent feasible. The assessment of mutagenic, carcinogenic, reproductive toxicity and developmental toxicity hazards is an essential component of the control of these risks. Not all test methods for the assessment of genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity are equally well developed, nor is their validity well established for the testing of medical devices.

Significant issues with test sample size and preparation, scientific understanding of disease processes and test validation can be cited as limitations of available methods. Since the previous edition of this document, many genotoxicity test methods have been updated with revised OECD guidelines. However, these generally provide clearer recommendations but little alteration in test methods. Scientifically sound alternatives to the proposed testing can be acceptable insofar as they address relevant matters of safety assessment of the medical device.

In the selection of tests needed to evaluate a particular medical device, a careful assessment of expected human uses and potential interactions with biological systems is important, particularly in such areas as reproductive toxicity and developmental toxicity.

This document presents test methods and evaluation strategies for the identification of specific biological harms. Testing is not always necessary or appropriate in evaluating biological risks associated with exposure to medical device materials but, where it is appropriate, it is important that maximum test sensitivity is achieved.

In view of the multitude of possible outcomes and the importance of factors such as extent of exposure, species differences and mechanical or physical considerations, risk assessment is typically performed on a case-by-case basis. Suggestions for risk consideration and integration of ISO 10993-17 and ISO 10993-18[7] are provided.

Biological evaluation of medical devices —

Part 3: Evaluation of genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity

1 Scope

This document specifies strategies for risk estimation and evaluation of biological harms with respect to:

— genotoxicity;

— carcinogenicity;

— reproductive toxicity; and

— developmental toxicity.

This document is applicable when the need to evaluate a medical device for potential genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity has been established.

This document is not applicable to active pharmaceutical ingredients of device-drug combination products or biological components of device-biologic combination products which are covered by regulations.

NOTE Guidance on selecting relevant biological effects for medical devices is covered in ISO 10993-1.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 10993-1:2025, *Biological evaluation of medical devices — Part 1: Requirements and general principles for the evaluation of biological safety within a risk management process*

ISO 10993-2, *Biological evaluation of medical devices — Part 2: Animal welfare requirements*

ISO 10993-12:2021, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO 10993-17, *Biological evaluation of medical devices — Part 17: Toxicological risk assessment of medical device constituents*

ISO 14971:2019, *Medical devices — Application of risk management to medical devices*

OECD 471:2020, *OECD Guideline for the Testing of Chemicals — Bacterial Reverse Mutation Test*

OECD 473:2016, *OECD Guideline for the Testing of Chemicals — In vitro Mammalian Chromosome Aberration Test*

OECD 487:2016, *OECD Guideline for the Testing of Chemicals — In vitro Mammalian Cell Micronucleus Test*

OECD 490:2016, *OECD Guideline for the Testing of Chemicals — In vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993-1, ISO 10993-12 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
carcinogenicity test
test to determine cancer outcomes after long term or life-span exposure of animals

Note 1 to entry: Outcomes are typically based on tumorigenic potential and other factors.

3.2
developmental toxicity evaluation
methods to determine adverse effects on the developing organism during prenatal development or postnatally to the time of sexual maturation

3.3
genotoxicity test
test using cells, bacteria, yeast, fungi, animals or other biological systems to determine whether gene mutations, chromosomal alterations or other genetic changes are caused by the test sample

3.4
reproductive toxicity evaluation
methods to evaluate the potential effects of test samples on male or female reproductive function during any stage of development or fertility

3.5
sham-incubated vehicle
vehicle control exposed to the same extraction time and temperature as the test sample extract

3.6
threshold of toxicological concern
TTC
level of exposure for constituents, below which there would be no appreciable risk to human health

Note 1 to entry: The constituent is defined as a chemical or compound present in or on a finished medical device or material(s) of construction. Constituents can be intentionally present (e.g. an additive such as an antioxidant) or unintentionally present (e.g. an impurity).

[SOURCE: ISO/TS 21726:2019, 3.5[11], modified — Note 1 to entry has been added.]

4 Assessment strategies

4.1 General

ISO 10993-1 indicates circumstances where the potential for genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity are relevant biological harms for consideration in an overall biological safety evaluation. Evaluation of the biological harms shall consider the following factors:

— whether assessment is necessary based on patient population (e.g. life expectancy, sensitive population) and the anticipated impact of test results on risk management judgements,

— whether assessment is necessary based on the worst-case quantity, type of contact and duration of exposure,

NOTE Quantity can be influenced by device size and number used per patient.

— an analysis of the chemical constituents of the device material(s), including manufacturing process residues and degradation products or metabolites, to identify causes of concern. The identification can be based on chemical-specific toxicity data if available; otherwise, structure-activity relationships or previous demonstration of relevant toxicity in the chemical class can be used,

— existing information relevant to the genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity evaluation of the medical device, and

— previous use of equivalent (see ISO 10993-1:2025, 6.7) materials and processing in relevant applications.

The decision to waive in vitro genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity testing shall be justified. The decision to conduct in vivo testing shall be justified.

If exposure to constituents identified in accordance with ISO 10993-18[7] is determined to be within acceptable levels based on a toxicological risk assessment conducted in accordance with ISO 10993-17, no additional testing needs to be performed to address the relevant risks.

4.2 Other considerations

Toxicity evaluation can be warranted for additional states of the device, such as wear debris generated from the device, absorbable materials, or materials that cure in situ (e.g. cements, adhesives, pre-polymer mixtures) unless toxicological risk assessment based on material information determines no cause for concern. For guidance on test sample preparation for in situ curing devices, see ISO 10993-12.

NOTE Material information can include chemical composition, degradation products, debris size and morphology, pre-polymerization, post-polymerization products, etc.

5 Genotoxicity assessment

5.1 General

Assessment of genotoxic risk should be made:

a) by conducting toxicological risk assessment based on chemical characterization according to ISO 10993-17 and ISO 10993-18[7]; or

b) by performing genotoxicity testing in the standard test battery according to 5.2.2.

In certain cases (e.g. a material which has not been used in a legally marketed device for its intended use), both toxicological risk assessment of medical device constituents based on chemical characterization and genotoxicity testing of a medical device can be appropriate.

NOTE 1 Genotoxicity testing can also be appropriate when chemical characterization data is insufficient for a toxicological risk assessment.

If the device is made with and releases nanomaterials, additional evaluation shall be considered.

NOTE 2 See ISO/TR 10993-22[9] and Annex D for more information.

If chemical characterization of the device identifies genotoxicants which are not easily detected using non-targeted analytical approaches described in ISO 10993-18[7] (e.g. glutaraldehyde, formaldehyde), targeted analysis of genotoxic residuals shall be conducted. Where compositional profiling or analytical chemistry reveals the presence of chemical constituents with inadequate genotoxicity data and where quantitative structure activity relationship (QSAR) information is inconclusive (see ISO 10993-17), testing of individual chemicals or device components containing the chemical should be considered. In either case, the test sample shall be justified and documented.

5.2 Testing strategy

5.2.1 General

No single test is capable of detecting all types of genotoxic agents. Therefore, the usual approach is to conduct a battery of in vitro and, under certain circumstances, also in vivo tests.

Genotoxicity tests are designed to detect the two major classes of genetic damage:

— gene mutations (DNA single base pair changes, small deletions or insertions), and

— chromosomal damage [structural aberrations such as translocations, small or large deletions and insertions, and gain or loss of whole chromosomes (aneuploidy) or parts of chromosomes].

NOTE Bacterial reverse mutation assays have been shown to detect relevant small-scale mutational changes produced by the majority of genotoxic carcinogens detected by rodent bioassays.

5.2.2 In vitro genotoxic test battery

When genotoxicity testing is performed, it shall be conducted in accordance with Annex B and the test battery shall include:

a) a test for gene mutations in bacteria (see OECD 471), modified for use with medical devices, see Clause B.2, and

b) one of the following three validated in vitro mammalian cell tests:

   1) an in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells (see OECD 473), modified for medical devices, in accordance with Clause B.3 and B.4.2, or

   2) an in vitro mouse lymphoma $tk^{+/-}$ assay using L5178Y ($tk^{+/-}$) cells (see OECD 490), modified for medical devices, including detection of small (slow growing) and large colonies, see Clause B.3 and B.4.1, or

   3) an in vitro mammalian cell micronucleus test for chromosomal damage and aneugenicity (see OECD 487), modified for medical devices, see Clause B.3 and B.4.3.

NOTE 1 If the medical device is known to contain nanomaterials testing according to OECD 471 is not recommended, see Annex D.

All tests (bacterial and in vitro mammalian tests) shall be performed in the presence and absence of metabolic activation. Metabolic activation is achieved by addition of a co-factor-supplemented post-mitochondrial fraction (S9).

NOTE 2 An example for a S9 recipe is given in Annex E.

In vitro mammalian tests shall include both a short-term exposure with and without S9 and a long-term exposure without S9.

NOTE 3 See B.3.2.3 for descriptions of short-and long-term exposure.

Results from both chromosomal aberration assays and the in vitro mouse lymphoma $tk^{+/-}$ assay (MLA) have a relatively high level of congruence for compounds that are regarded as genotoxic but yield negative results in the bacterial reverse mutation assay. The MLA detects the broadest set of genetic damage, including both small-scale and large-scale genetic damages. The micronucleus assay detects chromosomal damage in the form of whole chromosome loss or chromosomal fragments and uniquely detects aneuploidy. The three tests are equally acceptable as an in vitro mammalian genotoxicity test (see OECD 473, OECD 487 and OECD 490).

The hypoxanthine guanine phosphoribosyl transferase gene mutation assay (HGPRT/HPRT) method (see OECD 476[84]) is not included in the recommended test battery for device testing. This test can be appropriate for medical devices containing nanomaterials (see Annex D).

5.2.3 In vivo genotoxicity testing

In vivo genotoxicity tests are not part of the recommended genotoxicity test battery because they are relatively insensitive and not likely to detect genotoxicity at the concentrations of substances generally found in medical devices or in medical device extracts[73][74]. These concentrations can be insufficient to meet the limit dose required by the test methods. However, under certain circumstances, an in vivo test can be useful.

These circumstances can include:

a) assessment of nanomaterial or other particulates suspected of sequestration in particular organs or tissues (see Annex D, Reference [30] and ISO/TR 10993-22[9]); or

b) when additional relevant factors [e.g. toxicokinetics (see ISO 10993-16[6]), organ-specific toxicity] need to be considered.

In vivo tests are valid only with data demonstrating the test sample reached the target cells, organs, or tissues. Mutational analysis in target organs of rats or mice can be determined using one of several rodent transgenic mutation assays (see OECD 488[27]); chromosomal damage can be assessed in the erythrocyte micronucleus assay (see OECD 474[24]), the in vivo comet assay (see OECD 489[28]), or the in vivo chromosomal aberration assay (see OECD 475[25]). See Clause B.5 for additional considerations for in vivo assays.

All animal studies shall be performed in a facility meeting the requirements of ISO 10993-2.

5.2.4 Follow-up evaluation

If in vitro genotoxicity tests are performed in accordance with 5.2.2 and if the tests are negative, the test sample can be considered non-genotoxic and further testing is not necessary.

Equivocal results (i.e. elevated results that do not meet criteria for a positive result), can require repeating tests with a modified protocol such as adjusting dilution of the test sample or extract.

When any single in vitro test is positive or equivocal, then the following options shall be considered:

a) Identification of genotoxic constituents by chemical characterization (information gathering or analytical chemistry testing) or other testing, with appropriate steps taken to eliminate or manage any identified risks.

b) If confounding factors (e.g. osmolality, pH, cytotoxicity) are suspected to have caused the positive response or equivocal in an initial genotoxicity test, the dosing or test conditions may be modified with a justification to address confounding factors.

c) Presume that a genotoxicity risk exists and manage the risk in accordance with ISO 14971.

5.3 Test sample preparation

Test samples should be extracted in vehicles compatible with the test system (see Annex A). Use of extraction vehicles that are incompatible with the test system requires additional manipulation (e.g. solvent removal) that can alter the composition of the extract and, therefore, affect the results of the test. Use of extraction vehicles that are incompatible with the test system shall be justified.

Extraction shall be performed in accordance with ISO 10993-12 unless otherwise justified.

Tests can be performed on solutions (e.g. soluble or liquid devices), suspensions (e.g. Method A in Annex A), extracts (e.g. Method B in Annex A) of the finished product, medical device components or individual chemicals from the medical device. If individual chemicals are tested, then the appropriate OECD guidelines shall be followed.

NOTE Some authorities having jurisdiction prefer Method C in Annex A while others do not accept Method C.

6 Carcinogenicity assessments

6.1 General

The objective of carcinogenicity studies is to determine tumorigenic potential of medical devices or medical device constituents in animals and to evaluate the relevant risk to patients. Mechanisms of carcinogenesis can be genotoxic or non-genotoxic. Medical devices shall be evaluated for both types of carcinogenicity, preferably by alternative approaches, such as chemical characterization and toxicological risk assessment, that do not use in vivo testing.

Animal testing for carcinogenicity is technically challenging. It requires implantation of many medical devices, representative samples of the medical device, or the application of concentrated medical device extracts to achieve the elevated doses necessary for a valid test in a reasonable number of animals. For these reasons, animal testing is generally not justified when the risks can be adequately assessed by other means.

NOTE For additional information on animal carcinogenicity testing, see Annex C.

6.2 Evaluation strategy

6.2.1 General

Carcinogenicity should be evaluated initially using chemical characterization according to ISO 10993-18[7] and toxicological risk assessment according to ISO 10993-17. When medical devices or materials are not readily amenable to analytical chemistry testing and adequate chemical characterization information is available from information gathering, it is always preferable to use the chemical information and toxicological risk assessment including assessment of available genotoxicity data to evaluate carcinogenicity rather than performing an animal carcinogenicity study.

NOTE 1 Some authorities having jurisdiction can require consideration of carcinogenicity testing for novel materials (e.g. when data are not available to provide an adequate assessment).

NOTE 2 Evaluation of solid-state carcinogenesis due to release of particulates or fibres from a medical device can be partially addressed by toxicological risk assessment according to ISO 10993-17. This does not address all the risks. See Annex C for more information.

If the results of toxicological risk assessment indicate the risk of carcinogenicity is acceptable, no further assessment is required.

Carcinogenicity testing shall not be performed when risks can be adequately assessed or managed without generating new carcinogenicity test data or when the benefit-risk analysis obviates the need for a carcinogenicity assessment. If the toxicological risk of carcinogenicity is unacceptable, the risk shall be managed accordingly (see also ISO 14971).

If the medical device contains a chemical with inadequate toxicological data, biological testing to address carcinogenicity shall be justified and documented based on the need for additional information. The assessment should address both genotoxic (see 6.2.2) and non-genotoxic carcinogenicity (see 6.2.3) on the individual chemical identified in the toxicological risk assessment.

NOTE 3 The history of human clinical use can be considered in the risk assessment. The clinical evidence for carcinogenicity risk is generally not monitored over an adequate timeframe and this aspect of medical device safety can be unknown.

6.2.2 Genotoxic carcinogens

The carcinogenicity risk from genotoxic carcinogens can be established by genotoxicity testing (if unknown) or by toxicological risk assessment (of identified genotoxins). Genotoxicity testing requires a battery of tests (see 5.2.2).

Where genotoxicity of a medical device is established, either based on genotoxicity testing or prior material knowledge of the medical device, additional evaluation of carcinogenic risk shall be performed in accordance