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Third edition
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Biological evaluation of medical devices —

Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity

Évaluation biologique des dispositifs médicaux —

Partie 3: Essais concernant la génotoxicité, la cancérogénicité et la toxicité sur la reproduction

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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.

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 documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received. www.iso.org/patents

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

The committee responsible for this document is ISO/TC 194.

This third edition of ISO 10993-3 cancels and replaces the second edition (ISO 10993-3:2003), which has been technically revised.

The major technical changes are the following:

a) test strategy changed by inclusion of a *in vivo* test and a follow-up evaluation;

b) new Annex A " Guidance on selecting an appropriate sample preparation procedure in genotoxicity testing" included;

c) Inclusion of further *in vitro* and *in vivo* test for evaluating the genotoxic potential of medical devices;

d) new Annex B "Flowchart for follow-up evaluation" included;

e) Annex E changed to "Considerations for carcinogenicity studies performed as implantation studies" and made normative;

f) new Annex F "*In vitro* tests for embryo toxicity" included.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1: Evaluation and testing within a risk management process*

— *Part 2: Animal welfare requirements*

— *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4: Selection of tests for interactions with blood*

— *Part 5: Tests for in vitro cytotoxicity*

— *Part 6: Tests for local effects after implantation*

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 9: Framework for identification and quantification of potential degradation products*

— *Part 10: Tests for irritation and skin sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymeric medical devices*

— *Part 14: Identification and quantification of degradation products from ceramics*

— *Part 15: Identification and quantification of degradation products from metals and alloys*

— *Part 16: Toxicokinetic study design for degradation products and leachables*

— *Part 17: Establishment of allowable limits for leachable substances*

— *Part 18: Chemical characterization of materials*

— *Part 19: Physico-chemical, morphological and topographical characterization of materials [Technical specification]*

— *Part 20: Principles and methods for immunotoxicology testing of medical devices* [Technical specification]

The following part is under preparation:

— *Part 33: Supplement to* ISO 10993-3:— *Guidance on tests to evaluate genotoxicity* [Technical Report]

The following definitions apply in understanding how to implement an ISO International Standard and other normative ISO deliverables (TS, PAS, IWA).

— "shall" indicates a requirement;

— "should" indicates a recommendation;

— "may" is used to indicate that something is permitted;

— "can" is used to indicate that something is possible, for example, that an organization or individual is able to do something.

ISO/IEC Directives, Part 2 (sixth edition, 2011), 3.3.1, defines a requirement as an "expression in the content of a document conveying criteria to be fulfilled if compliance with the document is to be claimed and from which no deviation is permitted."

ISO/IEC Directives, Part 2 (sixth edition, 2011), 3.3.2, defines a recommendation as an "expression in the content of a document conveying that among several possibilities one is recommended as particularly suitable, without mentioning or excluding others, or that a certain course of action is preferred but not necessarily required, or that (in the negative form) a certain possibility or course of action is deprecated but not prohibited."

Introduction

The basis for biological evaluation of medical devices is often empirical and driven by the 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 minimised to the greatest extent feasible. The assessment of mutagenic, carcinogenic and reproductive hazards is an essential component of the control of these risks. Not all test methods for the assessment of genotoxicity, carcinogenicity or reproductive 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. For example, the biological significance of solid state carcinogenesis is poorly understood. It is expected that on-going scientific and medical advances will improve our understanding of and approaches to these important toxicological effects. At the time this document was prepared, the test methods proposed were those most acceptable. Scientifically sound alternatives to the proposed testing may be acceptable insofar as they address relevant matters of safety assessment.

In the selection of tests needed to evaluate a particular medical device, there is no substitute for a careful assessment of expected human uses and potential interactions of the medical device with various biological systems. These considerations will be particularly important in such areas as reproductive and developmental toxicology.

This part of ISO 10993 presents test methods for the detection of specific biological hazards, and strategies for the selection of tests, where appropriate, that will assist in hazard identification. Testing is not always necessary or helpful in managing toxicological 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 have to be performed on a case-by-case basis.

Biological evaluation of medical devices —

Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity

1 Scope

This part of ISO 10993 specifies strategies for risk estimation, selection of hazard identification tests and risk management, with respect to the possibility of the following potentially irreversible biological effects arising as a result of exposure to medical devices:

— genotoxicity;

— carcinogenicity;

— reproductive and developmental toxicity.

This part of ISO 10993 is applicable when the need to evaluate a medical device for potential genotoxicity, carcinogenicity, or reproductive toxicity has been established.

NOTE Guidance on selection of tests is provided in ISO 10993-1.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

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

ISO 10993-6, *Biological evaluation of medical devices — Part 6: Tests for local effects after implantation*

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

ISO 10993-18, *Biological evaluation of medical devices — Part 18: Chemical characterization of materials*

OECD 414, *Prenatal Development Toxicity Study*

OECD 415, *One-Generation Reproduction Toxicity Study*

OECD 416, *Two-generation Reproduction Toxicity*

OECD 421, *Reproduction/Developmental Toxicity Screening Test*

OECD 451, *Carcinogenicity Studies*

OECD 453, *Combined Chronic Toxicity/Carcinogenicity Studies*

OECD 471, *Bacterial Reverse Mutation Test*

OECD 473, *In vitro Mammalian Chromosome Aberration Test*

OECD 476, *In vitro Mammalian Cell Gene Mutation Test*

OECD 487, *In Vitro Mammalian Cell Micronucleus Test*

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.

3.1
carcinogenicity test
test to determine the carcinogenic potential of medical devices, materials, and/or extracts using multiple exposures for a major portion of the life span of the test animal

3.2
energy-depositing medical device
device intended to exert its therapeutic or diagnostic effect by the delivery of electromagnetic radiation, ionising radiation or ultrasound

Note 1 to entry: This does not include medical devices that deliver simple electrical current, such as electrocautery medical devices, pacemakers or functional electrical stimulators.

3.3
genotoxicity test
test using mammalian or non-mammalian cells, bacteria, yeasts, fungi or whole animals to determine whether gene mutations, changes in chromosome structure, or other DNA or gene changes are caused by the test samples

3.4
maximum tolerated dose
MTD
maximum dose that a test animal can tolerate without any adverse effects

3.5
reproductive and developmental toxicity test
test to evaluate the potential effects of test samples on reproductive function, embryonic morphology (teratogenicity), and prenatal and early postnatal development

3.6
test sample preparation
residual, extractables, leachables or biodegradable device materials that are resuspended in a vehicle compatible with the test system

4 Requirements for test strategies

4.1 General

ISO 10993-1 indicates circumstances where the potential for genotoxicity, carcinogenicity and reproductive toxicity is a relevant hazard for consideration in an overall biological safety evaluation. Testing to investigate these hazards shall be justified on the basis of a risk assessment. In determining if genotoxicity, carcinogenicity and reproductive toxicity testing of the device is warranted an assessment of risk shall address the following factors

— 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 on the basis of structure-activity relationships or previous demonstration of relevant toxicity in the chemical class,

— the mechanistic basis of the toxic response under consideration, if available,

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

— the extent of previous use of comparable materials in relevant applications,

— consideration of residuals from the final finished device with respect to how well they are characterized and their potential biological activity (e.g. structure-activity relationships, or previous demonstration of relevant outcomes).

— exposure route,

— patient population,

— extent and duration of localized (at the site of implantation or use) and systemic exposure,

— the anticipated impact of test results (or lack of testing) on risk management judgements, and

— changes in the type or amount of residuals that the patient will be exposed to, either through an increase in device exposure, or an increase in devices size when compared to an equivalent device.

Commonly used risk assessment tools (e.g. TTC) may be helpful in evaluating these factors.

Where an analysis of the composition of device materials reveals the presence of chemical constituents that are of concern but for which inadequate toxicity data are available, consideration shall be given to testing individual chemical. Individual chemicals shall be tested in preference to compounded materials or extracts, where this would improve the risk estimate. Where testing of a device material is indicated testing shall be conducted on the final product (including sterilization if applicable), or representatives from the final products, or materials processed in the same manner as the final product (including sterilization if applicable). The decision to test, and the nature of the test sample, shall be justified and documented.

Testing may be warranted for additional states of the device such as, wear debris generated from the device or materials that cure *in situ* (e.g. cements, adhesives and pre-polymer mixtures) unless toxicological risk assessment determines no cause for concern from additional device/material states. For guidance on *in situ* curing devices see ISO 10993-12.

4.2 Additional requirements for carcinogenicity testing

For carcinogenicity testing, in addition to 4.1, the following factors shall be addressed:

— physical characteristics (e.g. particle size and shape, pore size, surface continuity, surface condition, device thickness);

— results from genotoxicity, implantation and other studies.

4.3 Additional requirements for reproductive toxicity testing

For reproductive testing, in addition to 4.1, the total direct or indirect cumulative contact duration with reproductive tissue, the embryo/foetus or the germ cells shall be addressed.

Any information from published literature on the effect of device materials on male/female reproductive organs or from subacute/chronic study on the histopathology of reproductive system should also form the basis before a full scale reproductive toxicity testing is performed.

5 Genotoxicity tests

5.1 General

Before a decision to perform a genotoxicity test is made, ISO 10993-1 shall be taken into account. The rationale for a test programme, taking into consideration all relevant factors given in 4.1 to 4.3, shall be justified and documented.

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

— Gene mutations (point mutations);

— Chromosomal damage [structural aberrations such as translocations, small or large deletions and insertions, and numerical chromosomal aberrations (aneuploidy)].

5.2 Test strategy

5.2.1 General

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

Bacterial reverse mutation assays have been shown to detect relevant genetic changes produced by the majority of genotoxic carcinogens detected by rodent assays. Certain classes of genotoxin, e.g. alkyl halides, are not detected.

The potential of test materials to produce DNA damage in bacterial systems might not be relevant to their likely effects in eukaryotic cells, and therefore, testing in mammalian cell test systems shall be performed unless otherwise justified. Several mammalian cell systems are in use: systems that detect gross chromosomal damage (*in vitro* tests for structural and numerical chromosomal aberrations), systems that detect primarily gene mutations (HPRT mutation assay), and a system that detects gene mutations and clastogenic effects [mouse lymphoma thymidine kinase (tk) assay with both colony number and size determination]. *In vitro* tests for chromosomal damage and the mouse lymphoma tk assay yield results that are equivalent. Results from both tests have a relatively high level of congruence for compounds that are regarded as genotoxic but yield negative results in the bacterial reverse mutation assay. Therefore, the chromosome aberration test and the mouse lymphoma tk assay are currently considered equally acceptable when either is used with the bacterial reverse mutation assay in a standard battery for genotoxicity testing.

5.2.2 Test battery

When genotoxicity testing is performed, the test battery shall include

a) a test for gene mutations in bacteria (OECD 471), modified for medical devices to allow, for example, testing with extracts from devices, see ISO/TR 10993-33:—, Clause 6, and either

b) an *in vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells (OECD 473), modified for medical devices, see ISO/TR 10993-33:—, Clause 7, or

c) an *in vitro* mouse lymphoma tk assay (OECD 476), modified for medical devices, including detection of small (slow growing) and large colonies, see ISO/TR 10993-33:—, Clause 9, or

d) an *in vitro* mammalian cell micronucleus test for chromosomal damage and aneugenicity (OECD 487), modified for medical devices, see ISO/TR 10993-33:—, Clause 8.

When additional relevant factors (such as genotoxic mechanism and pharmacokinetics) that can influence the genotoxic activity of a compound, need to be considered an *in vivo* test may be performed if justified. An *in vivo* test for chromosomal damage in rodent haematopoietic cells could be either an analysis of chromosomal aberrations in bone marrow cells or an analysis of micronuclei in bone marrow

or peripheral blood erythrocytes [see ISO/TR 10993-33:—, Clause 10 (OECD 474) or ISO/TR 10993-33:—, Clause 11 (OECD 475)].

Where applicable, the *in vivo* test for chromosomal damage in rodent haemapoietic cells shall be performed using two extracts (see ISO 10993-12 or Annex A). The preferred application route of polar vehicles is intravenously. The preferred application route for the non-polar vehicles is intraperitoneally.

An *in vivo* assay is not necessary, if the user can demonstrate that the quantities of extractables from the test article are less than the amount of material that would induce a positive response with a potent well-characterized *in vivo* micronucleus genotoxin.

An example is cisplatin (CAS no. 15663-27-1), which was shown a positive response at 0,3 mg/kg, see Reference.[35]

5.2.3 Follow-up evaluation

If genotoxicity testing is performed in accordance with 5.2.2 and if the results of the two *in vitro* tests are negative, further genotoxicity testing in animals is unnecessary.

If any test is positive, the following step-wise procedure is applicable (see also Annex B).

Step 1: Identification of confounding factors in results from the initial set of genotoxicity tests, if available.

a) Identification of confounding factors (e.g. non-physiological conditions, interaction of test article with culture medium, auto-oxidation and cytotoxicity).

b) Identification of metabolic effects (e.g. nature of the exogenous metabolic system, nature of the metabolic profile, unique metabolites).

c) Identification of impurities by chemical characterization (i.e. materials ingredient research or analytical testing).

Step 2: Weight of evidence (WOE) assessment with mechanism and mode of action (MOA) to be considered.

a) Direct DNA reactive versus non direct DNA reactive mode of action.

b) Aneuploidy and polyploidy issues. Is an aneuploidy mechanism involved?

Step 3: Decision point.

Determine whether the extract from the medical device or chemical of concern is a genotoxin and if,

a) the interpretation of results and WOE/MOA analysis within a toxicological risk assessment framework present a low/negligible concern for patients under the expected usage, or

b) the interpretation of results and WOE/MOA analysis within a toxicological risk assessment framework suggest there may be potential risks for patients under expected usage.

If the determination is a) no further additional tests or evaluation are needed.

If the decision is b), then continue to step 4.

Step 4: Perform risk management.

Either manage risks assuming a genotoxic hazard or select the appropriate *in vitro* and/or *in vivo* follow-up testing.

Step 5: Select and run additional *in vitro* and/or *in vivo* test.

Any *in vivo* test shall be chosen on the basis of the most appropriate end point identified by the *in vitro* tests.

in vivo tests commonly used are

— micronucleus test in rodents (OECD 474),

— metaphase analysis in rodent bone marrow (OECD 475),

— transgenic mutagenicity tests (OECD 488).

The decision as to the most appropriate test system shall be justified and documented.

NOTE Recently, a draft OECD guideline for the testing of chemicals on rodent alkaline single cell gel electrophoresis (Comet) assay is under development for genotoxicity testing. This test might prove valuable for medical device testing, but at the time of publication of this International Standard the OECD Guideline was not published.[1)]

An attempt shall be made to demonstrate that the test substance has reached the target organ. For micronucleus test in rodents or metaphase analysis in rodent bone marrow, the bioavailability can be proved by one of the following approaches

— analytical quantification of specific extract compounds in the blood or serum,

— test extract induced cytotoxicity to the bone marrow cells,

— intravenous route of exposure (for polar vehicles).

If the target organ exposure cannot be demonstrated, a second *in vivo* test in another target organ shall be performed to verify the lack of *in vivo* genotoxicity.

Step 6: Reinterpret all of the accumulated data and determine if the test article is genotoxic.

In some cases, positive *in vitro* tests may not be relevant. The following should be considered in determining the overall relevance of the *in vitro* results. This list is not exhaustive but is given as an aid for decision making process.

a) Only one of the original two *in vitro* tests performed had a positive result.

b) Further *in vitro* investigation using similar mechanistic end points do not confirm the positive result.

c) Mechanistic information indicates that positive *in vitro* results are not relevant to *in vivo* situations (e.g. high cytotoxicity, osmolality, etc).

d) *In vivo* testing including evidence that the test sample reached the target organ did not demonstrate a genotoxic effect.

The overall WOE and interpretation of the entire data set shall be documented with the final conclusion. In some cases, site-specific or genetic end point specific tests might be necessary. In most cases, these tests do not have internationally recognized protocols.

5.3 Sample preparation

Unless the sample can be dissolved in a solvent compatible with the test system, appropriate extraction solvents shall be chosen on the basis of its ability to maximize extraction of the material or medical device to a level at which the concentration of genotoxic residues would be sufficient to produce a positive response in the test system, but without degradation of the device or the test sample. The test system vehicle(s) shall be chosen on the basis of its compatibility with the genotoxicity test system. Tests shall be performed on solutions, suspensions (e.g. Method A in Annex A), extracts (e.g. Method C in Annex A) or exaggerated extracts (e.g. Method B in Annex A) of the finished device (including sterilization if applicable), device material, device component or the individual chemicals of the device.

1) OECD Draft-Guideline for the testing of chemicals – In vivo Mammalian Alkaline Comet Assay, available at: http://www.oecd.org/

Device materials should include all final formulation and processing, unless otherwise justified. It is generally not appropriate to conduct testing on raw materials, as formulation and processing could change the potential for toxicity of the final device.

The rationale for choosing to test individual chemicals shall be justified and documented. The rationale shall include considerations of interactions and synergistic effects.

Where relevant, the test material should be extracted with the two solvents (see ISO 10993-12 or Annex A).

Any decision to omit testing with one class of solvent shall be justified and documented.

6 Carcinogenicity tests

6.1 General

Before a decision to perform a carcinogenicity test is made, ISO 10993-1 shall be taken into account. The decision to perform a test shall be justified on the basis of an assessment of the risk of carcinogenesis arising from the use of the medical device. Carcinogenicity testing shall not be performed when risks can be adequately assessed or managed without generating new carcinogenicity test data.

These tests may be designed to examine simultaneously in a single study both chronic toxicity and carcinogenicity. When chronic toxicity and carcinogenicity are to be evaluated in a single study, particular care needs to be taken at the study design stage to ensure the dose groups are appropriate. This helps to prevent or minimize premature mortality from chronic/cumulative systemic toxicity compromising the statistical evaluation of data derived from animals surviving to the end of the study period (i.e. normal life-span).

NOTE *In vitro* cell transformation systems are available for carcinogenicity pre-screening [e.g. Syrian hamster embryo (SHE) cell transformation assay and Balb3T3 cell transformation assay]. At the time of publication of this International Standard an OECD Guideline was not published. Additional information on cell transformation test systems is given in Annex D.

6.2 Evaluation strategy

Carcinogenicity testing of genotoxic materials shall be scientifically justified. In most instances for genotoxic materials, a carcinogenic hazard can be presumed and the risk managed accordingly.

In the absence of evidence to rule out carcinogenic risks for non-genotoxic materials, situations in which the need for carcinogenicity testing shall be considered can include the following:

— materials for which the degradation time is greater than 30 days;

— materials introduced in the body and/or its cavities with a cumulative contact of greater than 30 days.

Circumstances where testing cannot be justified include:

— materials with significant and adequate data on human use or exposure;

— materials that are expected to give rise to solid state carcinogenesis (see Annex E)

— methodological constraints or other circumstances that would limit the predictive value of a test.

To determine if a device has significant human-use history, the assessment should include an evaluation that addresses if the device undergoes a similar manufacturing process, is used to treat a similar patient population, at a similar treatment site and with a smaller or similar accumulated exposure. Human use history should document whether information is available from monitoring for adverse events, particularly cancer risk, in the human use population.