INTERNATIONAL STANDARD

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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.

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 10993-3 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This second edition cancels and replaces the first edition (ISO 10993-3:1992), which has been technically revised.

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

— *Part 1: Evaluation and testing*

— *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 8: Selection and qualification of reference materials for biological tests*

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

— *Part 10: Tests for irritation and delayed-type hypersensitivity*

— *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*

Future parts will deal with other relevant aspects of biological testing.

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 minimized 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 in 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 ongoing scientific and medical advances will alter our understanding of and approaches to these important toxicity test methods. At the time this part of ISO 10993 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 hazard identification but, where it is appropriate, it is important that maximum test sensitivity be achieved. Most tests included in this part of ISO 10993 refer to Guidelines for Testing of Chemicals, prepared by the Organization for Economic Cooperation and Development (OECD).

The interpretation of findings and their implications for human health effects are beyond the scope of this part of ISO 10993. Because 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 has 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 hazard identification and tests on medical devices for the following biological aspects:

— genotoxicity,

— carcinogenicity, and

— reproductive and developmental toxicity.

This part of ISO 10993 is applicable for evaluation of a medical device whose potential for genotoxicity, carcinogenicity or reproductive toxicity has been identified.

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

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 10993-1:1997, *Biological evaluation of medical devices — Part 1: Evaluation and testing*

ISO 10993-2:1992, *Biological evaluation of medical devices — Part 2: Animal welfare requirements*

ISO 10993-6:1994, *Biological evaluation of medical devices — Part 6: Tests for local effects after implantation*

ISO 10993-12:2002, *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[1)], *Prenatal Development Toxicity Study*

OECD 415, *One-Generation Reproduction Toxicity Study*

OECD 416, *Two-Generation Reproduction Toxicity*

1) Organization for Economic Cooperation and Development.

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*

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 tumorigenic potential of medical devices, materials and/or extracts using either single or multiple exposures over a major portion of the life span of the test animal

NOTE These tests may be designed to examine both chronic toxicity and tumorigenicity in a single experimental study. When chronic toxicity and carcinogenicity are evaluated within a single study, care in study design with emphasis on dose selection should be exercised. This will help to ensure that premature mortality from chronic/cumulative toxicity does not compromise the statistical evaluation of animals that survive until scheduled study termination (i.e. normal life-span).

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

NOTE 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 or fungi to determine whether gene mutations, changes in chromosome structure, or other DNA or gene changes are caused by the test samples

NOTE These tests can include whole animals.

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

4 Genotoxicity tests

4.1 General

Before a decision to perform a genotoxicity test is made, ISO 10993-1 and the chemical characterization of materials (ISO 10993-18) shall be taken into account. The rationale for a test programme, taking into consideration all relevant factors, shall be documented.

ISO 10993-1 indicates circumstances where the potential for genotoxicity is a relevant hazard for consideration in an overall biological safety evaluation (see ISO 10993-1:1997, Table 1). Testing for genotoxicity, however, is not necessary for medical devices, and components thereof, made only from materials known to show no genotoxicity. Testing for genotoxicity is indicated where a review of the composition of the materials reveals the possible presence in the final medical device of compounds that might interact with genetic material, or when the chemical composition of the medical device is unknown. In such circumstances, the genotoxic potential of suspect chemical components should be assessed, bearing in mind the potential for synergy, in preference to carrying out genotoxicity tests on the material or medical device as a whole.

When the genotoxicity of a medical device has to be experimentally assessed, a series of *in vitro* tests shall be used. This series shall include either two tests if 4.2.1.2 is performed which uses the mouse lymphoma assay incorporating colony number and size determination, or three tests if 4.2.1.1 is performed. When tests are performed, at least two tests, investigating different end-points, shall use mammalian cells.

4.2 Test strategy

4.2.1 Genotoxicity testing shall be performed on the basis of an initial decision to test in accordance with either Option 1 (4.2.1.1) or Option 2 (4.2.1.2).

4.2.1.1 Option 1

a) a test for gene mutations in bacteria (OECD 471); and

b) a test for gene mutations in mammalian cells (OECD 476); and

c) a test for clastogenicity in mammalian cells (OECD 473)

4.2.1.2 Option 2

a) a test for gene mutations in bacteria (OECD 471); and

b) a test for gene mutations in mammalian cells (OECD 476), specifically a mouse lymphoma assay incorporating colony number and size determination in order to cover both endpoints (clastogenicity and gene mutations).

4.2.2 If the results of all *in vitro* tests performed in accordance with 4.2.1 are negative, further genotoxicity testing in animals is not normally justified and should not be performed, in the interest of preventing undue use of animals.

In vivo testing shall be performed in accordance with ISO 10993-2.

4.2.3 If any of the *in vitro* tests is positive, either *in vivo* mutagenicity tests shall be performed (see 4.2.4) or the presumption shall be made that the compound is mutagenic.

4.2.4 Any *in vivo* test shall be chosen on the basis of the most appropriate endpoint identified by the *in vitro* tests. An attempt shall be made to demonstrate that the test substance has reached the target organ. If this cannot be demonstrated, a second *in vivo* test in another target organ may be required to verify the lack of *in vivo* genotoxicity.

In vivo tests commonly used are:

a) micronucleus test in rodents (OECD 474) or

b) metaphase analysis in rodent bone marrow (OECD 475) or

c) unscheduled DNA synthesis test with mammalian liver cells (OECD 486).

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

4.2.5 If other *in vivo* test systems to investigate genotoxicity are used in order to obtain additional information, the rationale for this shall be justified and documented.

4.3 Sample preparation

4.3.1 Where genotoxicity tests are carried out on the material or a medical device or as a whole, sample preparation shall be in accordance with ISO 10993-12. Tests shall be performed on extracts, exaggerated extracts or the individual chemical compounds of the material/medical device. The highest test concentration shall be within OECD guidelines. If exaggerated extraction conditions are used, care shall be taken that this does not alter the chemical characteristics.

4.3.2 An appropriate solvent shall be chosen on the basis of its compatibility with the test system and its ability to maximize extraction of the material or medical device. The rationale for the choice of solvent shall be documented.

4.3.3 Where relevant, two appropriate extractants shall be used, one of which is a polar solvent, the second a non-polar solvent or liquid appropriate to the nature and use of the medical device, both of which are compatible with the test system.

4.4 Test methods

4.4.1 *In vitro* genotoxicity tests

Test methods for *in vitro* genotoxicity tests shall be chosen from the OECD Guidelines for Testing of Chemicals.

Preferred test methods are: OECD 471, OECD 473, OECD 476, OECD 479 and OECD 482. It may be necessary to consider, in the design and selection of tests, that a number of materials or substances can influence the test, e.g. antibiotics and antiseptics. If this is relevant, the rationale for the decision shall be documented.

4.4.2 *In vivo* genotoxicity tests

Test methods for *in vivo* genotoxicity tests shall be chosen from the OECD Guidelines for Testing of Chemicals.

Preferred test methods are: OECD 474, OECD 475, OECD 478, OECD 483, OECD 484, OECD 485 and OECD 486.

NOTE Recently, transgenic animal test systems have been developed for genotoxicity testing. These tests may prove valuable for medical device testing, but their use has not been validated at the time of publication of this part of ISO 10993. References on test systems are given in the bibliography for transgenic animals.

5 Carcinogenicity tests

5.1 General

Before a decision to perform a carcinogenicity test is made, ISO 10993-1 and ISO 10993-18 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.

NOTE There are suitable *in vitro* cell transformation systems that may be used for carcinogenicity prescreening. Cell transformation tests have so far not been described in International Standards. Additional information on cell transformation test systems are given in Annex A.

5.2 Test strategy

5.2.1 In the absence of evidence to rule out carcinogenic risks, situations in which the need for carcinogenicity testing shall be considered may include the following:

a) resorbable materials and medical devices for which the resorption time is greater than 30 days, unless there are significant and adequate data on human use or exposure;

b) materials and medical devices introduced in the body and/or its cavities with a permanent or cumulative contact of greater than 30 days, except when significant and adequate human-use history is available.

Carcinogenicity testing of genotoxic materials is not scientifically justified. For genotoxic materials, a carcinogenic hazard shall be presumed and the risk managed accordingly.

5.2.2 When in accordance with ISO 10993-1, chronic toxicity and carcinogenicity have been considered, and it is determined that testing is necessary, tests shall be performed in accordance with OECD 453, if possible.

5.2.3 When in accordance with ISO 10993-1, only a carcinogenicity study has been considered, and it is determined that testing is necessary, tests shall be performed in accordance with OECD 451.

5.2.4 One animal species is sufficient for testing medical devices. The choice of species shall be justified and documented.

NOTE Recently, transgenic animal tests have been developed for carcinogenicity testing, but they have not been validated for medical devices at the time of publication of this part of ISO 10993. References on test systems are given in the Bibliography for transgenic animal tests as alternatives to lifetime carcinogenicity tests.

5.3 Sample preparation

Sample preparation shall be in accordance with ISO 10993-12. Whenever possible, the medical device shall be tested in a form representative of its “ready-to-use” state.

5.4 Test methods

5.4.1 If carcinogenicity tests are necessary as part of an evaluation of biological safety, these studies shall be performed with defined chemicals or characterized extracts of medical devices. The performance of implantation studies (see Annex C) shall be justified, and the role in the evaluation of human risk shall be described and documented.

5.4.2 If an implantation study is to be performed, consideration shall be given to the clinical use of the medical device in selecting the implant site.

5.4.3 If testing of an extract is considered relevant, the carcinogenicity tests shall be performed in accordance with OECD 451 or OECD 453.