INTERNATIONAL STANDARD

ISO 10993-3

First edition
1992-12-15

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

Reference number
ISO 10993-3:1992(E)

Contents

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

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.

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.

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

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

— *Part 1: Guidance on selection of tests*

— *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 cytotoxicity: in vitro methods*

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

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 8: Clinical investigation*

— *Part 9: Degradation of materials related to biological testing*

— *Part 10: Tests for irritation and sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

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

Annex A of this part of ISO 10993 is for information only.

Introduction

The basis for biocompatibility evaluation of medical devices is often empirical and driven by the relevant concerns for human safety. 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 and approaches to these important toxicity test methods. At the time the document was prepared, the test methods proposed were those most acceptable. Sound scientific alternatives to the proposed testing should be acceptable insofar as they address relevant matters of safety assessment.

In the selection of tests needed to evaluate a particular device, there is no substitute for a careful assessment of expected human uses and potential interactions of the 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 therefore maximum test sensitivity is required. The interpretation of findings and 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 such factors 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 tests for the following biological aspects:

— genotoxicity,

— carcinogenicity, and

— reproductive and developmental toxicity.

These are relevant in the biological evaluation of some categories of medical devices (see note 1). Guidance on selection of tests is provided in ISO 10993-1. Where the need for the evaluation of the potential for genotoxicity, carcinogenicity or reproductive toxicity has been identified, they should be evaluated in accordance with this part of ISO 10993.

Most tests included in this part of the International Standard refer to the OECD guidelines for testing of chemicals. Reference to these tests is made by the term "OECD guideline(s)" followed by the appropriate test number(s).

At the time of testing, these tests are to be performed according to current OECD guidelines.

NOTE 1 The term "devices" corresponds to the definition given in ISO 10993-1 and covers materials, as well as dental materials and devices. The definition is in accordance with the CEN standard document.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

ISO 10993-2:—[1], *Biological evaluation of medical devices — Part 2: Animal welfare requirements.*

***OECD Guidelines for testing of chemicals* — Selected assays**

— *In vitro* genotoxicity tests

471 *Genetic Toxicology:* Salmonella typhimurium, *Reverse Mutation Assay.*

472 *Genetic Toxicology:* Escherichia coli, *Reverse Mutation Assay.*

473 *Genetic Toxicology:* In vitro *Mammalian Cytogenetic Test.*

476 *Genetic Toxicology:* In vitro *Mammalian Cell Gene Mutation Test.*

479 *Genetic Toxicology:* In vitro *Sister Chromatid Exchange Assay in Mammalian Cells.*

480 *Genetic Toxicology:* Saccharomyces cerevisiae, *Gene Mutation Assay.*

481 *Genetic Toxicology:* Saccharomyces cerevisiae, *Mitotic Recombination Assay.*

1) To be published.

482 *Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells* In vitro.

— ***In vivo* genotoxicity tests**

474 *Genetic Toxicology: Micronucleus Test.*

475 *Genetic Toxicology:* In vivo *Mammalian Bone Marrow Cytogenetic Test — Chromosomal Analysis.*

478 *Genetic Toxicology: Rodent Dominant Lethal Test.*

483 *Genetic Toxicology: Mammalian Germ-Cell Cytogenetic Assay.*

484 *Genetic Toxicology: Mouse Spot Test.*

485 *Genetic Toxicology: Mouse Heritable Translocation Assay.*

— **Carcinogenicity tests**

451 *Carcinogenicity Studies.*

453 *Combined Chronic Toxicity/Carcinogenicity Studies.*

— **Tests for reproductive toxicity**

414 *Teratogenicity.*

415 *One-Generation Reproduction Toxicity Study.*

Rules Governing Medicinal Products in the European Community. Volume 3. Guidelines on the Quality, Safety and Efficacy of Medicinal Products for Human Use. Commission of the European Community 1989. ISBN 92-825-9619-2.

3 Definitions

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

3.1 genotoxicity test: Test that applies 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 materials, devices and/or extracts from materials.

NOTE 2 Tests on whole animals may also address these endpoints.

3.2 carcinogenicity test: Test to determine the tumorigenic potential of devices, materials, and/or extracts to either a single or multiple exposures over a period of the total life-span of the test animal.

NOTE 3 These tests may be designed to examine both chronic toxicity and tumorigenicity in a single experimental study.

3.3 reproductive and developmental toxicity tests: Tests to evaluate the potential effects of devices, materials, and/or extracts on reproductive function, embryonic development (teratogenicity), and prenatal and early postnatal development.

3.4 maximum implantable dose (MID): Maximum amount of implant material (dose) that a test animal can tolerate without any adverse physical or mechanical effects.

NOTE 4 To avoid unnecessary morbidity in animals on a long-term test, preliminary testing may be necessary.

3.5 energy-depositing device: Device intended to exert its therapeutic or diagnostic effect by the absorption of electromagnetic, ionic or ultrasonic radiation.

NOTE 5 This does not include devices which deliver simple electrical current, such as electrocautery devices, pacemakers or functional electrical stimulators.

4 Genotoxicity tests

4.1 General

When the genetic toxicity of a medical device has to be experimentally assessed, a series of *in vitro* tests shall be used. This series shall include at least three assays. At least two of these should preferably use mammalian cells as a target. The tests should preferably cover the three levels of genotoxic effects: DNA effects, gene mutations and chromosomal aberrations.

NOTE 6 OECD tests 471 and 473 have proven useful in the first instance, supported where necessary by test 476.

In vivo testing on animals shall only be carried out in accordance with subclause 4.1 of ISO 10993-2.

Medical devices shall be tested for genotoxicity as specified in ISO 10993-1:1992, except those made only from materials known to show no genotoxicity, when, moreover, all major components of extracts can be identified by suitable analytical methods and have been shown to have no genetic toxicity (see also table 1 of ISO 10993-1:1992).

4.2 Sample preparation

Any material or device shall be in its "ready-to-use" form (i.e. as a final product) prior to any extraction or test procedure. Tests shall be performed either on extracts or the dissolved material using appropriate media.

Where meaningful, two appropriate extractants shall be used, one of which is a physiological medium, the

second a solvent such as dimethylsulfoxide (DMSO), which is reasonably compatible with the test system.

WARNING — DMSO is known to be cytotoxic in selected assay systems at greater than 5 g/l concentrations of aqueous solvent.

The highest reasonably possible surface area per volume of extractant (expressed in square centimetres per millilitre) shall be used.

Materials and devices which are cured *in situ* shall be tested in the cured as well as in the non-cured state.

Extraction shall be performed in closed containers with minimum headspace.

To ensure comparability of results, the extraction temperature should preferably be 37 °C and the extraction time at least 24 h.

When biphasic release characteristics are to be expected, this shall be taken into account.

NOTE 7 A general guideline on sample preparation is under way (ISO 10993-12; see page iii) and may amend or partly substitute this section on sample preparation.

4.3 Test methods

4.3.1 *In vitro* genotoxicity

Test methods shall normally be chosen from the OECD Guidelines for testing of chemicals: 471, 472, 473, 476, 479, 480, 481 and 482.

NOTE 8 Some devices incorporate substances designed to have an effect on cells, e.g. antibiotics or antiseptics that are designed to incorporate an effect on cells.

4.3.2 *In vivo* genotoxicity

If scientifically indicated or *in vitro* test results indicate potential genotoxicity, then *in vivo* genotoxicity tests shall be undertaken. Test methods shall normally be chosen from the OECD Guidelines for testing of chemicals: 474, 475, 478, 483, 484 and 485.

NOTE 9 Recently transgenic animal test systems are being developed for genotoxicity testing. These tests may prove valuable for implant testing but their use had not been validated at the time of publication of this International Standard. References on test systems employing transgenic animals are given in A.1.

5 Carcinogenicity tests

5.1 General

Carcinogenicity tests shall be undertaken as indicated in ISO 10993-1.

Situations suggesting the need for carcinogenicity testing may include the following:

a) resorbable materials and devices, unless there are significant and adequate data on human use or exposure;

b) materials and devices where positive results have been obtained in genetic toxicity testing on mammalian cells;

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

In those cases where carcinogenicity testing is required but no effects have occurred in genotoxicity tests, clinical testing may be performed concurrently with carcinogenicity testing.

Where implantation does not represent the most appropriate route of exposure, scientifically justified alternatives should be considered.

5.2 Sample preparation

Whenever possible the device shall be tested in its "ready-to-use" form. Otherwise a suitably formed implant shall be made of the test material, with appropriate consideration of potential solid state carcinogenicity (Oppenheimer effect, see annex A.3, [15]).

NOTE 10 A general guideline on sample preparation is under way (ISO 10993-12; see page iii) and may amend or partly substitute this section on sample preparation.

5.3 Test methods

Carcinogenicity tests shall be performed in accordance with OECD Guidelines 451 or 453 after suitable modifications for implantable materials.

There will ordinarily be two dose levels, the maximum implantable dose (MID), and a fraction thereof (usually one half of the MID). The controls will generally include polyethylene implants or other materials whose lack of carcinogenic potential is documented in a comparable form and shape.

In carcinogenicity testing on rodents, the maximum implantable dose (MID) of a material or device should be applied. Where possible, this dose should be expressed as multiple of the worst case human exposure in milligrams per kilogram.

Tissues evaluated should include the implantation site and adjacent tissues.

NOTES

11 Suitable cell transformation systems may be used for carcinogenicity prescreening. Cell transformation tests have

so far not been subject to International Standards or national standards. References on cell transformation test systems are given in A.2.

12 There is also some evidence that two-step cell transformation assays can detect carcinogens which are non-genotoxic, but it is at this time not possible to conclude that all non-genotoxic carcinogens can be detected by cell transformation assays. Therefore, carcinogenicity tests have to be performed as lifetime studies *in vivo* on at least one appropriate rodent species.

6 Reproductive toxicity tests

6.1 General

Reproductive toxicity tests should normally be considered for the following:

a) intrauterine devices (IUDs), or any other long-term contact devices likely to come into direct contact with reproductive tissues or the embryo/foetus;

b) energy-depositing devices;

c) resorbable or leachable materials and devices.

There is no need for the testing of resorbable devices or devices containing leachable moieties where there is adequate and reassuring data from absorption, metabolism, distribution and on the reproductive toxicity of all major components identified in extracts. Individual compounds known to cause reproductive toxicity should not be present as significant components of extracts of materials or devices.

6.2 Sample preparation

In the case of energy-depositing devices, whole-body irradiation of the animals with a multiple of the dose to be expected in humans should be applied.

When possible, IUDs, resorbable devices or devices containing leachable moieties shall be tested in their "ready-to-use" form. Otherwise a suitably formed implant shall be made of the test material.

The maximum implantable dose (MID) of a material or device should be applied. Where possible this dose should be expressed as a multiple of the worst case human exposure (in milligrams per kilogram).

NOTE 13 A general guideline on sample preparation is under way (ISO 10993-12; see page iii) and may amend or partly substitute this section on sample preparation.

6.3 Test methods

Assessment of effects on this first generation (F1) should be made according to absorption-kinetic data and OECD Guidelines 414 and 415. As the OECD guidelines were not intended for implantable devices the following modifications shall be considered:

— dose (in the case of energy-depositing devices),

— route of application,

— exposure time (elevated blood levels during organogenesis when possible).

NOTE 14 Depending on intended human use and material characteristics, peri-/post-natal studies may be indicated (see also *Rules Governing Medicinal Products in the European Community. Volume 3*).

If information derived from other tests indicates potential effects on the male reproduction system, then appropriate tests for male reproductive toxicity shall be conducted.

NOTE 15 Recently, *in vitro* reproductive test systems have been developed. They may be useful as a prescreening test method for reproductive toxicity. References to *in vitro* reproductive test systems are included in A.4.

Annex A
(informative)

Bibliography

A.1 Literature on transgenic animals

[1] KOHLER, SW., PROVOST, GS., KRETZ, PL., DYCAICO, MI., SORGE, JA. and SHORT, JM. Development of a short-term *in vivo* mutagenesis assay: the effect of methylation on the recovery of a lambda phage shuttle vector from transgenic mice. *Nucleic Acid Research.* 1990, vol. 18, p. 3007-3013.

[2] SHORT, JM., KOHLER, SW. and PROVOST, GS. The use of lambda phage shuttle vectors in transgenic mice for development of a short term mutagenicity assay. In *Mutation and the environment.* Wiley-Liss: New York, 1990. p. 355-367.

A.2 Literature on cell transformation assays

[3] Advances in Modern Environmental Toxicology. Vol. 1. *Mammalian Cell Transformation by Chemical Carcinogens.* N. Mishra, V. Dunkel, and M. Mehlman (eds). Senate Press: Princeton Junction (New Jersey, 08550), 1981.

[4] *Transformation Assays of Established Cell Lines: Mechanisms and Application.* T. Kakunaga and H. Yamasaki (eds). Proceedings of a Workshop Organized by IARC in Collaboration with the US National Cancer Institute and the US Environmental Protection Agency, Lyon 15-17 Feb. 1984. IARC Scientific Publication No. 67.

[5] BARRETT, JC., OHSHIMURA, M., TANAKA, N. and TSUTSUI, T. Genetic and Epigenetic Mechanisms of Presumed Nongenotoxic Carcinogens. In *Banbury Report 25: Nongenotoxic Mechanisms in Carcinogenesis,* 1987, p. 311-324.

[6] OSHIMURA, M., HESTERBERG, TW., TSUTSUI, T. and BARRETT, JC. Correlation of Asbestos-induced Cytogenetic Effects with Cell Transformation of Syrian Hamster Embryo Cells in Culture. *Cancer Res.* Nov. 1984, vol. 44, p. 5017-5022.

[7] BARRETT, JC., OSHIMURA, M., TANAKA, N. and TSUTSUI, T. Role of Aneuploidy in Early and Late Stages of Neoplastic Progression of Syrian Hamster Embryo Cells in Culture. In *Aneuploidy.* Wicki L. Dellargo, Peter E. Voytek and Alexander Hollaender (eds). Plenum Publishing, 1985.

[8] FITZGERALD, DJ. and YAMASAKI, H. Tumor promotion: models and assay systems. *Teratogenesis Carcinog. Mutagen.,* 1990, vol. 10, No. 2, p. 89-102.

[9] KUROKI, T. and MATSUSHIMA, T. Performance of short-term tests for detection of human carcinogens. *Mutagenesis,* 1987, vol. 2, No. 1, p. 33-7.

[10] RAY, VA., KIER, LD., KANNAN, KL., HAAS, RT., AULETTA, AE., WASSOM, JS., NESNOW, S. and WATERS, MD. An approach to identifying specialized batteries of bioassays for specific classes of chemicals: class analysis using mutagenicity and carcinogenicity relationships and phylogenetic concordance and discordance patterns. 1. Composition and analysis of the overall data base. A report of phase II of the *U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res,* 1987, vol. 3, p. 197-241.

[11] DUNKEL, VC., SCHECHTMAN, LM., TU, AS., SIVAK, A., LUBET, RA. and CAMERON, TP. Interlaboratory evaluation of the C3H/10T1/2 cell transformation assay. *Environ. Mol. Mutagen.,* 1988, vol. 12, No. 1, p. 21-31.

[12] JONES, CA., HUBERMAN, E., CALLAHAM, MF., TU, A., HALLOWEEN, W., PALLOTA, S., SIVAK, A., LUBET, RA., AVERY, MD., KOURI, RE., SPALDING, J. and TENNANT, RW. An interlaboratory evaluation of the Syrian hamster embryo cell transformation assay using eighteen coded chemicals. *Toxicology* in vitro, 1988, vol. 2, No. 2, p. 103-116.