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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

[Revision of second edition (ISO 10993-3:2003)]

ICS 11.100.20

ISO/CEN PARALLEL PROCESSING

This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO-lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five-month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

In accordance with the provisions of Council Resolution 15/1993 this document is circulated in the English language only.

Conformément aux dispositions de la Résolution du Conseil 15/1993, ce document est distribué en version anglaise seulement.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Pour accélérer la distribution, le présent document est distribué tel qu'il est parvenu du secrétariat du comité. Le travail de rédaction et de composition de texte sera effectué au Secrétariat central de l'ISO au stade de publication.

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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* and by Technical Committee CEN/TC 206, *Biological evaluation of medical devices* in collaboration.

This third edition cancels and replaces the second edition (EN ISO 10993:2003), 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 within a risk management procedure*

— *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 delayed-type hypersensitivity*

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— *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: Method for the 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]*

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 ongoing scientific and medical advances will improve our understanding of and approaches to these important toxicity test methods. 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 hazard identification but, where it is appropriate, it is important that maximum test sensitivity is achieved.

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 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 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, *Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management procedure*

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 characterisation 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 Mamalian Chromosome Aberration Test*

OECD 474, *Mammalian Erythrocyte Micronucleus Test*

OECD 475, *Mammalian Bone Marrow Chromosome Aberration Test*

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

3 Terms and definitions

For the purposes of this part of ISO 10993, the definitions given in ISO 10993-1, ISO 10993-12 and the following definitions 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

NOTE 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 minimise 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).

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 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 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

3.6
test sample preparation
residual, extractables, leachables or (resorbable) 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. In determining if genotoxicity, carcinogenicity and reproductive toxicity testing of the device is warranted an assessment of risk shall address the following factors:

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

— material's history of use in patients in relation to a survey or study on reproductive outcome,

— device's intended use,

— exposure route,

— total direct or indirect cumulative contact duration with reproductive tissue, the embryo/foetus or the germ cells,

— patient population,

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

— manufacturing process (e.g. cleaning solvents, leachates, monomers, processing aids, release agents),

— causes of concerns (e.g. structure-activity relationship, previous demonstration of genotoxicity, carcinogenicity and reproductivity in the product class),

— degradation products or metabolites of the device material and

— biologic components,

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

Testing shall be conducted on the sterile final product, or representatives from the final product, or materials processed in the same manner as the final product (including sterilization). 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 10.4.3 of ISO 10993-12:2007.

4.2 Additional requirements for carcinogenicity testing

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

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

b) results from genotoxicity, implantation and other biocompatibility studies.

4.3 Additional requirements for reproductive toxicity testing

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

a) direct physical contact of the device with reproductive tissue or the embryo/foetus,

b) target organ for leachable chemicals is reproductive tissue or the embryo/foetus.

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 numerical chromosomal aberrations such as aneuploidy).

5.2 Test strategy

5.2.1 General

It is clear that 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 for genotoxicity.

Bacterial reverse mutation assays have been shown to detect relevant genetic changes produced by the majority of 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 supplementary testing in mammalian cell test systems is therefore advisable. 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, and a system that detects gene mutations and clastogenic effects (mouse lymphoma thymidine kinase (tk) assay). *In vitro* tests for chromosomal damage and the mouse lymphoma tk assay yield results that are similar. 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 other genotoxicity tests in a standard battery for genotoxicity testing.

Genotoxicity testing shall be performed on the basis of an initial decision to test in accordance with either 5.2.2 or 5.2.3.

5.2.2 *In vitro* test battery

a) A test for gene mutations in bacteria. Bacterial Reverse Mutation Assay, in accordance E.6 (also OECD 471);

and either

b) an *in vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells, Chromosome aberration test, in accordance with E.7 (also OECD 473);

or

c) an *in vitro* mouse lymphoma tk assay in accordance with E.8 (also, OECD 476), including the determination of the ratio of small and large colonies

5.2.3 *In vivo* testing

If the results of the two *in vitro* tests performed in accordance with 5.2.2 are negative, further genotoxicity testing in animals is not normally justified and shall not be performed in the interest of preventing undue use of animals.

If regulatory authorities require *in vivo* testing in the basic test battery, an *in vivo* test for chromosomal damage in rodent haemapoietic cells (see E.9 - OECD 474 or E.10 - OECD 475) should be considered. *In vivo* tests should be conducted if the user cannot adequately address one or more of the following

a) demonstrate the material has a long history of patient use, the final finished medical device residuals are well characterized and the residuals do not express features that may be of concern (e.g. structure-activity relationship, previous demonstration of genotoxicity),

b) explain that the *in vivo* test results are uninformative because the user demonstrated there is inadequate residual present to conduct the *in vivo* test. For example, if the amount of device material residual is less than the amount that would induce a positive response with a potent, well-characterized, *in vivo* micronucleus genotoxin such as, cisplatin,

c) -demonstrate that the medical device does not increase the amount of residuals that the patient will be exposed, either through an increase in device exposure or an increase in device size when compared to an equivalent marketed device.

To request the waiver of the *in vivo* tests, the user should provide *in vitro* data together with the scientific rationale.

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

5.2.4 Follow-up evaluation

If any *in vitro* test is positive, the following evaluation should be carried out as a step-wise procedure within a toxicological risk assessment framework.

The follow-up evaluation should be carried out as a step-wise procedure:

Step 1: Interpretation of results from initial set of genotoxicity tests and additional available data.

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