American National Standard

ANSI/AAMI/ISO 10993-3:2003

Biological evaluation of medical devices— Part 3: Tests for genotoxicity, carcinogenicity, and reproductive toxicity

AAMI Association for the Advancement of Medical Instrumentation

The Objectives and Uses of AAMI Standards and Recommended Practices

It is most important that the objectives and potential uses of an AAMI product standard or recommended practice are clearly understood. The objectives of AAMI's technical development program derive from AAMI's overall mission: the advancement of medical instrumentation. Essential to such advancement are (1) a continued increase in the safe and effective application of current technologies to patient care, and (2) the encouragement of new technologies. It is AAMI's view that standards and recommended practices can contribute significantly to the advancement of medical instrumentation, provided that they are drafted with attention to these objectives and provided that arbitrary and restrictive uses are avoided.

A voluntary *standard* for a *medical device* recommends to the manufacturer the information that should be provided with or on the product, basic safety and performance criteria that should be considered in qualifying the device for clinical use, and the measurement techniques that can be used to determine whether the device conforms with the safety and performance criteria and/or to compare the performance characteristics of different products. Some standards emphasize the information that should be provided with the device, including performance characteristics, instructions for use, warnings and precautions, and other data considered important in ensuring the safe and effective use of the device in the clinical environment. Recommending the disclosure of performance characteristics often necessitates the development of specialized test methods to facilitate uniformity in reporting; reaching consensus on these tests can represent a considerable part of committee work. When a drafting committee determines that clinical concerns warrant the establishment of *minimum* safety and performance criteria, referee tests must be provided and the reasons for establishing the criteria must be documented in the rationale.

A *recommended practice* provides guidelines for the use, care, and/or processing of a medical device or system. A recommended practice does not address device performance *per se*, but rather procedures and practices that will help ensure that a device is used safely and effectively and that its performance will be maintained.

Although a device standard is primarily directed to the manufacturer, it may also be of value to the potential purchaser or user of the device as a fume of reference for device evaluation. Similarly, even though a recommended practice is usually oriented towards health care professionals, it may be useful to the manufacturer in better understanding the environment in which a medical device will be used. Also, some recommended practices, while not addressing device performance criteria, provide guidelines to industrial personnel on such subjects as sterilization processing, methods of collecting data to establish safety and efficacy, human engineering, and other processing or evaluation techniques; such guidelines may be useful to health care professionals in understanding industrial practices.

In determining whether an AAMI standard or recommended practice is relevant to the specific needs of a potential user of the document, several important concepts must be recognized:

All AAMI standards and recommended practices are *voluntary* (unless, of course, they are adopted by government regulatory or procurement authorities). The application of a standard or recommended practice is solely within the discretion and professional judgment of the user of the document.

Each AAMI standard or recommended practice reflects the collective expertise of a committee of health care professionals and industrial representatives, whose work has been reviewed nationally (and sometimes internationally). As such, the consensus recommendations embodied in a standard or recommended practice are intended to respond to clinical needs and, ultimately, to help ensure patient safety. A standard or recommended practice is limited, however, in the sense that it responds generally to perceived risks and conditions that may not always be relevant to specific situations. A standard or recommended practice is an important *reference* in responsible decision-making, but it should never *replace* responsible decisionmaking.

Despite periodic review and revision (at least once every five years), a standard or recommended practice is necessarily a static document applied to a dynamic technology. Therefore, a standards user must carefully review the reasons why the document was initially developed and the specific rationale for each of its provisions. This review will reveal whether the document remains relevant to the specific needs of the user.

Particular care should be taken in applying a product standard to existing devices and equipment, and in applying a recommended practice to current procedures and practices. While observed or potential risks with existing equipment typically form the basis for the safety and performance criteria defined in a standard, professional judgment must be used in applying these criteria to existing equipment. No single source of information will serve to identify a particular product as "unsafe". A voluntary standard can be used as one resource, but the ultimate decision as to product safety and efficacy must take into account the specifics of its utilization and, of course, cost-benefit considerations. Similarly, a recommended practice should be analyzed in the context of the specific needs and resources of the individual institution or firm. Again, the rationale accompanying each AAMI standard and recommended practice is an excellent guide to the reasoning and data underlying its provision.

In summary, a standard or recommended practice is truly useful only when it is used in conjunction with other sources of information and policy guidance and in the context of professional experience and judgment.

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Requests for interpretations of AAMI standards and recommended practices must be made in writing, to the Manager for Technical Development. An official interpretation must be approved by letter ballot of the originating committee and subsequently reviewed and approved by the AAMI Standards Board. The interpretation will become official and representation of the Association only upon exhaustion of any appeals and upon publication of notice of interpretation in the "Standards Monitor" section of the *AAMI News.* The Association for the Advancement of Medical Instrumentation disclaims responsibility for any characterization or explanation of a standard or recommended practice which has not been developed and communicated in accordance with this procedure and which is not published, by appropriate notice, as an *official interpretation* in the *AAMI News.*

American National Standard

ANSI/AAMI/ISO 10993-3:2003
(Revision of ANSI/AAMI/ISO 10993-3:1993)

Biological evaluation of medical devices—Part 3: Tests for genotoxicity, carcinogenicity, and reproductive toxicity

Approved 8 October 2003 by
Association for the Advancement of Medical Instrumentation

Approved 23 October 2003 by
American National Standards Institute, Inc.

Abstract: Specifies strategies for hazard identification and tests on medical devices for genotoxicity, carcinogenicity, and reproductive and developmental toxicity.

Keywords: biological evaluation, genotoxicity, carcinogenicity, medical devices, reproductive and developmental toxicity

AAMI Standard

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Glossary of equivalent standards

International standards adopted in the United States may include normative references to other international standards. For each international standard that has been adopted by AAMI (and ANSI), the table below gives the corresponding U.S. designation and level of equivalency to the international standard. (Note: Documents are sorted by international designation.)

Other normatively referenced international standards may be under consideration for U.S. adoption by AAMI; therefore, this list should not be considered exhaustive.

International designation	U.S. designation	Equivalency
IEC 60601-1-2:2001	ANSI/AAMI/IEC 60601-1-2:2001	Identical
IEC 60601-2-04:2002	ANSI/AAMI DF80:2003	Major technical variations
IEC 60601-2-21:1994 and Amendment 1:1996	ANSI/AAMI/IEC 60601-2-21 & Amendment 1:2000 (consolidated texts)	Identical
IEC 60601-2-24:1998	ANSI/AAMI ID26:1998	Major technical variations
ISO 5840:1996	ANSI/AAMI/ISO 5840:1996	Identical
ISO 7198:1998	ANSI/AAMI/ISO 7198:1998/2001	Identical
ISO 7199:1996	ANSI/AAMI/ISO 7199:1996/(R)2002	Identical
ISO 10993-1:2003	ANSI/AAMI/ISO 10993-1:2003	Identical
ISO 10993-2:1992	ANSI/AAMI/ISO 10993-2:1993/(R)2001	Identical
ISO 10993-3:2003	ANSI/AAMI/ISO 10993-3:2003	Identical
ISO 10993-4:2002	ANSI/AAMI/ISO 10993-4:2002	Identical
ISO 10993-5:1999	ANSI/AAMI/ISO 10993-5:1999	Identical
ISO 10993-6:1994	ANSI/AAMI/ISO 10993-6:1995/(R)2001	Identical
ISO 10993-7:1995	ANSI/AAMI/ISO 10993-7:1995/(R)2001	Identical
ISO 10993-8:2000	ANSI/AAMI/ISO 10993-8:2000	Identical
ISO 10993-9:1999	ANSI/AAMI/ISO 10993-9:1999	Identical
ISO 10993-10:2002	ANSI/AAMI BE78:2002	Minor technical variations
ISO 10993-11:1993	ANSI/AAMI 10993-11:1993	Minor technical variations
ISO 10993-12:2002	ANSI/AAMI/ISO 10993-12:2002	Identical
ISO 10993-13:1998	ANSI/AAMI/ISO 10993-13:1999	Identical
ISO 10993-14:2001	ANSI/AAMI/ISO 10993-14:2001	Identical
ISO 10993-15:2000	ANSI/AAMI/ISO 10993-15:2000	Identical
ISO 10993-16:1997	ANSI/AAMI/ISO 10993-16:1997/(R)2003	Identical
ISO 10993-17:2002	ANSI/AAMI/ISO 10993-17:2002	Identical
ISO 11134:1994	ANSI/AAMI/ISO 11134:1993	Identical
ISO 11135:1994	ANSI/AAMI/ISO 11135:1994	Identical
ISO 11137:1995 and Amdt 1:2001	ANSI/AAMI/ISO 11137:1994 and A1:2002	Identical

International designation	U.S. designation	Equivalency
ISO 11138-1:1994	ANSI/AAMI ST59:1999	Major technical variations
ISO 11138-2:1994	ANSI/AAMI ST21:1999	Major technical variations
ISO 11138-3:1995	ANSI/AAMI ST19:1999	Major technical variations
ISO TS 11139:2001	ANSI/AAMI/ISO 11139:2002	Identical
ISO 11140-1:1995 and Technical Corrigendum 1:1998	ANSI/AAMI ST60:1996	Major technical variations
ISO 11607:2003	ANSI/AAMI/ISO 11607:2000	Identical
ISO 11737-1:1995	ANSI/AAMI/ISO 11737-1:1995	Identical
ISO 11737-2:1998	ANSI/AAMI/ISO 11737-2:1998	Identical
ISO TR 13409:1996	AAMI/ISO TIR13409:1996	Identical
ISO 13485:2003	ANSI/AAMI/ISO 13485:2003	Identical
ISO 13488:1996	ANSI/AAMI/ISO 13488:1996	Identical
ISO 14155-1:2003	ANSI/AAMI/ISO 14155-1:2003	Identical
ISO 14155-2:2003	ANSI/AAMI/ISO 14155-2:2003	Identical
ISO 14160:1998	ANSI/AAMI/ISO 14160:1998	Identical
ISO 14161: 2000	ANSI/AAMI/ISO 14161:2000	Identical
ISO 14937:2000	ANSI/AAMI/ISO 14937:2000	Identical
ISO 14969:1999	ANSI/AAMI/ISO 14969:1999	Identical
ISO 14971:2000 and A1:2003	ANSI/AAMI/ISO 14971:2000 and A1:2003	Identical
ISO 15223:2000	ANSI/AAMI/ISO 15223:2000	Identical
ISO 15223/A1:2002	ANSI/AAMI/ISO 15223:2000/A1:2001	Identical
ISO 15225:2000	ANSI/AAMI/ISO 15225:2000	Identical
ISO 15674:2001	ANSI/AAMI/ISO 15674:2001	Identical
ISO 15675:2001	ANSI/AAMI/ISO 15675:2001	Identical
ISO TS 15843:2000	ANSI/AAMI/ISO TIR15843:2000	Identical
ISO TR 15844:1998	AAMI/ISO TIR15844:1998	Identical
ISO TR 16142:1999	ANSI/AAMI/ISO TIR16142:2000	Identical
ISO 25539-1:2003	ANSI/AAMI/ISO 25539-1:2003	Identical

Committee representation

Association for the Advancement of Medical Instrumentation

Biological Evaluation Committee

The adoption of ISO 10993-3:2003 as an American National Standard was initiated by the AAMI Biological Evaluation Committee, which also functions as a U.S. Technical Advisory Group (TAG) to the relevant work in the International Organization for Standardization (ISO). U.S. representatives from the AAMI Genotoxicity, Carcinogenicity, and Reproductive Toxicity Working Group (U.S. Sub-TAG for ISO/TC 194/WG 6), chaired by Nirmal Mishra, DVM, PhD of the U.S. Food and Drug Administration and Robert Przygoda of Johnson & Johnson, played an active part in developing the ISO standard.

At the time this document was published, the **AAMI Biological Evaluation Committee** had the following members:

Cochairs:	Donald E. Marlowe
	Peter W. Urbanski
Members:	James M. Anderson, MD, PhD, Case Western Reserve University
	Eric R. Claussen, PhD, Becton Dickinson & Company
	Roger Dabbah, PhD, U.S. Pharmacopeial Convention, Inc.
	Lawrence H. Hecker, PhD, Abbott Laboratories
	Edward Mueller, MS, Annapolis, MD
	Barry F.J. Page, Garner, NC
	Melvin E. Stratmeyer, PhD, U.S. Food and Drug Administration/Center for Devices and Radiological Health/OST
	Paul J. Upman, PhD, NAMSA
	Peter W. Urbanski, Medtronic, Inc.
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	Donald E. Marlowe, U.S. Food and Drug Administration/Center for Devices and Radiological Health
	Sharon J. Northup, PhD, U.S. Pharmacopeial Convention, Inc.

At the time this document was published, the **AAMI Genotoxicity, Carcinogenicity, and Reproductive Toxicity Working Group** had the following members:

Cochairs:	Nirmal Mishra, V, DVM, PhD
	Robert Przygoda, PhD
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	Gloria H. Frost, PhD, Cardinal Health Medical Products and Services Group
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	Chad Summers, Nelson Laboratories, Inc.

NOTE—Participation by federal agency representatives in the development of this standard does not constitute endorsement by the federal government or any of its agencies.

Background of ANSI/AAMI adoption of ISO 10993-3:2003

As indicated in the foreword to the main body of this document (page viii), the International Organization for Standardization (ISO) is a worldwide federation of national standards bodies. The United States is one of the ISO members that took an active role in the development of this standard.

International standard ISO 10993-3 was developed by Technical Committee ISO/TC 194, Biological evaluation of medical devices, to specify strategies for hazard identification and tests on medical devices for genotoxicity, carcinogenicity, and reproductive and developmental toxicity.

U.S. participation in this ISO TC is organized through the U.S. Technical Advisory Group for ISO/TC 194, administered by the Association for the Advancement of Medical Instrumentation (AAMI) on behalf of the American National Standards Institute (ANSI). The U.S. made a considerable contribution to this International Standard.

AAMI encourages its committees to harmonize their work with International Standards in the area of biological evaluation of medical devices as much as possible. Upon review of ISO 10993-3, the AAMI Biological Evaluation Committee and the AAMI Genotoxicity, Carcinogenicity, and Reproductive Toxicity Working Group proposed the adoption of 10993-3:2003 verbatim as a revision of ANSI/AAMI/ISO 10993-3:1993.

This edition of ISO 10993-3 is different from the 1992 edition in that it changes the recommendations for sample preparation. The new edition provides options for genotoxicity testing and provides additional guidance for deciding when to perform genotoxicity, carcinogenicity, or reproductive tests. Also, the references were updated in the bibliography and several informative annexes were added.

AAMI and ANSI procedure require that American National Standards be reviewed and, if necessary, revised within five years to confirm currency or reflect technological advances that have occurred since publication, as appropriate.

AAMI (and ANSI) have adopted other ISO standards. See the Glossary of Equivalent Standards for a list of ISO standards adopted by AAMI, which gives the corresponding U.S. designation and the level of equivalency with the ISO standard.

The concepts incorporated in this standard should not be considered inflexible or static. This standard, like any other, must be reviewed and updated periodically to assimilate progressive technological developments. To remain relevant, it must be modified as technological advances are made and as new data comes to light.

Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Standards Department, AAMI, 1110 N. Glebe Road, Suite 220, Arlington, VA 22201-4795.

NOTE—Beginning with the ISO foreword on page viii, this American National Standard is identical to ISO 10993-3:2003.

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*

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*

[1)] Organization for Economic Cooperation and Development.

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 program, 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 endpoints, 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.

5.4.4 Tissues evaluated shall include relevant tissues from the list indicated in OECD 451 or OECD 453, as well as the implantation and adjacent tissues.

6 Reproductive and developmental toxicity tests

6.1 General

6.1.1 Before a decision to perform reproductive and developmental toxicity tests is made, ISO 10993-1 and ISO/DIS 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 reproductive and developmental toxicity arising from the use of the medical device.

6.1.2 There is no need for the reproductive toxicity testing of resorbable medical devices or medical devices containing leachable substances if there are adequate and reassuring data from absorption, metabolism, and distribution studies or on the lack of the reproductive toxicity of all components identified in extracts of materials or medical devices.

6.1.3 Reproductive and developmental toxicity testing is not required where an acceptable biological risk assessment of the medical device takes into account the fact that the risk of reproductive and developmental toxicity has been ruled out.

6.2 Test strategy

In the absence of evidence to rule out reproductive/developmental risks, reproductive/developmental tests shall be considered. This may include tests on the following:

a) prolonged- or permanent-contact devices likely to come into direct contact with reproductive tissues or the embryo/fetus;

b) energy-depositing medical devices;

c) resorbable materials or leachable substances.

If testing is required, this shall start with OECD 421 in order to provide initial information on possible effects on reproduction and/or development. Positive results with these tests are useful for initial hazard assessment and contribute to decisions with respect to the necessity for and timing of additional tests.

If additional tests are considered necessary, they shall be performed in accordance with OECD 414, OECD 415, or OECD 416, as appropriate.

6.3 Sample preparation

6.3.1 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.

6.3.2 In the case of energy-depositing medical devices, whole-body exposure of the animals is appropriate. A multiple of the predicted human exposure to the reproductive organs shall be applied.

6.3.3 The highest dose used in the animals is either the maximum tolerated dose or that limited by the physical constraints of the animal model. This dose shall be expressed as a multiple of the estimated maximum human exposure (in mass and/or surface area of dose per kilogram of subject).

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

6.4 Test methods

6.4.1 Assessment of effects on the first generation (F1) or even second generation (F2) shall be made in accordance with OECD 414, OECD 415 or OECD 416, and OECD 421. As the OECD guidelines were not intended for medical devices, the following modifications shall be considered:

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

— route of application (implant, parenteral, other);

— extraction media (aqueous and non-aqueous extracts);

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

NOTE—Depending on intended human use and material characteristics, peri-/post-natal studies may be indicated.

6.4.2 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—Recently, *in vitro* reproductive test systems have been developed. They can be useful as a prescreening test method for reproductive and developmental toxicity. References to *in vitro* reproductive test systems are included in the bibliography for reproductive/developmental toxicity testing.

7 Test report

7.1 The test report shall include at least the following details, where relevant:

a) description of material and/or medical device, including intended use (e.g. chemical composition, processing, conditioning, and surface treatment);

b) description and justification of test methods, test conditions, test materials, and test procedures;

c) description of analytical methods, including quantification limits;

d) statement of compliance to appropriate good laboratory practices;

e) test results, including summary;

f) statistical methods;

g) interpretation and discussion of results.

7.2 Further details as specified in the relevant OECD guidelines shall be included in the test report, if applicable.

Annex A
(informative)

Cell transformation test system

Cell transformation test systems may be used for carcinogenicity prescreening.

Guidance is given in [12] for *in vitro* cell transformation tests. Further references on cell transformation test systems are given in the bibliography for cell transformation assays.

There is also some evidence that two-step cell transformation assays can detect carcinogens which are non-genotoxic, but at this time it is not possible to conclude that all non-genotoxic carcinogens can be detected by cell transformation assays. Therefore, cell transformation test systems cannot be used as an alternative to lifetime carcinogenicity studies in at least one appropriate rodent species.

Annex B
(informative)

Rationale of test systems

B.1 Genotoxicity tests

The primary function of genotoxicity tests is to investigate, using test cells or organisms, the potential of products to induce genetic changes in man that may be transmitted via the germ cells to future generations. Scientific data generally support the hypothesis that DNA damage in somatic cells is a critical event in the initiation of cancer. Such damage can result in mutations, and tests to detect genotoxic activity may also identify chemicals that have the potential to lead to carcinogenesis. Thus, some of the tests are useful for the investigation of putative carcinogenic activity.

While in classical toxicology tests several pertinent parameters or endpoints can be observed within one experimental design, the same is not true for genetic toxicology. The diversity of the genetic endpoints usually precludes the detection of more than one of them in a single test system.

Approximately fifteen different tests are cited in test guidelines. The selection of the most appropriate of these to meet a particular requirement is governed by a number of factors. These include the type of genetic change it is required to detect, or the metabolic capability of the test system.

It must be emphasized that there is no international agreement on the best combination of tests for a particular purpose, though there have been attempts to harmonize the selection of the most appropriate tests. It may also be helpful to note that there are other mutagenicity tests in use or in development which, although without an OECD Guideline, may also be useful. The existence of the ICH/S2B agreement for pharmaceuticals should be noted.

Chemicals that interact with DNA produce lesions which, after the influence of various repair processes, may lead to genetic changes at the gene level, e.g. gene or point mutations, small deletions, mitotic recombination, or various microscopically visible chromosome changes, and tests are available to investigate each of these events.

Current short-term tests cannot, of course, mimic all the stages in the carcinogenic process and are frequently assumed to detect only the event leading to the initiation phase, i.e. the ability to induce a mutagenic or clastogenic DNA lesion. The main value of these procedures, therefore, lies in their ability to identify substances that may, under certain exposure conditions, either cause cancer by a predominantly genotoxic mechanism or induce the initial phase of the carcinogenic process. It is apparent, from the complexity of the carcinogenic process compared with the relative simplicity of short-term tests, that, although they provide useful qualitative information, considerable caution is required in their interpretation in terms of carcinogenic activity.

Since no single test has proved capable of detecting mammalian mutagens and carcinogens with an acceptable level of precision and reproducibility, it is usual scientific practice to apply these tests in "batteries." Initial information on the mutagenicity of a substance can be obtained using tests that measure gene mutations and chromosomal damage. Because separate procedures are required to investigate these endpoints, a battery of tests is needed.

B.2 Carcinogenicity studies

The objective of a long-term carcinogenicity study is to observe test animals, for a major portion of their life span, for the development of neoplastic lesions, during or after exposure to various doses of a test substance by an appropriate route. Such a test requires careful planning and documentation of the experimental design (see Annex C), a high quality of pathology, and unbiased statistical analysis.

B.3 Reproductive/developmental toxicity tests

Reproductive toxicity tests cover the areas of reproduction, fertility, and teratogenicity. It has been found that many substances can affect fertility and reproduction, often in an insidious manner without other signs of toxicity. Fertility can be affected in males and females, and effects can range from slightly decreased reproductive capability to complete sterility.

Teratogenicity deals with the adverse effects of a substance on the developing embryo and fetus. Reproductive toxicity has an important bearing on the health of mankind. Test techniques are developing and the concept of combined tests, covering all aspects of reproductive toxicology, appears promising.

Annex C
(informative)

Role of implantation carcinogenicity studies

C.1 General

Tumors induced by implants are well known in experiments using rats. This phenomenon is called "foreign body carcinogenesis" or "solid state carcinogenesis." The phenomenon is summarized as follows.

Tumors usually develop around or near an implant with a frequency that is dependent on several factors:

a) the size of the implant (large implants generally produce more sarcomas than small ones);

b) their form (discs are reported to be among the most efficient);

c) their smoothness (those with rough surfaces are less carcinogenic than those with smooth surfaces);

d) the continuity of the surface area (the larger the holes or pores in the implant, the lower the tumor incidence);

e) for certain materials, their thickness (thicker implants produce more sarcomas);

f) the length of time the implant remains in the tissue.

The same material that produces tumors as a film or sheet will, for the most part, produce fewer or no tumors when implanted as a powder, a thread, or a porous material.[33], [34]

On the other hand, many reports indicate a difference of incidence of tumor formation among different materials of similar shape and size using the same animal experimental protocol.

Mechanistic understandings were summarized in an IARC Monograph.[35]

C.2 The process and rationale of decision

Under these circumstances, the Working Group has reconsidered the current guideline in ISO 10993-3 on the design of carcinogenicity studies.

The Working Group were presented with data obtained using a specified protocol including a defined and consistent shape for all implanted materials.[36] This protocol involved 2 year subcutaneous implantation of a film implant of dimensions 10 mm × 20 mm × (0.5 mm to 1.0 mm) in 30 to 50 male Wistar or F344 rats at a number of establishments. These data demonstrated a significant increase in the number of tumors detected in test animals compared to sham-operated controls for all the materials tested, including nominal negative controls. The proportion of test animals with tumors ranged from 7 % for silicone to 70 % for polyethylene, however there was only a little variation (5 %, 7 %, and 10 %) when studies were repeated with silicone. The group also reviewed a presentation on a new hypothesis suggesting that solid state carcinogenesis may be related to interference of gap-junctional intercellular communication caused by cell/material interactions.[37] The group considered this theory promising but considered its relevance to carcinogenic risk to humans as ambiguous.

During the discussion, representatives from European, Japanese, and U.S. regulatory bodies agreed that no decision on carcinogenic risk has been made on the basis of solid state carcinogenesis alone. In the few examples known, where decisions on carcinogenic risk were made using solid state carcinogenesis results, there had always been supporting data, such as positive mutagenicity data.

The conduct of carcinogenicity studies by implantation requires surgically invasive procedures on both test and control (sham-operated) animals. Thus there is a significant animal welfare cost in conducting such a study. In considering the methodology for carcinogenicity studies while undertaking the revision of this part of ISO 10993, the Working Group considered that they could no longer justify requiring carcinogenicity studies to be performed by implantation under the present ambiguous relevance to human risk. The supporting rationale was the lack of any clear role for these implantation studies in decisions affecting the evaluation of biological safety combined with the marked animal welfare cost.

If carcinogenicity studies are deemed necessary (see 5.4.1), however, the method provided in C.3 may assist in the interpretation of carcinogenicity studies performed by implantation. If such studies are performed, the need for the study design should be justified and its role in the evaluation of human risk described.

C.3 Carcinogenicity studies performed as implantation tests

If this optional procedure is performed, the following protocol shall be followed.

While a single maximum implantable dose (MID) group may be sufficient, two dose groups including the MID and a fraction thereof (usually one-half of the MID) are recommended. The negative control group will generally receive a comparable shape and form of a clinically acceptable material or reference control material whose lack of carcinogenic potential has been documented, e.g. polyethylene implants.

In carcinogenicity tests on rodents, the MID of a material or medical device shall be applied. If possible, this dose shall be expressed as a multiple of the worst-case human exposure, in milligrams per kilogram.

The mass and/or surface area that determines the implant dose shall exceed the expected clinical exposure. The rationale for dose selection shall be documented. When appropriate, a suitably formed implant in accordance with ISO 10993-6 shall be made of the test material(s), with appropriate consideration being given for the possibility of inducing solid state carcinogenicity (Oppenheimer effect; see bibliography for genotoxicity and carcinogenicity testing).[31]

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