



มาตรฐานผลิตภัณฑ์อุตสาหกรรม

THAI INDUSTRIAL STANDARD

มอก. 2395 เล่ม 3–2551

ISO 10993-3 : 2003

การประเมินทางชีวภาพของเครื่องมือแพทย์

เล่ม 3 : การทดสอบเพื่อประเมินความเป็นพิษต่อระบบพันธุกรรม

การก่อมะเร็ง และความเป็นพิษต่อระบบสืบพันธุ์

**BIOLOGICAL EVALUATION OF MEDICAL DEVICES -
PART 3 : TESTS FOR GENOTOXICITY, CARCINOGENICITY AND
REPRODUCTIVE TOXICITY**

สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

กระทรวงอุตสาหกรรม

ICS 11.100.20

ISBN 978-974-292-577-2

มาตรฐานผลิตภัณฑ์อุตสาหกรรม
การประเมินทางชีวภาพของเครื่องมือแพทย์

เล่ม 3 : การทดสอบเพื่อประเมินความเป็นพิษต่อระบบ
พันธุกรรม การก่อมะเร็ง และความเป็นพิษต่อระบบสืบพันธุ์

มอก. 2395 เล่ม 3 – 2551

สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม
กระทรวงอุตสาหกรรม ถนนพระรามที่ 6 กรุงเทพฯ 10400
โทรศัพท์ 0 2202 3300

ประกาศในราชกิจจานุเบกษา ฉบับประกาศและงานทั่วไป เล่ม 126 ตอนพิเศษ 21ง
วันที่ 10 กุมภาพันธ์ พุทธศักราช 2552

คณะกรรมการวิชาการคณะที่ 687

มาตรฐานการทดสอบทางชีวภาพของวัสดุอุปกรณ์ทางการแพทย์และทันตกรรม

ประธานกรรมการ

นางสุวรรณมา จารุณูช

กรมวิทยาศาสตร์การแพทย์

กรรมการ

นางสาวศิริพรรณ เอี่ยมรุ่งโรจน์

สำนักงานคณะกรรมการอาหารและยา

-

คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

รองศาสตราจารย์ชาญยุทธ ศุภชาติวงศ์

คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

นางสาวภาวิณี พันยนิติศาสตร์

ศูนย์เนื้อเยื่อชีวภาพกรุงเทพ

นางสุมาลี ปรารค์ประทานพร

องค์การเภสัชกรรม

นายทวีทรัพย์ ชัยสมบูรณ์พันธ์

สมาคมพิษวิทยาแห่งประเทศไทย

กรรมการและเลขานุการ

นางสาวศุสึพร ศรีพัฒนาพิพัฒน์

สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

เนื่องจากเครื่องมือแพทย์ที่ใช้ต้องมีความปลอดภัยต่อร่างกาย การพิจารณาความเสี่ยงต่ออันตรายที่ร้ายแรง และที่ไม่สามารถแก้ไขกลับคืนสภาพเดิมได้ เช่น การก่อกลายพันธุ์ การก่อมะเร็ง อันตรายต่อระบบสืบพันธุ์ เป็นสิ่งสำคัญ ดังนั้นเพื่อให้เกิดความปลอดภัยในการใช้เครื่องมือแพทย์ จึงกำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม การประเมินทางชีวภาพของเครื่องมือแพทย์ เล่ม 3 : การทดสอบเพื่อประเมินความเป็นพิษต่อระบบพันธุกรรม การก่อมะเร็ง และความเป็นพิษต่อระบบสืบพันธุ์ขึ้น

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ กำหนดขึ้นโดยรับ ISO 10993-3 : 2003 Biological evaluation of medical devices-Part 3 : Tests for genotoxicity, carcinogenicity and reproductive toxicity มาใช้ในระดับเหมือนกันทุกประการ (identical) โดยใช้ ISO ฉบับภาษาอังกฤษเป็นหลัก

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ กำหนดขึ้นเพื่อให้ทันกับความต้องการของผู้ใช้ และจักได้แปลเป็นภาษาไทย ในโอกาสอันควร หากมีข้อสงสัยโปรดติดต่อสอบถามที่สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

คณะกรรมการมาตรฐานผลิตภัณฑ์อุตสาหกรรมได้พิจารณามาตรฐานนี้แล้ว เห็นสมควรเสนอรัฐมนตรีประกาศตาม มาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ. 2511



ประกาศกระทรวงอุตสาหกรรม

ฉบับที่ 3893 (พ.ศ. 2551)

ออกตามความในพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม

พ.ศ. 2511

เรื่อง กำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม

การประเมินทางชีวภาพของเครื่องมือแพทย์

เล่ม 3 : การทดสอบเพื่อประเมินความเป็นพิษต่อระบบพันธุกรรม การก่อมะเร็ง

และความเป็นพิษต่อระบบสืบพันธุ์

อาศัยอำนาจตามความในมาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ.2511 รัฐมนตรีว่าการกระทรวงอุตสาหกรรมออกประกาศกำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม การประเมินทางชีวภาพของเครื่องมือแพทย์ เล่ม 3 : การทดสอบเพื่อประเมินความเป็นพิษต่อระบบพันธุกรรม การก่อมะเร็ง และความเป็นพิษต่อระบบสืบพันธุ์ มาตรฐานเลขที่ มอก. 2395 เล่ม 3-2551 ไว้ ดังมีรายการละเอียดต่อท้ายประกาศนี้

ประกาศ ณ วันที่ 23 กรกฎาคม พ.ศ. 2551

สุวิทย์ คุณกิตติ

รัฐมนตรีว่าการกระทรวงอุตสาหกรรม

มาตรฐานผลิตภัณฑ์อุตสาหกรรม

การประเมินทางชีวภาพของเครื่องมือแพทย์

เล่ม 3 : การทดสอบเพื่อประเมินความเป็นพิษต่อระบบพันธุกรรม การก่อมะเร็ง และความเป็นพิษต่อระบบสืบพันธุ์

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ กำหนดขึ้นโดยรับ ISO 10993-3 : 2003 Biological evaluation of medical devices–Part 3 : Tests for genotoxicity, carcinogenicity and reproductive toxicity มาใช้ในระดับเหมือนกันทุกประการ (identical) โดยใช้ ISO ฉบับภาษาอังกฤษเป็นหลัก

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดวิธีการสำหรับการชี้บ่งและการทดสอบอันตรายที่เกิดขึ้นจากเครื่องมือแพทย์ด้านความเป็นพิษต่อระบบพันธุกรรม การก่อมะเร็ง และความเป็นพิษต่อระบบสืบพันธุ์ โดยกำหนดข้อกำหนดทั่วไป ลำดับการทดสอบ การเตรียมตัวอย่าง วิธีทดสอบ และการรายงานผล รายละเอียดให้เป็นไปตาม ISO 10993-3 : 2003

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 414¹⁾, *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.

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/foetus;
- 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 foetus. 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

Tumours 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.

Tumours 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 sarcoma 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 tumour 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 tumours as a film or sheet will, for the most part, produce fewer or no tumours 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 tumour 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 tumours detected in test animals compared to sham-operated controls for all the materials tested, including nominal negative controls. The proportion of test animals with tumours 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 whilst 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]).

Bibliography

General bibliography

- [1] OECD 474, *Mammalian Erythrocyte Micronucleus Test*
- [2] OECD 475, *Mammalian Bone Marrow Chromosome Aberration Test*
- [3] OECD 478, *Genetic Toxicology — Rodent Dominant Lethal Test*
- [4] OECD 479, *Genetic Toxicology — In vitro Sister Chromatid Exchange Assay in Mammalian Cells*
- [5] OECD 480, *Genetic Toxicology — Saccharomyces cerevisiae — Gene Mutation Assay*
- [6] OECD 481, *Genetic Toxicology — Saacharomyces cerevisiae — Miotic Recombination Assay*
- [7] OECD 482, *Genetic Toxicology — DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro*
- [8] OECD 483, *Mammalian Spermatogonial Chromosome Aberration Test*
- [9] OECD 484, *Genetic Toxicology — Mouse Spot Test*
- [10] OECD 485, *Genetic Toxicology — Mouse Heritable Translocation Assay*
- [11] OECD 486, *Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells In vivo*
- [12] *Official Journal of the European Communities*, L 133/73, May 1988, concerning *in vitro* cell transformation tests.

Bibliography for transgenic animals

- [13] GORELICK, N. J. Overview of mutation assays in transgenic mice for routine testing. *Environmental and Molecular Mutagenesis*, 1995, **25**, pp. 218-230
- [14] PROVOST, G.S., ROGERS, B.J., DYCAICO, M.J., and CARR, G. Evaluation of the transgenic Lambda/LacI mouse model as a short-term predictor of heritable risk. *Mutation Research*, 1997, **388**, pp. 129-136
- [15] KRISHNA, G., URDA, G., and THEISS, J. Principles and practice of integrating genotoxicity evaluation into routine toxicology studies: a pharmaceutical industry perspective. *Environmental and Molecular Mutagenesis*, 1998, **32**, pp. 115-120
- [16] MACGREGOR, J.T. Transgenic animal models for mutagenesis studies: role in mutagenesis research and regulatory testing. *Environmental and Molecular Mutagenesis*, 1998, **32**, pp. 106-109
- [17] KOHLER, S.W., *et al.* Development of a short-term *in vitro* mutagenesis assay: The effect of methylation on the recovery of a lambda phage shuttle vector from transgenic mice. *Nucleic Acid Research*, 1990, **18**, pp. 3007-3013
- [18] SHORT, J.M., KOHLER, S.W. and PROVOST, G.S. 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, pp. 355-367

Bibliography for cell transformation assays

- [19] LEBOEUF, R.A., KERCKAERT, K.A., AADEMA, M.J., and ISFORT, R.J. Use of the Syrian hamster embryo and BALB/c 3T3 cell transformation for assessing the carcinogenic potential of chemicals. *IARC Science Publications*, 1999, **146**, pp. 409-425
- [20] LEBOEUF, R.A. *et al.* The pH 6.7 hamster embryo cell transformation assay for assessing the carcinogenic potential of chemicals. *Mutation Research*, 1996, **356**, pp. 65-84
- [21] AARDEMA, M.J., ISFORT, R.J., THOMPSON, E.D., and LEBOEUF, R.A. The low pH Syrian hamster embryo (SHE) cell transformation assay: a revitalized role in carcinogenic prediction. *Mutation Research*. 1996. **356**, pp. 5-9
- [22] ISFORT, R.J. and LEBOEUF, R.A. The Syrian hamster embryo (SHE) cell transformation system: a biologically relevant in vitro model – with carcinogen predicting capabilities – of *in vivo* multistage neoplastic transformation. *Critical Reviews in Oncology*, 1995, **6**, pp. 251-260
- [23] *Advances in Modern Environment Toxicology*, Vol. 1. Mammalian Cell Transformation by Chemical Carcinogens. N. Mishra, V. Dunkel, and M. Mehlman (eds). Senate Press: Princeton Junction, NJ, 1981
- [24] 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*
- [25] BARRET, J.C., OHSIMURA, M., TANAKA, N. and TSUTSUI, T. Genetic and Epigenetic Mechanisms of Presumed Nongenotoxic Carcinogens. In Banbury Report 25: *Nongenotoxic Mechanisms in Carcinogenesis*, 1987, pp. 311-324
- [26] OSHIMURA, M., HESTERBERG, T.W., TSUTSUI, T. and BARRETT, J.C. Correlation of asbestos-induced cytogenetic effects with cell transformation of Syrian hamster embryo cells in culture. *Cancer Res.*, Nov. 1984, **44**, pp. 5017-5022
- [27] BARRETT, J.C., 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*. W. L. Dellargo, P. E. Voytek and A. Hollaender (eds). Plenum Publishing, 1985
- [28] FITZGERALD, D.J. and YAMASAKI, H. Tumor promotion: Models and assay systems. *Teratogenesis Carcinog. Mutagen.*, 1990, **10** (2), pp. 89-102
- [29] KUROKI, T. and MATSUSHIMA, T. Performance of short-term tests for detection of human carcinogens. *Mutagenesis*, 1987, **2** (1), pp. 333-7
- [30] RAY, V.A. *et al.* 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 database. A report of phase II of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat Res*, 1987, **3**, pp. 197-241
- [31] DUNKEL, V.D. *et al.* Interlaboratory evaluation of the C3H/10T1/2 cell transformation assay. *Environ. Mol. Mutagen.*, 1988, **12** (1), pp. 12-31
- [32] JONES, C.A. *et al.* An interlaboratory evaluation of the Syrian hamster embryo cell transformation assay using eighteen coded chemicals. *Toxicology in vitro*, 1988, **2** (2), pp. 103-116

Bibliography for genotoxicity and carcinogenicity testing

- [33] *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, Some Monomers, Plastics, and Synthetic Elastomers, and Acrolein, p. 41, 1979
- [34] *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 74, Surgical Implants and Other Foreign Bodies, pp. 225-228, 1999
- [35] *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 74, Surgical Implants and Other Foreign Bodies, pp. 282-297, 1999
- [36] NAKAMURA A. *et al.* Difference in tumor incidence and other tissue responses to polyetherurethanes and polydimethylsiloxane in long-term subcutaneous implantation into rats, *J. Biomed. Mater. Res.*, 1992, **26**, pp. 631-650
- [37] TSUCHIYA T and NAKAMURA A. A new hypothesis of tumorigenesis induced by biomaterials: Inhibitory potentials of intercellular communication play an important role on the tumor-promotion stage, *J. Long-term Effects Med. Implants*, 1995, **5**, pp. 232-242
- [38] Department of Health. *Guidelines for the testing of chemicals for mutagenicity*. London: HMSO, 1989. (Report on Health and Social Security Subjects No. 35)
- [39] Department of Health. *Guidelines for the evaluation of chemicals for carcinogenicity*. London: HMSO, 1992. (Report on Health and Social Security Subjects No. 42)
- [40] OPPENHEIMER, B.S., OPPENHEIMER, E.T. and STOUT, A.P. Sarcomas induced in rats by implanting cellophane. *Proc. Soc. Exp. Biol. Med.*, 1948, **67** (33)
- [41] BRAND, K.G., JOHNSON, K.H. and BUON, L.C. Foreign Body, Tumorigenesis CRC Crit. Rev. In *Toxicology*, October 1976, p. 353
- [42] BRAND, L. and BRAND, K.G. Testing of Implant Materials for Foreign Body Carcinogenesis. In *Biomaterials*, 1980, p. 819. G.D. Winter, D.F. Gibbons, H. Plenk Jr. (eds). *Advances in Biomaterials*, Volume 3, New York. J. Wiley, 1982
- [43] *Biological Bases for Interspecies Extrapolation of Carcinogenicity Data*. Hill TA., Wands, RC., Leukroth RW. Jr. (eds). (Prepared for the Center for Food Safety and Applied Nutrition, Food and Drug Administration, Department of Health and Human Services, Washington, D.C.) July 1986, Bethesda (MD): Life Science Research Office, Federation of American Societies for Experimental Biology
- [44] *National Toxicology Program Report of the BTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation*, August 1984, Board of Scientific Counselors
- [45] ASTM F 1439-39 *Standard guide for performance of lifetime bioassay for the tumorigenic potential of implant materials*
- [46] CARERE A. *et al.*, Methods and testing strategies for evaluating the genotoxic properties of chemicals, European Commission Report EUR 15945 EN, ISSN 1018-5593, Luxemburg (1995)
- [47] FORAN J.A. (ed.), *Principles for the selection of doses in chronic rodent bioassays*, ILSI Risk Science Institute, Washington DC, USA, ISBN 0.944398-71-5, 1997

Bibliography for reproductive/developmental toxicity testing

- [48] *Guideline for toxicity studies of drugs*, Chapter 4: Reproductive and developmental toxicity studies. First edition. Editorial Supervision by New Drugs Division, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, 1990 Yakuji Nippo Ltd

- [49] GABRIELSON, J.L. and LARSSON, K.S. Proposal for improving risk assessment in reproductive toxicology. *Pharmacol. Toxicol.*, 1990, **66**, pp. 10-17
- [50] NEUBERT, D. *et al.* Results of *in vivo* and *in vitro* Studies for Assessing Prenatal Toxicity. *Environmental Health Perspectives*, 1986, **70**, pp. 89-103
- [51] SADLER, T.W., HORTON, W.E. and WARNER, C.W. Whole Embryo Culture: A Screening Technique for Teratogens? *Teratogenesis, Carcinogenesis, and Mutagenesis*, 1982, **2**, pp. 243-253
- [52] *In vitro Methods in Developmental Toxicology: Use in Defining Mechanisms and Risk Parameters*. G.L. Kimmel and D.M. Kochhar (eds.). Boca Raton (Florida): CRC Press, 1990
- [53] *In vitro* Embryotoxicity and Teratogenicity Tests. F. Homburger and A.H. Goldberg (eds.). *Concepts in Toxicology*, Vol. 3. Karger, Basel, 1985
- [54] BRENT, R.L. Predicting Teratogenic and Reproductive Risks in Humans from Exposure to Various Environmental Agents Using *In vitro* Techniques and *In vivo* Animal Studies. *Congen. Anom.*, 1988, **28** (Suppl.), S41-S55
- [55] TSUCHIYA, T., NAKAMURA, A., IIO, T. and TAKAHASHI, A. Species Differences between Rats and Mice in the Teratogenic Action of Ethylenethiourea: *In vivo/In vitro* Tests and Teratogenic Activity of Sera Using an Embryonic Cell Differentiation System. *Toxicol. Appl. Pharmacol.*, 1991, **109**, pp. 1-6
- [56] TSUCHIYA, T., *et al.* Embryo lethality of new herbicides is not detected by the micromass teratogen tests. *Arch. Toxicol.*, 1991, **65**, pp. 145-149
- [57] KISTLER, A., TSUCHIYA, T., TSUCHIYA, M. and KLAUS, M. Teratogenicity of arotinoids (retinoids) *in vivo* and *in vitro*. *Arch. Toxicol.*, 1990, **64**, pp. 616-622
- [58] TSUCHIYA, T., *et al.* Comparative Studies of Embryotoxic Action of Ethylenethiourea in Rat Whole Embryo and Embryonic Cell Culture. *Teratology*, 1991, **43**, pp. 319-324
- [59] Report of the *in vitro* teratology task force, Organized by the Division of Toxicology, Office of Toxicological Sciences, Center for Food Safety and Applied Nutrition, Food and Drug Administration. *Environmental Health Perspectives*, 1987, **72**, pp. 200-235
- [60] BASS, R., *et al.* Draft guideline on detection of toxicity to reproduction for medical products. *Adverse Drug React. Toxicol. Rev.*, 1991, **9** (3), pp. 127-141
- [61] BROWN *et al.*, Screening chemicals for reproductive toxicity: the current approaches - Report and recommendations of an ECVAM/EST workshop (ECVAM Workshop 12), *ATLA*, 1995, **23**, pp. 868-882
- [62] SPIELMANN, H., Reproduction and development, *Environmental Health Perspective*, **106** (Suppl. 2), 1998, pp. 571-576

Bibliography for transgenic animal tests as alternatives to life-time carcinogenicity tests

- [63] GULEZIAN, D., *et al.* Use of transgenic animals for carcinogenicity testing: considerations and implications for risk assessment. *Toxicol. Pathol.*, 2000, **28**, pp. 482-499
- [64] STORER, R.D. Current status and use of short/medium term models for carcinogenicity testing of pharmaceuticals - Scientific perspective. *Toxicol. Lett.*, 2000, **112-113**, pp. 557-566
- [65] DASS, S.B., BUCCI, T.J., HEFLICH, R.H. and CASCIANO, D.A. Evaluation of the transgenic p53⁺/⁻ mouse for detecting genotoxic liver carcinogens in a short-term bioassay. *Cancer Lett.*, 1999, **143**, pp. 81-85
- [66] TENNANT, R.W., *et al.* Genetically altered mouse models for identifying carcinogens. *IARC Science Publications*, 1999, **146**, pp. 123-150
- [67] MAHLER, J.F., *et al.* Spontaneous and chemically induced proliferative lesions in TG.AC transgenic and p53-heterozygous mice. *Toxicol. Pathol.*, 1998, **26**, pp. 501-511