INTERNATIONAL STANDARD

ISO 10993-11

Second edition
2006-08-15

Biological evaluation of medical devices —

Part 11: Tests for systemic toxicity

Évaluation biologique des dispositifs médicaux —

Partie 11: Essais de toxicité systémique

Reference number
ISO 10993-11:2006(E)

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Published in Switzerland

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 10993-11 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This second edition cancels and replaces the first edition (ISO 10993-11:1993) 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 9: Framework for 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*

— *Part 19: Physico-chemical, morphological and topographical characterization*

— *Part 20: Principles and methods for immunotoxicology testing of medical devices*

Introduction

Systemic toxicity is a potential adverse effect of the use of medical devices. Generalized effects, as well as organ and organ system effects can result from absorption, distribution and metabolism of leachates from the device or its materials to parts of the body with which they are not in direct contact. This part of ISO 10993 addresses the evaluation of generalized systemic toxicity, not specific target organ or organ system toxicity, even though these effects may result from the systemic absorption and distribution of toxicants.

Because of the broad range of medical devices, and their materials and intended uses, this part of ISO 10993 is not overly prescriptive. Whilst it addresses specific methodological aspects to be considered in the design of systemic toxicity tests, proper study design must be uniquely tailored to the nature of the device's materials and its intended clinical application.

Other elements of this part of ISO 10993 are prescriptive in nature, including those aspects that address compliance with good laboratory practices and elements for inclusion in reporting.

While some systemic toxicity tests (e.g. long term implantation or dermal toxicity studies) can be designed to study systemic effects as well as local, carcinogenic or reproductive effects, this document focuses only on those aspects of such studies, which are intended to address systemic effects. Studies which are intended to address other toxicological endpoints are addressed in ISO 10993-3, ISO 10993-6, ISO 10993-10 and ISO/TS 10993-20.

Pyrogenicity (see Annex F) represents an additional systemic effect which has historically been included in this part of ISO 10993. However, efforts are being taken to address pyrogenicity in a dedicated, stand-alone standard.

Finally, toxicology is an imperfect science. The outcome of any single test should not be the sole basis for making a determination of whether a device is safe for its intended use.

Biological evaluation of medical devices —

Part 11:
Tests for systemic toxicity

1 Scope

This part of ISO 10993 specifies requirements and gives guidance on procedures to be followed in the evaluation of the potential for medical device materials to cause adverse systemic reactions.

2 Normative references

The following referenced documents are indispensable for the application of this document. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

ISO 10993-12, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

3 Terms and definitions

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

3.1
dose
dosage
amount of test sample administered (e.g. mass, volume) expressed per unit of body weight or surface area

3.2
dose-effect
relationship between the dosage and the magnitude of a defined biological effect either in an individual or in a population sample

3.3
dose-response
relationship of dosage to the spectrum of effects related to the exposure

NOTE There are two types of dose-response relationships. The first type is the response of an individual to a range of doses. The second type is the distribution of responses of a population of individuals to a range of doses.

3.4
leachable substance
chemical removed from a device or material by the action of water or other liquids related to the use of the device

NOTE Examples of leachable substances are additives, sterilant residues, process residues, degradation products, solvents, plasticizers, lubricants, catalysts, stabilizers, anti-oxidants, colouring agents, fillers and monomers.

3.5
limit test
use of a single group treated at a suitable dosage of test sample in order to delineate the presence or absence of a toxic hazard

3.6
systemic toxicity
toxicity that is not limited to adverse effects at the site of contact between the body and the device

NOTE Systemic toxicity requires absorption and distribution of a toxicant from its entry point to a distant site at which deleterious effects are produced.

3.7
acute systemic toxicity
adverse effects occurring at any time after single, multiple or continuous exposures of a test sample within 24 h

3.8
subacute systemic toxicity
adverse effects occurring after multiple or continuous exposure between 24 h and 28 d

NOTE Since this term is semantically incorrect, the adverse effects occurring within the specified time period may also be described as a short-term repeated exposure systemic toxicity study. The selection of time intervals between 14 d and 28 d is consistent with most international regulatory guidelines and considered a reasonable approach. Subacute intravenous studies are generally defined as treatment durations of > 24 h but < 14 d.

3.9
subchronic systemic toxicity
adverse effects occurring after the repeated or continuous administration of a test sample for a part of the lifespan

NOTE Subchronic toxicity studies are usually 90 d in rodents but not exceeding 10 % of the lifespan of other species. Subchronic intravenous studies are generally defined as treatment durations of 14 d to 28 d.

3.10
chronic systemic toxicity
adverse effects occurring after the repeated or continuous administration of a test sample for a major part of the life span

NOTE Chronic toxicity studies usually have a duration of 6 months to 12 months.

3.11
test sample
material, device, device portion, component, extract or portion thereof that is subjected to biological or chemical testing or evaluation

4 General considerations

4.1 General

Selection of the appropriate test(s) for a device shall be in accordance with ISO 10993-1, giving due consideration to mode and duration of contact.

Testing shall be performed on the final product and/or representative component samples of the final product and/or materials. Test samples shall reflect the conditions under which the device is normally manufactured and processed. If deviations are necessary, they shall be recorded in the test report, together with their justification. For hazard identification purposes, it may be necessary to exaggerate exposure to the test samples.

Physical and chemical properties of the test sample including, for example, pH, stability, viscosity, osmolality, buffering capacity, solubility and sterility, are some factors to consider when designing the study.

When animal tests are considered, to satisfy the provisions of ISO 10993-2, all reasonably and practically available replacement, reduction and refinement alternatives should be identified and implemented. For *in vivo* acute toxicity testing, *in vitro* cytotoxicity data are useful in estimating starting doses [9].

4.2 Selection of animal species

There is no absolute criterion for selecting a particular animal species for systemic toxicity testing of medical devices. However, the species used shall be scientifically justified and in line with the provisions of ISO 10993-2. For acute oral, intravenous, dermal and inhalation studies of medical devices the mouse or rat is preferred with the option of the rabbit in the case of dermal and implantation studies. Non-rodent species may also need to be considered for testing, recognizing that a number of factors might dictate the number or choice of species for study.

It is preferred that a single animal species and strain is used when a series of systemic toxicity studies of different durations are performed, e.g. acute, subacute, subchronic and/or chronic systemic toxicity. This controls the variability between species and strains and facilitates an evaluation related solely to study duration. Should multiple species or strains be used, justification for their selection shall be documented.

4.3 Animal status

Generally, healthy purpose-bred young adult animals of known origin and with defined microbiological health status should be used. At the commencement of the study, the weight variation of animals used within a sex should not exceed ± 20 % of the mean weight. When females are used, they should be nulliparous and non-pregnant. Animal selection shall be justified.

4.4 Animal care and husbandry

Care and handling of animals shall conform to accepted animal husbandry guidelines. Animals shall be acclimatized to the laboratory conditions prior to treatment and the period of time documented. Control of environmental conditions and proper animal care techniques are necessary for meaningful results. Dietary constituents and bedding materials that are known to produce or influence toxicity should be properly characterized and their potential to influence test results taken into account.

4.5 Size and number of groups

4.5.1 Size of groups

The precision of the systemic toxicity test is dependent to a large extent on the number of animals used per dose level. The degree of precision needed and, in turn, the number of animals per dose group needed depends on the purpose of the study.

Group sizes should logically increase with the duration of treatment, such that at the end of the study enough animals in every group are available for thorough biological evaluation. However, the minimum number of animals should be used consistent with obtaining meaningful results (see ISO 10993-2). Recommended minimum group sizes, all routes considered, are given in Table 1.

Table 1 — Recommended minimum group sizes

Study type	Rodent	Non-rodent
Acute [a]	5	3
Subacute	10 (5 per sex) [a]	6 (3 per sex) [a]
Subchronic	20 (10 per sex) [a]	8 (4 per sex) [a]
Chronic	40 (20 per sex) [b, c]	c

[a] Testing in a single sex is acceptable. When a device is intended for use in only one sex, testing should be done in that sex.

[b] The recommendation refers to one dose-level group testing. Where additional exaggerated dose groups are included the recommended group size may be reduced to 10 per sex.

[c] Expert statistical consultation for chronic study group size is recommended. The number of animals tested should be based on the minimum required to provide meaningful data. Enough animals must remain at the termination of the study to ensure proper statistical evaluation of the results.

4.5.2 Number of groups

One dose group treated at a suitable dosage of test sample in a single species could delineate the presence or absence of a toxic hazard (i.e., limit test). However, other multi-dose or dose response studies require multiple groups to delineate the toxic response.

Group numbers may increase when attempting to exaggerate the dose. The following examples for exaggerating dose should be considered:

— multiples of the clinical surface area exposure;

— multiples of the duration of exposure;

— multiples of the extractable fraction of the individual chemicals;

— multiple administrations within a 24-h period.

Other methods to exaggerate the dose may be acceptable. The method used shall be justified.

4.5.3 Treatment controls

Depending on the objective of the study, the nature of the test article and the route of exposure, negative, vehicle and/or sham-treated controls should be incorporated into all systemic toxicity studies. These controls shall mimic the test sample preparation and treatment procedure.

4.6 Route of exposure

Medical devices or their leachable substances may gain access to the body by multiple routes of exposure. The test route of exposure shall be the most clinically relevant to the use of the device, where possible. If an alternative route of exposure is necessary, it shall be justified. Examples of routes of administration can be found in Annex A.

4.7 Sample preparation

Guidance on sample preparation and stability is given in ISO 10993-12.

4.8 Dosing

4.8.1 Test sample administration

Procedures should be designed to avoid physiological changes or animal welfare problems not directly related to the toxicity of the test material. If a single daily dose of a sufficient volume or concentration is not possible, the dose may be given in smaller fractions over a period not exceeding 24 h.

Test samples shall be delivered at a physiologically acceptable temperature. In general, room or body temperature is a common practice. Deviations shall be justified.

Vehicles administered by a parenteral route should be physiologically compatible. When necessary, sample filtration to remove particulates should be used and documented.

Restraint of animals in repeated exposure systemic toxicity studies should generally be limited to between 4 h and 6 h per day. The nature and the duration of restraint should be the minimum required to meet the scientific objectives and should not of themselves compromise the welfare of the test animals. Deviations shall be justified.

When restraint is required animals should be acclimatized to the restraint device prior to test sample administration.

4.8.2 Dosage volumes

Guidance on dosage volume is summarized in Annex B. When multiple dosage groups are used, variability in the test volume may be minimized by adjusting the concentration to ensure a constant volume at all doses. Use of dosage volumes greater than those given in Annex B shall be justified.

Large dose volumes administered by the oral route should be avoided because they have been shown to overload the stomach capacity and pass immediately into the small bowel. Large volumes may also reflux into the oesophagus.

Intramuscular administration is also volume-limited, depending on size of the animal and the muscular site. Species-specific intramuscular administration volumes are addressed in Annex B.

Bolus intravenous injection volumes are usually given over a short period of approximately 1 min. The rate of injection is an important factor and it is suggested that, for rodents, the rate shall not exceed 2 ml/min.

Slow or timed injection, or intravenous infusion, may be required for large volume administration. Regardless of the calculated rate, the rate of fluid administration shall be stopped or decreased if the animal demonstrates a marked change in clinical condition.

Slow intravenous injection rates may be necessary for test samples limited by solubility or irritancy.

Continuous infusion may be used if clinically indicated. The volume and rate of administration will depend on the substance being given and take into account standard fluid therapy practice. As a guide, the volume administered on a single occasion will be < 10 % of the circulating blood volume over 2 h. Minimal effective restraint of test animals is a key factor to be considered for prolonged infusion.

For subcutaneous administration of test article, refer to Annex B. The rate and extent of absorption depends on the test sample formulation.

4.8.3 Dosage frequency

The dosage frequency should be based on clinical relevancy. Exaggerated procedures shall be clearly described and justified.

In acute systemic toxicity studies, the animals should be exposed to the test sample in a single dose or with multiple fractions of the dose given within a 24 h period.

In repeated exposure studies the animals should be dosed with the test sample daily, seven days each week for the duration of the test. Other dosage regimens may be acceptable but shall be justified.

4.9 Body weight and food/water consumption

Body weight change and changes in food and water consumption may be attributed to the effects of a test article. Consequently, individual weights of the animals shall be determined shortly before the test sample is administered (e.g. usually within 24 h for single or acute dosing, and no more than 7 d for repeated exposure studies), at regular intervals throughout the study and at study termination. When dosing by body weight, the most recent body weight should be utilized.

Measurements of food and water consumption, as appropriate, shall be considered for longer-term repeated exposure studies.

4.10 Clinical observations

Clinical observations should be performed by trained individuals to ensure consistent reporting. The frequency and duration of observation should be determined by the nature and severity of the toxic reactions, rate of onset and recovery period. Increased frequency of observation may be necessary in the early phase of a study, especially acute studies. The time at which signs of toxicity appear and disappear, their duration and the time of death are important, especially if there is a tendency for adverse clinical signs or deaths to be delayed. Humane endpoints should be used in order to avoid unnecessary suffering. General clinical observations shall consider the peak period of anticipated effects after dosing.

Observations shall be recorded systematically as they are made. Records shall be maintained for each animal.

Cage-side observations for viability or overt clinical signs shall be recorded at least once each day using common laboratory descriptors of clinical effects (see Annex C).

Morbidity and mortality observations shall be recorded at least twice daily for long-term repeated exposure studies. A more extensive screening for adverse clinical signs may be considered on at least a weekly basis for longer-term repeated exposure studies.

4.11 Clinical pathology

Haematology and clinical chemistry analyses are performed to investigate toxic effects in tissues, organs and other systems. When indicated, these analyses shall be performed on blood samples obtained from repeated exposure study animals at least just prior to, or as a part of, the procedure for scheduled animal termination. Fasting of animals prior to blood sampling may be necessary in some cases. When scientifically indicated, urinalysis can be performed during the last week of a long-term repeated exposure study using timed (e.g. 16 h to 24 h) urine volume collection.

Suggested haematology, clinical chemistry and urinalysis parameters for evaluation are listed in Annex D.

4.12 Anatomic pathology

When clinically indicated, gross pathological evaluations should be considered for acute systemic toxicity studies.

All animals in repeated exposure studies shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents. Selected organs for weighing should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible to avoid drying.

Annex E suggests the tissues that should be weighed and preserved in an appropriate fixation medium for histopathological examination.

A summary of minimum observations for each type of study is given in Table 2.

Table 2 — Summary of observations

Observation	Acute	Subacute	Subchronic/chronic [a]
Body weight change	+	+	+
Clinical observations	+	+	+
Clinical pathology	b	a, b	+
Gross pathology	b	+	+
Organ weights	b	+	+
Histopathology	b	a, b	+

[a] Chronic systemic toxicity testing is generally a time extension of subchronic testing, justified by the human exposure period. Many of the same parameters are recorded and reported. Group sizes may be increased to include satellite groups for which some, or all, of these observations may be made.

[b] Consideration should be given to these measurements when clinically indicated or if longer exposure testing is not anticipated. Lists of suggested blood and organ/tissue analyses are included in Annex D and Annex E.

4.13 Study designs

Study designs are listed in subsequent sections of this part of ISO 10993. Expert consultation for study design is recommended.

4.14 Quality of investigation

Good laboratory practices deal with the organization, process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported. These practices are intended to promote the quality and validity of the test data. They also support the global harmonization effort by facilitating the memoranda of understanding between trading nations. Systemic toxicity studies shall be conducted following such principles.

5 Acute systemic toxicity

5.1 General

Acute systemic toxicity provides general information on health hazards likely to arise from an acute exposure by the intended clinical route. An acute toxicity study might be an initial step in establishing a dosage regimen in subacute/subchronic and other studies and may provide information on the mode of toxic action of a substance by the intended clinical exposure route. Subsequent to test sample administration in acute systemic

toxicity testing, observations are made of effects (e.g. adverse clinical signs, body weight change, gross pathological findings) and deaths. Animals showing severe and enduring signs of distress and pain need to be euthanized immediately. Corrosive or irritating materials known to cause marked pain or distress should be reported as such and need not be tested.

NOTE ICCVAM and ECVAM are currently validating *in vitro* cytotoxicity tests as an alternative to acute toxicity testing.

5.2 Study design

5.2.1 Preparations

Healthy young adult animals are acclimatized to the laboratory conditions for at least 5 d prior to the test. Shorter durations shall be justified. Animals are then randomized and assigned to the treatment groups.

5.2.2 Experimental animals

5.2.2.1 Selection of species

Typically, a rodent species (rat, mouse) will be used. Characteristics of the model (age, weight, etc.) are as described in 4.2 and 4.3. If non-rodent species are used their use shall be scientifically justified.

5.2.2.2 Number and sex

The number and type of group, animals per group, and sex are as described in 4.5.

5.2.2.3 Housing and feeding conditions

The temperature and the relative humidity in the experimental animal rooms should be appropriate for the species, e.g. (22 ± 3) °C and 30 % to 70 % RH, for mice. Typically, the artificial lighting sequence should be 12 h light, 12 h dark.

For feeding, standardized commercial laboratory diets may be used with an unlimited supply of drinking water. Animals should be caged in-groups by sex or individually, as appropriate; for group housing not more than five animals shall be housed per cage.

5.2.3 Test conditions

5.2.3.1 Dose levels

Dose levels shall be as described in 4.8.

Animals in the control group should be handled in an identical manner to the test group subjects with the exception of not being dosed with the test sample.

5.2.3.2 Procedure

The animals receive a single dose of the test sample or, when necessary, multiple doses within a single 24 h period. Signs of toxicity should be recorded as they are observed including the time of onset, degree and duration.

Regular observation of the animals is necessary to ensure that animals are not lost from the study due to cannibalism, autolysis of tissues or misplacement. At the end of the study all surviving animals are euthanized. Any moribund animals should be removed and euthanized when noticed to exhibit such behaviour.

The observation schedules and humane endpoints applied should preclude the possibility of animals being found dead as a direct consequence of test sample toxicity.

5.2.4 Body weights

Body weight measurements should be made immediately before dosing, daily for the first three days after dosing, weekly after the first dose if indicated by study duration, and at the end of the study.

5.2.5 Clinical observations

The observation period for an acute systemic toxicity study shall be at least 3 d, or longer when deemed appropriate. Specifics of frequency and observation type are described in 4.10 and Annex C. In all cases, observations shall be made at a frequency, and appropriate actions taken, to minimize the loss of animals to the study, e.g. necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals. Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern, using the descriptors provided in Annex C.

5.2.6 Pathology

5.2.6.1 Clinical pathology

Clinical pathology evaluations shall be considered when clinically indicated. The following examinations should be made.

a) Haematology, as described in Annex D, should be considered for investigation at the end of the test period.

b) Clinical biochemical determination on blood, as listed in Annex D, should be considered at the end of the test period. Test areas which are considered appropriate to acute exposure studies are liver and kidney function. Additional clinical biochemistry may be utilized where necessary to extend the observation of the observed effects.

Urinalysis is not necessary on a routine basis but only when there is an indication based on expected or observed toxicity. Suggested parameters are listed in Annex D.

5.2.6.2 Gross pathology

Gross pathological evaluations shall be considered when clinically indicated. This should include an examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. When appropriate, consideration should also be given to recording the weight of the brain, liver, kidneys, adrenals and testes, which should be weighed wet as soon as possible after dissection to avoid drying and subsequent falsely low values.

5.2.6.3 Histopathology

Full histopathology is not typically carried out on organs and tissues from animals in the acute systemic toxicity study, unless indicated specifically by unique gross necropsy findings.

5.3 Evaluation criteria

5.3.1 General

Depending on the test design utilized, the following evaluation criteria apply.

a) For pharmacopoeia-type testing

— If during the observation period of an acute systemic toxicity test none of the animals treated with the test sample shows a significantly greater biological reactivity than animals treated with the vehicle control, the sample meets the requirements of this test.

— Using five animals, if two or more animals die, or if behaviour such as convulsions or prostration occurs in two or more animals, or if a body weight loss greater than 10 % occurs in three or more animals, the sample does not meet the requirements of the test.

— If any animals treated with the sample show only slight signs of biological reactivity, and not more than one animal shows gross symptoms of biological reactivity or dies, repeat the testing using groups of ten animals.

— On the repeat test, if all ten animals treated with the sample show no scientifically meaningful biological reactivity above the vehicle control animals during the observation period, the sample meets the requirements of this test.

b) For non-pharmacopoeia acute systemic toxicity tests

The option exists to perform evaluations using more extensive methods including clinical and anatomic pathology, which may eliminate the need for a repeat test. Acute exposure may include a re-evaluation if there are equivocal differences from concurrent controls. Differences should be explained and the study extended to include an additional five animals, if applicable.

5.3.2 Evaluation of results

The findings of an acute systemic toxicity study should be evaluated in conjunction with the findings of preceding studies, if available, and considered in terms of the toxic effects and the gross necropsy findings, if observed. The evaluation shall include the relationship between the dose of the test substance and the presence or absence and the incidence and severity of abnormalities, including behavioural and clinical abnormalities, gross lesions, body weight changes, effects on mortality and any other general or specific effects.

5.4 Final report

The following information, where applicable, shall be contained in the final test report for the acute systemic toxicity study.

a) Test substance;

— physical nature, purity and physiochemical properties, as appropriate;

— other identification data.

b) Vehicle (if appropriate);

— justification for choice of vehicle if other than those listed in ISO 10993-12.

c) Test animals;

— species/strain used;

— number, age and sex of animals;

— source including microbiological status (e.g. barrier raised, conventional), housing conditions (temperature, humidity, bedding, lighting, diet, etc.);

— weights at the start of the test.

d) Test conditions;

— rationale for dose selection;

— details of test substance formulation/preparation; achieved concentrations; stability and homogeneity, if appropriate;

— details of the administration of the test substance;

— conversion from test substance concentration (ppm) to the actual dose (mg/kg BW), if applicable;

— details of food, water and bedding quality.

e) Results;

— data may be summarized in tabular form, showing for each control and test group the number of animals at the start of the test, the number of animals showing adverse clinical signs, and the number of animals displaying body weight changes;

— body weight/body weight change;

— food and water consumption, if applicable;

— toxic response data by sex and dose level, including signs of toxicity;

— nature, severity and duration of clinical observations (whether reversible or not);

— neurobehavioural assessments, if applicable;

— haematological tests utilized and results with relevant baseline data, if applicable;

— clinical biochemistry tests utilized and results with relevant baseline data, if applicable;

— urinalysis tests utilized and results with relevant baseline data, if applicable;

— terminal body weight and organ weight data, if applicable;

— necropsy findings;

— detailed description of all histopathological findings, if applicable;

— statistical evaluation of results where used and a discussion of their biological significance.

f) Discussion of results.

g) Conclusions.

h) Quality assurance statement.

An acute systemic toxicity study will provide information on the effects of acute exposure to a test substance. Extrapolation of the results of the study to humans is valid to a limited degree but it can provide useful information on permissible exposure.

6 Repeated exposure systemic toxicity (subacute, subchronic and chronic systemic toxicity)

6.1 General

While acute toxicity deals with the adverse effects of single doses (or limited exposure), a more common form of human exposure to many medical devices is in the form of repeated or continuous exposures. Effects from

repeated or continuous exposure may potentially occur due to accumulation of chemicals in tissues or by other mechanisms, and it is important to identify any potential for these by long-term testing (subacute, subchronic, chronic).

Repeated exposure systemic toxicity tests provide information on health hazards likely to arise from a prolonged exposure by the intended clinical route. It might also provide information on the mode of toxic action of a substance by the intended clinical exposure route.

Repeated exposure systemic toxicity studies will provide detailed information on toxic effects, target organs, reversibility or other effects and may serve as the basis for safety estimation. Results of these studies provide important information that is reflected in the extent of the guidance of clinical and anatomic pathology investigations.

Repeated exposure studies do not generally provide a retest criterion. Rather, group sizes are designed to accommodate a statistical assessment of the recorded observations (see Table 1).

Because of the variable durations for repeated exposure studies, test samples shall be prepared as required, to assure their stability.

6.2 Study design

6.2.1 Preparations

Healthy young adult animals are acclimatized to the laboratory conditions for at least 5 d prior to the test. Animals are then randomized and assigned to the treatment groups.

6.2.2 Experimental animals

6.2.2.1 Selection of species

Typically the rodent (rat, mouse) will be used. Characteristics of the model (age, weight, etc.) are described in 4.2 and 4.3. When non-rodent species are used they shall be scientifically justified.

6.2.2.2 Number and sex

The number and type of groups, animals per group, and sex are as described in 4.5.1. When scientifically justified, consideration should be given to the use of satellite animals treated with the high dose level along with satellite controls for a predetermined period beyond the terminal euthanasia. This group, with its controls, may be used to examine treatment effects including reversibility, persistence or delayed toxic effects. For subchronic studies the satellite animals shall be maintained for not less than 28 d.

6.2.2.3 Housing and feeding conditions

The temperature and the relative humidity in the experimental animal rooms should be appropriate for the species, e.g. (22 ± 3) °C and 30 % to 70 % RH, for rats. Typically, the artificial lighting sequence should be 12 h light, 12 h dark.

For feeding, standardized commercial laboratory diets may be used with an unlimited supply of drinking water. Animals may be caged in groups by sex or individually, as appropriate; for group housing not more than five animals should be housed per cage.

6.2.3 Test conditions

6.2.3.1 Dose levels

The experimental animal and other resource investment of the repeated exposure systemic toxicity study, in addition to the goal of establishing human safety, warrants consideration of multiple groups to examine dose-response effects. Dose levels shall be as described in 4.8.

The dose to use for toxicity tests of medical devices shall be defined in relation to the results of risk assessment, balancing the clinical exposure dose with the use of safety factors, as applicable. For longer duration studies, efforts should be made to include at least three dose levels and appropriate controls. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects.

Unlike classical chemical studies of repeated exposure systemic toxicity, repeated exposure studies with medical devices often do not result in a dose-response effect, thus a toxic effect at the highest dose level is not mandatory. Nevertheless, utilizing a range of doses will provide a useful estimation of the margin of human safety.

6.2.3.2 Procedure

The animals are dosed with the test sample ideally on 7 d/week, over the duration of the study. For longer term repeated exposure studies, dosing on a 5 day/week basis is acceptable but should be documented and justified.

6.2.4 Body weights

Body weight measurements should be made immediately before dosing, weekly after the first dose if indicated by study duration, and at the end of the study.

6.2.5 Clinical observations

The observation period for a repeated dose systemic toxicity study shall be appropriate for the duration of the study. Specifics of frequency and observation type are described in 4.10 and Annex C. In all cases, observations shall be made at a frequency, and appropriate actions taken, to minimize the loss of animals to the study, e.g. necropsy or refrigeration of those animals found dead and isolation or euthanasia of weak or moribund animals. Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern, using the descriptors provided in Annex C.

Typically, ophthalmologic examinations, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study, preferably in all animals but at least in the high dose and control groups. If changes in the eyes are detected, all animals should be examined. Exception to examination should be documented and justified.

6.2.6 Pathology

6.2.6.1 Clinical pathology

The following examinations should be made.

a) Haematology, as described in Annex C, should be investigated at the end of the test period. Depending on the length of the study, more frequent sampling should be considered.

b) Clinical biochemical determination on blood should be carried out at the end of the test period. Depending on the length of the study, more frequent sampling should be considered. Test areas that are considered appropriate to all repeated exposure studies are electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests may be influenced by observations on the mode of

action of the test substance. Suggested determinations are listed in Annex D. Additional clinical biochemistry may be utilized where necessary to extend the observation of the observed effects.

Urinalysis is not necessary on a routine basis but only when there is an indication based on expected or observed toxicity. Suggested parameters are listed in Annex D.

Historical data for normal values are useful for establishing baseline levels and for comparison with concurrent study controls. If historical baseline data are deemed inadequate, consideration should be given to the collection of this information for animals of the same age, sex, strain and source, preferably within the same laboratory.

6.2.6.2 Gross pathology

All animals should be subjected to full gross necropsy, which includes examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus and uterus should be weighed wet as soon as possible after dissection to avoid drying and subsequent falsley low values. The organs and tissues listed in Annex E should be preserved in a suitable medium for possible future histopathological examination.

6.2.6.3 Histopathology

a) Full histopathology should be carried out on organs and tissues from animals in the control and high dose groups.

b) All gross lesions should be examined.

c) The lungs of animals in the low and intermediate dose groups, if used, should be subjected to histopathological examination for evidence of infection, since this provides a convenient assessment of the state of health of the animals. Consideration should also be given to histopathological examination of the liver and kidneys in these groups. Further histopathological examination may not be required routinely on the animals in these groups but must always be carried out in organs which showed evidence of lesions in the high dose group.

d) When a satellite group is used, histopathology may be performed on tissues and organs identified as showing effects in the treated groups.

e) In general, for chronic studies, sentinel animals should be used for monitoring the occurrence of infectious agents. Serology or histology of sentinel groups may be performed as indicated.

6.3 Evaluation criteria

6.3.1 General

Data may be summarized in tabular form, showing, for each test group, the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion. Statistical evaluations should be performed but biological relevance should be considered first. All generally accepted statistical methods may be used; the statistical methods should be selected during the design of the study.

6.3.2 Evaluation of results

The findings of a repeated exposure study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects and the necropsy and histopathological findings. The evaluation shall include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioural and clinical abnormalities, gross lesions, microscopic changes, identified in target organs, effects on mortality and any other general or specific effects.

6.4 Final report

The information given in 5.4 shall be contained in the final report for the repeated exposure systemic toxicity study. In addition the following information shall be provided:

— haematological tests utilized and results with relevant baseline data;

— clinical biochemistry tests utilized and results with relevant baseline data;

— histopathological findings;

— a statistical evaluation of results where used and a discussion of their biological significance.

A long-term systemic toxicity study will provide information on the effects of repeated exposure to a test substance. Extrapolation of the results of the study to humans is valid to a limited degree but it can provide useful information on permissible human exposure.

Annex A
(informative)

Routes of administration

A.1 General

Several routes of administration are listed in A.2 to A.10. Other routes of administration may be more clinically relevant and should be utilized. The most relevant route of administration shall be used. If an alternative route of administration is used it shall be justified. Expert consultation is suggested when designing appropriate studies.

A.2 Dermal

Tests for systemic toxicity by the dermal route may be appropriate for surface devices. Consideration should be given to limiting animal oral access to the test sample.

A.3 Implantation

Tests for systemic toxicity by implantation may be appropriate for implanted devices. The test may be appropriate for direct testing of a material by application to a general or specific area. Shape and texture of the test article should be taken into consideration. Methods for implantation can be found in ISO 10993-6.

A.4 Inhalation

Tests for systemic toxicity by the inhalation route may be appropriate for devices with a contact environment conducive to volatile chemical vapour leaching or for an aerosol/particulate test sample with potential for inhalation. Protocol specifics for this route of administration may be found in most dedicated texts for inhalation toxicology.

A.5 Intradermal

Tests for systemic toxicity by the intradermal route may be appropriate for a device with an intradermal contact environment conducive to chemical leaching. Test samples are typically administered directly to the intradermal region by injection. The use of multiple treatment sites should be clearly described and justified.

A.6 Intramuscular

Tests for systemic toxicity by the intramuscular route may be appropriate for devices with a muscle tissue contact environment conducive to chemical leaching. Test samples are typically administered directly to the muscle tissue by injection or surgical implantation. Sites need to be chosen to minimize the loss of function or the possibility of pain from nerve damage caused by muscle fibre tension from the injected or implanted test sample. Sites should be rotated for repeated dose studies since, for example, non-aquaous formulations may remain as a depot for > 24 h. The use of multiple treatment sites should be clearly described and justified.

A.7 Intraperitoneal

Tests for systemic toxicity by the intraperitoneal route may be appropriate for devices with a fluid-path or peritoneal cavity contact environment conducive to chemical leaching. This is also an appropriate route when the extract should not be given intravenously, such as with non-polar oil extracts and where particulates might be present. This route is preferable to filtering for an intravenous injection. Test samples are typically administered directly to the peritoneal cavity. Dose frequency calculations should consider that test articles administered by this route are absorbed primarily through the portal circulation and therefore must pass through the liver before reaching general circulation. Care should be taken not to inject into the stomach or intestinal tract.

A.8 Intravenous

Tests for systemic toxicity by the intravenous route may be appropriate for devices with a direct or indirect fluid-path or blood contact environment conducive to chemical leaching. Test samples are typically placed in or administered directly to the vascular system. If particulates are present, delivery by the intraperitoneal route or sample filtration should be considered. Recommended dosage volumes and rates of administration for intravenous studies with the most commonly used laboratory animal species are listed in Annex B.

Care should be taken to minimize the possibility of extra vascular injection of test sample. For injection taking 5 min or more, consideration should be given to the use of a butterfly needle or an intravenous cannula.

A.9 Oral

Tests for systemic toxicity by the oral route may be appropriate for devices contacting the oral mucosa directly or indirectly, or for products with other enteral application. Test samples are typically administered by gavage. Experimental animals should generally be fasted prior to test sample administration. The period of fasting may range from hours to overnight, with the shorter periods for animals with higher metabolic rates. Following the period of fasting, the animals should be weighed and then the test sample administered in a single dose based on body weight. After the test sample has been administered, food may be withheld for an additional 3 h to 4 h. Where a dose is administered in fractions over a certain period, it may be necessary to provide the animals with food and water depending on the length of the period.

A.10 Subcutaneous

Tests for systemic toxicity by the subcutaneous route may be appropriate for a device with a subcutaneous contact environment conducive to chemical leaching. Test samples are typically administered directly to the subcutaneous region by injection or by implantation. The use of multiple treatment sites should be clearly described and justified.

Annex B
(informative)

Dosage volumes

B.1 General

The principles of humane animal research require that all reasonable efforts be made to minimize or eliminate all adverse physiological or pathological effects. The values listed in Table B.1 are the maximum limits reported in the literature. These values should not be taken as a recommendation in this part of ISO 10993 but investigators should apply upper limits with regard to factors such as body weight/surface area, rate of administration, physical-chemical and biological properties of the test sample, and animal strain. Attempts should be made to minimize the dosage volume while taking into consideration these adjustment factors.

Table B.1 — Maximum dosage volumes for test sample administration

Species	Subcutaneous ml/kg	Intramuscular ml/kg	Intraperitoneal ml/kg	Gavage ml/kg	Intravenous ml/kg
Rat	20	1	20	50	40
Mouse	50	2	50	50	50
Rabbit	10	1	20	20	10
Dog	2	1	20	20	10
Monkey	5	1	20	15	10

NOTE Regulations of individual countries may supersede the maximum volumes listed above. It is generally recommended that intramuscular administrations in rodents should not exceed 0,1 ml/site (mouse) and 0,2 ml/site (rat).

B.2 Dosage volume references

See Bibliography, Part 2 [10-15].

Annex C
(informative)

Common clinical signs and observations

Table C.1 — Common clinical signs and observations

Clinical observation	Observed sign	Involved system(s)
Respiratory	Dyspnea (abdominal breathing, gasping), apnoea, cyanosis, tachypnea, nostril discharges	CNS, pulmonary, cardiac
Motor activities	Decrease/increase somnolence, loss of righting, anaesthesia, catalepsy, ataxia, unusual locomotion, prostration, tremors, fasciculation	CNS, somatomotor, sensory, neuromuscular, autonomic
Convulsion	Clonic, tonic, tonic-clonic, asphyxial, opisthotonos	CNS, neuromuscular, autonomic, respiratory
Reflexes	Corneal, righting, myotact, light, startle reflex	CNS, sensory, autonomic, neuromuscular,
Ocular signs	Lacrimation, miosis, mydriasis, exophthalmos, ptosis, opacity, iritis, conjunctivitis, chromodacryorrhea, relaxation of nictitating membrane	Autonomic, irritation
Cardiovascular signs	Bradycardia, tachycardia, arrhythmia, vasodilation, vasoconstriction,	CNS, autonomic, cardiac, pulmonary
Salivation	Excessive	Autonomic
Piloerection	Rough hair	Autonomic
Analgesia	Decrease reaction	CNS, sensory
Muscle tone	Hypotonia, hypertonia	Autonomic
Gastrointestinal	Soft stool, diaorrhea, emesis, diuresis, rhinorrhea	CNS, autonomic, sensory, GI motility, kidney
Skin	Edema, Erythema	Tissue damage, irritation

Annex D
(informative)

Suggested haematology, clinical chemistry and urinalysis measurements

D.1 Haematology

— Clotting potential (PT, APTT)

— Haemoglobin concentration

— Haematocrit

— Platelet count

— Red blood cell count

— White blood cell count

— WBC differential

D.2 Clinical chemistry

— Albumin

— ALP

— ALT

— AST

— Calcium

— Chloride

— Cholesterol

— Creatinine

— GGT

— Glucose

— Inorganic phosphorus

— Potassium

— Sodium

— Total bilirubin

— Total protein

— Triglycerides

— Urea nitrogen

— Additional enzymes, as scientifically appropriate

— Total immunoglobulin levels may be considered as an indicator for immunotoxicity

D.3 Urinalysis (timed collection, e.g., 16 h to 24 h)

— Appearance

— Bilirubin

— Glucose

— Ketones

— Occult Blood

— Protein

— Sediment

— Specific gravity or osmolality

— Volume

— Other scientifically appropriate tests if test article is suspected to cause specific organ toxicity (generally requires refrigerated sample collection)