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Biological evaluation of medical devices —

Part 11: Tests for systemic toxicity

Évaluation biologique des dispositifs médicaux —

Partie 11: Essais de toxicité systémique

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 194 *Biological and clinical evaluation of medical devices*.

This third edition cancels and replaces the second edition (ISO 10993-11:2006), which has been technically revised with the following changes:

a) reduction in group size for chronic toxicity testing in Table 1;

b) a new Annex F was added;

c) the original Annex F was moved to Annex G;

d) a new Annex H was added;

e) the Bibliography was updated.

A list of all parts in the ISO 10993 series can be found on the ISO website.

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 document 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 document is not overly prescriptive. While it addresses specific methodological aspects to be considered in the design of systemic toxicity tests, proper study design has to be uniquely tailored to the nature of the device's materials and its intended clinical application.

Other elements of this document 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 end points are addressed in ISO 10993-3, ISO 10993-6, ISO 10993-10 and ISO/TS 10993-20.

Prior to conducting a systemic toxicity study, all reasonably available data and scientifically sound methods in the planning and refinement of the systemic toxicity study design should be reviewed. This includes the suitability of use of input data such as existing toxicological data, data from chemical characterization studies and/or other biological tests (including *in vitro* tests and less invasive *in vivo* tests) for the refinement of study design, dose selection, and/or selection of pathological end points to cover in the evaluation of a study. For the repeated exposure systemic toxicity study in particular, the use of scientifically sound study design, the use of pilot studies and statistical study design and the use of unbiased, quantitative end points/methods in the pathological (including histopathological) and clinical chemistry methods are important so as to obtain data which have sufficient scientific validity.

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 document 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 documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

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

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

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 1 to entry: 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 1 to entry: 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 1 to entry: 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 within 72 h after single, multiple or continuous exposures of a test sample for 24 h

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

Note 1 to entry: 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 1 to entry: 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 for rodents and non-rodents, respectively.

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 1 to entry: 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

Before a decision to perform a systemic toxicity test is made, ISO 10993-1 shall be taken into account. The decision to perform a test shall be justified on the basis of an assessment of the risk of systemic toxicity. 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, all reasonably and practically available replacement, reduction and refinement alternatives should be identified and implemented to satisfy the provisions of ISO 10993-2. For *in vivo* acute toxicity testing, *in vitro* cytotoxicity data are useful in estimating starting doses.

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 rodent (mouse or rat) is preferred with the option of the rabbit (lagomorph) in the case of dermal and implantation studies. Other 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 are 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	30 (15 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 for rodents 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 non-rodent group size is recommended. The number of animals tested should be based on the minimum required to provide meaningful data. Enough animals shall 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.

The number of treatment groups may be increased when attempting to characterize a dose response using exaggerated doses. The following examples for exaggerating the dose should be considered:

— multiples of the clinical surface area of exposure;

— multiples of the duration of exposure;

— multiples of the extractable fraction or 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. When medical devices and/or test samples in the form of nanomaterials are to be evaluated sample filtrations shall not be performed. (see ISO/TR 10993-22).

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 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 specified 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 end points, as defined by national or international animal welfare guidelines, 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.

