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Biological evaluation of medical devices —

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

Évaluation biologique des dispositifs médicaux —

Partie 20: Principes et méthodes relatifs aux essais d'immunotoxicologie des dispositifs médicaux

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Contents

Page

Foreword

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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 10993-20 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

ISO/TS 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 of materials*

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

Introduction

International and European Standards are the main focus for demonstration of the safety and compliance of medical devices. There has been increasing attention over the past few years on the potential for medical devices to cause changes in the immune system. It was felt necessary to provide guidance on how to address adverse effects of medical devices on the immune system. As there are no standardized tests available, this document provides a framework on how to approach the evaluation of immunotoxicity.

The intention of this document is:

— to summarize the current state of knowledge in the area of immunotoxicology, including information on methods of assessment of immunotoxicity and their predictive value;

— to identify what the problems are and how they have been dealt with in the past.

For clinical indications of immune alterations due to medical devices, an extensive literature review has been performed, primarily through Medline. The key areas which have been researched are:

— immunosuppression;

— immunostimulation;

— hypersensitivity;

— chronic inflammation;

— autoimmunity.

These key words are linked with the following materials:

— plastics and other polymers;

— metals;

— ceramics, glasses and composites;

— biological materials.

NOTE See also Table 1 for possibilities of interaction of materials with the immune system.

Biological evaluation of medical devices —

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

1 Scope

This part of ISO 10993 presents an overview of immunotoxicology with particular reference to the potential immunotoxicity of medical devices. It gives guidance on methods for testing for immunotoxicity of various types of medical devices.

This part of ISO 10993 is based on several publications written by various groups of immunotoxicologists over the last few decades in which the development of immunotoxicology as a separate entity within toxicology took place.

The current state of knowledge with regard to immunotoxicity is described in Annex A. A summary of clinical experience to date with immunotoxicology associated with medical devices is given in Annex B.

NOTE See also Bibliographic Reference [11].

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 14971, *Medical devices — Application of risk management to medical devices*

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 10993-6, *Biological evaluation of medical devices — Part 6: Tests for local effects after implantation*

ISO 10993-10, *Biological evaluation of medical devices — Part 10: Tests for irritation and delayed-type hypersensitivity*

ISO 10993-11:2006, *Biological evaluation of medical devices — Part 11: Test for systemic toxicity*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
immunotoxicology
study of the adverse health effects that result, directly or indirectly, from the interaction of xenobiotics with the immune system

3.2
medical device
any instrument, apparatus, appliance, material or other article, including software, whether used alone or in combination, intended by the manufacturer to be used on human beings solely or principally for the purpose of:

— diagnosis, prevention, monitoring, treatment or alleviation of disease;

— diagnosis, monitoring, treatment, alleviation of, or compensation for an injury or handicap;

— investigation, replacement or modification of the anatomy or of a physiological process;

— control of conception

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which can be assisted in its function by such means.

NOTE 1 Devices are different from drugs and their biological evaluation requires a different approach.

NOTE 2 Use of the term "medical device" includes dental devices.

3.3
xenobiotic
substance foreign to the human body or living organisms

3.4
immunogenic
able to stimulate cells of the immune system resulting in an antigen specific immune response

4 Risk assessment and risk management

Risk assessment includes hazard identification, dose response assessment and exposure assessment, which together allow characterization of the risk. Based on this risk characterization, risk management shall be applied.

Because of the difficulties in predicting immunotoxicity of new chemicals and materials, effort and interest need to be focused on the assessment and management of risks arising from known immunotoxic chemicals contained in medical devices. Application of risk management to medical devices shall be performed in accordance with ISO 14971. Possible immunotoxic hazards of the chemicals contained in the medical device shall be identified first by an extensive literature search. Examples of such hazards are the production of anaphylactic shock by chlorohexidine in medicines and by proteins in latex rubber. Subsequently the overall risk management/reduction procedures shall be considered, together with the various possible actions that could be taken to further reduce remaining risks such as indicating contra-indications on the label, product recall, design-change and restrictions of use or application.

5 Identification of hazards

Immunological hazards should be identified by assessing exposure to medical device materials to identify the presence of (potentially) immunotoxic agents. There are many sources from which information on immunological hazards can be obtained. These sources include but are not limited to:

— material characterization;

— residue characterization;

— characterization of the leachable materials;

— characterization of drugs and other substances added to the medical device;

— characterization of exposure duration and route;

— observations made during previous exposure to chemicals, drugs or materials;

— toxicity testing.

Most immunological reactions identified to date relate to the additives to materials. Therefore exposure assessment for these chemicals is important in order to identify the immunological hazard. Details of potential outcomes with various materials from different types of medical devices are given in Table 1.

Table 1 — Potential responses of the immune system

Medical device categorization by			Immune system responses					
Nature of body contact		Contact duration						
Category	Contact	A:limited (⩽ 24 h) B:prolonged (> 24 h to 30 d) C:permanent (> 30 d)	Irritation/ acute inflammation	Chronic inflammation	Immunosuppression	Immunostimulation	Hypersensitivity	Autoimmunity
Surface device	Skin	A	×	–	–	×	×	–
		B	×	×	–	×	×	–
		C	×	×	×	×	×	×
	Mucosal membrane	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
	Breached or compromised surface	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
External communicating device	Blood path, indirect	A	×	–	–	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
	Tissue, bone, dentin communicating implant devices	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
Implant device	Tissue, bone and other body fluids	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×

NOTE This table is a framework for consideration of the potential interaction of materials from different types of medical devices with various parts of the immune system, and is not a checklist for testing.

Effects on the immune system (immunotoxicity) occur due to an encounter of immunologically competent cells with foreign substances that are toxic and kill the cells, or result from foreign substances that interact with the early events of the immune response and alter subsequent responses. Prediction of the likelihood of immunotoxicity is difficult but can be based on known events in immunology.

First of all, for a substance to stimulate the immune response, it must be recognized as foreign to the host. The likelihood of being immunogenic is greatest with proteins, then polysaccharides, then nucleic acids and then lipids. Small molecular weight substances are generally not immunogenic. However, these substances may become immunogenic by binding to host proteins and altering the structure of the protein. These substances are usually referred to as haptens.

It is possible that polymeric materials, ceramic materials, and metallic materials may have leachable, wear or degradation products that bind to host proteins. Materials of biological origin, such as collagens, natural latex proteins, albumins and animal tissues are known to stimulate the immune response, and efforts must be taken to make these materials non-immunogenic. In order for large substances (size $>$ 1 000 000 daltons) to be immunogenic, they must be broken down and delivered as smaller substances.

The foregoing are examples of substances and materials which may have immunogenic potential and thus should be considered for their adverse effects on the immune system.

Body contact: every body contact listed in ISO 10993-1 is capable of manifesting an inappropriate immune response (immunotoxicity). Skin and mucous membranes are particularly likely to develop Type I and Type IV reactions. Other routes are likely to give systemic responses including Type I and Type IV reactions.

Duration of contact: in general, the longer the material is in contact with the body, the greater the likelihood of immunogenic substances forming. However, some chemicals will act rapidly and immune responses from materials in contact with the body for less than 24 hours can be immunogenic.

6 Methods of assessment of immunotoxicity

6.1 General

Immunotoxicity testing can be carried out using *in vivo* and *in vitro* assays. In contrast to *in vivo* immunotoxicity testing, possibilities for *in vitro* testing are limited as the models lack the complexity of the intact immune system. The value of *in vitro* methods in assisting extrapolation of animal data to man (by elucidating mechanisms of toxicity) is further limited because they are not yet sufficiently developed and standardized. However, they can be useful as mechanistic studies.

An important focus of immunotoxicology is the detection and evaluation of undesired effects of substances by means of tests on rodents. When animal tests are considered, to satisfy the provisions of ISO 10993-2 all reasonable and practically available replacement, reduction and refinement alternatives should be identified and implemented. Although there are validated laboratory tests, in many cases the biological significance and predictive value of immunotoxicity tests require careful consideration. The potential for effects on the immune system can be indicated by alterations in lymphoid organ weight or histology, changes in total or differential peripheral leukocyte counts, depressed cellularity of lymphoid tissues, increased susceptibility to infections by opportunistic organisms or neoplasia. The prime concern within the area of immunotoxicology is therefore to identify such changes and assess their significance with regard to human health.

In the context of immunotoxicity two kinds of assays can be distinguished: non-functional and functional. Non-functional assays have a descriptive character in that they measure, in morphological and/or quantitative terms, alterations in the extent of lymphoid tissue, the number of lymphoid cells and levels of immunoglobulins or other markers of immune function. In contrast, functional assays determine activities of cells and/or organs, such as proliferative responses of lymphocytes to mitogens or specific antigens, cytotoxic activity and specific antibody formation (e.g. in response to sheep erythrocytes).

A new development in this area is the application of “-omics” for the detection of alterations in the expression of genes involved in immune functions.

The evaluation of immunotoxicological hazards should be planned in accordance with the flow chart given in Annex C. Examples of tests for and indicators of immune responses are given in Table 2.

Although there are specific materials that are known or suspected to be immunotoxic, immunotoxicity testing related to immunosuppression or immunostimulation shall initially be limited to those assays carried out in the phase of general toxicity testing. Only those agents that show evidence of causing immunosuppression or immunostimulation shall be subjected to further investigation. Sub-acute tests are useful for obtaining general indications of potential immunosuppression or immunostimulation. If they are performed, they shall be carried out in accordance with ISO 10993-11.

6.2 Inflammation

Agents can interact with components of the non-specific arm of the immune system, i.e. granulocytes, macrophages and other cell types that are capable of producing and releasing inflammatory mediators. It should be noted that after implantation of a foreign body, a local inflammatory response is quite common. The duration and degree of the response determines whether it indicates an adverse effect. The most direct and adequate method of assessing the degree of induction of inflammation after exposure to agents is histopathology of the injection or implantation site of the agent. Chronic inflammation associated with immunotoxicity is a lesion which is predominant in lymphocytic cells as opposed to the foreign body reaction which is composed of macrophages and foreign body giant cells at the tissue/material interface. Initial tests for local inflammation are described in ISO 10993-6. Other useful tests include serum assays for C-reactive protein and acute phase protein.

6.3 Immunosuppression

For the detection of immunosuppression a tiered approach is warranted in order to reflect the complexity of the immune system with its variety of functions and components. This tiered approach comprises a first tier of immunosuppression testing using non-functional assays, followed by a second tier, that includes functional assays. This tiered approach does not provide the most sensitive approach available as functional assays are more sensitive than non-functional assays. The rationale for including less sensitive indicators as a first tier and more sensitive indicators as a second tier is not because it best assesses the immune system, but rather because it reduces the need for additional test animals.

In the first tier, indications for immunosuppression are induced alterations in, for instance, weight of immune organs, in cell numbers and/or cell populations and in immunoglobulins.

In the second tier, more specific immune function assays can then be utilized, such as determination of the influence of the agent on NK cell activity and/or on immune function during active immunization, for instance, the assay of antigen-specific antibody production after sensitization. In some guidelines some of these assays are already included in the first tier (antibody response to T-cell dependent antigens such as sheep red blood cells).

The real consequence of immunosuppression can probably be best determined by assessing effects on resistance against infection in bacterial, viral and/or parasitic animal models, and/or effects on resistance against tumours. The importance of these types of assays is that they assess the immune system as a complete and functional entity. However, since it is not practical to evaluate all immunologically relevant parameters in a single toxicity or immunosuppression study, the most important predictive parameters need to be identified and a practical approach chosen to assess immunosuppression for a particular agent.

As the general malaise of an individual also affects the immune system, immunosuppression is considered to exist when immune alterations are detected at dose levels inducing no overt general toxicity. Therefore, immunosuppression testing is best performed in the context of general toxicity testing, since general toxicity testing uses a range of doses of an agent and evaluates all major organ systems.

For the detection of general toxicity of chemicals after sub-acute exposure OECD 407[1] was recently adapted to include several immunotoxicological parameters for the determination of an immunotoxic effect of the compound under investigation.

Table 2 — Examples of tests for and indicators of the evaluation of immune responses

Immune responses	Functional assays	Non-functional assays		
		Soluble mediators	Phenotyping	Other[a]
Tissue/Inflammatory	Implant/systemic ISO 10993-6 and ISO 10993-11	N.A.	Cell surface markers	Organ weight analysis
Humoral response	Immunoassays (e.g. ELISA) for antibody responses to antigen plus adjuvant[b] Plaque-forming cells Lymphocyte proliferation Antibody dependent cellular cytotoxicity Passive cutaneous anaphylaxis Direct anaphylaxis	Complement (including C3a and C5a anaphylatoxins) Immune complexes	Cell surface markers	
Cellular Responses				
T-cells	Guinea pig maximization test Mouse local lymph node assay Mouse ear swelling test Lymphocyte proliferation Mixed lymphocyte reaction	Cytokine patterns indicative of T cell subset (Th1, Th2)	Cell surface markers (helper and cytotoxic T cells)	
NK cells	Tumour cytotoxicity	N.A.	Cell surface markers	
Macrophages and other monocytes	Phagocytosis Antigen presentation	Cytokines (IL1, TNFα, IL6, TGFβ, IL10, γ-interferon)	MHC markers	
Dendritic cells	Antigen presentation to T-cells	N.A.	Cell surface markers	
Vascular endothelial cells	Activation			
Granulocytes (Basophils, Eosinophils, Neutrophils)	Degranulation Phagocytosis	Chemokines, bioactive amines, inflammatory cytokines, enzymes	N.A.	Cytochemistry
Host resistance	Resistance to bacteria viruses and tumours	N.A.	N.A.	
Clinical symptoms	N.A.	N.A.	N.A.	Allergy, skin rash, urticaria, oedema, lymphadenopathy, inflammation

[a] Animal models of some human auto-immune diseases are available. However, routine testing for induction of auto-immune diseases by materials/devices is not recommended.

[b] Most commonly used tests. Functional assays are generally more important than tests for soluble mediators or phenotyping.

6.4 Immunostimulation

Immunostimulation does not, in most cases, lead to diminished resistance to infectious diseases; in contrast, immunostimulation can have consequences in terms of exacerbation of existing allergic or auto-immune phenomena.

Assays that are used for detection of immunosuppression are generally also suitable for detection of immunostimulation. The consequences of exposure to those agents that have been shown to stimulate the immune system non-specifically can be best studied using animal models in which allergy or auto-immunity is induced. As is the case with host resistance models, allergy and auto-immunity models are generally quite cumbersome. There are no validated animal models for testing allergy and auto-immunity, which allow extrapolation of animal data to humans.

Besides the immunostimulation properties of the material itself, immunostimulation activity of contaminations, such as pyrogens, shall also be considered, as stated in Annex F of ISO 10993-11:2006.

6.5 Hypersensitivity

Agents can be recognized by the immune system on the basis of their antigenic properties. As such, these agents can act as allergens, inducing hypersensitivity. The most common forms of hypersensitivity are delayed-type hypersensitivity (Type IV) and immediate-type hypersensitivity (Type I). There is no good predictive test for Type I hypersensitivity.

Delayed-type hypersensitivity comprises antigen-specific cellular inflammatory responses. Tests for this are given in ISO 10993-10.

IgE mediates immediate-type hypersensitivity. Detection of specific IgE production can be assayed in several ways. Classical assays for induction of immediate-type hypersensitivity include the passive anaphylaxis assay.

6.6 Auto-immunity

Exogenous agents can alter components of the host so that they are recognized by the immune system as non-self. Such conditions generally require highly specific combinations of agent and host; animal research has revealed that auto-immune diseases are highly genetically dependent. It is therefore unlikely that the potential for induction of auto-immunity would be detected in a general toxicological screening assay.

There are no validated animal models for testing allergy and auto-immunity, which allow extrapolation of animal data to human health.

A model for predictive testing of auto-immunity has been proposed. This is a modification of the popliteal lymph node assay. In this assay the proliferative response of the draining lymph node has been considered indicative of the induction of sensitization, including auto-immunity. The extension of the test with simultaneous administration of T-cell dependent and T-cell independent antigens (reporter antigens) has added value to the assay, in the sense that induction of responses by neo-antigens can be detected. However, the assay needs further validation.

7 Extrapolation of data provided by pre-clinical assays

The problem of extrapolation of *in vitro* and animal data to humans is complicated by the immunological redundancy and/or reserve which is characteristic of the immune system, so immunotoxicological effects might not necessarily result in health effects. In addition, testing is complicated by the need to utilize distinct tests based upon the site examined (e.g. systemic, lung, skin) and the immunopathology of concern (i.e. hypersensitivity, immune regulation, auto-immunity, or inflammation), the latter phenomena not being identified in conventional general toxicity testing.

The increased understanding of the cellular, molecular and genetic events responsible for mounting appropriate immune responses, and of the immune mediators involved in these events, has provided opportunities for the utilization of more streamlined and informative tests.

Annex A
(informative)

Current state of knowledge

A.1 Immunology

The immune system provides protection against agents that threaten an individual's health, notably infectious agents causing disease, but also other environmental agents and neoplasia. It acts through mechanisms such as immune surveillance and production of immunoglobulins, cytokines and interleukins. It provides immune surveillance against newly arising neoplastic cells and regulates homeostasis of leucocyte maturation. It is a highly evolved organ system, the functions of which are provided by two major mechanisms. The first is a non-specific mechanism not requiring prior contact with the inducing agent and lacking in specificity. The second is a specific or adaptive mechanism directed specifically against an eliciting agent. The adaptive system depends on innate systems (e.g. complement, clotting and fibrinolytic systems) for effectiveness. It also depends on antibody/antigen reactions, T-cells, cytokines and chemokines.

Mononuclear phagocytes (i.e. blood monocytes and tissue macrophages), granulocytes and foreign body giant cells are phagocytic cells involved in non-specific resistance. Lymphoid cells, macrophages and their cytokine products are all involved in various aspects of specific host resistance. Replenishment and renewal of the cellular elements of the immune system constitute a major task of lymphoid tissue and occur in the primary lymphoid organs (bone marrow, thymus).

The B-lymphocyte pathway produces B-cells which differentiate into plasma cells which secrete antibodies with specific antigen binding capacity. At the early stage of differentiation, B-cells have only cell-bound antibodies, which can bind to a specific antigen, but they do not secrete any soluble antibodies into the plasma. In order for the B-cells to differentiate further into plasma cells, a number of important processes have to take place. The antigen is internalized into the cell and undergoes a digestive process. Fragments of the digested antigen then become bound to specialized molecules, human leucocyte antigen (HLA), which are then transported to the surface of the B-lymphocyte and displayed on its surface.

T-lymphocytes have immunologically specific receptors that recognize and bind to a complex of the displayed HLA molecule and the bound antigenic fragments. In many immune responses, B-cells require interactions with T-cells in order to complete all their differentiation steps into antibody-secreting plasma cells.

Once T-cells have been activated, they secrete a series of cytokines, which are chemical messengers that are critical to mobilization and mediation of inflammatory and immunological processes. They provide activating and inhibitory signals that exert profound effects on other cells in the immune and haemopoietic systems and in connective tissue. The specific immune response is thus the trigger for a series of down-stream effects, such as inflammation, coagulation, fibrinolysis and activation of vascular endothelial cells.

The adaptive immune system can respond to an invading organism or agent in the following different functional ways:

a) a humoral immune response comprising an antibody reaction to an antigen on the surface of bacteria, viruses etc.;

b) a cellular immune response against antigens, which is mediated by T-cells, macrophages and monocytes.

These two different mechanisms can act simultaneously and interact with each other. Both involve lymphocyte activity. B-lymphocytes, which have immunoglobulin (Ig) receptors, differentiate into plasma cells, which then manufacture antibodies specific to the encountered antigen. After binding antigen at a T-cell receptor T-lymphocytes become primed (antigen specific) sensitized T-cells that can produce various kinds of cytokines depending on the antigen encountered.

A.2 Immunotoxicology

The interaction with an immunotoxic agent can alter the delicate balance of the immune system, which can result in undesirable effects such as:

— immunosuppression, resulting in alterations of host defence mechanisms against pathogens or neoplasia;

— allergy;

— auto-immunity.

The term “immunotoxic agent” (hereinafter referred to as an “agent”) is used to indicate chemicals (e.g. drugs) or biological molecules, including their degradation products, and, in certain circumstances, physical factors (e.g. radiation). In the context of this part of ISO 10993 such agents include materials used in the production of medical devices and/or chemicals present as residues within medical devices.

Immunotoxicity can take several forms including:

a) damage to, or functional impairment of, one or more components of the immune system such that immune function is suppressed and normal host resistance compromised;

b) the stimulation by chemicals or proteins of specific immune responses that result in the development of allergic sensitization and allergic disease;

c) the provocation, directly or indirectly, of anti-self responses, leading to auto-immunity and auto-immune disease.

In the case of immunotoxicity due to a direct effect on the immune system, the systemic or local (e.g. skin, lung) immune system acts as a target for the agent, and the result can be an increased incidence or severity of infectious disease or neoplasia. For example, Epstein-Barr virus infection can progress to B-cell lymphoma, or UV-B exposure can progrerss to skin cancer in immune-suppressed transplant patients. Direct immunotoxicity leading to enhancement or suppression of the immune system can also have an impact on immune responses to antigens that are not related to the immunotoxic agent, and thus have an impact on allergies and autoimmunity, for instance by exacerbation of these responses.

Immunotoxicity can also result from indirect effects. For example, hydralizine-induced lupus is due to an effect on the complement system, leading to complement deficiency.

Immunotoxicity can thus be due to the effect of the agent at a variety of points, either in the immune or haemopoietic systems or downstream of these.

Immunotoxicity can also result from an agent inducing or modifying the activity of the immune system. For instance, in the case of allergy, the immune system responds to chemical (hapten)-host protein conjugates or high molecular weight compounds. The most likely health consequences of the latter are respiratory tract allergies (e.g. asthma, rhinitis), gastrointestinal allergies or allergic contact dermatitis.

Auto-immunity can occur as a result of an agent-induced alteration in either the host tissue, endocrine function or immune regulation. Auto-immune diseases are diseases of immune disregulation manifested by the production of antibodies to self or modified self-antigens, or by tissue destruction from T-lymphocytes or macrophages reacting to endogenous self-antigens. Auto-immune diseases do not necessarily occur as a result of auto-immunity. Agents can bind to tissue or serum proteins and an immune response can be generated against these modified self-antigens, leading to cell injury or cell death. Immunotoxicity resulting in hypersensitivity and auto-immunity (immune disregulation) generally shows a high degree of variability between individuals in the exposed population and, because of species differences, is difficult to mimic in animal models.

The pathogenic steps that lead to an auto-immune reaction are not completely understood; however certain factors have been clearly identified as playing an important role, including the following:

— genetic makeup;

— gender;

— age;

— exposure to environmental agents.

NOTE Few of these environmental agents have been identified, but they might include certain infections.

At least four mechanisms for the induction of auto-immune disease are recognized:

— hidden antigens, i.e. normally intracellular substances that are recognized as foreign if released into the circulation;

— self antigens, which can become immunogenic as a result of chemical, physical or biological alteration;

— foreign antigens, which can induce an immune response that cross-reacts with normal self antigens;

— mutational changes, which can occur in immuno-functional cells.

It should be noted that disregulation of the immune system, which would not by itself lead to the induction of auto-immunity, might have an impact on the expression of latent auto-immunity already present.

The differentiation between direct toxicity and toxicity due to an immune response to a compound is to a certain extent artificial. Some compounds can exert a direct toxic action on the immune system as well as inducing a specific immune response. In animals, heavy metals, e.g. mercury, manifest immunosuppressive activity and cause hypersensitivity and auto-immunity.

A.3 Human health consequences of changes in the immune system

The potential for adverse health effects in humans due to alterations in the immune system is a matter of increasing scientific and public concern. In humans, a number of agents has been shown in volunteer studies, or after accidental exposure, to have immunomodulatory properties, as shown by various tests. However, the true biological impact of those changes has not been documented stringently. That modest immunomodulation can be of clinical importance in humans is evidenced by stress-related decreases in vaccination titres, and increased Herpes simplex symptoms after exposure to ultraviolet radiation. The full impact of drug-induced immunodeficiency can be appreciated from the increased incidence of infectious diseases (particularly those caused by opportunistic pathogens) and certain types of neoplastic diseases, seen with the use of immunosuppressive agents for control of transplant rejection reactions.

Many of the immune changes seen in humans after exposure to immunomodulating agents can be subtle and sporadic, and effects on health can be difficult to discern. The structure and function of the immune system can manifest changes, but these might not have any apparent clinical effects on health, owing to the action of compensatory mechanisms. This implies that exposed individuals might not show obvious health effects, but that the effects might be manifested in an increased vulnerability to common diseases. Thus, the effects might be detectable at a population level, for example as an increased prevalence of allergies and of common infections, such as the common cold, influenza and otitis media. These effects might occur especially in sub-populations that are more vulnerable to the risks of exposure to immunotoxic agents, such as children and the elderly.

In addition it should be recognized that the immune status of populations is extremely heterogeneous. Age, race, gender, pregnancy, stress and the ability to cope with stress, co-existent disease and infections, nutritional status, tobacco smoking and other life style factors, medication and seasonal differences contribute to this heterogeneity.

Despite this heterogeneity and the redundancy inherent in the function of the immune system, a decrease in the capability of the immune system to react to its full potential is clearly undesirable, as adaptive compensatory systems might be needed to deal with other more threatening situations. On the other hand, an increase (especially if persistent) in activity of the immune system carries a risk of more serious consequences (tissue damage, anaphylaxis) which occur in allergy and/or auto-immunity. The severe impact of allergic and auto-immune responses due to exposure to exogenous agents in humans is especially evident in the case of exposure to chemicals and drugs.

Annex B
(informative)

Clinical experience with medical devices

The literature review (see introduction) has revealed indications of the existence of immunotoxicity in humans from various materials as follows.

— Type I hypersensitivity reactions occur with certain biological materials (e.g. latex proteins). Individual cases have also been reported with plastics and polymers (e.g. acrylics/acrylates), and metal salts (e.g. salts of nickel and chromium). (This has also been reported with dental amalgams.) It is not always possible to differentiate between 'classical' Type I hypersensitivity (i.e. mediated via IgE antibodies) and direct action on mast cell degranulation by the toxic substance.

— There are several reports of Type IV hypersensitivity reactions associated with low molecular weight organic molecules (e.g. thiurams and other additives/residues in latex, and bisphenol A in dental resins), and plastics/polymers (e.g. acrylates and additives to polymer coatings in pacemaker leads and formaldehyde released in polymerising dental materials).

— Metals and metal salts in medical devices are occasionally associated with Type IV hypersensitivity reactions.

— Chronic inflammation of the foreign body type occurs with implants composed of many types of materials e.g. poly(dimethylsiloxane) (silicone), poly(tetrafluoroethylene) (PTFE), poly(methylmethacrylate) and polyester. However, for any particular material, it is difficult to establish a causal relationship between chronic inflammation and serious sequelae such as auto-immunity. With silicones, for which such a possibility has been extensively investigated, a marked fibrotic response can occur but the evidence to date does not indicate any systemic disease.

— Immunosuppression resulting from certain metals (e.g. nickel and mercury) is suspected in some subjects. However, systematic studies relevant to medical devices/materials in humans are uncommon.

— Certain clinical reports (as well as laboratory animal studies) suggest immunostimulation, specifically adjuvant activity, in the case of silicone, but this might be due to an antigen sparing (depot) effect rather than to direct immunotoxicity.

— Complement activation, with generation of anaphylatoxins, is a common immunotoxic effect associated with solid materials contacting blood (e.g. cellulose-based and synthetic haemodialysis/cardiopulmonary bypass materials, polyester/PTFE block copolymers for vaccines).

— Auto-immunity has been associated with certain metals that are used in implanted medical devices (e.g. mercury and gold). However, convincing evidence that any material causes auto-immune disease (as opposed to a humoral and/or cellular auto-immune response) is difficult to obtain, even in animal models of human disease.

Hypersensitivity (both Types I and IV) is the most commonly reported immunotoxic effect. Certain non-human natural products are both immunogenic and activate complement (e.g. collagen). Other materials (e.g. crystalline silica and charcoal immuno-adsorbents, and low molecular weight organic additives) also have shown immunotoxic effects (e.g. complement activation with generation of anaphylatoxins, and Type IV hypersensitivity reactions, respectively).

In the literature, case reports or small group studies are most common. Notable exceptions are larger clinical studies showing hypersensitivity reactions, for example to certain metals or latex, and studies of populations of women with breast implants (which have, to date, shown no evidence of immunotoxicity). Apart from these, systematic studies of the potential for medical device materials to cause immunotoxic effects in humans are

generally lacking. This might explain the fact that reports of immunotoxicity arising from medical devices is not often encountered. This could also be due, in part, to effective screening out of potentially immunotoxic materials at the early stages of product development. The results of such screening studies might not appear in the scientific literature.