INTERNATIONAL STANDARD

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Biological evaluation of medical devices —

Part 7: Ethylene oxide sterilization residuals

Évaluation biologique des dispositifs médicaux —

Partie 7: Résidus de stérilisation à l'oxyde d'éthylène

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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 10993-7 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This second edition cancels and replaces the first edition (ISO 10993-7:1995) 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 within a risk management system*

— *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 skin sensitization*

— *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* [Technical Specification]

— *Part 20: Principles and methods for immunotoxicology testing of medical devices* [Technical Specification]

Introduction

Requirements for the development, validation and routine control of an ethylene oxide sterilization process for medical devices are given in International Standards developed by ISO/TC 198. Certain requirements relating to medical devices for biological testing, selection of tests, and the allocation of devices to categories are dealt with in a variety of International Standards developed by ISO/TC 194. The specific requirement for ethylene oxide and other sterilization process residuals was referred to ISO/TC 194. Other International Standards delineate particular requirements for biological testing for specific products.

As noted in the introduction to ISO 11135-1:2007, when determining the suitability of ethylene oxide (EO) for sterilization of medical devices, it is important to ensure that the levels of residual EO, ethylene chlorohydrin (ECH) and ethylene glycol (EG) pose a minimal risk to the patient in normal product use. Therefore, it is important that the use of alternative materials and sterilization processes be considered during product design and development. EO is known to exhibit a number of biological effects. In the development of this part of ISO 10993, consideration was given to these effects, which include irritation, organ damage, mutagenicity and carcinogenicity in humans and animals, and reproductive effects in animals. Similar consideration was given to the harmful effects of ECH and EG. In practice, for most devices, exposure to EO and ECH is considerably lower than the maximum values specified in this part of ISO 10993.

Moreover, when the choice for EO sterilization has been made, irrespective of the provisions of this part of ISO 10993, exposure to EO residues should be minimized. Requirements herein are in addition to the biological evaluation and testing requirements for each individually designed medical device as indicated in ISO 10993-1. The biological evaluation and testing requirements, combined with the EO-sterilization process residue limits, form the justification that an EO-sterilized device is acceptable for use. Maximum allowable residues for ethylene chlorohydrin (ECH), when ECH has been found to be present in medical devices sterilized with EO, are also specified. Local effects (e.g., irritation) have been considered and are incorporated in the tolerable contact limit (TCL) as given in 4.3.5.2 and Annex G for EO, and in 4.3.5.3 and Annex H for ECH.

Biological evaluation of medical devices —

Part 7:
Ethylene oxide sterilization residuals

1 Scope

This part of ISO 10993 specifies allowable limits for residual ethylene oxide (EO) and ethylene chlorohydrin (ECH) in individual EO-sterilized medical devices, procedures for the measurement of EO and ECH, and methods for determining compliance so that devices may be released. Additional background, including guidance and a flowchart showing how this document is applied, are also included in the informative annexes.

EO-sterilized devices that have no patient contact (e.g., *in vitro* diagnostic devices) are not covered by this part of ISO 10993.

NOTE This part of ISO 10993 does not specify limits for ethylene glycol (EG).

2 Normative references

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

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

ISO 10993-3, *Biological evaluation of medical devices — Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

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

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

ISO 10993-17:2002, *Biological evaluation of medical devices — Part 17: Establishment of allowable limits for leachable substances*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993-1, ISO 10993-17 and the following apply.

3.1
simulated-use extraction
extraction to demonstrate compliance with the requirements of this part of ISO 10993, by evaluating residue levels available to the patient or user from devices during the routine use of a device with water extraction to simulate product use

1) To be published. (Revision of ISO 10993-1:2003)

3.2
exhaustive extraction
extraction until the amount of EO or ECH in a subsequent extraction is less than 10 % of that detected in the first extraction, or until there is no analytically significant increase in the cumulative residue levels detected

NOTE As it is not possible to demonstrate the exhaustive nature of residual recovery, the definition of exhaustive extraction adopted is as above.

4 Requirements

4.1 General

NOTE Information on the derivation of the limits in this part of ISO 10993 as well as other important background information and guidance relevant to the use of this document is contained in the informative annexes.

This clause specifies maximum allowable residues for ethylene oxide (EO) for each individual medical device sterilized with EO. As noted in the introduction to ISO 11135-1:2007, when determining the suitability of EO for sterilization of medical devices, it is important to ensure that the levels of residual EO, ethylene chlorohydrin (ECH) and ethylene glycol (EG) pose a minimal risk to the patient in normal product use. Moreover, when the choice for EO sterilization has been made, irrespective of the provisions of this standard, exposure to EO residues should be minimized. Maximum allowable residues for ECH, when ECH has been found to be present in medical devices sterilized with EO, are also specified. Local effects (e.g., irritation) have been considered and are incorporated in the tolerable contact limit (TCL) as discussed in 4.3.5.2 and Annex G for EO, and 4.3.5.3 and Annex H for ECH. No device limits are specified for EG because a risk assessment (Annex I) indicates that calculated allowable levels are higher than those likely to occur in a medical device. However, the potential exists for acute haemodynamic and haemolytic effects to occur following rapid intravenous administration of hyperosmolar compounds like EG. Ethylene oxide sterilization of medical devices would not be expected to produce hyperosmolar solutions. Methods for the determination of EO and ECH are given in 4.4.

The requirements in this part of ISO 10993 are in addition to the biological testing requirements set out in ISO 10993-1. For devices sterilized using ethylene oxide, attention shall be paid in particular to ISO 10993-3 and ISO 10993-10. All applicable requirements of ISO 10993-1 shall take into account the EO residual level at the time of release for each individually designed medical device.

Results of the biological assessment of the device may dictate more stringent limits than those specified in 4.3, which are designed to protect against systemic effects.

4.2 Categorization of devices

In establishing the maximum daily doses of EO and ECH that a medical device is allowed to deliver to patients, devices shall be categorized according to the duration of contact.

Devices shall be placed into one of three exposure categories in accordance with ISO 10993-1:—, 5.3:

a) limited exposure (A) – devices whose cumulative single, multiple or repeated use or contact is up to 24 h;

b) prolonged exposure (B) – devices whose cumulative single, multiple, or repeated long-term use or contact is likely to exceed 24 h but not 30 d;

c) permanent contact (C) – devices whose cumulative single, multiple or repeated long-term use or contact exceeds 30 d.

If a material or device can be placed in more than one duration category, the more rigorous testing and/or evaluation considerations should apply. With multiple exposures, the decision into which category a device is placed should take into account the potential cumulative effect, bearing in mind the period of time over which these exposures occur.

NOTE As it is applied in this part of ISO 10993, “multiple use” is defined to mean repeated use of the same device type, e.g. dialyser cartridges.

4.3 Allowable limits

4.3.1 General

For each medical device, the maximum allowable doses of EO and ECH delivered to patients shall not exceed the values given below for the exposure category that the device has been placed into in accordance with 4.2.

The limits for permanent contact and prolonged exposure devices are expressed as maximum average daily doses. These limits carry additional constraints for the first 24 h of the exposure period and, in the case of the permanent contact devices, for the first 30 days. These constraints place limitations on the amount of EO and ECH that can be delivered to the patient during these early time periods. If data are available, consideration should be given for proportioning the limits downward if multiple devices with the residue of concern are used at one time, or proportioning the limits upward when device use is only for a part of the exposure period of concern. These concomitant exposure factors (CEF) and proportional exposure factors (PEF) are given in ISO 10993-17. The procedure that was used to establish the allowable limits is described in Annex G for EO, in Annex H for ECH, and the rationale for considering the establishment of allowable limits for EG is described in Annex I.

4.3.2 Permanent contact devices

The average daily dose of EO to patient shall not exceed 0,1 mg/d. In addition, the maximum EO dose shall not exceed:

— 4 mg in the first 24 h;

— 60 mg in the first 30 d;

— 2,5 g in a lifetime.

The average daily dose of ECH to patient shall not exceed 0,4 mg/d. In addition, the maximum ECH dose shall not exceed:

— 9 mg in the first 24 h;

— 60 mg in the first 30 d;

— 10 g in a lifetime.

4.3.3 Prolonged exposure devices

The average daily dose of EO to patient shall not exceed 2 mg/d. In addition, the maximum EO dose shall not exceed:

— 4 mg in the first 24 h;

— 60 mg in the first 30 d.

The average daily dose of ECH to patient shall not exceed 2 mg/d. In addition, the maximum ECH dose shall not exceed:

— 9 mg in the first 24 h;

— 60 mg in the first 30 d.

4.3.4 Limited exposure devices

The average daily dose of EO to patient shall not exceed 4 mg.

The average daily dose of ECH to patient shall not exceed 9 mg.

4.3.5 Tolerable contact limits for surface contacting devices and implants

4.3.5.1 Overview

The tolerable contact limit (TCL) is expressed in units of micrograms per square centimetre for EO and milligrams per square centimetre for ECH. The unit of square centimetre represents the surface area of the patient-device interface.

NOTE The intent of this subclause is to prevent localized irritation due to EO or ECH released from the device.

4.3.5.2 Tolerable contact limit for EO

Either the EO TCL for surface contacting devices and implants shall not exceed 10 µg/cm^2 or it shall exhibit negligible irritation as specified in ISO 10993-10.

4.3.5.3 Tolerable contact limit for ECH for surface contacting devices

Either the ECH TCL for surface contacting devices and implants shall not exceed 5 mg/cm^2 or it shall exhibit negligible irritation as specified in ISO 10993-10.

4.3.6 Special situations

For multi-device systems the limits shall apply to each individual patient-contact device.

Residue of EO in intraocular lenses shall not exceed 0,5 µg EO per lens per day, or 1,25 µg per lens. Prorate limits for other intraocular devices are set on the basis of the mass of the device, with the mass of an intraocular lens taken as 20 mg. The acceptability of ECH levels in intraocular devices made from viscoelastic materials that contain chlorine may need to be evaluated, as the level of ECH that results in ocular toxicity is about four times greater than the corresponding EO level.

For blood cell separators used in patient and donor blood collection, the maximum allowable dose of EO is 10 mg and the maximum allowable dose of ECH shall not exceed 22 mg.

For blood oxygenators and blood separators, the maximum allowable dose of EO to patient is 60 mg and the maximum allowable dose of ECH shall not exceed 45 mg.

For devices used in cardiopulmonary bypass procedures, the maximum allowable limits shall be 20 mg for EO and 9 mg for ECH.

For extracorporeal blood purification devices, the EO and ECH limits specified shall be 4,6 mg/device, but the allowable EO dose for a lifetime may be exceeded.

For drapes that are intended to contact only intact skin, the maximum allowable limits shall be the TCL of 10 µg/cm^2 for EO and 5 mg/cm^2 for ECH, or the drapes shall exhibit negligible irritation as specified in ISO 10993-10.

NOTE The rationale for specifying EO limits for certain devices that are at variance with the general requirements appears in Annex F.

A flowchart providing guidance for the application of this part of ISO 10993 to the determination of EO residuals in medical devices is presented in Annex C.

4.4 Determination of EO and ECH residuals

4.4.1 General

4.4.1.1 Procedure

The procedure for determining compliance with 4.3 consists of extracting the residue from samples, determining the amount of residue, determining the contact surface of the device, and analysing and interpreting the data.

DANGER — Analysts and others who obtain samples should perform all work involving the use of the chemicals and solvents required for these methods in a fume cupboard whilst wearing appropriate protective clothing, and should review the Material Safety Data information for each chemical prior to such use. Healthcare workers using EO-sterilized medical devices shall take appropriate precautions to protect against exposure to residues, which may be required by local occupational health and safety regulations.

4.4.1.2 Ethylene oxide

This is a flammable gas that is irritating to body surfaces and highly reactive. It is mutagenic under many conditions, has fetotoxic and teratogenic properties, can adversely effect testicular function and can produce injury to many organ systems in the body. In cancer studies in animals, inhalation exposure produced several types of neoplastic changes including leukaemia, brain tumours and mammary tumours while ingestion or subcutaneous administration produced tumours only at the site of contact. One investigator has reported higher cancer and mortality rates in some subpopulations of exposed workers. However, the results of several studies in workers have shown even weaker associations. See References [177], [178] and [181]. In 1994 the International Agency for Research on Cancer (IARC) reclassified EO as a human carcinogen (class 1) based mainly on its mechanism of action. See Reference [75].

4.4.1.3 Ethylene chlorohydrin

This is a flammable liquid that is irritating to body surfaces, acutely toxic and readily absorbed through the skin in toxic amounts. It has weak mutagenic potential, has some potential to produce fetotoxic and teratogenic changes and can produce injury to several organ systems in the body including lungs, kidneys, central nervous system and cardiovascular system. It was negative in cancer bioassays in animals.

4.4.2 Determination of residue

A valid method of extraction and measurement shall be used to determine the amount of EO and, where necessary, ECH delivered to the patient.

If ECH is not detected based on the results of analyses performed using the methods given in either K.4.2 or K.4.7, no further monitoring for ECH is required.

NOTE Many gas chromatography (GC) methods that use a capillary column instead of a packed column will produce EO, ECH and EG results during a single sample run.

The guiding principle in selecting appropriate extraction methods (4.4.6) for the quantitative determination of EO and, where necessary, ECH is the evaluation of the dose to the patient in order to show compliance with the requirements set out in 4.3.

Where residues are shown to be within the requirements for products tested by exhaustive extraction, there is no need to further challenge the device by simulated-use extraction, provided all applicable limits in 4.3 are met. When exhaustive extraction is used, particular attention shall be paid to the limits expressed for the first 24 h and for the first 30 days in 4.3.

Many analytical methods for these EO-sterilization residuals have been described and are reviewed in the Bibliography. However, the enormous diversity of materials and methods of construction of sterile medical

devices may, in certain cases, still present problems in determining residual EO and ECH levels using the methods given in the Bibliography. Therefore, any method that has been shown to be analytically sound (i.e. demonstrated accuracy, precision, linearity, sensitivity, and selectivity) may be used, provided that it has been validated. Annex A contains general validation requirements for gas chromatographic methods.

4.4.3 Product sampling and sample "blank"

4.4.3.1 Product sampling

Samples to be used for residual analysis shall be selected in such a manner as to be truly representative of the product. When selecting samples, attention shall be given to the many factors described in Annex D. Since many of these factors influence not only the initial levels of residuals in device components but also the rate of residue dissipation, they shall also be considered when test samples are drawn from a processed load and sent to the laboratory for analysis. Removal of the product samples from the processed load soon after a sterilization cycle is completed and shipment to a laboratory far from the sterilization site or storage in the laboratory for later analysis can jeopardize correlations of residual levels on the samples with those on the rest of the load. Moreover, if samples cannot be drawn from the load and handled so that the effect on aeration conditions for the sample will be negligible, an experiment to establish the relationship between the sample aeration and load aeration at various seasons of the year shall be carried out.

Precautions shall be taken to minimize or control the effects of laboratory conditions on the rate of aeration for test samples that have been removed from a product load (see D.1.5). In addition, operator and analyst safety shall be ensured. Samples should remain with the product load until the day of analysis or until test samples are retrieved and immediately frozen. The time between removal of samples from a controlled aeration area and the beginning of extraction should be held to a minimum. Samples shall be sealed, shipped and stored frozen when analysis is delayed. Samples shall be shipped on dry ice on overnight delivery. Dry ice shall remain in the shipping container throughout the shipment and be present when the package is opened in the laboratory. Test samples may also be taken directly from the product load at the desired aeration interval and immediately placed into a headspace vial, which is sealed and then shipped to the laboratory for analysis. As an alternative, samples may be extracted and the extraction fluid shipped to the analytical laboratory for analysis. If the extraction fluid is water, then shipment shall be done such that the fluid is kept at ice-cold temperatures (< 10 °C) until arrival. Testing should be carried out to measure hydrolysis of EO to EG.

Samples to be analysed shall be placed in a fume cupboard and removed from the packaging. Samples shall be prepared according to any applicable pre-use instructions in the product labelling. Extractions shall be started as soon as possible after the device has been removed from the packaging or pre-use preparations have been completed.

4.4.3.2 Sample "blank"

To ensure that no other sample matrix components with the same retention time as any of the residues being determined are present a "blank" sample shall be evaluated for the possible presence of such interferences by the extraction of a non-sterilized sample using the identical procedure being applied to the EO-sterilized samples. In the event of materials being extracted from such a "blank" with conflicting or overlapping retention times in the GC analysis, chromatographic conditions shall be modified to separate the interfering peak from the analyte peak, or an alternative analytical procedure shall be used.

4.4.4 Sample/fluid ratios

The volume of fluid used to extract residues from devices, or representative sections of them, shall be sufficient to maximize extraction efficiency while maintaining detection sensitivity. The nature and size of the device sample therefore determines what constitutes the optimal fluid volume for extraction. Therefore, to maximize analytical sensitivity, a minimum amount of extraction fluid should be used depending on the extraction method required and size of the sample. Devices composed of highly absorbent materials or those from which residues are extracted by filling may require sample/extraction fluid ratios reflecting increased fluid volume. In any case, sample/extraction fluid ratios shall not undermine detection sensitivity.

4.4.5 Extraction time and conditions

The aim of product extraction is to indicate the worst-case amount that could be delivered to the patient in actual use of the device: on a daily basis for limited exposure items; on a daily and up to monthly basis for prolonged exposure items; on a daily, monthly, and up to a lifetime basis for permanent contact items. As indicated in Annexes E and F, exhaustive extraction as described below can be a useful alternative for permanent contact devices, provided that shorter-term constraints are ensured.

4.4.6 Product extraction

4.4.6.1 Overview

There are two basic extraction methods used for the determination of EO-sterilization residuals in medical devices: simulated-use extraction, which is the reference method; exhaustive extraction, which represents an acceptable alternative in certain situations. The choice of extraction method shall be based on the intended use of the device. Examples of suggested extraction methods are shown in Annex K.

The extraction method chosen shall represent the intended use of the product with the greatest challenge to the patient and not solely expeditious analysis or to minimize the apparent concentration of residuals.

Extraction temperatures and times shall be determined based on the nature of the patient's exposure and the patient's duration of contact with the device as described in 4.2 and 4.3. See ISO 10993-12 for extraction temperatures.

The analyst is cautioned that for certain devices, simulated-use extraction may result in relatively large elution volumes. Should this occur it might significantly increase the limit of detection for the residual material to the point where a determination of compliance with this part of ISO 10993 is compromised.

Small devices shall be extracted in a suitable container. When a device is too large to be extracted in its entirety, it may be necessary to extract several representative portions of the device components in order to ensure confidence in the data derived.

These representative portions may be selected in one of two ways. If several varied materials are used, the proportion of each component, as compared with the total sample mass, should parallel the ratio of that component to the total mass of the device being tested. An alternative method would be to select one of the components for testing, subsequent to an evaluation demonstrating that it represented the worst case with regard to residual content. The method chosen shall be validated.

4.4.6.2 Simulated-use extraction (reference method)

Simulated-use aqueous extraction is the reference method in that it is the only method that produces results directly comparable to the limits specified in 4.3. These limits are expressed in terms of delivered dose of EO and ECH to patients.

Since it is necessary to evaluate the residue levels available to the patient, or other end user, from devices in routine use, extraction methods that simulate use are required. Simulated-use extraction shall be carried out under conditions that provide the greatest challenge to the intended use.

For example, many blood-contacting and parenteral devices can be extracted with water by filling or flushing the blood or fluid path (whichever is appropriate). Samples shall be extracted for a time equivalent to or exceeding the maximum time for single use, and at temperatures that provide the greatest realistic simulated challenge.

To determine the dose of EO and, where necessary, ECH delivered to the patient or user over the course of normal product use, simulated-use aqueous extraction procedures are used.

NOTE The amounts of EO (or ECH) extracted by simulating normal product use are not necessarily similar to the total product residual content.

Water (see [92]) is commonly used for the recovery of residual EO, ECH (and EG if there is any concern about hydrolysis of EO) in simulated-use extractions. Water is used for elution of EO residuals from the sample rather than to dissolve the sample material itself. If the intent is to simulate product use by filling the device, the device should be filled so as to eliminate any air pockets: extract devices that are wholly or partially in contact with the body during use at 37 °C (body temperature); extract devices having no immediate body contact during use (e.g., hypodermic syringes) at 25 °C (room temperature). See also ISO 10993-12. If the assay is not performed immediately, the extract should be decanted from the sample and sealed in a poly-(tetrafluoroethylene) (PTFE)-lined, septum-capped vial. The headspace in the vial of any standard solution or extract shall be less than 10 % of the total volume. The extract can be stored in the refrigerator for several days (see Annex F) but, where water extraction is used, caution shall be taken, as EO may convert to EG or ECH (or both) during the extraction period as well as during storage of the extract (see [35]). The analyst shall evaluate the possibility of this conversion to EG and/or ECH at the analysis site when extracting the sample with water.

4.4.6.3 Exhaustive extraction (acceptable alternative method)

4.4.6.3.1 Overview

Exhaustive extraction represents an acceptable alternative and can provide useful information. It produces results that would tend to represent a dose greater than or equal to one the patient may receive. Because such an extraction precludes measurement of dose as a function of time, it does not ensure that the mass of residue is not delivered to the patient on the first day or during the first month of exposure. However, when all applicable limits in 4.3 are met and residues are shown to be within the requirements for products tested by exhaustive extraction, there is no need to further challenge the device by simulated-use extraction. When exhaustive extraction is used, particular attention shall be paid to the limits expressed for the first 24 h and for the first 30 d in 4.3.

Exhaustive extraction methods are intended to recover the entire residual content of a device. For EO determination, extraction procedures used include thermal extraction followed by headspace gas analysis, solvent extraction procedures, with either headspace gas analysis of the solvent extract, chromatography of the solvent extract, or preparation of the bromohydrin derivative of EO which is determined using a more sensitive GC detector such as an electron capture detector.

4.4.6.3.2 Residual ethylene oxide

A variety of extraction fluids has been used for the exhaustive recovery of residual EO. Thermal desorption followed by headspace gas analysis, as described in K.4.3, is an example of a procedure that does not use an extraction fluid. When conducted as described, headspace methods are considered exhaustive since they are designed to recover all of the residual EO from the sample. However, headspace methods may not be feasible or preferred for intact testing of large or complex devices. The analyst shall exercise caution in the execution of headspace methods when evaluating residue levels in polymer materials such as poly-(methylmethacrylate) to ensure total recovery of EO.

For solvent extraction procedures, selection of a suitable extraction fluid depends on the material composition of the device and its components. To facilitate complete recovery of EO from the sample, fluids that dissolve the sample material are generally preferred in an exhaustive extraction, provided that interfering substances are not also put into solution by the procedure. Solvent extraction procedures that are combined with headspace gas analysis are described in K.4.4 and such procedures may be able to separate EO from co-extracted interfering chemicals from the sample matrix. Several extraction fluids have been evaluated through interlaboratory comparison testing, see References [112], [113] and [114].

Prudent analytical procedure dictates that, in the initial analysis of a given material, more than one extraction procedure shall be used to validate quantitative recovery whenever an exhaustive extraction is to be performed. For devices containing a relatively small amount of residual EO, the commonly used methods may not be capable of extracting these small amounts, even after relatively long extraction times.

4.4.6.3.3 Residual ethylene chlorohydrin

Water is typically used to extract residual ECH from medical devices using methods similar to those described for determining residual EO.

4.4.7 Data analysis and interpretation

4.4.7.1 Calculation of amount of residue extracted

The concentration of residue observed in the extracts, C_e, is converted to the amount delivered to a patient, in milligrams, M_d, as follows.

Residue extracted by simulated use may be calculated as follows:

$$M_d = \sum_{1}^{n} (C_{en} \times V_{en}) \quad (1)$$

Residue extracted by exhaustive extraction may be calculated as follows:

$$M_d = \sum_{1}^{n} (C_{en} \times V_{en}) \times \frac{m_d}{m_s} \quad (2)$$

where

M_d is the extract residue, in milligrams;

n is the number of extractions;

C_e is the amount of EO in milligrams per millilitre of extract as derived from the standard curve;

V_e is the extract volume, in millilitres;

m_d is the entire device mass, in grams;

m_s is the mass of sample, in grams.

NOTE This applies only if a portion of the device is extracted.

4.4.7.2 Calculation of average delivered dose, M_{add}, for comparison to allowable limits in 4.3

For permanent contact devices, the average delivered dose, M_{add}, in milligrams per day, is as follows:

$$M_{add} = \frac{M_d}{25\,000} \quad (3)$$

where

25 000 is the number of days per lifetime;

M_d is the extract residue, in milligrams.

Permanent contact devices shall also meet the prolonged exposure and limited exposure limits as calculated below.

For prolonged exposure devices

$$M_{add} = \frac{M_d}{30} \quad (4)$$

where

30 is the number of days per month;

M_d is the extract residue, in milligrams.

Prolonged exposure devices shall also meet the limited exposure limits as calculated below.

For limited exposure devices:

$$M_{add} = M_d \quad (5)$$

5 Product release

5.1 General

A product is in compliance with this part of ISO 10993 when it meets the requirements for EO and, if applicable, ECH. If sufficient experimental data on residue diffusion kinetics are available, it may be possible to group devices for quality assurance testing based on similarity of materials, manufacturing processes and use (see Annex D).

For release of batches of EO-sterilized product, one of the two methods in 5.2 and 5.3 respectively shall be used.

5.2 Release of products without dissipation curve data

When dissipation curve data are not available on a product, the product may be released if it is in compliance with this part of ISO 10993 and the data were obtained from testing carried out according to appropriate procedures delineated in Annex K and meet the requirements for EO and, if applicable, ECH set out in 4.3.

5.3 Procedure for product release using residue dissipation curves

Dissipation curves are used to estimate the post-sterilization time required for products, or families of similar products, to reach residue limits, principally for EO, in compliance with 4.3. Products shall be released to the marketplace according to predetermined post-sterilization times and conditions defined by experimental dissipation curves so that the target EO residue levels for the device, as set out in 4.3, are ensured. The product aeration concerns documented in Annex D are to be considered by pooling data from sterilization loads taken from aeration or quarantine storage at different times of the year if aeration temperatures differ. Re-sterilization of product and the presence of other EO-sterilized medical devices in adjacent areas shall also be considered when obtaining experimental data to generate such dissipation curves.

Release of products manufactured and sterilized under controlled conditions, as described in ISO 11135-1, may be carried out if data are pooled from a minimum of three sterilization lots run at different times. Dissipation of EO from most materials and devices follows first-order kinetics, i.e. (ln[EO])α (time after sterilization). A plot of the natural logarithm of the experimentally determined EO concentration against time after sterilization is linear. Release shall then be based on the time after sterilization when the mean regression line intersects the maximum allowable residue. This approach may be used for products which are not sterilized in sufficient quantity (numbers of sterilization runs) for the procedure described below to be applied, or may be used while the dissipation curve data described is being collected. Various alternative methods can be used; for example, if dissipation curves are established whereby samples are tested after the residual limits have been met, interpolation of the dissipation curve can be used to establish the release of the product after sterilization.

Regression analysis of pooled data from sufficient time points for at least three lots of the same product to establish the nature of the dissipation curve will enable product to be released at the calculated upper 95 % prediction limit, L_p, for the allowed residue limit for the product. Time-concentration curves for devices made from combinations of dissimilar materials may not fit this simple pattern over the entire range and may need to be handled differently.

Formulae for calculating the prediction limit, L_p:

$$x_o = \frac{y_o - a}{b} \tag{6}$$

$$L_p = x_o + t_\alpha \times \sqrt{\frac{(S_\alpha)^2}{b^2} \times \left[1 + \frac{1}{n} + \frac{(y_o - y_\mu)^2}{b^2 \times \sum (x_i - x_\mu)^2}\right]} \tag{7}$$

where

x_o is the calculated average value of the release time corresponding to the EO limit;

y_o is the logarithmic value of the EO limit;

a is the intercept of the linear regression line obtained from the plot ln[EO]α time;

b is the slope of the regression line;

L_p is the prediction limit for a single individual of the product;

t_α is the student t value at significance α with $n - 2$ degrees of freedom;

$(S_\alpha)^2$ is the residual variance of the regression line;

y_μ is the average of logarithmic EO values;

n is the number of values;

x_i is the individual time after sterilization at which measurements are made;

x_μ is the average of the times after sterilization;

$\Sigma (x_i - x_\mu)^2$ is the sum of squares for x (time).

All data obtained for release of medical devices in compliance with this part of ISO 10993 shall be obtained from experiments and data analyses carried out following valid standard operating procedures.

When sterilization process parameters listed in Annex D are changed, an audit shall be made of the product residue. When this audit shows an increase in the level of residual EO, new residue dissipation curves shall be obtained to ensure product acceptability. When this audit shows a decrease in the level of residual EO, consideration should be given to the generation of new dissipation curves.

NOTE Verification of dissipation curves is typically done during routine sterilization revalidation in accordance with ISO 11135-1.

Annex A
(normative)

Evaluation of gas chromatograms

A.1 General

This annex discusses the minimum requirements for the analytical procedures employed for EO and ECH measurements. These requirements apply for both packed and capillary GC column systems.

A.2 Background

These requirements are discussed in reference books on GC and should be reviewed by analysts before their use of any of the procedures. Also recommended is a review of the articles concerning detection limits, see References [15], [35] and [74].

A.3 Symbols

The symbols in Table A.1 are used in Figures A.1 and A.2.

Table A.1 — Symbols

Symbol	Description
f	distance from peak maximum to leading edge of peak
k'	capacity factor
R	resolution
T	tailing factor
t	retention time of the relevant residue peak (EO or ECH)
t_a	retention time for a non-retained component, such as air, which is not retarded in its passage through the column
t_1, t_2	retention time of chromatographic peaks 1 and 2, where t_1 is EO (or ECH) and t_2 is an immediately adjacent peak
W_1, W_2	respective widths extrapolated to the baseline for peaks 1 and 2 in the same units as the retention time
$W_{0,05}$	peak width at 5 % of height

A.4 Minimum requirements

A.4.1 For these procedures, it is recommended that the following minimum requirements be met for these parameters (see Figures A.1 and A.2).

Resolution, R, calculated as follows

$$R = 2\frac{(t_2 - t_1)}{(W_2 + W_1)} \qquad \text{(A.1)}$$

shall be $\geqslant$ 2,0 for peak area or peak height quantitation.

Alternatively, the following equation may be useful to calculate the capacity factor, k', which shall be greater than 1,5 for well-resolved peaks:

$$k' = \frac{t}{t_a} - 1 \tag{A.2}$$

Tailing, T, given by the following equation, shall be less than or equal to 1,8 for the EO and ECH peaks:

$$T = \frac{W_{0,05}}{2f} \tag{A.3}$$

A.4.2 Relative deviation of the standard curve (RSD) should not exceed 5 % for EO and ECH for the range of standards used, see References [13] and [14].

$$\text{RSD} = \left(\frac{\sigma}{\lambda}\right) \times 100 \tag{A.4}$$

$$\sigma^2 = \frac{\left(\frac{\Sigma_y^2 - \Sigma^2}{n}\right) - S \times \left(\Sigma_{xy} - \frac{\Sigma \times \Sigma_y}{n}\right)}{n-2} \tag{A.5}$$

$$\lambda = \frac{\Sigma_y}{n} \tag{A.6}$$

where

n is the total number of samples evaluated;

y is the chromatographic peak area or peak height;

λ is the mean;

x is the concentration of the standard;

σ is the standard deviation;

σ^2 is the variance;

S is the slope of the least squares regression line for the standard curve.

These criteria are calculated for triplicate analyses of at least three standards prepared to cover the expected linear dynamic range of each of the standard curves used in the analysis of EO and ECH.

A.5 Chromatographic baseline

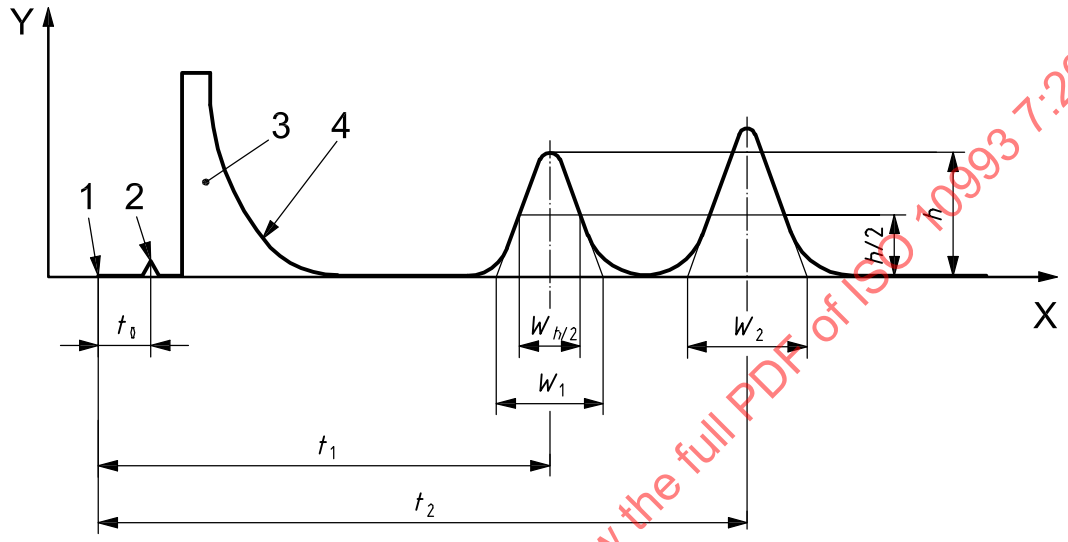
In addition, it is recommended that the chromatographic baseline return to within 5 % of the initial baseline between chromatographic runs.

A.6 Resources

The following sources of information are suggested when corrective changes in these analytical procedures are indicated:

— the manufacturer's manual for the gas chromatograph used;

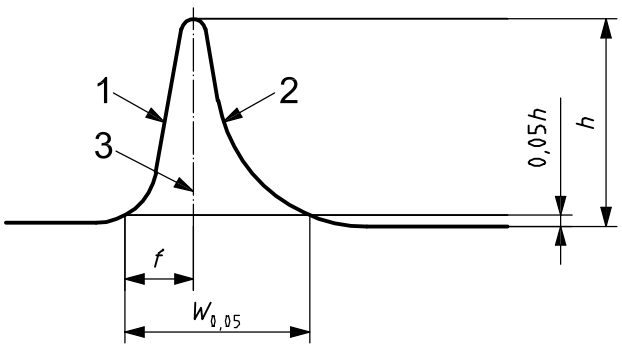
— the various textbooks on GC.



Key

X time
Y detection response
1 injection
2 air peak
3 solvent peak
4 solvent tail

Figure A.1 — Chromatographic separation of two substances



Key

1 peak front
2 peak tail
3 peak maximum

Figure A.2 — Asymmetrical chromatographic peak

Annex B
(informative)

Gas chromatographic determination for EO and ECH

B.1 Chromatographic procedures

B.1.1 Preparation of standards

Analysts should establish the stability of the standards they use to calibrate the chromatographic procedure(s) used and ensure that standards are not used past their established expiry point.

B.1.2 General

The following paragraphs outline the procedure for preparation of GC standards. Two alternatives are commonly available:

a) use of prepared standards from commercial sources;

b) preparation of standards either volumetrically, by diluting known volumes of EO gas or gravimetrically, by diluting a known mass of liquid EO. In all cases, prepare a standard curve of peak height or peak area response versus EO concentration.

NOTE Peak area response compiled by the software of computer-controlled GC instrumentation is more precise than measuring peak height in determining EO concentrations.

Examples of procedures used for the preparation of EO and ECH standards are provided in Annex J.

B.2 Criteria for validating gas chromatographic methods

B.2.1 General overview

Many methods are suitable for quantitatively analysing extracts for ethylene oxide. A number of procedures for exhaustive extraction followed by GC for the determination of EO have been described. There are probably just as many unpublished methods for determining residual ethylene oxide. Because of the diversity in medical devices, published methods may not be suitable for all devices. Therefore, any method that has been shown to be analytically sound and meets the performance criteria described in this part of ISO 10993 can be used.

Analytically sound means that the method demonstrates sufficient accuracy, precision, selectivity, linearity, ruggedness and sensitivity to determine the specified level of EO in a device which is intended to be analysed in relation to the residue limits shown in 4.3 and is applicable to the device which is intended to be analysed.

A number of analytical methods for assessing levels of EO and ECH residues have been reviewed from the literature (see Bibliography). For a more detailed discussion of each method, the original literature should be consulted. The following are recommended criteria for validating a method.

B.2.2 Accuracy

Accuracy is a measure of the closeness of test results obtained by the test method, to the true value. Accuracy is expressed in terms of recovery, the measured value expressed as a percentage of the accepted or true value. It requires the comparison of the test method measurement with a known value. The known value can be prepared from an analyte of known purity or from spiked samples.

Spiked samples as the means of determining accuracy can be reported as percent recovery of a known added amount of analyte in the sample. However, for EO, this method for determining accuracy is extremely difficult to carry out because of the volatility of this compound. As an alternative, the use of commercially available certified standards is recommended. Thus, the measure of accuracy becomes the mean measured results divided by the accepted true value together with the confidence interval. In either case, the percent recovery can be calculated as

$$R = \frac{R_o \times 100}{a \text{ or } t_v} \tag{B.1}$$

where

R is the recovery in percent;

R_o is the result obtained;

a or t_v is the accepted or true value.

Accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (i.e. three replicates each at three different concentrations).

B.2.3 Precision

B.2.3.1 Overview

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Precision contains three components: repeatability, intermediate precision and reproducibility.

B.2.3.2 Repeatability

Repeatability can be assessed using a minimum of nine determinations covering the specified range of standards used (i.e., three replicates each at three different concentrations). Data generated from method accuracy as in B.2.2 above can be used for repeatability assessment.

Repeatability can be calculated as the relative standard deviation (coefficient of variation) of the peak area as specified in Equation (A.4).

The % RSD for EO and ECH should not exceed 5 % for the range of the standards used. The % RSD is calculated as described in A.4.2.

B.2.3.3 Intermediate precision

Intermediate precision can be assessed by establishing the effects of random events on the precision of the analytical procedure. Examples of random effects include days, analysts, equipment, etc. It is not necessary to study these events individually. The use of an experimental design (matrix) is encouraged.

As a minimum, data generated as described in B.2.2, accuracy, for two separate events is recommended to indicate the intermediate precision of the test method. The standard deviation, relative standard deviation (coefficient of variation), and confidence interval should be reported.

B.2.3.4 Ruggedness/reproducibility

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagent, different elapsed assay times, different assay temperatures, different days, etc. Ruggedness is normally expressed as the lack of influence on the test results of operational and environmental variables of the analytical method. Ruggedness is a measure of reproducibility of the test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst.

Since the method of validation would be performed in an individual laboratory to introduce a new column or new method, this part of the validation can be done by a combination of different analysts, different days, different instruments, etc. Reproducibility is not normally expected if intermediate precision is accomplished. The interlaboratory studies are not important in this part.

B.2.4 Linearity

Linearity is a measure of the correlation between the method response and the concentration of the analyte. Linearity should be established across the range of standards used. Regression analysis of the standard concentration versus peak area or peak height should be performed using a minimum of five concentrations.

The analyst should determine the linearity of the calibration data, along with the reproducibility of the slope and intercept. The minimum correlation coefficient for the standard curve should be 0,95.

B.2.5 Method detection limit (MDL)

B.2.5.1 Overview

The method detection limit is the smallest amount that can be detected with a reasonable confidence. The detection limit can be determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

There are many ways to determine the method detection limit. Approaches other than those listed below may be acceptable.

B.2.5.2 MDL based on signal-to-noise

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio of 3:1 is generally accepted.

B.2.5.3 MDL based on the standard deviation of the response

To determine the method detection limit, make a known standard of the analyte of interest near the estimated MDL and determine the standard deviation for seven injections of the standard.

$$\text{MDL} = s \times t \tag{B.2}$$

where

s is the standard deviation of injections;

t is the student t value at $n - 1$ degrees of freedom at the 99 % confidence level.

B.2.6 Quantitation limit (QL)

B.2.6.1 Overview

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

There are many ways to determine quantitation limit. Approaches other than those listed below may be acceptable.

B.2.6.2 QL based on signal-to-noise

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably quantified. A signal-to-noise ratio of 10:1 is generally accepted.

B.2.6.3 QL based on the standard deviation of the response

The quantitation limit can be expressed as

$$QL = 5 \times MDL \tag{B.3}$$

Annex C
(informative)

Flowchart and guidance for the application of this part of ISO 10993 series of standards to the determination of EO and ECH residuals in medical devices

C.1 Background

This annex provides guidance on the application of certain parts of the ISO 10993 series to the biological evaluation of medical devices that have been sterilized with ethylene oxide (EO). This annex primarily addresses the application of this part of ISO 10993, but limited guidance is also given for other parts of the ISO 10993 series.

This part of ISO 10993 specifies the requirements for establishing allowable limits for EO residues and analytical procedures to show that an EO-sterilized device is in compliance with the allowable limits. Maximum allowable limits for ethylene chlorohydrin residues where ECH has been found to be present in medical devices sterilized with EO are also specified. No exposure limits are set for ethylene glycol because risk assessment indicated that when EO residues are controlled, it is unlikely that biologically significant residues of EG would be present. Dose to patient is the basis for establishing the allowable limits and the reference method for showing compliance with this part of ISO 10993. The second paragraph of the Introduction notes that alternative materials and sterilization methods should be considered during product development and design to minimize exposure to EO residues.

In addition to meeting the requirements of this part of ISO 10993, an EO-sterilized device must meet the biological testing requirements of the other parts of the ISO 10993 series. The requirements of the other parts of the ISO 10993 series should also be considered.

There are certain circumstances (e.g., major surgery) where the lifesaving nature of the therapy significantly alters the risk-benefit analysis of the use of an EO-sterilized medical device. The exposure limits given in 4.3 are based on risks and benefits associated with less critical circumstances. In consequence, there is scope for relaxation of the proposed limits in life-threatening situations where it is not possible to meet the specified limits.

This annex includes a flow chart that is intended to assist a user in understanding the steps necessary to apply this document. The flow chart shows the decision points and provides guidance for choosing the appropriate actions where alternatives are given in the document. Some of the guidance represents a practical means of applying the document to different products based on factors such as: nature of exposure; duration of exposure; frequency of use; special situations of use (e.g., as cited in 4.3.6); product size. The flow chart is supplemented by more detailed text. In addition, Table C.1 provides a succinct summary of the allowable limits for medical devices in various categories.

Subclause 4.4 gives the requirements for determining EO and ECH residues, and analytical procedures are described in Annex B. Extraction conditions for the determination of residual EO are given in Annex E. Guidance on developing an appropriate simulated-use extraction procedure is given in C.3. This enables users to develop and document the rationale for an appropriate simulated-use extraction procedure for their EO-sterilized products.

The analytical laboratory should work with the device manufacturer to demonstrate that the simulated-use extraction is carried out under conditions that provide the greatest challenge to the intended use. Product use simulation should be carried out assuming that the device is assigned to the most stringent category probable for the duration of exposure and should take into consideration both tissue(s) exposed and temperature of exposure.

This text should be used in conjunction with the flow chart in Figure C.1.

Table C.1 — Summary of allowable limits for EO and ECH (limits per device)

Device category	EO	ECH
Limited (< 24 h)	4 mg	9 mg
Prolonged (> 24 h < 30 d)	60 mg/30 d	60 mg/30 d
Permanent (> 30 d)	2,5 g/lifetime	10 g/lifetime
Tolerable contact limit (TCL)	10 µg/cm^2 or negligible irritation	5 mg/cm^2 or negligible irritation
Intraocular lens	0,5 µg/lens/d 1,25 µg/lens	4 × EO limits suggested
Blood cell separator (apheresis)	10 mg	22 mg
Blood oxygenators	60 mg	45 mg
Cardiopulmonary bypass devices	20 mg	9 mg
Blood purification devices (hemodialysers)	4,6 mg	4,6 mg
Drapes contacting intact skin	10 µg/cm^2 or negligible irritation	5 mg/cm^2 or negligible irritation

C.2 Guidance

C.2.1 Use of alternative materials and sterilization methods should have been considered during product development and design with the aim of minimizing exposure to residues. The rationale and basis for this decision should be documented.

C.2.2 If the device has no patient contact [2], this part of ISO 10993 does not apply.[3]

C.2.3 If this is a multi-device system, the limits apply to each individual patient-contact device.

C.2.4 If the device is in a special category, the following apply.

a) If the device is an intraocular lens, the limits are 0,5 µg/lens/d, not to exceed 1,25 µg total [4]. Limits for other intraocular devices can be prorated on the basis of the mass of the device, with the mass of an intraocular lens taken as 20 mg. When EO residues are controlled as specified for intraocular devices, it is unlikely that significant amounts of ECH will be present. This may not be true for intraocular devices made from viscoelastic materials that contain chlorine. In such cases, References [44], [118], [119] and [120]) indicate that the level of ECH that results in ocular toxicity is about four times greater than the corresponding EO level. This should be taken into consideration when evaluating the acceptability of ECH levels associated with these devices.

b) If the device is a blood cell separator used in donor and patient blood collection, determine EO and ECH residues.[4] The maximum allowable limit for EO and ECH shall not exceed 10 mg and 22 mg per device, respectively. If these limits are exceeded, simulate product use to determine EO residues by extracting the device at 37 °C for up to 24 h, but not less that 1 h. (see C.3.2 and C.3.3). If EO from simulated use exceeds 10 mg and/or ECH from simulated use exceeds 22 mg, reduce EO and/or ECH; otherwise, the EO and ECH residue requirements for this device are met, provided the requirements noted in footnote 7) to C.2.9 have been addressed.

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2) Examples include *in vitro* diagnostic devices, back table covers, Mayo stand covers, light handles, etc.

3) Employee exposure limitations may be required by local occupational health regulations.

4) An exhaustive extraction procedure, as specified in Table E.1 and defined in 3.2, is required to determine EO residues. The analyst shall verify and document the procedure used.

c) If the device is a blood oxygenator or blood separator, determine EO and ECH residues. [5] The maximum allowable dose of EO to patient shall not exceed 60 mg and the maximum allowable dose of ECH shall not exceed 45 mg. If it does, determine EO residues by simulating product use by extracting the device at 37 °C for up to 24 h but not less than 1 h (see C.3.2 and C.3.3). If the daily dose of EO and/or ECH from simulation of product use exceeds 60 mg and/or 45 mg, respectively, reduce EO and/or ECH. Otherwise, if the daily dose of EO is not more than 60 mg and/or the daily dose of ECH is less than 45 mg, the EO and ECH residue requirements for this device are met.

d) If the device is used in a cardiopulmonary bypass procedure, determine EO and ECH residues. The maximum allowable daily dose of EO to patient shall not exceed 20 mg and the maximum allowable dose for ECH shall not exceed 9 mg.

e) If the device is a blood purification device, the EO and ECH limits shall not exceed 4,6 mg per device, but the allowable EO and ECH dose for a lifetime may be exceeded.

f) If the device is a drape contacting intact skin, the TCL shall be 10 µg/cm^2 for EO and 5mg/cm^2 for ECH or the drape shall have negligible irritation as specified in ISO 10993-10.

C.2.5 If the device is not in a special category as described in C.2.4, determine EO and ECH residues. [6]

C.2.6 For permanent exposure devices (those contacting the patient for longer than 30 d to a lifetime), proceed as follows.

a) If the measured EO and ECH residuals are not more than 2,5 g and 10 g respectively, go to C.2.6 b). Otherwise, use appropriate temperatures (either 37 °C or 25 °C) and times (based on anticipated use time) with water as the extracting medium to simulate product use (see C.3). If the measured dose of EO is not more than 2,5 g or the measured dose of ECH is not more than 10 g, where ECH has been found, go to C.2.6 b). Otherwise, reduce EO and/or ECH.

b) If the measured EO and ECH are not more than 60 mg, go to C.2.6 c). Otherwise, use appropriate temperatures (either 37 °C or 25 °C) for 30 d with water as the extracting medium to simulate product use (see C.3). If the measured EO and ECH dose, where ECH has been found, is not more than 60 mg, go to C.2.6 c). Otherwise, reduce EO and/or ECH as in C.2.6 a) and C.2.6 c).

c) If the measured EO and ECH are not more than 4 mg and 9 mg, respectively, go to C.2.9. Otherwise, use appropriate temperatures (either 37 °C or 25 °C) for 24 h with water as the extracting medium to simulate product use (see C.3). If the measured EO and ECH doses from simulated use are not more than 4 mg or 9 mg respectively, go to C.2.9. Otherwise, reduce EO and/or ECH.

C.2.7 For prolonged exposure devices (those contacting the patient for more than 24 h up to 30 d), proceed as follows.

If the measured EO and/or ECH are not more than 60 mg, go to C.2.6 c). Otherwise, use appropriate temperatures (either 37 °C or 25 °C) and times (based on anticipated use time) with water as the extracting medium to simulate product use (see C.3). If the measured EO and ECH dose, where ECH has been found, is not more than 60 mg, go to C.2.6 c). Otherwise, reduce EO and/or ECH.

C.2.8 For limited exposure devices (those contacting the patient for up to 24 h), proceed as follows.

If the measured EO and ECH residues are not more than 4 mg and 9 mg, respectively, go to C.2.9. Otherwise, use appropriate temperatures (either 37 °C or 25 °C) and times (based on anticipated use time,

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5) An exhaustive extraction procedure may be impractical for these products, in which case proceed directly to the simulated-use procedure.

6) An exhaustive or simulated-use extraction procedure as specified in Table E.1 and defined in 3.1 and 3.2 is necessary for determining EO residues. The analyst verifies and documents the procedure used. For very large products, an exhaustive extraction procedure may be impractical. In such cases, continue at C.2.6 and follow the requirement to use a simulated-use procedure for the appropriate duration category.

but with a minimum of 1 h) with water as the extracting medium to simulate product use (see C.3). If the measured EO and ECH doses from simulated use are not more than 4 mg and 9 mg, respectively, go to C.2.9. Otherwise reduce EO and/or ECH.

C.2.9 The device shall not be irritating with the amount of EO and ECH to be allowed on the device at release. If the device is a surface-contacting device or an implantable device, this means that the tolerable contact limits (TCL) for EO and ECH shall not exceed 10 µg/cm^2 and 5 mg/cm^2, respectively, or that the device shall have negligible irritation as specified in ISO 10993-10. Otherwise, the evaluation of the device according to this part of ISO 10993 has been completed [7)].

C.3 Simulated-use extraction procedure

C.3.1 Extraction fluid

Water should be used for simulated-use extraction of EO residues (see [92]).

C.3.2 Extraction temperature

Extract devices wholly or in part in contact with the body during use at 37 °C and extract devices having no immediate body contact during use (e.g., hypodermic syringes) at 25 °C. When devices are extracted at 37 °C, evaluate the conversion of EO to EG.

C.3.3 Extraction time

Consider the expected reasonable worst case range of times over which the device use is recommended or expected when establishing extraction times. In addition, it may be useful to collect data to establish the extraction rate of EO and ECH from the device at the use temperature established in C.3.2 (4.4.6.2). Evaluate these data or other pertinent information to determine an extraction time appropriate for the device that takes into account the available data. The minimum extraction time is one hour.

C.3.4 Extraction of device

Where pre-treatment of the device is required prior to use, perform this pre-treatment before the device is extracted. Where the device is filled for extraction, do this in a manner that eliminates entrained air pockets. Extract the device with water at the temperature and for the time established. Where use of the device involves circulation of fluids (e.g., blood, dialyser fluid), extract the device using water to simulate the fluid's circulating in a manner consistent with product use. Note that where blood is returned from the device to the patient, it must be assumed that any EO residuals will stay in the body. Hence, water simulating blood passing from a device into a patient should not be recirculated. Document the rationale for the conditions established.

C.3.5 Grouping of devices

Devices of similar design but different sizes may be grouped and the worst case selected for testing as representative of the group. Document the rationale for this decision.

C.3.6 Device kits and trays

Initially determine residues for each EO- and ECH-absorbing patient-contact device in kits and trays, and establish the worst-case device or devices. Additional data can then be collected using such worst cases. Document the rationale for the decision.

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7) Meeting both the biological testing requirements for each individually designed medical device as indicated in ISO 10993-1 and the EO-sterilization process residual limits form the justification that an EO-sterilized device is acceptable for use with regard to its biological evaluation.

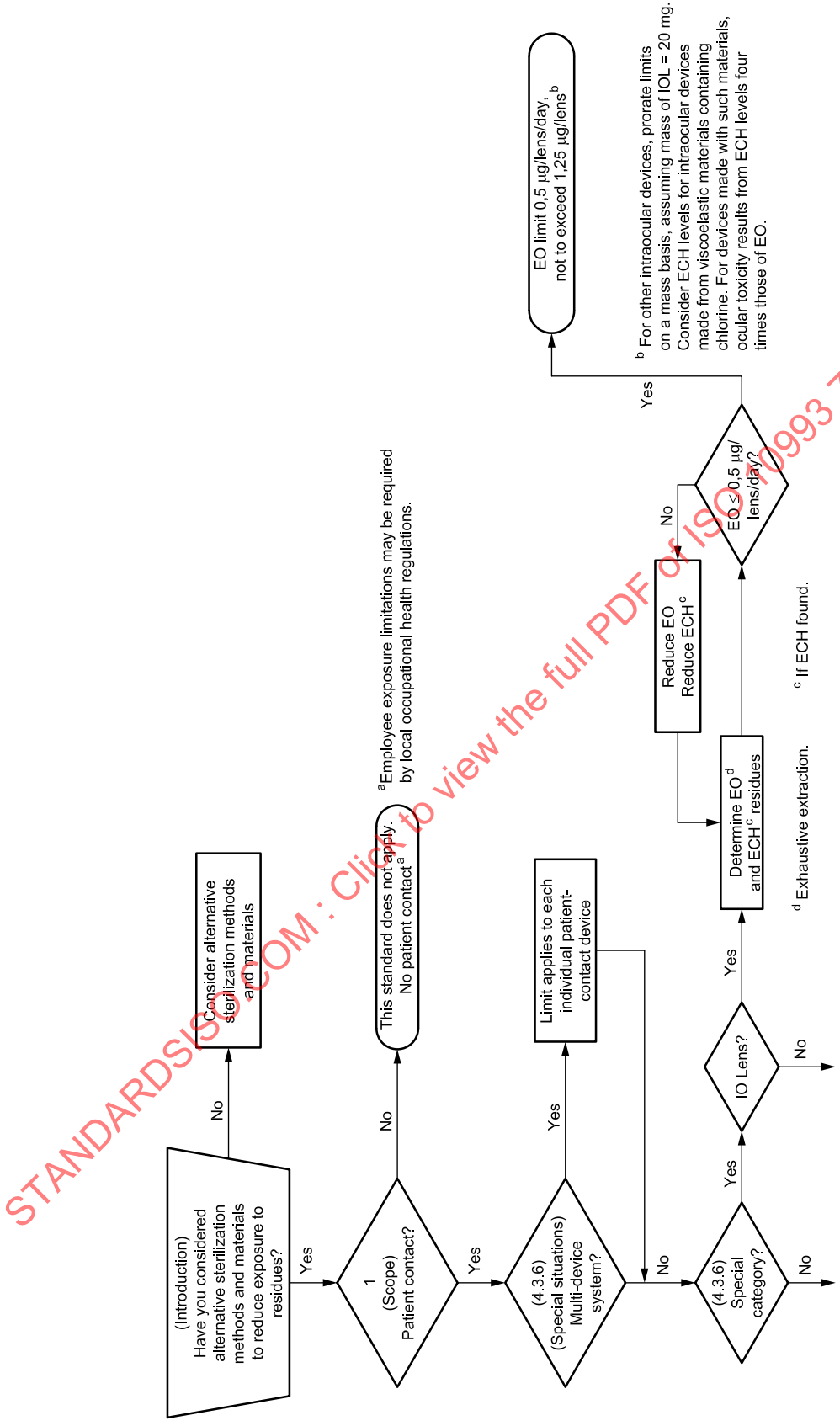


Figure C.1 — Flow chart to assist in understanding the steps necessary to apply this part of ISO 10993
(*continued on Figures C.2 and C.3*)

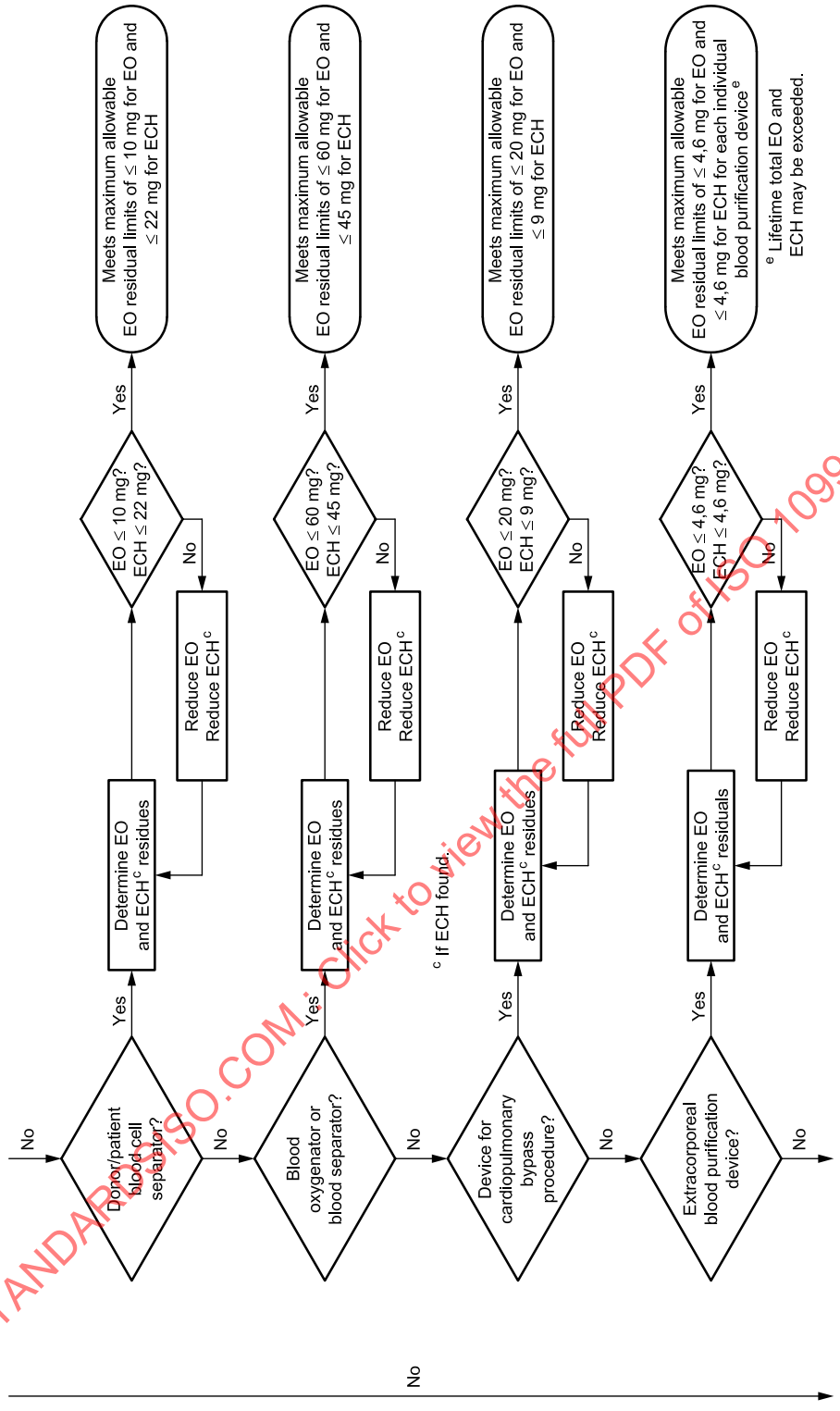


Figure C.2 — Flow chart to assist in understanding the steps necessary to apply this part of ISO 10993
(*continued from Figure C.1 and continued on Figure C.3*)

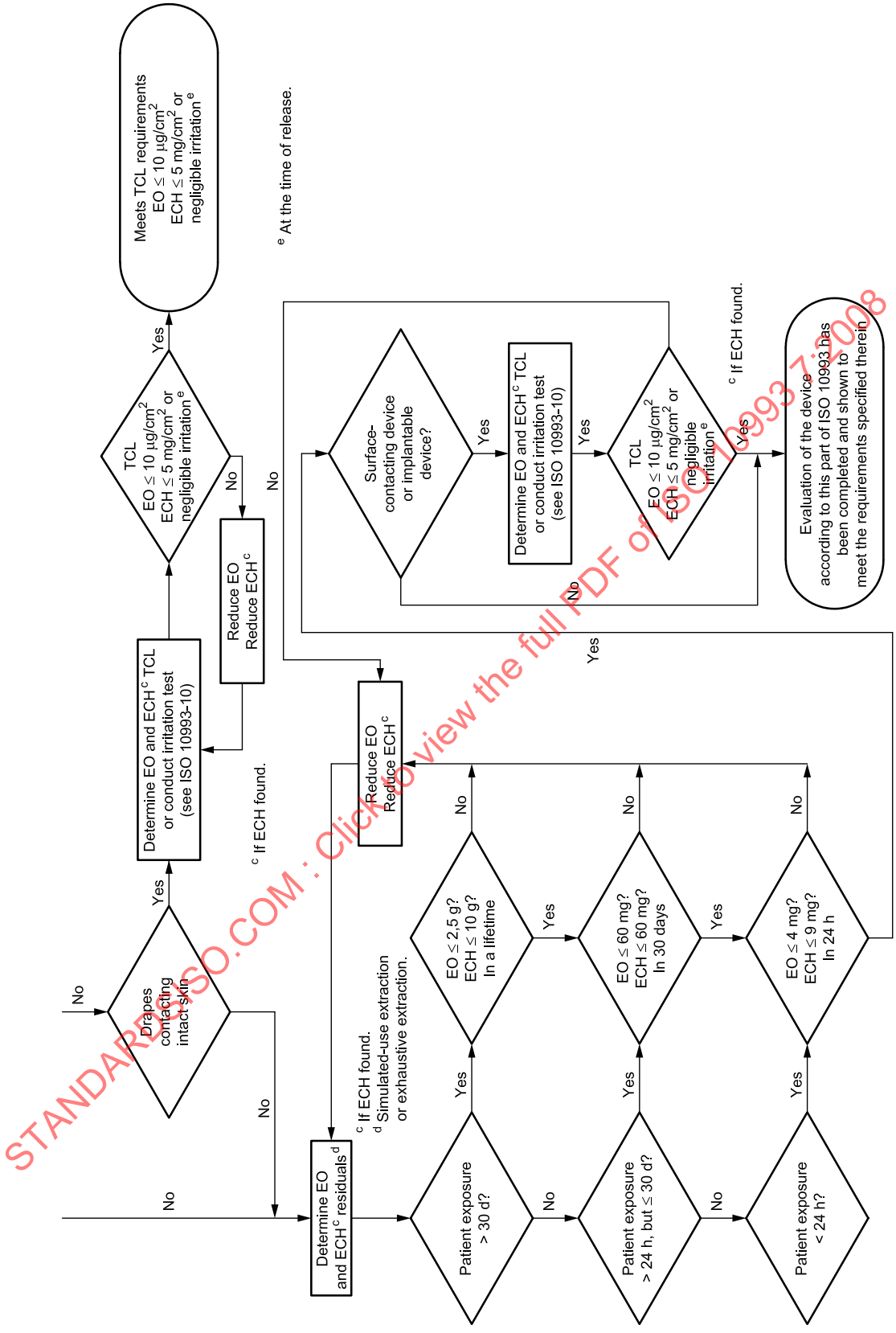


Figure C.3 — Flow chart to assist in understanding the steps necessary to apply this part of ISO 10993
(*continued from Figure C.2*)

Annex D
(informative)

Factors influencing product residual

D.1 Sterilization process parameters

D.1.1 General overview

Sterilization process parameters are defined in ISO 11135-1. However, to properly analyse residues in EO-exposed devices, it is necessary to recognise those parameters that have an effect on residue content. An understanding of EO kinetics may make it possible to address a family of like devices through the analysis of a "worst-case" representative. Recognition of a family of similar products (that is, similar in size and use, material composition, packaging, EO exposure, water content, and exposure to environmental conditions) may preclude the necessity of analysing each item of the product line. The following parameters affect residue content and may allow analysis of one or more "worst-case" representatives.

D.1.2 Material composition

Materials vary considerably in their ability to absorb, retain and release EO. When conversion of EO to ECH is possible, two similar devices made of different materials are likely to have very different residue profiles. For example, materials that contain a source of free chloride ions exhibit a wide degree of variation in the concentration of ECH formed.

Similarly, a single device composed of two dissimilar materials may require a representative sample of both materials to ensure accurate analysis. Composition and size may be particularly important when considering the simulation of normal product use.

D.1.3 Packaging

Packaging materials vary widely in their abilities to allow penetration and dissipation both of EO gas and the other possible residues, which may in turn affect ECH residue levels. Packing density and the density of the shipping container are other sources of variability.

D.1.4 Ethylene oxide sterilization cycle

Process conditions under which the device is exposed to EO will affect the residue levels. These conditions include gas concentration, exposure time, temperature, type of cycle (that is, pure EO or EO mixtures), humidity (including the quality of the water source), re-evacuations and air washes, and the product and load density or the configuration of the product load in the sterilizer.

D.1.5 Aeration

Residual EO in devices may vary as a function of aeration temperature, load density and configuration, air flow, loading pattern, surface area of products being aerated and aeration time. Some materials demonstrate aeration rates which can roughly double (aeration time reduced by one half) for each 10 °C increase in aeration temperature.

Factors such as humidity, temperature, and air flow may influence ECH formation depending on EO content in the product after removal from the sterilizer.

Analysts should be aware of seasonal variations in aeration rates when samples are stored under laboratory conditions which differ from the ambient warehouse conditions. Under certain circumstances, which can best be determined by experience, it may be necessary to hold samples prior to analysis under conditions that approximate the lowest temperature at which the product is likely to be stored during aeration.

D.1.6 Sample retrieval

Caution should be exercised when product samples are routinely removed for analysis from the sterilization load soon after the sterilization process is completed. Caution should also be exercised when the product sample or an extract thereof is shipped to an analysis site remote from the sterilization site. In such cases, the errors associated with attempting to correlate the residue amounts on samples and on the rest of the load should be recognized and an experiment to establish the relationships between these conditions carried out.

D.2 Controlling variables

Given sufficient experimental evidence on residue diffusion kinetics (e.g., the rate of EO gas dissipation from the packaging for the range of given devices), it may be possible to group devices for quality assurance testing based on similarities of materials, manufacturing processes and use. For such a classification system to work, the variables discussed above must be controlled. Lack of control may yield data about residue levels that are applicable only to the samples analysed.

Annex E
(informative)

Extraction conditions for determination of residual EO

Extraction conditions for the determination of residual EO to demonstrate compliance with this part of ISO 10993 are shown in 4.4.

Table E.1 represents suggested extraction conditions that could facilitate laboratory operations.

Specific methods for simulated-use and exhaustive extraction are given in 4.4.6.2 and 4.4.6.3.

The guiding principle in selecting appropriate extraction methods for the determination of EO is the evaluation of the dose to the patient in order to show compliance with the requirements set out in this part of ISO 10993, using simulated use wherever possible. For devices in the prolonged exposure category, it is important to note that the device must also meet the residue requirements of the limited exposure category, and that devices in the permanent contact category must also meet the residue requirements of the prolonged exposure and limited exposure categories, whichever extraction condition is used. Where residues are shown to be within these requirements for products tested by exhaustive extraction, there is no need to further challenge the device by simulated-use extraction.

Table E.1 — Suggested extraction conditions

Device contact duration (see 4.3)		
Permanent contact (> 30 d)	Prolonged exposure (24 h to 30 d)	Limited exposure (< 24 h)
Exhaustive extraction	Simulated use	Simulated use

Where an exhaustive extraction procedure as defined in 3.2 is specified it may be impractical for large and/or complex devices. In such cases it may be necessary to extract representative portions of the device, then extrapolate the results to the entire device. See also 4.4.6.

In certain exceptional situations where simulated-use extraction may be neither feasible nor practical (e.g., for large, surface-contacting devices such as gowns or drapes), the dose of EO transferred to the patient may be estimated on a weight- or surface-area-proportional basis using, for example, the transfer reduction factor approach described in the section *Exposure per use* in [154].

Annex F
(informative)

Rationale for the provisions of this part of ISO 10993

F.1 General

This annex specifies the rationale for establishing allowable limits for ethylene oxide sterilization residues in medical devices on the basis of duration of contact. Included is the basis for establishing limits for ethylene oxide (EO), ethylene chlorohydrin (ECH) and ethylene glycol (EG).

F.2 Rationale for special situations

F.2.1 General

There are certain circumstances, for example major surgery, where the life-saving nature of the therapy significantly alters the risk-benefit analysis. The exposure limits given are based on risks and benefits associated with less critical circumstances. Therefore, ISO 10993-17 allows for device benefit alterations in allowable limits on a case-by-case basis. In consequence, there is scope for relaxation of limits in life-threatening situations where it is not possible to meet the specified limits. Similarly, there may also be a need to tighten limits where warranted by risks in specific situations.

During the development of this part of ISO 10993, six special situations were recognized in which the limits of 4.3 would not be practical due to limitations of the devices themselves, or in which human data indicated that the dose levels shown in 4.3 are not applicable. Human data are available from patient exposure to intraocular lenses which must be addressed by revision of the residue requirements for such devices. Blood cell separators used in donor and patient blood collection can be used multiple times and donors and patients have been shown to become sensitized to EO. Allowable limits for EO for these devices must be lowered to minimize the possibility of sensitization. During treatment of blood with oxygenators or blood separators or cardiopulmonary bypass devices it is recognised that the medical benefit outweighs the risk and this is addressed in considering the allowable short-term limits for these devices. In the case of extracorporeal blood purification set-ups, long-term use could potentially lead to the maximum lifetime dose requirement being exceeded and this is also addressed. In the case of drapes contacting intact skin, no systemic toxicity is anticipated and patient safety should be adequately protected by meeting the TCL or irritation test requirements.

F.2.2 Intraocular lens limits

The residue limits for intraocular lenses (implant devices in the eye) is 0,5 µg EO per lens per day. This limit is not based on the permanent contact limit with an average daily dose of 0,1 mg (100 µg) per day for a lifetime. Rather, it is a special case in which the maximum delivered dose cannot exceed a ceiling value of 0,5 µg per lens per day. This is necessary to prevent documented irritation responses of EO to ocular tissue (see References [43], [116], [117], [143] and [164]). Prorate limits are used for other intraocular devices on the basis of the mass of the device, with the mass of an intraocular lens taken as 20 mg.

When EO residuals are controlled as specified here for intraocular devices, it is unlikely that significant amounts of ECH will be present. This may not be true for intraocular devices made from viscoelastic materials that contain chlorine. In such cases, References [43], [115], [116] and [117] indicate that the level of ECH that results in ocular toxicity is about four times greater than the corresponding EO level. This should be taken into account when evaluating the acceptability of ECH levels associated with these devices.

F.2.3 Blood cell separators used in donor or patient blood collection

The maximum allowable limit for EO is 10 mg per device. The maximum allowable limit for ECH shall not exceed 22 mg per device. These devices are used for apheresis collection. This limit takes into account the multiple use of such devices in individual donors or patients.

In this case the default assumption of five devices used simultaneously appeared somewhat conservative. If one were to assume only two devices as a reasonable worst case, the UTF would increase from 0,2 to 0,5. This would raise the allowable limit to 10 mg EO (rounded down from 10,5 mg). See Equations F.1 and F.2.

For EO

$$\mathrm{TE} = \mathrm{TI} \times M_{\mathrm{B}} \times \mathrm{UTF} = 0{,}3\ \mathrm{mg/kg/d} \times 70\ \mathrm{kg} \times 0{,}5 = 10{,}5\ \frac{\mathrm{mg}}{\mathrm{d}} \qquad \text{(F.1)}$$

For ECH

$$\mathrm{TE} = \mathrm{TI} \times M_{\mathrm{B}} \times \mathrm{UTF} = 0{,}64\ \mathrm{mg/kg/d} \times 70\ \mathrm{kg} \times 0{,}5 = 22{,}4\ \frac{\mathrm{mg}}{\mathrm{d}} \qquad \text{(F.2)}$$

F.2.4 Blood oxygenators and blood separators

The exposure limit for such devices is 60 mg for EO and 45 mg for ECH in a 24 hour period. These devices are used in severe operations such as open heart surgery. Such procedures are used on individual patients no more than once or twice in a lifetime. Since these devices are used for a day or less, the default UTF of 0,2 appears overly conservative. A UTF of 1,0 appears more reasonable. At this UTF, the allowable limit would increase to 21 mg EO and 45 mg ECH. See Equations F.3 and F.4. The EO limit reflects manufacturer's current ability to remove EO from these rather large devices. Under such circumstances this further three-fold relaxation of the EO limit is warranted.

For EO

$$\mathrm{TE} = \mathrm{TI} \times M_{\mathrm{B}} \times \mathrm{UTF} = 0{,}3\ \mathrm{mg/kg/d} \times 70\ \mathrm{kg} \times 1 = 21\ \frac{\mathrm{mg}}{\mathrm{d}} \qquad \text{(F.3)}$$

Given one day or less of use: 21 mg/d × 1 d = 21 mg/device

For ECH

$$\mathrm{TE} = \mathrm{TI} \times M_{\mathrm{B}} \times \mathrm{UTF} = 0{,}64\ \mathrm{mg/kg/d} \times 70\ \mathrm{kg} \times 1 = 44{,}8\ \frac{\mathrm{mg}}{\mathrm{d}} \qquad \text{(F.4)}$$

Given one day or less of use: 44,8 mg/d × 1 d = 44,8 mg/device

F.2.5 Devices used in cardiopulmonary bypass procedures

The exposure limit for such devices is 20 mg for EO in a 24 hour period. These devices are used in severe operations such as open heart surgery. Such procedures are used on individual patients no more than once or twice in a lifetime. Since these devices are used for a day or less, the default utilization factor (UTF) of 0,2 appears overly conservative. A UTF of 1,0 appears more reasonable. At this UTF, the allowable limit would increase to 21 mg EO. The EO limit reflects manufacturers' current ability to remove EO from these rather large devices. Under such circumstances this further three-fold relaxation of the EO limit is warranted. The limits for ECH apply.

For EO

$$TE = TI \times M_B \times UTF = 0{,}3 \text{ mg/kg/d} \times 70 \text{ kg} \times 1 = 21 \frac{\text{mg}}{\text{d}} \quad \text{(F.5)}$$

NOTE This is rounded to 20 mg/d for these devices.

Given one day or less of use: 20 mg/d × 1 d = 20 mg/device

F.2.6 Extracorporeal blood purification devices

These devices are used in patients multiple times often over many years. In setting the allowable limits for these devices, consideration is given to the benefit derived from blood purification. The maximum allowable limit for each device used on a patient was set by considering the use of thirteen (13) such devices over each month, and setting the maximum allowable limit as one thirteenth of the maximum allowable limit over 30 d, which is 4,6 mg for EO and 4,6 mg for ECH. The maximum allowable EO dose of 2,5 g for a lifetime may be exceeded, provided that the allowable limit for EO of 4,6 mg for each use is met. In addition, the maximum allowable ECH dose of 10 g for a lifetime may be exceeded, provided that the allowable limit for ECH of 4,6 mg for each use is met. To exceed the 2,5 g lifetime dose of EO, a patient undergoing blood purification would need to be exposed to 4,6 mg of EO thirteen times every month and such exposure would need to continue for 3,5 years. Similarly, ECH lifetime exposure could be exceeded after about fourteen (14) years of use by end-stage renal disease patients.

For EO:

— Lifetime dose of 2,5 g = 2 500 mg.

— Maximum allowable dose of EO from use of 13 extracorporeal blood purification devices per month is 60 mg.

— So it will take 2 500 mg/(60 mg/month) = 42 months or about 3,5 years to reach the maximum allowable lifetime EO dose for the use of such devices.

For ECH:

— Lifetime dose of 10 g = 10 000 mg.

— Maximum allowable dose of ECH from use of 13 extracorporeal blood purification devices per month is 60 mg.

— So it will take 10 000 mg/(60 mg/month) = 167 months or about 14 years to reach the maximum allowable lifetime ECH dose for the use of such devices.

F.2.7 Drapes contacting intact skin

Drapes that contact intact skin provide benefits to patients with minimal risk. Surgical drapes are used to minimize the spread of infective agents to and from the patient, thereby contributing to a reduction in post-operative infections. Medical devices that contact intact skin have not been shown to cause systemic toxicity. The tolerable contact limit (TCL) values for EO and ECH are based on local toxic effects. Thus the TCL values of 10 µg/cm^2 for EO and 5 mg/cm^2 for ECH or the device having negligible irritation when tested according to ISO 10993-10 are the appropriate binding limits for drapes that contact intact skin.

F.3 Rationale for 4.4

F.3.1 General

This clause provides the general rationale for each of the major parts of 4.4.

F.3.2 Product extraction

The critical parameter in the regulation of EO-sterilization residues is the dose the patient or user may receive from use of devices so sterilized. In order to assess this patient or user dose, extraction procedures are required which simulate normal product use. In some cases, this may be achieved by simply filling the product with water, whereas in other cases more complicated simulations including continuous fluid flow may be required. It is recognised that, should the requirements be met by determining the total residue present in the product, by exhaustive extraction, there may be no need to simulate product use.

The definition of exhaustive extraction used includes the concept that extraction should continue until the last extraction step performed produces a yield of the analyte which is less than 10 % of the yield of the analyte in the first extraction of the sample. This concept fails when the yield of the first extraction is very small, as in the case of a device with little residue or a sample that releases the analyte at a very slow rate. In such cases, extraction should continue until the increase in the cumulative total of the analyte extracted in the several extraction steps is small relative to the analytical uncertainties.

F.3.3 Analytical methods

F.3.3.1 Stability of EO in solution

Each laboratory should conduct its own stability study to determine the shelf life of its ethylene oxide residuals standards. The effective standard should be not less than a specified percentage of the original concentration upon the final day of the validated stability shelf life. Otherwise, all standards should be made on a daily basis.

During the interlaboratory comparison study of the EO method described in K.4.4 (see [140]), a study was made of the stability of standard solutions of EO in ethanol. Solutions of EO at concentrations of 25 µg/ml, 50 µg/ml and 100 µg/ml were prepared and stored both at refrigerator temperature and at 40 °C. These solutions were analysed at different times over periods of up to six weeks. The study showed that, at 40 °C, the EO concentration was reduced to 70 % of the original concentration after 2 weeks for the 50 µg/ml and 100 µg/ml standards, whereas all of the standards studied were stable to within 10 % of the original concentration after storage at refrigerator temperature (5 °C) for up to 60 d.

F.3.3.2 Stability of ECH in solution

Prior to the interlaboratory comparison study of ECH, eleven laboratories participated in a study of the stability of ECH standards. Aqueous solutions of ECH were prepared by one laboratory and shipped to all participants. The solutions were stored at refrigerator temperature upon arrival. These solutions were analysed at different periods of time, such as immediately after arrival, 1 week after, and 2, 3, 4, 8 and 12 weeks after arrival, by various types of column. The study showed that there is no significant difference in the concentration in the first 2 weeks. It was concluded that ECH standard solutions are stable when stored at refrigerator temperature for at least 14 d.

F.3.3.3 Linearity of standard curve

Ideally, the procedures described in this part of ISO 10993 would be applicable over the range of concentrations required to meet the limits specified in 4.3. The method precision, detection limits, quantitation limits, and the linearity of the calibration curve, should be validated.

F.3.4 Rationale for 4.4.7.1, data analysis and interpretation

The proper treatment of data is presented to permit the analyst to calculate the product residual level and from this the potential dose to patient. This permits release of product based on conformance with the requirements listed in 4.3.

Annex G
(informative)

Establishment of allowable limits for EO

G.1 General

The approach described in ISO 10993-17 was used to derive limited, prolonged, and permanent tolerable intake (TI) values for EO. Separate TI values were not calculated for various routes of exposure. The derived TI values for EO were converted to allowable limit and device limit values and compared to the limits from ISO 10993-7:1995. For the limited exposure category, the derived TI and corresponding device limit from the evaluation presented herein have been accepted. For the prolonged and permanent contact categories, the existing limits from the 1995 edition have been retained, although the derived TI values and corresponding device limits from the evaluation presented herein support higher levels. The rationale for retaining the current limits is the successful clinical history since adoption of the 1995 edition, and the ability of manufacturers to comply with these limits. Concomitantly, there is no current clinical or manufacturing reason to raise the existing limits for the prolonged and permanent categories to the levels supported by the evaluation described herein.

A limited/prolonged exposure category TI of 0,3 mg/kg/d was derived based on the results in References [82], [83], [84], [169], [170] and [171]. Data from these studies were previously used as support for the prolonged exposure limit for EO. A modifying factor (MF) of 30 was applied to the data, based on a UF1 of 30 to account for interindividual variability and a UF2 of 1 to account for interspecies difference in potency. Justification is provided for the selection of values for UF1 and UF2.

A permanent exposure category TI of 0,02 mg/kg/d was derived based on cancer effects and was derived using dose-response modelling of human data. Other approaches for cancer risk assessment were also explored for derivation of the cancer-based TI. A permanent non-cancer TI of 0,03 mg/kg/d can be derived based on the adverse effects on spermatogenesis seen after long-term inhalation exposure of cynomolgus monkeys to EO (see References [107], [108] and [109]) and an MF of 60. The MF used to derive the permanent exposure non-cancer TI includes a UF3 for LOAEL-to-NOAEL extrapolation.

G.2 Introduction

Since the publication of the first edition of this part of ISO 10993 in 1995, new data have become available on the adverse effects of EO in humans and experimental animals. In addition, data have become available to reduce uncertainty in assessing the relative sensitivity of humans and experimental animals to this compound and the variability of response within the human population to EO. Furthermore, new tools (e.g., benchmark dose and physiologically based pharmacokinetic modelling) have become available to assist in more accurately assessing the risk posed by exposure to EO. This risk assessment serves as the basis for the selection of TI used in this part of ISO 10993.

G.3 Methods

G.3.1 General

The approach described in ISO 10993-17 was used to derive TI values for EO for various durations of exposure.

The potential exists for patients to be exposed to EO released from medical devices for short or long durations; as a result, it was necessary to derive limited/prolonged and permanent TI values for this

compound. In addition, it is possible for patients to be exposed to EO via various routes of exposure. Although patients are typically exposed to EO via parenteral routes of exposure in clinical settings, very little toxicity data are available to derive TI values for these routes of exposure. In contrast, there is a large database of data available on the effects of EO in experimental animals and humans following inhalation exposure. To use this rich resource of inhalation toxicity data for setting parenteral TI values for EO, a method was developed for route-to-route extrapolation to derive estimates of internal dose following inhalation exposure.

G.3.2 Route-to-route extrapolation of dose

A fairly large number of toxicity studies have been conducted on EO; however, relatively few of these were conducted using parenteral routes of exposure. Nevertheless, the extent to which EO is absorbed following inhalation exposure is known; therefore, it should be possible to estimate the internal dose of EO based on knowledge of the exposure concentration of EO and the extent to which the compound is absorbed via the respiratory tract.

Absorbed dose can also be estimated based on knowledge of the exposure concentration, ventilation rate in the exposed species, duration of exposure, and extent of absorption via the inhalation route. Using the data in References [186] and [22], estimates of the relative absorption of airborne EO at various exposure concentrations were established (Table G.1).

Table G.1 — Absorbed dose of EO in rats exposed to various concentrations of EO in air

Exposure concentration (ppm)	Percent absorbed %
10	94
33	74
50	68
100	61
1 000	36

Calculation of absorbed dose in cynomolgus monkeys is based on the mean ventilation rate (0,83 m^3/d) derived from the values reported by Fisher[52] for methanol-exposed cynomolgus monkeys.

G.3.3 Non-cancer risk assessment approach

TI values for non-cancer effects of EO were derived by dividing the most relevant NOAEL or LOAEL values from critical studies by uncertainty factors to account for data on the variability in response to EO in human populations (UF1), potential species difference in potency (UF2) and data deficiencies (UF3). ISO 10993-17 emphasises the use of scientific data, when available, to derive uncertainty factors applied to the data from key toxicity studies when deriving TI values. Consistent with this philosophy, data on the variability in response to EO in human populations and on the potency of EO across species was used in deriving values for UF1 and UF2, respectively. Factors that were taken into account when selecting a value for UF1 include the polymorphic expression of the enzymes that metabolize EO in the human population, the ability of various disease states to inhibit these enzymes, and variability in the ability to repair DNA damage. Consideration of these factors resulted in the selection of a value for UF1 that is greater than the default value of 10 that is typically used for this parameter. In contrast, scientific data and the results of physiologically based pharmacokinetic (PBPK) modelling suggest that the potency of EO varies little across species and therefore a value of less than 10 is appropriate for UF2, compared to the default of 10 that is typically used for this parameter.

G.3.4 Cancer risk assessment approach

Application of a weight-of-evidence test indicates that EO is a genotoxic carcinogen and that tumours seen in animals are relevant for humans. ISO 10993-17 allows for various approaches to be considered when deriving a cancer-based TI value for a genotoxic carcinogen. Accordingly, cancer-based TI values have been derived using multiple approaches, namely, simple linear extrapolation from the LOAEL, application of UFs to the LOAEL, and application of dose-response modelling.

G.3.5 Effects not considered in deriving TI values for EO

The TI values for EO based on cancer or non-cancer effects are not necessarily protective for immunological effects such as hypersensitivity reactions and anaphylaxis, nor are they necessarily protective for effects such as haemolysis. Other approaches may be necessary to protect patients against these effects that have been associated with exposure to EO.

G.4 Non-cancer-based TI values for EO

G.4.1 Overview

Derivation of a non-cancer-based TI value for EO involves:

— selection of appropriate NOAEL and LOAEL values from the literature and

— selection of uncertainty factors to account for intra-individual variability in the human population, interspecies differences in potency and deficiencies in the data.

These steps are described in G.4.2 and G.4.3, respectively.

G.4.2 Selection of critical studies

G.4.2.1 Limited/prolonged exposure category

Adequate single-dose toxicity data are not available to establish a limited exposure category TI for EO. However, ISO 10993-17 notes all available data should be considered in the context of understanding the overall toxicity profile of the substance. The basic approach is that acute data (for example data from studies of 14 d or less) should be used to set limited exposure or short-term limits.

Therefore, data from longer-term studies were used to establish the limited exposure category TI.

Table G.2 summarises the most relevant data for the derivation of the limited/prolonged exposure category TI for EO; however, it should be noted that many studies other than those listed in following tables were reviewed in the process of setting TI values for EO.

Woodard and Woodard[203] described a study in which dogs received SC injections of EO at doses of 6 mg/kg, 18 mg/kg and 54 mg/kg (later adjusted to 36 mg/kg) for 30 consecutive days; however, because of the small number of animals used, these results cannot be used with confidence to establish the limited/prolonged TI.

Table G.2 — Studies used to derive limited/prolonged exposure category TI for EO

Species	Route	Exposure	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects at LOAEL	Study
Dog	SC	6, 18 or 54 mg/kg daily × 30 d	6	18	Weight loss, coagulation changes, increased liver, kidney and spleen weights	[203]
Rabbit	IV	9, 18 and 36 mg/kg daily on GD 4-16	9	18	Decreased maternal weight gain	[82]
Rats	Inhalation	10, 33 or 100 µg/kg 6 h/d on GD 6-15	9	27,5	Depression of foetal body weight	[169]

A similar NOAEL value for EO was derived from the inhalation study conducted by Snellings[169]. Decreased foetal body weight was observed following exposure of pregnant Fischer 344 rats to 100 ppm EO for 6 h/d on days 6 to 15 of gestation. No adverse effects were seen following exposure to 33 ppm EO. Using the absorbed dose data from the study in Reference [22], the absorbed dose equivalent to 33 ppm in Reference [169] is

$$33 \text{ ppm} \times 1{,}8 \text{ mg/m}^3\text{/ppm} \times 0{,}29 \text{ m}^3\text{/d} \times 6/24 \times 0{,}74/0{,}35 \text{ kg} = 9{,}1 \text{ mg/kg/d}$$

Identical NOAEL values in the studies in References [82] and [169] increase the confidence in using this value as the basis for the limited/prolonged exposure category TI values.

G.4.2.2 Permanent exposure category

The lowest absorbed dose associated with adverse non-cancer effects following long-term inhalation exposure of experimental animals to EO is 2,0 mg/kg/d, based on the results reported by Lynch[107]. These investigators exposed cynomolgus monkeys to 0 ppm EO, 50 ppm EO or 100 ppm EO for 7 h/d, 5 d/week for 24 months. Statistically significant decreases in sperm counts and sperm motility were seen in both EO-exposed groups, compared to controls.

Based on the mean ventilation rate measured in methanol-exposed cynomolgus monkeys in the Fisher *et al.* study[52] and the assumption that the percent of EO absorbed at 50 ppm is the same in rats and monkeys, the absorbed dose following exposure to 50 ppm EO is

$$50 \text{ ppm} \times 1{,}8 \text{ mg/m}^3\text{/ppm} \times 0{,}83 \text{ m}^3\text{/d} \times 7/24 \times 5/7 \times 0{,}68/5{,}3 \text{ kg} = 2{,}0 \text{ mg/kg/d}$$

The effects on sperm count and motility seen in the Lynch study are consistent with those seen in EO-exposed humans [5] and with other sperm parameters reported in experimental animals (e.g. Reference [128]). Furthermore, statistically significant decreases in sperm count, motility or morphology are considered to be adverse effects for the purpose of setting exposure limits for compounds (see References [126] and [188]). Finally, there is no mechanistic reason to suggest that the results seen in cynomolgus monkeys would not be relevant for humans. As a result, it is valid to use the results reported in Reference [107] to derive a permanent parenteral TI for EO based on non-cancer effects.

G.4.3 Selection of uncertainty factors for non-cancer effects

See Table G.3.

Table G.3 — Uncertainty factors for TI derivation

Uncertainty factor designation	Range	Magnitude of default UF	Description
UF1, inter-individual variability in the human population	1 to 10	10	To account for the variability in response between the mean of the healthy population and the response in some proportion of a sensitive subpopulation.
UF2, inter-species extrapolation	1 to 10	10	To account for the possibility that humans are more sensitive to the adverse effects of a compound than experimental animals are.
UF3, quality and relevance of the experimental data	1 to 100	None	To account for limitations in the toxicological data available for TI derivation, including absence of NOAEL value, absence of NOAEL from a long-term study, and lack of data from a clinically relevant route of exposure.

G.4.3.1 Inter-individual variability (UF1)

G.4.3.1.1 Overview

ISO 10993-17 notes that it is preferable to have actual data to assess human variation in order to define the magnitude of the value selected for UF1. Fortunately, data are available to characterize the variability of the response to EO of various biomarkers in human populations, primarily in occupational cohorts. For example, Fuchs[54] observed “remarkable individual differences in susceptibility” in EO-exposed workers to single strand breaks of DNA in peripheral mononuclear blood cells. These investigators identified two subpopulations among workers occupationally exposed to EO, a “higher sensitive” group and a “lower sensitive” group. Among non-smokers in the lower sensitive group, the lowest concentration of EO (4 hour TWA) associated with DNA single strand breaks was 3,5 mg/m^3. Among non-smokers in the higher sensitive group, the lowest concentration of EO associated with DNA single strand breaks was 0,6 mg/m^3. Therefore, a UF1 value of at least 6 (3,6/0,6) is necessary to protect sensitive individuals in the “higher sensitive” group from this specific genotoxic effect. Various factors may be responsible for this variability in response to EO, including polymorphic expression of the enzymes responsible for EO metabolism (the theta 1 isoform of glutathione transferase and epoxide hydrolase), and variability in DNA repair mechanisms. In addition, there are factors that may increase the sensitivity of critically ill and injured patients to the adverse effects of EO, relative to the general population, such as inhibition of metabolic enzymes that play a role in the detoxification of EO and reduction in the levels of co-factors necessary for the enzyme reactions to occur (e.g., glutathione). Consequently, the variability seen in response to EO in the general population may not necessarily reflect the response variability seen in a patient population. Therefore, the variability in response seen in the healthy adult populations occupationally exposed to EO (see [54]) may under-represent the variability seen among patients exposed to EO.

Each of the factors that may play a role in contributing to variability in the human population to adverse effects associated with EO exposure will be explored in this section for the purpose of identifying a value for UF1 that will be appropriately protective for sensitive subpopulations.

G.4.3.1.2 Polymorphism of EO detoxification enzymes

G.4.3.1.2.1 General considerations

Ethylene oxide is metabolized, and consequently, detoxified, in rodents and humans by two enzymes: the theta 1 isoform of glutathione transferase (GSTT1) and epoxide hydrolase (EH). Both of these enzymes are polymorphically expressed in the human population (e.g. References [182], [183] and [184]). A consequence of these polymorphisms is that a certain percentage of the human population is expected to have a reduced capacity to metabolize EO, relative to the rest of the population. Since EO is detoxified by these enzymes, one

would expect poor metabolizers of EO to be at increased risk of adverse effects, relative to the rest of the population. Considerable attention has been paid to the role GSTT1 polymorphism may have in the variability in response seen in human populations to EO (e.g. Reference [50]). However, EO is primarily metabolized by EH in humans; consequently it may be assumed that GSTT1 polymorphism is not expected to influence the variability in response of the human population to a significant degree. Despite the assumed principal role of EH to the metabolism of EO in humans, little attention has been paid to the potential role of EH polymorphism or inhibition on EO metabolism and, subsequently, on the risk posed by exposure to EO. The impact of polymorphisms of GSTT1 and EH on the response to EO in the human population is discussed G.4.3.1.2.2 and G.4.3.1.2.3.

G.4.3.1.2.2 Role of GSTT1 polymorphism in the variability of the response of the human population to EO

The frequency of the GSTT1 null genotype can be as high as 54 % in some populations (see Reference [6]), but most papers report values in the 17 % to 25 % range (e.g. Reference [158]), depending on the population. Since the GSTT1 null genotype is associated with reduced GSTT1 enzyme activity, a significant percentage of the population may be at increased risk of EO-associated adverse effects.

The GSTT1 null genotype exerts a specific influence on levels of haemoglobin adducts in EO-exposed individuals (see Table G.4).

Table G.4 — Effect of GSTT1 polymorphism on haemoglobin adduct levels in conjugator vs. non-conjugator populations

Study	Mean difference in response between GSTT1+ and GSTT1 null populations
[53]	3
[130]	2
[182]	1,5
[50]	1,5
[205]	2,1

The results shown in Table G.4 suggest that populations with a GSTT1 null genotype have a 1,5- to 3-fold higher internal dose of EO; however, comparison of the mean difference between the two populations will underestimate the difference between the dose associated with the mean response in a GSTT1+ and a lower-bound percentile of the GSTT1 null population.

Although the data show a clear dependence of GSTT1 expression on levels of haemoglobin adducts in EO-exposed persons, the data on the influence of GSTT1 polymorphism on induction of SCE are mixed. Hallier[64] reported that induction of SCE in peripheral lymphocytes of GSTT1 null individuals was much greater than that in GSTT1; however, Schroder[163] and Wiencke[202] reported very modest increases in background SCE levels in GSTT1 null individuals compared to those with a GSTT1 genotype. In aggregate, these results suggest that GSTT1 polymorphism leads to increased levels of Hgb adducts, but only modestly increases the genotoxic effects of EO in poor conjugators.

It is important to note that the GSTT1 null genotype is associated with an increased risk of some cancers (e.g., References [48] and [207]), but not necessarily with cancers associated with ethylene oxide exposure.

G.4.3.1.2.3 Role of EH polymorphism in the variability of the response of the human population to EO

Similarly to GSTT1, the expression of EH is polymorphically expressed in the human population (see References [69] and [144]), as a result, variability in EH activity in humans can be significant. For example, Mertes[123] found a 63-fold variability in the metabolism of EH substrates by human liver samples; however, 90 % of the samples deviated by less than a factor of 3 from the median. Kitteringham *et al*.[90] have summarized the impact that EH polymorphism has on EH activity in the human population and they note:

> "To generalize from these studies, it appears that no individual is entirely deficient in HYL1 [microsomal epoxide hydrolase], but there does exist some degree of intersubject variation in hepatic activity, although the majority of the population would be encompassed by a 10-fold range."

If the entire range of EH activity in the human population (including poor metabolizers and rapid metabolizers) can be encompassed by a factor of 10, the difference in activity between the mean of the population and most poor metabolizers can probably be described by a factor of 5, depending on the shape of the distribution.

The variability in EH activity seen in the human population has been associated with an increased risk of some cancers, but not necessarily with cancers associated with EO exposure. For example, McGlynn *et al*.[121] have observed a 2-fold increase of hepatocellular carcinomas in a Chinese population with a polymorphism that results in lower EH activity. In addition, reduced ability to metabolize epoxides via epoxide hydrolase has been associated with an increased risk of foetal hydantoin syndrome and other toxicities associated with the use of anticonvulsants (see Reference [93]). Presumably, since EO is detoxified by the EH pathway, individuals with reduced EH activity as a result of reduced polymorphic expression of the enzyme could be at increased risk of EO-associated adverse effects relative to individuals in the population that metabolizes epoxides efficiently.

G.4.3.1.3 Inhibition of EO detoxification enzymes

G.4.3.1.3.1 Inhibition in disease states

Epoxide hydrolase activity is inhibited during certain disease states, such as endotoxemia and traumatic shock. Administration of bacterial endotoxin to rats inhibited both EH activity (see Reference [49]) and EH gene expression (see Reference [36]). Microsomal EH was inhibited approximately 50 % in an animal model of trauma (see Reference [60]). Presumably, the metabolism of EO could be impaired in disease states that result in reduced EH capacity.

G.4.3.1.3.2 Inhibition by drugs and other compounds

The anticonvulsant drugs, valproic acid and valpromide, have been shown to inhibit EH activity in humans at therapeutic concentrations[88]. This inhibition is thought to play a role in the increased teratogenicity seen following co-exposure to valproic acid and other antiseizure drugs in patients with epilepsy.

Significance of enzyme inhibition

The physiologically based pharmacokinetic (PBPK) model of EO developed by Fennell and Brown[51] can be used to specifically assess the impact of GSTT1 and EH inhibition on the internal dose of EO. A sensitivity analysis conducted by these investigators of the impact that each parameter has on modelled venous blood concentrations of EO revealed that the changes in the values for the GST V_{max} parameter in the model had a significant impact on venous blood concentration of EO in mice and rats, but not humans. Conversely, changes in the values for the EH V_{max} parameter in the model had a significant impact on venous blood concentration of EO in humans, but not mice and rats. The sensitivity coefficient for the EH V_{max} parameter in humans was about −0,4 %. Therefore, for every 1 % reduction in the value for the EH V_{max} parameter, the venous blood concentration of EO would be expected to increase by 0,4 %. Consequently, the 50 % inhibition of EH that may occur in some disease states (e.g., trauma, sepsis), would be associated with a 20 % increase in venous blood concentration of EO. Inhibition of GSTT1 would be expected to have little impact on venous blood concentrations of EO in humans. Therefore, although inhibition of EH can lead to important clinical consequences (e.g., drug interactions), the impact of EH inhibition on estimated internal doses of EO in humans can be accounted for by a factor of 2 or less, based on the results of the PBPK modelling exercise.

G.4.3.1.4 Glutathione levels

Detoxification of EO via the GSTT1 pathway requires sufficient levels of endogenous glutathione to be available in the tissues as a co-factor. A number of studies have shown that critically ill or post-operative patients typically have lower tissue levels of reduced glutathione (GSH) than healthy persons. For example, Wernerman[197] found that surgery and critical illness reduce glutathione levels by 40 %. As a result, critically ill patients could be at increased risk of developing EO-associated effects, compared to healthy persons.

G.4.3.1.5 Polymorphism of DNA repair capacity

Another pharmacodynamic factor, polymorphism in genes associated with DNA repair, as well as carcinogen metabolism, can have an impact on cancer risk (see Reference [73]). Presumably, individuals with inefficient DNA repair mechanisms may be at higher risk of EO-associated adverse effects than most individuals in the general population. Some experimental data exist to support this assertion. Nivard[138] found up to 20-fold enhanced mutation rates in the absence of maternal nucleotide excision repair (NER) in Drosophila exposed to high concentrations of EO compared to repair proficient conditions. However, this increased mutation rate was not seen in Drosophila at lower doses. Therefore, although inefficient DNA repair may place some individuals at increased risk of EO-associated adverse effects, it is not possible to assess DNA repair polymorphism data in a quantitative manner to identify a value for UF1 for EO.

Aggregate variability

As discussed above, various pharmacokinetic factors can result in a reduced capacity to metabolize and subsequently to detoxify EO in some patients. These factors include polymorphic expression of GSTT1 and EH, inhibition of EH by drugs and other compounds, and reduced EH activity in certain disease states. In addition, various pharmacodynamic factors, such as lower levels of glutathione in tissues of critically ill patients and polymorphism in DNA repair capacity may make the target tissues of some individuals more susceptible to damage by EO. It is not possible to use these data in a quantitative way to select a value for UF1; however, these factors, when viewed in aggregate, may inform the process of selecting a UF to characterize inter-individual variability in response to EO.

It is possible to justify a value for UF1 of at least 6, based on the variability present in the haemoglobin biomarker data reported by Fuchs[54]; however, this value probably under-represents the difference in sensitivity between the mean response in a healthy population and the response of sensitive individuals in a population of critically ill patients.

Knudsen[91] considered the impact of multiple factors that influence metabolic capacity on the magnitude of the UF to account for inter-individual variability and noted:

> "In risk assessment the safety 'factor' of 10 is generally accepted to allow for variation in individual susceptibility. Reviewing the literature justifies the factor of 10 when considering single polymorphisms. However in an individual with several susceptible metabolism genotypes as well as other determinants of susceptibility, e.g. defective DNA repair, poor-nutritional state, etc. the risk may increase far above a safety factor of 10."

The combined effect of these factors is unknown; however, the data collectively suggest that a value for UF1 that is greater than the default of 10 would be appropriate. As a result, it appears that the most sensitive populations would be adequately protected by a UF1 of 30 to account for inter-individual variability.

G.4.3.2 Inter-species differences (UF2)

G.4.3.2.1 Overview

Before species differences in potency are considered when deriving a value for UF2, it is important to ask whether the results seen in experimental animals exposed to EO are relevant for humans. As discussed in G.4.2.1 and G.4.2.2, the critical endpoints for TI derivation for EO are reduced weight gain in rabbits (limited/prolonged exposure category LOAEL) and altered spermatogenesis and testicular effects in cynomolgus monkeys (prolonged exposure category LOAEL for non-cancer effects). Reduced weight gain in experimental animals is a general effect that is considered to be adverse and relevant for establishing TI values. Spermatogenesis in non-human primates is organizationally similar to the process that occurs in humans, with regard to length of the spermatogenesis cycle, duration of spermatogenesis, and number of mitotic divisions (see References [124] and [195]). Consequently, non-human primates have been described as an appropriate model for experimental studies of human spermatogenesis. By analogy, it can be assumed that EO-induced effects on this process seen in cynomolgus monkeys would be applicable for humans. Since EO is thought to exert its carcinogenic effect as a direct acting genotoxic carcinogen, the effects seen in experimental animals are directly applicable for humans.

Based on allometric principles, humans are assumed to be more sensitive to the adverse effects of chemical compounds than experimental animals (see Reference [127]). As a result, a default UF of 10 is recommended in ISO 10993-17 to account for the assumed difference in potency of a compound between experimental animals and humans. However, several lines of evidence suggest that the potency of ethylene oxide is equivalent among species. As described in more detail below, the results of physiologically based pharmacokinetic (PBPK) modelling suggest that an internal dose in mice, rats and humans is expected to be the same following inhalation exposure to a given concentration of EO. The results of the PBPK modelling exercise are supported by data on species equivalence in internal dose of EO and similar compounds (propylene oxide, styrene oxide) following inhalation exposure. These factors all support the selection of a value of 1 for UF2 for use in deriving TI values for EO.

G.4.3.2.2 Results of PBPK modelling

Using the PBPK model mentioned previously, Fennell and Brown[51] found that estimated internal doses of EO [area under the curve (AUC) in blood] were equivalent in mice, rats and humans following inhalation of low concentrations of EO for 6 h (Table G.5).

Table G.5 — Estimated internal dose following inhalation of EO

EO concentration (ppm)	**Species** AUC (mg × h/l)		
	Mouse	Rat	Human
1	0,044	0,059	0,056
10	0,44	0,59	0,57

Species similarities in response

Using haemoglobin adduct levels as an index of internal dose, Ehrenberg and Tornqvist[45] found that the increment in adduct levels was consistent across species exposed to the same concentration of EO. Similarly, the internal dose of EO from exposure to the same EO concentration was similar across species (Table G.6).

Table G.6 — Inter-species comparisons of internal dose of EO[45]

Dose metric	**Species**		
	Mouse	Rat	Human
HOEtVal adduct increment from 1 ppmh	12	16	12
Dose in blood (μMh ppm^{-1})	0,5	0,35	0,3

Cross species comparison of potency for similar epoxides

Segerback[165] reported that *in vivo* DNA adduct levels following inhalation exposure to propylene oxide were equivalent across species. From a pharmacodynamic perspective, Bjorge *et al.*[23] found similar levels of single strand DNA breaks in isolated human and rat testicular cells exposed *in vitro* to styrene oxide. Given the similarities in structure and mechanism of action between propylene oxide, styrene oxide and EO, these findings lend support to the conclusion that EO is equipotent across species.

G.4.3.2.3 Species differences in DNA repair rates

DNA repair appears to proceed at similar rates across species, lending further support to the selection of a value of 1 for UF2. For example, human and rat cells repair DNA lesions produced by methyl methanesulfonate (MMS) at similar rates (see Reference [142]). Since EO and MMS exert effects on germ cells via a similar mechanism (see Reference [192]), it can be assumed that DNA repair rates for EO will be equivalent across species.

Based on similarities across species in modelled or measured internal dose of EO following exposure to the same concentration of EO, and species similarities in DNA repair rates, scientific justification exists for selecting a value of 1 for the UF2 parameter.

G.4.3.3 Quality and relevance of experimental data (UF3)

UF3 accounts for limitations in the toxicological data available for TI derivation, including absence of NOAEL value, absence of NOAEL from a long-term study, and lack of data from a clinically relevant route of exposure.

Use of route-to-route extrapolation approaches to address the lack of data from clinically relevant routes of exposure was covered in G.3 and should not be explicitly used to derive a value for UF3. However, a value for UF3 is needed to address the lack of a NOAEL value in Reference [107].

In the absence of experimental data to define a NOAEL value in cynomolgus monkeys, one approach to estimate this value would be to:

— estimate the internal dose of EO at the NOAEL in mice;

— scale this dose to an equivalent internal dose in cynomolgus monkeys;

— compare the values.

Based on the results in Reference [186], the internal dose corresponding to exposure of rats to 10 ppm EO for 6 h is 2,7 mg/kg. The estimated internal dose of EO in cynomolgus monkeys following exposure to EO for 6 h at the LOAEL in Reference [107] is 3,3 mg/kg (not normalized for 5 d exposure per week). Since EO doses are assumed to scale across species as a direct function of body weight, the ratio of the internal dose at the monkey LOAEL and the internal dose at the rat NOAEL is 3,3/2,7 = 1,2. This ratio is valid assuming pharmacodynamic equivalence in response of sperm among species to the effects of EO.

Therefore, a UF of 3 applied to the LOAEL should be adequate in estimating the NOAEL in Reference [107]. This value is consistent with the LOAEL-to-NOAEL UF of 3 applied by the US FDA (2000) to derive the parenteral TI value for DEHP. Further, Abdel-Rahman and Kadry[3] found a median ratio of 3,5 for the oral LOAEL-to-NOAEL ratios for 24 chemicals and reported with 96 % of the ratios below 10. Consequently, there is direct scientific support for a UF of 3 applied to the LOAEL reported in Reference [107] for derivation of a TI for EO and there are precedents for the selection of this value.

G.4.4 Derivation of non-cancer TI values for EO

Justification is provided above to support the selection of the following values for each of the uncertainty factors necessary to derive a non-cancer TI for EO:

— UF1 inter-individual variability 30

— UF2 inter-species extrapolation 1

— UF3 data deficiency 3 (if NOAEL is lacking)

As a result, the aggregate MF to be used when a NOAEL is available is 30 and 90 when only a LOAEL is available.

Application of the selected MFs to the NOAEL or LOAEL values from the critical studies yields the non-cancer TI values for EO shown in Table G.7.

Table G.7 — Derivation of non-cancer TI (limited/prolonged exposure category)

Study	NOAEL/LOAEL (mg/kg/d)	UF	TI (mg/kg/d)
[169]	9 (NOAEL)	30	0,3
[82]	9 (NOAEL)	30	0,3
[107]	2 (LOAEL)	90	0,02

G.5 Cancer-based TI values for EO

G.5.1 General overview

ISO 10993-17 offers flexibility in selecting the approach that is most appropriate for setting a cancer-based TI, depending on the available data and regulatory norms. Since EO exerts its carcinogenic effects via a genotoxic mechanism, a linear extrapolation approach is generally considered to be the most appropriate method to estimate low-dose risks. This linear extrapolation approach can employ statistical, dose-response models to estimate the dose associated with a given risk in humans at low doses or can involve simple linear extrapolation from the lowest dose associated with increased cancer risk in humans or experimental animals to the dose associated with zero risk. Alternatively, a LOAEL or NOAEL/UF approach, similar to that used for non-cancer risk assessment, has been advocated by some regulatory agencies, particularly those in Europe. Finally, a non-linear, biologically based approach for EO risk assessment has been proposed, but this approach has not been fully validated or accepted.

Cancer-based TI values for EO have been derived using the following approaches:

— linear extrapolation from human data;

— linear extrapolation from animal data;

— application of UFs to LOAEL values;

— dose-response modelling.

G.5.2 Approach 1: Linear extrapolation from human data

Gaylor[56] calculated an excess incidence rate of leukaemia of 0,043 in workers in Reference [71] exposed to 20 ppm EO for an average of 3,9 years.

Absorbed dose associated with 0,043 excess cancer risk:

$$20 \text{ ppm} \times 1{,}8 \text{ mg/m}^3\text{/ppm} \times 10 \text{ m}^3\text{/d} \times 0{,}8 \text{ (absorption factor)} \times 5/7 \div 70 \text{ kg} = 2{,}94 \text{ mg/kg/d}$$

Unit cancer risk:

$$0{,}043/2{,}94 \text{ mg/kg/d} = 0{,}015 \text{ (mg/kg/d)}^{-1}$$

Dose associated with 10^{-4} excess cancer risk:

$$10^{-4}/0{,}015 \text{ (mg/kg/d)}^{-1} = 0{,}006\,7 \text{ mg/kg/d}.$$

G.5.3 Approach 2: Linear extrapolation from animal data

An increased incidence of leukaemia was noted in male rats at both exposure doses in Reference [108]. The excess incidence of leukaemia in low dose (50 ppm) rats was 0,072 (0,11 in exposed vs. 0,038 in control). The absorbed dose at this exposure concentration was:

$$50 \text{ ppm} \times 1{,}8 \text{ mg/m}^3\text{/ppm} \times 0{,}29 \text{ m}^3\text{/d} \times 0{,}68 \text{ (absorption factor)} \times 5/7 \times 7/24 \div 0{,}35 \text{ kg} = 10{,}56 \text{ mg/kg/d}$$

Unit cancer risk:

$$0{,}072/10{,}56 \text{ mg/kg/d} = 0{,}006\ 8 \text{ (mg/kg/d)}^{-1}$$

Dose associated with 10^{-4} excess cancer risk:

$$10^{-4}/0{,}006\ 8 \text{ (mg/kg/d)}^{-1} = 0{,}015 \text{ mg/kg/d}.$$

G.5.4 Approach 3: Uncertainty factor approach

An increased incidence of leukaemia, brain tumours and mesothelioma was observed in rats exposed to 33 ppm of EO for 2 y in References [172] and [173]. This exposure concentration is equivalent to:

$$33 \text{ ppm} \times 1{,}8 \text{ mg/m}^3\text{/ppm} \times 0{,}29 \text{ m}^3\text{/d} \times 0{,}68 \times 5/7 \times 6/24 \div 0{,}35 = 6{,}0 \text{ mg/kg/d}$$

Application of an MF of 90 to this LOAEL dose yields a cancer-based TI of 0,07 mg/kg/d.

G.5.5 Approach 4: Linear dose-response modelling of human data

ISO 10993-17 instructs that when human data are available to assess the risk posed by exposure of patients to a carcinogenic compound, these data are preferred over animal data. Seilken and Valdez-Flores[166] derived inhalation unit risk values (risk associated with exposure to 1 µg/m^3 EO) using a modelling approach of the dose-response data in the Union Carbide Corporation (UCC)[181] and the U.S. National Institute for Occupational Safety and Health (NIOSH)[176] datasheets. See Table G.8.

Table G.8 — Equivalent dose associated with 10^{-4} risk based on unit risk values derived by Reference [166]

Data set	Unit risk $(\mu g/m^3)^{-1}$	Equivalent dose associated with 10^{-4} risk [a] (mg/kg/d)
UCC	$5{,}1 \times 10^{-7}$	0,020
NIOSH	$5{,}8 \times 10^{-7}$	0,019

[a] Conversion to mg/kg/d based on assumed ventilation rate of 10 m^3/d for a 5-day working week, and a body weight of 70 kg.

G.5.6 Comparison of cancer-based TI value

See Table G.9.

Table G.9 — Comparison of cancer-based TI values for EO

Approach	Cancer-based TI (mg/kg/d)
Approach 1: Linear extrapolation (human data) [a]	0,007
Approach 2: Linear extrapolation (animal data) [a]	0,015
Approach 3: Uncertainty factor (animal data) [b]	0,07
Approach 4: Linear dose-response modelling (human data) [a]	0,020

[a] Based on 10^{-4} excess cancer risk.

[b] Based on MF of 90.

The cancer-based TI values derived using Approaches 1, 2 and 4 range from 0,007 to 0,02 mg/kg/d. The cancer based TI derived using Approach 4 was calculated using dose-response modelling and presumably is a more accurate representation of the dose-response relationship in humans. The TI derived using Approach 4 serves as the basis for the cancer-based TI.

Data from Reference [54] support the selection of this cancer-based TI. In this study, the lowest concentration of EO associated with DNA single strand breaks in workers in the "higher sensitive" group was 0,6 mg/m^3. This EO concentration is equivalent to an absorbed dose of:

$$0{,}6 \text{ mg/m}^3 \times 10 \text{ m}^3\text{/d} \div 70 \text{ kg} = 0{,}085 \text{ mg/kg/d}$$

A cancer-based TI of 0,02 mg/kg/d should be adequate to protect against genotoxic effects in a sensitive population.

G.5.7 Comparison of TI values for EO

ISO 10993-17 instructs the user to compare non-cancer- and cancer-based TI values and select the lower value as the basis of the permanent exposure category TI. See Table G.10.

Table G.10 — Comparison of non-cancer- and cancer-based TI values for EO

Approach	TI (mg/kg/d)
Cancer-based Linear dose-response modelling (human data)	0,020
Non-cancer – permanent Uncertainty factor (Lynch *et al.*[107] 1982 data)	0,022

As shown in Table G.10, the non-cancer- and cancer-based TI values for the permanent exposure category are essentially identical.

G.6 Calculation of tolerable exposure (TE) levels

G.6.1 Tolerable exposure TE

TI values are modified to account for the manner in which a particular device is used and to enable practical calculation of individual device limits. The tolerable exposure (TE) is the product of the TI, body mass (m_b), and a utilization factor (UTF):

$$TE = TI \times m_b \times UTF$$

The body mass factor commonly used in the absence of specific patient population information is 70 kg.

The utilization factor UTF is the product of a factor that accounts for exposure to EO from several devices, or a concomitant exposure factor (CEF), and a factor to proportionally account for situations where a device is not used for the entire duration period, termed a proportional exposure factor (PEF):

$$UTF = CEF \times PEF$$

In the absence of specific information, default values for the CEF and PEF correspond to 0,2 and 1,0 respectively.

G.6.2 Limited exposure TE

TE = 0,30 mg/kg/d × 70 kg × 0,2

TE equals 4,2 mg/d, rounded down for ease in calculating individual device limits to 4 mg/d.

Therefore, the average daily dose of EO would not exceed 4 mg/d (see G.1).

G.6.3 Prolonged exposure TE

TE = 0,30 mg/kg/d × 70 kg × 0,2

TE equals 4,2 mg/d, rounded down for ease in calculating individual device limits to 4 mg/d.

Therefore, the average daily dose of EO would not exceed 4 mg/d. The current limit of 2,0 mg/d has been retained.

G.6.4 Permanent exposure TE

TE = 0,02 mg/kg/d × 70 kg × 0,2

TE equals 0,28 mg/d, rounded down for ease in calculating individual device limits to 0,3 mg/d.

Therefore, the average daily dose of EO would not exceed 0,3 mg/d. As noted in 4.3.2, the current limit of 0,1 mg/d has been retained.

G.6.5 Calculation of Tolerable Contact Limit (TCL)

G.6.5.1 Rationale

Because EO can be irritating, calculation of a TCL is relevant. A TCL is necessary for EO-sterilized surface-contacting and implantable devices. The approach described in ISO 10993-17 was used to derive TCL values for EO.

G.6.5.2 Selection of critical studies

A number of studies (References [12], [117], [168] and [179]) contain dose-response data that may be used to derive a TCL for EO.

Matsumoto[117] EO sterilized segments of cardiac and urinary catheters and allowed them to aerate for 6 h, 24 h, 48 h, 72 h, 96 h or 168 h. The amount of EO remaining on the catheters was determined following aqueous extraction for three days. Two-centimetre sections of the catheters were implanted subcutaneously in rats and the animals were sacrificed at 24 h, 48 h, 72 h and 1 week after implantation. The NIL and MIL on the cardiac catheter was 0,46 and 1,02 mg EO/gm catheter, respectively.

Andersen[12] also conducted a study of EO-induced irritation from implanted materials; however, the amount of EO in the material was determined by the difference in weight before and at various times after sterilization. Because of the imprecision of this technique, these data will not be used to derive a TCL for EO.

Shupack[168] examined the local effects of EO-sterilized patches affixed to backs of human volunteers. The material that produced effects at the lowest levels of EO was a PVC block. Irritation was seen following contact with PVC blocks containing EO at 893 ppm. A NIL was not reported in the study for PVC blocks. The blocks used in the study weighed 719 mg, so the MIL is equivalent to 0,642 mg EO (0,893 mg EO/gm material × 0,719 gm PVC). Two square centimetres of material was in contact with the skin, so the MIL for this study expressed on a surface area basis was 0,32 mg/cm^2 (321 µg/cm^2).

Tanaka[179] conducted a dermal irritation study of EO-impregnated gauze patches, in rabbits. The highest dose that did not produce irritation was 0,75 mg/patch. The surface area of the patch was 1,77 cm^2, so the NIL expressed on a surface area basis was 0,424 mg/cm^2 (424 µg/cm^2).

Anand[11] saturated a cotton pellet with 0,5 ml of an EO solution, then placed the pellet in the cheek pouch of a hamster. Following a 4 h exposure, the highest concentration of EO that did not produce irritation after a 14 d observation period was 2 500 µg/ml. Since the effective surface area of a hamster cheek pouch is about 1,5 cm^2, the NIL expressed on a surface area basis is 833 µg/cm^2.

The NIL values obtained from these studies are summarised in Table G.11.

Table G.11 — Comparison of studies of irritation effects of EO

Study	Device/material	NIL or MIL (µg/cm^2)
[117]	Cardiac catheter	103
[168]	PVC block	321
[179]	Gauze patch	424
[11]	Cotton pellet	833

G.6.5.3 Selection of uncertainty factors for TCL derivation

As in the derivation of TI values for EO, uncertainty factors are used in the derivation of a TCL to account for inter-individual variability in the human population in the irritant response to a compound (UF4), inter-species differences in response to an irritant (UF5) and for deficiencies in the data (UF6).

G.6.5.4 Inter-individual variability (UF4)

Data are not available to establish a compound-specific UF4 for EO. Although the variability in the human population to a given dose of various contact irritants has been well established (e.g. Reference [21]), these data are not sufficient to provide support for a default value for UF4. Nevertheless, inter-individual variability is assumed to be minimal for the effects seen after implantation of EO-sterilized materials. Somewhat more variability can be expected for dermal contacting materials, especially if the skin is not intact. As a result, an UF4 value of 3 will be used to derive the TCL for EO when the irritation data are obtained from implantation or mucosal contact studies and an UF4 value of 5 when the studies involve EO effects on skin.

G.6.5.5 Inter-species differences (UF5)

Data are not readily available to derive a compound-specific UF5 value for EO. However, it is assumed that species-specific responses do not occur for the local effects produced by EO, especially for implanted materials. Therefore, a value of 1 will be used for UF5.

G.6.5.6 Data deficiencies

Various tissues differ in their relative sensitivity to local irritant effects. Therefore, the potential exists for EO-sterilized devices to come into contact with tissues (e.g., brain parenchyma) that may be more sensitive to the effects of EO than the sites used in the studies that are available for derivation of the TCL. A factor of 3 is employed to account for the potential that EO-sterilized materials may come into contact with sensitive tissues.

A NIL value was not identified in Reference [168]. A factor of 2 was used to account for the lack of a NIL.

As noted in ISO 10993-17, the critical concept in deriving either a TI or a TCL for a compound is “dose-to-patient” or bio-available dose. When local irritation studies are conducted by placing the material in contact with the skin or mucosa, a certain amount of the EO can remain in the device and a certain amount can be

volatilized. Either of these processes result in less EO being available to produce the irritant effect at the target tissue site. Data are not available on the bio-available dose of EO in Reference [11], [168] or [179], but it will be assumed that 50 % of the dose reaches the target site. As a result, a factor of 2 will be used to account for questions about bio-available dose in these studies.

Exposure in [11] took place for 4 h only; however, the potential exists for EO-sterilized materials to be in contact with tissues for more than 4 h. A factor of 2 was used to account for the potential that adverse effects could have been seen at lower doses of EO in contact with tissues for a longer period.

The UF4, UF5 and UF6 values applied to each study, the resulting MF, and the corresponding TCL values, are presented in Table G.12.

Table G.12 —Uncertainty factors and modifying factors derived for studies of the irritation of EO and the TCL derived from these data

Study	Site	NIL/MIL ($\mu g/cm^2$)	UF4	UF5	UF6	MF	TCL ($\mu g/cm^2$)
[117]	Implantation	103	3	1	3	10	10,3
[168]	Dermal	321	5	1	12	60	5,4
[179]	Dermal	424	5	1	6	30	14,1
[11]	Mucosa	833	3	1	12	36	23,1

Based on the values derived from these four diverse studies and considering the clinical relevance of the contacting tissues (mucosa and implantation), the lower TCL of 10 $\mu g/cm^2$ will be adequately protective for EO-induced local effects in various tissues.

G.7 Calculation of allowable limits

The allowable limit (AL) is the largest amount of EO deemed acceptable as a result of exposure from a medical device, and is expressed in units of milligrams per day. Allowable limits are readily converted to individual device limits and these calculations are highlighted in Clause G.8. The AL is the product of the TE and a benefit factor BF:

$$AL = TE \times BF$$

The benefit factor BF is appropriate in some instances where exposure to a particular leachable substance or residue is unavoidable upon use of a medical device, and there is significant health benefit arising from use of that particular medical device. Because there is no readily quantifiable and/or significant health benefit arising from use of EO-sterilized devices (as opposed to that device sterilized in an alternative manner), the BF is set at 1, except for some specific device categories discussed separately in Annex F. Therefore, for all exposure duration categories, the allowable limit is equal to the tolerable exposure value unless otherwise specified in Annex F.

G.8 Calculation of device limits

G.8.1 General considerations

The maximum amount of EO, expressed as mass from a medical device, is the product of the allowable limit, AL, and the number of days a device may be used in the specific exposure duration categories:

$$m_{dev,\ cat} = AL \times \text{days in category}$$

G.8.2 Limited contact devices

Medical devices in the limited exposure category may be used for up to 1 d:

$$m_{dev,\ lmt} = 4{,}0\ mg/d \times 1\ d = 4\ mg$$

G.8.3 Prolonged contact devices

Medical devices in the prolonged exposure category may be used from 2 d to 30 d. If the AL for the prolonged category derived herein were used, then the device limit would correspond to

$$m_{dev,\ prol} = 4{,}0\ mg/d \times 30\ d = 120\ mg$$

However, the current limit of 2,0 mg/d has been retained and therefore, the prolonged device limit corresponds to

$$m_{dev,\ prol} = 2{,}0\ mg/d \times 30\ d = 60\ mg$$

In addition, the maximum dose of EO should not exceed 4,0 mg in any one-day period.

G.8.4 Permanent contact devices

Medical devices in the permanent exposure category may be used from 31 d to 25 000 d. If the AL for the permanent category derived herein were used, then the device limit would correspond to

$$m_{dev,\ perm} = 0{,}28\ mg/d \times 25\ 000\ d = 7{,}0\ g$$

However, the current limit of 0,1 mg/d has been retained and therefore, the permanent device limit corresponds to

$$m_{dev,\ perm} = 0{,}1\ mg/d \times 25\ 000\ d = 2{,}5\ g$$

In addition, the maximum dose of EO should not exceed 60 mg in the first 30 d, or 4 mg in any one-day period.

G.8.5 Limit based on TCL value

For surface-contacting devices, a TCL-based limit is relevant. The formula for calculation of a mass limit based on the TCL is as follows:

$$m_{dev,\ BSC} = TCL \times A$$

where

$m_{dev,\ BSC}$ is the mass per device, i.e. maximum dose to patient, in milligrams;

TCL is the tolerable contact limit, in milligrams per square centimetre;

A is the surface area of medical device in contact with the body, in square centimetres.

Therefore, for individual devices, the approximate area in square centimetres would be multiplied by the TCL of 10 µg/cm^2 to arrive at the device limit.

EXAMPLE Device surface area in contact with the body = 100 cm^2:

$$m_{dev,\ BSC} = 10\ \mu g/cm^2 \times 100\ cm^2 = 1\ mg$$

Annex H
(informative)

Establishment of allowable limits for ECH

H.1 General

The acute toxicity data and repeated dose data demonstrate that ECH is readily accessible to the systemic circulation following skin, oral and parenteral exposure. Inspection of median lethal dosages (LD_{50}) and no-observed-adverse-effect levels (NOAELs) also suggests that the potency of ECH at specific time intervals, limited exposure, prolonged and permanent, is comparable by oral and parenteral routes of exposure. Based upon data generated in subchronic and chronic toxicity studies, ECH does not appear to become more potent as the duration of exposure is increased. While ECH is not notable for its target organ toxicity, specific target organ effects can vary with route and duration of exposure. The allowable daily dose limits that are discussed in the reactions that follow reflect these general observations. For the prolonged and permanent contact categories, the existing limits from the 1995 standard have been retained, although the derived TI values and corresponding device limits from the evaluation presented herein support higher levels. The rationale for retaining the current limits is the successful clinical history since adoption of the 1995 standard, and the ability of manufacturers to comply with these limits. Concomitantly, there is no current clinical or manufacturing reason to raise the existing limits for the prolonged and permanent categories to the levels supported by the evaluation described herein.

H.2 Introduction

The residue limits for ECH in medical devices contained in this annex were established using the methodology outlined in Clause 4 of ISO 10993-17:2002 to establish the Tolerable Intake (TI). The limits for ECH in medical devices were established based upon the evaluation of many literature reports. Acute toxicity data, target organ effects data and animal chronic toxicity data were deemed the most appropriate for the derivation of the limits themselves as discussed in H.4.

H.3 Methods

H.3.1 Overview

The approach described in ISO 10993-17 was used to derive TI values for ECH for various durations of exposure.

The potential exists for patients to be exposed to ECH released from medical devices for limited to permanent duration due to the extent of medical device exposure. As a result, it was necessary to derive separate limited, prolonged and permanent TI values for this compound. Although patients are typically exposed to ECH via parenteral routes of exposure in clinical settings, very little toxicity data are available to derive TI values for these routes of exposure. In contrast, there is a database available on the effects of ECH in experimental animals.

H.3.2 Route-to-route extrapolation of dose

There are limited data which reference the ECH exposure to patients via the inhalation route. ECH exposure results from the derivitization of EO to ECH with the addition of a chlorine molecule and therefore exists due to environmental factors. A route-to-route extrapolation of dose was not conducted as part of this risk assessment for ECH.

H.3.3 Non-cancer risk assessment approach

TI values for non-cancer effects of ECH were derived by dividing the most relevant NOAEL or LOAEL values from critical studies by uncertainty factors to account for data on the variability in response to EO in human populations (UF1), potential species difference in potency (UF2) and data deficiencies (UF3). ISO 10993-17 emphasizes the use of scientific data, when available, to derive uncertainty factors applied to the data from key toxicity studies when deriving TI values. Consistent with this philosophy, data on the variability in response to ECH in human populations and on the potency of ECH across species was used in deriving values for UF1 and UF2, respectively.

H.3.4 Cancer risk assessment approach

ECH has exhibited no potential to produce cancer in bioassays in animals and is not considered a possible human carcinogen by regulatory agencies or consensus groups. A cancer-based TI value was not calculated for ECH as part of this assessment.

H.3.5 Effects not considered in deriving TI values for ECH

It should be noted that the TI values for ECH based on non-cancer effects are not necessarily protective for immunological effects such as hypersensitivity reactions and anaphylaxis, nor are they necessarily protective for effects such as haemolysis. Other approaches may be necessary to protect patients against these effects that have been associated with exposure to ECH.

H.4 Non-cancer-based TI values for ECH

H.4.1 Selection of critical studies

H.4.1.1 Limited exposure limit

The allowable limit (AL) for the limited exposure limit of ECH for the duration of less than 24 h is 9 mg/d. This limit is based on the data collected from a subchronic intraperitoneal injection study in rats for 30 d of 6,4 mg/kg ECH as the no-observed-adverse-effect level (NOAEL) (see Reference [103]). This dose was derived from a one-tenth dose level of a previously conducted study by the same investigators, which led to an LD_{50} value of 64 mg/kg (see Reference [102]). Similar acute toxicity LD_{50} results were reported by several investigators (References [104], [116], [159], [162], [194] and [203]) in several species by various routes of administration. Acute toxicity data, including median lethal dosages (LD_{50}), were available and evaluated, although they were not appropriate for this assessment. The LD_{50} data are summarised in Table H.1.

Inspection of the data in Table H.1 suggests that the toxicity of ECH for limited exposure, i.e. less than 24 h, is nearly identical regardless of the route of exposure and is relatively similar across species.

Since the data reflect LD_{50} values in Table H.1, and not a NOAEL or a LOAEL, then the value determined as the no-observed-adverse-effect level (NOAEL) provided by Reference [103] was used as previously indicated. In that study, the dose of 6,4 mg/kg/d was chosen by the investigators to represent a one-tenth of the LD_{50} value in the original acute study. The subchronic study was successful in establishing a no-effect level and the value of 6,4 mg/kg/d was used here in conjunction with the guidelines of ISO 10993-17 to formulate the allowable limit (AL) for ECH using the appropriate uncertainty factors and modifying factors:

NOAEL = 6,4 mg/kg/d

Table H.1 — Median lethal doses (LD_{50}) for limited exposure allowable limit for ECH

Oral LD_{50} (mg/kg)	**Intravenous** LD_{50} (mg/kg)	**Intraperitoneal** LD_{50} (mg/kg)	**Subcutaneous** LD_{50} (mg/kg)	**Other** LD_{50} (mg/kg)
rat: 50 rat: 60 rabbit: 60 rat: 70 rat: 71,3 rat: 72 mouse: 80 mouse: 81,4 mouse: 91 mouse: 95 guinea pig: 110 mouse: 150 mouse: 180	rat: 67 rabbit: 80 rat: 84 rat: 100 rat: 110 mouse: 120	rat: 44 rat: 58 rat: 60 rat: 63 rat: 64 rat: 70 rabbit: 80 rabbit: 84,6 guinea pig: 85 guinea pig: 85,5 rabbit: 90 mouse: 97 mouse: 98,4 mouse: 120 mouse: 130	rat: 60 rat: 72 rabbit: 100 mouse: 120 mouse: 150	Skin rabbit: 67,8 guinea pig: 84

Uncertainty factors (UFs):

— UF1 (inter-individual variation among humans) = 10

— UF2 (inter-species variation) = 1

— UF3 (quality/relevance of the data) = 1

A default value of UF1 = 10 for inter-individual variation among humans is used since the value is derived from the median value in animals and the assumption is that there would be similar variation among humans.

A value of UF2 = 1 for the interspecies variation is based on the understanding from References [80] and [81] indicating that low concentrations of ECH are detoxified in the liver by glutathione conjugation to S-carboxymethlyglutathione. Detoxification is maintained as long as sufficient concentrations of glutathione are present. At higher exposures of ECH, glutathione concentrations would be depleted leading to a general overt toxicity. Since animals and humans have this same mechanism of detoxification and the concentration of 6,4 mg/kg/d results in a NOAEL, then the value of UF2 is appropriately set at 1.

A value of UF3 = 1 is appropriately set due to the relevance and strength of the data.

a) Modifying factor (MF):

— MF = UF1 × UF2 × UF3 or

— MF = 10 × 1 × 1 = 10

— TI = NOAEL/MF, or TI = 6,4 mg/kg/d/10 = 0,64 mg/kg/d

b) Utilization factor (UTF):

— UTF = CEF (concomitant exposure factor) × PEF (proportional exposure factor)

— CEF = 0,2

— PEF = 1

— UTF = 0,2 × 1 = 0,2

c) Tolerable exposure (TE):

— TE = TI × BW × UTF

— TE = 0,64 mg/kg/d × 70 kg × 0,2 = 9 mg/d

d) Allowable limit (AL):

— This indent includes the use of a benefit factor (BF), which is performed by applying ISO 10993-17 on a case-by-case basis. In this case achieving the TE is feasible for ECH and therefore the BF is defaulted to equal a value of 1. The calculation for the allowable limit is as follows:

— Allowable Limit = TE × BF

— AL = 9 × 1 = 9 mg/d for less than 24 h exposure

— The limit is acceptable in the context of NOAELs derived from the subchronic/reproductive toxicity data based on the low NOAEL of 6,4 mg/kg/d for a 70 kg adult for repeated administration.

H.4.1.2 Prolonged exposure limit

The AL for the exposure for 24 h to 30 d is 3,8 mg/d, not to exceed 9 mg/d in any given day or 114 mg in a 30 d period (3,8 mg × 30 d). This limit was based upon subchronic toxicity and reproductive effects data (teratogenicity) generated in several species. These data have been reported by many investigators (References [8], [10], [18], [38], [83], [85], [103], [145] and [203]). In repeated-dose, oral and parenteral studies lasting for varying time periods up to 403 days, ECH produced a variety of adverse effects including death (accompanied by increased relative organ masses, darkened mottled liver, haemorrhagic adrenals, haemorrhagic pituitary gland, haemorrhagic gastrointestinal tract, myocarditis, thyroid congestion and congestive pulmonary changes in one study). Additionally, ECH produced decreased body mass and growth, increased brain, adrenal, kidney, lung and thyroid mass, small testes or testicular injury, emesis, decreased haemoglobin, packed cell value and haematocrit, liver injury, ectopic haematopoiesis and bone marrow hypercellularity, and a shift in white blood cells towards lymphocytes. Dosages ranged from about 2,7 mg/kg/d to 93 mg/kg/d or more. Reproductive studies were solely teratology studies in which ECH was administered during various time periods of gestation. In these studies, ECH produced maternal toxicity, foetal toxicity and, in one study, an increase in foetal malformations. This latter effect was observed only in the offspring of mice given ECH intravenously at a dosage of 120 mg/kg/d, a dosage well into the acutely lethal range (see Reference [80]). The data that became the basis for the calculation of the limit for prolonged exposure are summarized in Table H.2.

Table H.2 — Data used to establish prolonged exposure limit for ECH

Study type	Oral NOAEL mg/kg/d [Reference]	Parenteral NOAEL mg/kg/d [Reference]
Subchronic	13 [145]	2,7 prorated from 6,4 three times weekly [103]
Reproductive	50 [38]	9 [83]

Inspection of these data suggests that NOAELs of ECH for prolonged exposure periods, i.e. 1 d to 30 d, are comparable regardless of the route or specific target organ or reproductive effects. Animals may be more sensitive to the general systemic toxicity of ECH than to its ability to produce adverse changes to reproduction. A subchronic study performed by Lawrence *et al.*[103] used the one-tenth dose of 6,4 mg/kg/d, which was calculated from the LD_{50} dose in their original study previously reported as 64 mg/kg. The study revealed that 6,4 mg/kg/d of ECH delivered 3 days per week for 30 d resulted in a calculated NOAEL for parenteral administration of 2,7 mg/kg/d. This dose in rats was used as the basis of the calculation of the allowable limit for prolonged exposure as follows:

NOAEL = 6,4 mg/kg/d × 3 d/7 d = 2,7 mg/kg/d

Uncertainty Factors (UFs):

— UF1 (inter-individual variation among humans) = 10

— UF2 (inter-species variation) = 1

— UF3 (quality/relevance of the data) = 1

Uncertainty factors used in this subclause are the same as previously used for the section on limited exposure since the date and rationales are the same.

a) Modifying factor (MF):

— MF = UF1 × UF2 × UF3

— MF = 10 × 1 × 1 = 10

— TI = NOAEL/MF, or TI = 2,7 mg/kg/d/10 = 0,27 mg/kg/d

b) Utilization factor (UTF):

— UTF = CEF (concomitant exposure factor) × PEF (proportional exposure factor)

— CEF = 0,2

— PEF = 1

— UTF = 0,2 × 1 = 0,2

c) Tolerable exposure (TE):

— TE = TI/MF × BW × UTF

— TE = 0,27 mg/kg/d × 70 kg × 0,2 = 3,8 mg/d

d) Allowable limit (AL):

— This indent includes the use of a benefit factor (BF), which is performed by applying ISO 10993-17 on a case by case basis. In this case, achieving the TE is feasible for ECH and therefore the BF is defaulted to equal a value of 1. The calculation for the allowable limit is as follows:

— Allowable Limit = TE × BF

— AL = 3,8 × 1 = 3,8 mg/d within a 30 d period

— This limit is considered to be adequately protective for a 70 kg man in light of the observation that ECH does not increase in toxicity after chronic vs. prolonged exposure. The limit is based on animal data.

H.4.1.3 Permanent exposure limit

The allowable limit for permanent exposure of 30 d or more to life is 10 g, not to exceed 9 mg/d in any given day or 114 mg in a month. This limit was based upon chronic toxicity, genotoxicity and carcinogenicity data that has been reported in References [81], [116] and [133]. In these studies, rats received ECH in drinking water until 24 months of age, rats received ECH by subcutaneous injection twice weekly for at least a year and rats and mice received ECH by dermal application for 103 weeks to 104 weeks. Dosages ranged from 0,086 mg/kg/d to 71 mg/kg/d or more. In these studies, no increases in tumour incidence related to ECH administration or evidence of chronic toxicity with the exception of a possible reduction in survival rates were found (see Reference [81]). The key data that became the basis for the calculation of prospective permanent exposure limits are summarised in Table H.3.

Inspection of these data suggests that the NOAEL for ECH for permanent exposure periods, i.e. 30 d to life, by oral and parenteral routes are comparable. These data are also comparable to those generated in subchronic and reproductive toxicity studies. Animals are more sensitive to the general systemic toxicity of ECH than to its potential, if any, to produce cancer.

Table H.3 — Data used to establish permanent exposure limit for ECH

Study type	Oral NOAEL	Parenteral NOAEL	Dermal NOAEL
Chronic	4 × LOAEL [81]	2,9 prorated from 10 twice weekly [116]	No Data
Carcinogenicity	16 [81]	No Data	71 prorated from 100 five times weekly [a] [133]
[a] Ethylene chlorohydrin produced no increases in tumour incidence at the highest dosage tested.			

The lowest-observed-adverse-effect level (LOAEL) for chronic toxicity, 2,9 mg/kg/d, administered subcutaneously to rats for at least one year, and for tumour production, 16 mg/kg/d orally to rats until 24 months of age, was used for the calculation of a prospective permanent exposure allowable limit of 10 g as follows:

LOAEL = 2,9 mg/kg/d

Uncertainty factors (UFs):

— UF1 (inter-individual variation among humans) = 10

— UF2 (inter-species variation) = 10

— UF3 (quality/relevance of the data) = 1

A default value of UF1 = 10 for inter-individual variation among humans is used since the value is derived from the median value in animals and the assumption is that there would be similar variation among humans.

A default value of UF2 = 10 for the inter-species variation is used since there is no clear understanding of the long-term effects on the metabolic activity following ECH exposure in humans. It is thought that low concentrations of ECH are detoxified in the liver by enzymatic glucuronidation to S-carboxymethlyglutathione (see Reference [81]) but this is not sufficient to extrapolate to lifetime exposure.

A value of UF3 = 1 is appropriately set due to the relevance and strength of the data.

a) Modifying factor (MF):

   — MF = UF1 × UF2 × UF3

   — MF = 10 × 10 × 1 = 100

   — TI = LOAEL/MF, or 2,9 mg/kg/d/100 = 0,029 mg/kg/d

b) Utilization factor (UTF):

   — UTF = CEF (concomitant exposure factor) × PEF (proportional exposure factor)

   — CEF = 0,2

   — PEF = 1

   — UTF = 0,2

c) Tolerable exposure (TE):

   — TE = TI/MF × BW × UTF

   — TE = 2,9 mg/kg/d/100 × 70 kg × 0,2 = 0,4 mg/d

d) Allowable limit (AL):

— This indent includes the use of a benefit factor (BF), which is performed by applying ISO 10993-17 on a case by case basis. In this case, achieving the TE is feasible for ECH and therefore the BF is defaulted to equal a value of 1. The calculation for the allowable limit is as follows:

— Allowable limit = TE × BF

— AL = 9 × 1 = 9 mg in any given day, and

— AL = 2,9 mg/kg/d/100 × 70 kg × 0,2 × 25 000 d = 10 g in a lifetime

Upon examination of these prospective limits, 9 mg/d and 10 g/lifetime, it was determined that 9 mg/d would be adequately protective of the adverse effects of ECH resulting from permanent exposure. The limit thus provides at least a 100-fold safety margin for a 70 kg adult from the potential adverse effects of ECH resulting from permanent exposure based on animal data.

H.4.2 Selection of uncertainty factors for non-cancer effects

Table H.4 — Uncertainty factors for TI derivation

Uncertainty factor designation	Range	Magnitude of default UF	Description
UF1, inter-individual variability in the human population	1 to 10	10	To account for the variability in response between the mean of the healthy population and the response in some proportion of a sensitive subpopulation
UF2, inter-species extrapolation	1 to 10	1	To account for the possibility that humans are more sensitive to the adverse effects of a compound than experimental animals are
UF3, quality and relevance of the experimental data	1 to 100	1	To account for limitations in the toxicological data available for TI derivation, including absence of NOAEL value, absence of NOAEL from a long-term study, and lack of data from a clinically relevant route of exposure

H.5 Calculation of Tolerable Contact Limit (TCL)

There are limited published data available for the irritation effects of ECH. Calculation of a TCL is relevant. It is assumed that a TCL derived limit is appropriate for surface-contacting devices and perhaps implantable devices.

A study was conducted by Guess[61] in which dermal administration of undiluted ECH led to an insignificant irritation response in a rabbit. Intradermal and intramuscular injection of ECH, however, led to strong irritation response at the site of injection. Dilution of the ECH solutions to intradermal tissue and penile mucosa resulted in a modulated response. At up to 80 % dilution there was no irritation response found by several investigators (see References [59], [61], [102] and [103]) showed that when ECH was administered dermally in the rabbit model at a mean concentration of 68 mg/kg there was little effect on the LD_{50}, but there was no local irritation effect observed. It is suggested that this is due to an extremely rapid absorption rate of the chemical which is then rapidly transformed in the liver to a toxic metabolite. Additional intradermal and Draze eye irritation tests by these investigators resulted in high irritation score for undiluted ECH. However, ECH solutions of 5 % and 1 % (by volume) showed little to no irritation, respectively. These data indicate that ECH is highly irritating to intradermal and ocular tissues. As a result, a TCL and an intradermal TCL will be derived for both non-irritating level (NIL) and minimally irritating level (MIL) concentrations of ECH, respectively.

The TCL is derived for ECH in the following manner. A modifying-factor approach is used to calculate the TCL. This approach incorporates the use of uncertainty factors (see above) to provide an acceptable margin of safety against irritation. The formula for calculating the TCL, in milligrams per square centimetre, using the modifying-factor approach is

$$\text{TCL} = \frac{(\text{NIL or MIL})}{(\text{MFTCL} \times A)}$$

where

MFTCL is the modifying factor (UF4 × UF5 × UF6);

NIL is the non-irritating level, in milligrams;

MIL is the minimally irritating level, in milligrams;

A is the body contact surface area, in square centimetres.

Justification is provided above to support the selection of the following values for each of the uncertainty factors necessary to derive a TCL:

— UF4 inter-individual variability = 10

— UF5 inter-species extrapolation = 1

— UF6 data deficiency = 1

MFTCL = 10 × 1 × 1 = 10

Lawrence *et al.*[103] administered dermally to rabbits a maximum of 80 % solution of ECH in a volume of 0,2 ml per 3,27 cm^2 (0,5 in^2) surface area. This is calculated to 160 mg of ECH per 3,27 cm^2 and resulted in no observed irritation in the skin and therefore the observed non-irritating level (NIL):

NIL = 80 % solution = 0,2 ml × 80 g/100 ml = 160 mg dosed

Therefore, the TCL corresponds to:

TCL = 160 mg/(10 × 3,27 cm^2) = 4,89 mg/cm^2

The TCL is thus considered to be 5 mg/cm^2.

A secondary intradermal irritation study in the rabbit was conducted by Lawrence *et al.*[103] using several dilutions of ECH. In this study all dilutions caused dramatic local dermal irritation leading to local tissue necrosis with minor exception. The dilutions of 1 % and 5 % ECH were both non-irritating and minimally irritating (doubtful), respectively, according to the standard scoring method used. This was suggested to be due to the retention of ECH in the local area, which was subsequently not biotransformed and therefore led to the toxic local tissue response. Not overlooking this, a secondary minimal irritation level (MIL) was calculated from these intradermal doses:

MIL = 0,5 % solution = 0,2 ml × 5 g/100 ml = 10 mg of intradermal ECH

This means that intradermal exposure is non-irritating at a dose of 10 mg per animal. For this purpose we use and approximation of 2,5 kg per rabbit. If we then apply the same uncertainty factors as the original TCL then the following calculation will be for a man:

Intradermal TCL = rabbit dose (mg/kg)/MFTCL × 70 kg man

Intradermal TCL = [(10 mg/2,5 kg)/100] × 70 kg man

Intradermal TCL = 17,5 mg/kg

This means that the intradermal MIL for a man would be 17,5 mg/kg.

Annex I
(informative)

Establishment of allowable limits for EG [8)]

I.1 Background

The residue limits for EG in medical devices were established using the methodology outlined previously for EO and ECH for non-cancer endpoints. EG is not a genotoxin (see References [17], [135] and [136]), it has not exhibited any potential to produce cancer in animal bioassays (see Reference [41]), and it is not considered a carcinogen (see References [135] and [136]). For these reasons and because for most materials used in the manufacture of EO-sterilized medical devices conversion of EO to EG would not be significant, it was not considered necessary to establish allowable limits for EG. This annex uses the same method as used for establishing allowable limits for EO and ECH to show that the allowable limits for EG would be significantly higher than those for EO and ECH and are not likely to be reached for most device materials.

Where certain natural materials (e.g., collagen, cotton, etc.) are incorporated in EO-sterilized medical devices it is possible that extremely high concentrations of EG may be seen. The manufacturer is cautioned to establish that, when such high EG levels are seen, this does not present a hazard to the patient or compromise the performance of the medical device.

I.2 General considerations

I.2.1 Overview

The acute toxicity data and repeated dose data demonstrate that, although EG is not very potent, it is accessible to the systemic circulation following oral and parenteral exposure. Inspection of median lethal dosages (LD_{50}s) and no-observed-effect levels (NOAELs) also suggests that the potency of EG at specific time intervals, limited exposure, etc. is comparable by oral and parenteral routes. Based upon data generated in subchronic and chronic toxicity studies, EG does not appear to become more potent as the duration of exposure is increased. The kidneys represent the primary target organ for EG.

I.2.2 Limited exposure

EG does not represent a practical health hazard from exposure to medical devices for exposures less than 24 h in duration. This conclusion is based upon acute toxicity data generated in several animal species and reports from the literature regarding poisoning following ingestion of EG or products containing EG in humans (see References [85], [101], [116], [160], [162], [203] and [204]). There are also numerous reports regarding human death resulting from ingestion of EG. Based upon these data, the estimated lethal dosage in humans of EG is 1,4 ml/kg (see Reference [160]) or about 111 g to an adult. However, as it is known that saturation of human metabolism of EG occurs at 125 mg/kg (see References [20] and [148]) and that human data is always more persuasive in terms of setting safety levels, this dose was used as the basis for the calculations of the allowable limit for limited exposure as follows.

NOAEL = 125 mg/kg/d

8) This information is included for completeness, as it is not considered necessary to set allowable limits for EG when EO limits are controlled to the levels specified in this part of ISO 10993.

Uncertainty factors (UFs):

— UF1 (inter-individual variation among humans) = 10 (default value)

— UF2 (inter-species variation) = 1 (human data available)

— UF3 (quality/relevance of the data) = 1 (relevant data)

Modifying factor (MF):

— MF = UF1 × UF2 × UF3 or MF = 10 × 1 × 1 or MF = 10

— $TI = \frac{NOAEL}{MF}$ or $TI = \frac{12{,}5\ mg/kg/d}{10}$ or TI = 12,5 mg/kg/d

Utilization factor (UTF):

— UTF = CEF (concomitant exposure factor) × PEF (proportional exposure factor)

— CEF = 0,2 (default value)

— PEF = 1 (single day exposure)

— UTF = 0,2

Tolerable exposure (TE):

— TE = TI × BW × UTF or TE = 12,5 mg/kg/d × 70 kg × 0,2 or TE = 175 mg/d

Allowable limit (AL):

— Benefit factor (BF) = 1 (default value)

— AL = TE × BF or AL = 175 mg/d × 1 or AL = 175 mg/d or 175 mg/device

After examination of this allowable limit, it was determined that it would be highly unlikely that humans could be exposed to this much EG from limited exposure to medical devices.

I.2.3 Prolonged exposure

The prolonged exposure limit for EG was based on a review of the subchronic toxicity and reproductive effects data (teratogenicity, dominant lethality and reproductive toxicity) generated in animals (see References [42], [55], [67], [115], [122], [137], [149], [150], [152], [153], [164], [185], [203] and [204]).

In repeated-dose oral and parenteral studies lasting for varying time periods up to 157 d, EG produced a variety of adverse effects resulting primarily from its metabolism to oxalate, that included oxaluria, renal injury (nephrosis, tubular dilation, inflammation), elevated urea nitrogen and creatinine, renal crystals, crystals in the brain, decreased growth, centrilobular degeneration in the liver, shift in white blood cells toward neutrophils and bone marrow hypercellularity and ectopic hematopoeisis. Dosages ranged from 50 mg/kg to 2 200 mg/kg or more. Reproductive studies included teratology studies in which EG was administered during various time periods of gestation and general studies in which the effects of EG on fertility, reproduction performance, teratogenicity and feotal development, and the potential of EG to produce dominant lethal effects were evaluated. These latter studies were multigenerational in duration.

Dosages ranged from 40 mg/kg to 5 000 mg/kg or more. In the teratology studies (all conducted by oral administration), EG produced maternal toxicity, embryo toxicity, foetal toxicity and abnormalities to skeletal and visceral tissues at dosages above 150 mg/kg. In the multigenerational studies (also conducted only by oral administration), a dosage of 1 840 mg/kg (estimated from a study in which EG was administered at a concentration of 0,5 % in drinking water (see Reference [97]) produced no adverse effects while a dosage greater than 1 000 mg/kg was required before indications of foetal toxicity (decreased pup weight), embryo toxicity (decreased litter size) and teratogenicity were produced.

Inspection of the data suggested that the no-observed-effect levels of EG for prolonged exposure periods, i.e., 1 d to 30 d, were comparable regardless of the route of exposure on specific target organs or reproductive effect. Animals appeared to be somewhat more sensitive to the general systemic toxicity of EG than to its ability to produce adverse changes to reproduction. To provide the greatest protection for the patient, the lowest NOEL, 50 mg/kg from a subcutaneous toxicity study in dogs (see Reference [203]), was used as the basis for the calculation of the allowable limit for prolonged exposure as follows:

NOAEL = 50 mg/kg/d

Uncertainty factors (UFs):

— UF1 (inter-individual variation among humans) = 10 (default value)

— UF2 (inter-species variation) = 5 (similarity in response)

— UF3 (quality/relevance of the data) = 1 (relevant data)

Modifying factor (MF):

— MF = UF1 × UF2 × UF3 or MF = 10 × 5 × 1 or MF = 50

— $\mathrm{TI} = \frac{\mathrm{NOAEL}}{\mathrm{MF}}$ or $\mathrm{TI} = \frac{50\ \mathrm{mg/kg/d}}{50}$ or TI = 1,0 mg/kg/d

Utilization factor (UTF):

— UTF = CEF (concomitant exposure factor) × PEF (proportional exposure factor)

— CEF = 0,2 (default value)

— PEF = 1 (default value)

— UTF = 0,2

Tolerable exposure (TE):

— TE = TI × BW × UTF or TE = 1,0 mg/kg/d × 70 kg × 0,2 or TE = 14 mg/d

Allowable limit (AL):

— Benefit factor (BF) = 1 (default value)

— AL = TE × BF or AL = 14 mg/d × 1 or AL = 14 mg/d or 420 mg/device

I.2.4 Permanent exposure

The permanent exposure limit was based on a review of the chronic toxicity and carcinogenicity data (see References [24], [25], [41], [116] and [129]). In these studies, rats, mice and monkeys received EG in the diet for two or three years and rats received EG by subcutaneous injection twice weekly for at least a year. In the oral studies, animals exhibited renal changes (sclerosis, calcification, nephritis, tubular cell hyperplasia), oxalate deposition, increased urea nitrogen and creatinine, reduced haematology parameters (haematocrit, haemoglobin and red blood cell count), mineralization of soft tissues, parathyroid hyperplasia and liver injury (fatty changes). These changes were not reported following subcutaneous administration nor were there any increases in the incidence of tumour formation in these studies. Dosages ranged from 8,6 mg/kg to 800 mg/kg or more.

Inspection of these data revealed some route sensitivity in the no-observed-effect levels for EG for permanent exposure period, i.e., 30 d to life; however, they are comparable to those generated in subchronic and

reproductive toxicity studies. To provide the greatest protection for the patient, the lowest NOEL for chronic toxicity, 40 mg/kg/d administered in the rats' diet for two years, was used as the basis for the calculation of the allowable limit for permanent exposure as follows:

NOAEL = 40 mg/kg/d

Uncertainty factors (UFs):

— UF1 (inter-individual variation among humans) = 10 (default value)

— UF2 (inter-species variation) = 5 (similar response)

— UF3 (quality/relevance of the data) = 1 (relevant data)

Modifying factor (MF):

— MF = UF1 × UF2 × UF3 or MF = 10 × 5 × 1 or MF = 50

— $TI = \frac{NOAEL}{MF}$ or $TI = \frac{40\ mg/kg/d}{50}$ or TI = 0,8 mg/kg/d

Utilization factor (UTF):

— UTF = CEF (concomitant exposure factor) × PEF (proportional exposure factor)

— CEF = 0,2 (default value)

— PEF = 1 (default value)

— UTF = 0,2

Tolerable exposure (TE):

— TE = TI × BW × UTF or TE = 0,8 mg/kg/d × 70 kg × 0,2 or TE = 11,2 mg/d

Allowable limit (AL):

— Benefit factor (BF) = 1

— AL = TE × BF or AL = 5,6 mg × 1 or AL = 11,2 mg/d or 280 mg/device

I.2.5 Tolerable contact limit (TCL)

Global tolerable contact limits were not developed for EG as the local concentration and specific routes of exposure play a key role in determining the potential for local irritancy. It is recommended that local irritation of EG be addressed by application of ISO 10993-10 and ISO 10993-4. A review of the literature indicated that EG has an overall low potential for skin irritancy. A single exposure to 10 % EG was negative in a human patch test (see Reference [96]) while in another human study, repeated exposure indicated that EG was a minor skin irritant (see Reference [168]). The non-irritating concentration for acute eye irritation ranged from 0,4 % to 5 % (see References [118], [119] and [120]) while the non-irritating concentration for repeated ocular exposure was 20 % (see Reference [120]).

Annex J
(informative)

Preparation of EO and ECH standards

J.1 Preparation of EO standards

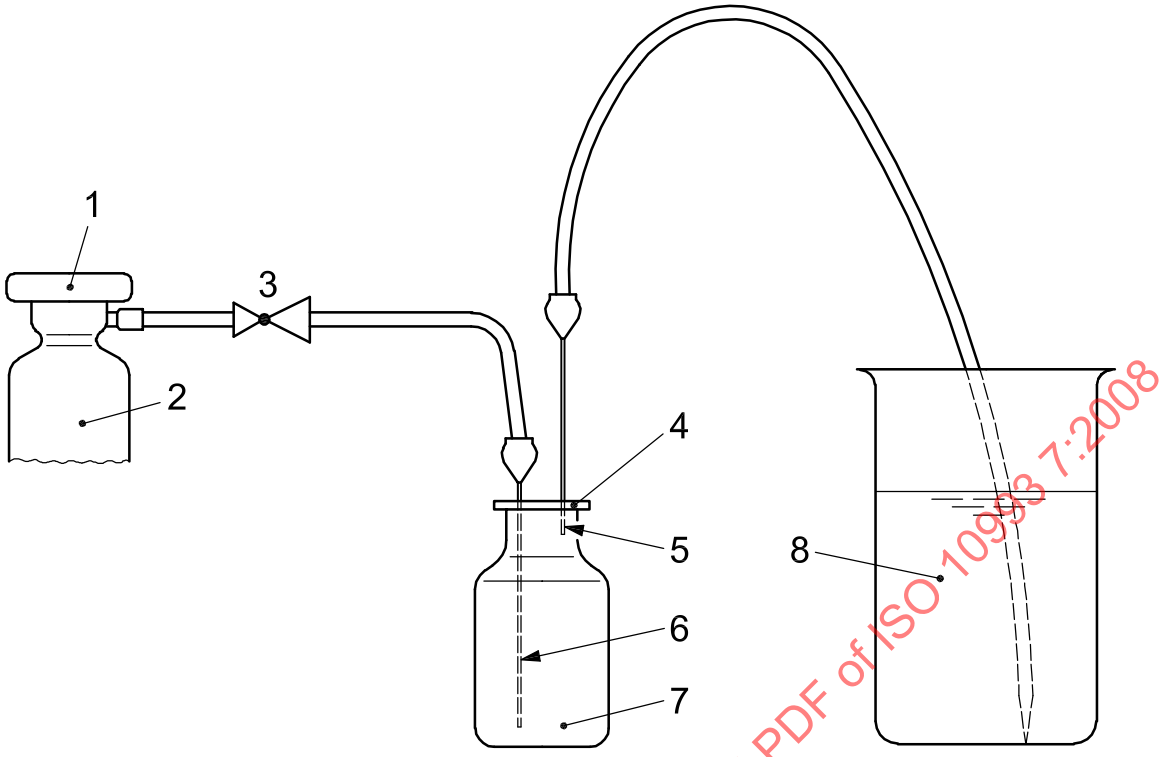
J.1.1 Collection of EO gas

Connect the EO standard gas cylinder to a serum vial (approximately 30 ml capacity) as shown in Figure J.1. Vent the vial by placing a hypodermic needle through the septum, keeping the point near the top of the vial. Connect a length of polyvinyl chloride tubing to the outlet needle 2 and submerge the end of the tubing in a beaker of water.

DANGER — To protect the analyst, it is extremely important that this procedure be carried out under an exhaust hood (fume cupboard). See 4.4.1.1.

Place another length of tubing on the EO cylinder regulator and connect to a hypodermic needle. Insert the second needle, or inlet needle 1, through the vial septum, and push the point down to the bottom. Start the EO flow through the system so that bubbles emerge from the vent tube at the rate of one per second. Purge the vial for about 15 min. Remove the inlet needle from the vial, and allow the EO gas in the vial to equilibrate to atmospheric pressure by removing the vent needle from the vial as the last bubble emerges from the vent tube in the beaker. Using the ideal gas law approximation, it can be shown that the concentration of EO in the vial is 1,83 µg/µl at 760 mm Hg [9] and 20 °C.

9) 1 mm Hg = 133 322 Pa or 760 mm Hg = 101 325 KPa.



Key

1 main valve
2 EO gas lecture bottle
3 second control valve
4 crimp cap with PFTE septum
5 EO outlet needle 2
6 EO inlet needle 1
7 serum vial (30 ml)
8 beaker with water (300 ml)

Figure J.1 — Apparatus for the preparation of EO standards

The concentration of EO (C_{EO}), in µg/µl, according to the ideal gas law, may be calculated for any given temperature, T (°C), and pressure, p (mmHg), using the following equation:

$$C_{EO} = 0{,}706 \frac{p}{273 + T}$$

where 0,706 is the inverse of the gas constant, R, for EO, expressed in grams kelvin per millimetre of mercury per litre.

J.1.2 EO standard dilutions for headspace methods

Dilute the standard from J.1.1 in a vial (nominal 15 ml) whose volume has been previously determined to the nearest 0,01 ml (the same size that will be used in the sample analysis) and that is first purged with dry nitrogen for 1 min. Remove about 10 µl of EO gas from the first vial with a gas-tight syringe. Remove the syringe from the vial and depress the plunger to the desired volume of 10 µl with the needle pointing upward.

Place the nitrogen-flushed vial on to the upward-pointing syringe needle and inject the 10 µl of EO into the vial. Do not flush the syringe; immediately remove it from the vial. The vial now contains 18,3 µg of EO at 20 °C and 760 mmHg. Adjust the concentration of EO for the ambient conditions described in J.1.1.

Inject duplicate 100 µl aliquots of the gas from the second standard vial on to the column of the gas chromatograph to obtain a response from the instrument. Prepare more highly concentrated standards by diluting larger aliquots of the pure EO gas from the first vial. Since the vials contain freely-available EO gas, the standards need not be heated as is required for the samples.

Store stock standard solutions in a refrigerator when not in use or if purchased, at the conditions specified by the manufacturer (see Annex F). Establish storage stability and shelf life for EO stock. Prepare calibration standards fresh daily. Discard after use.

J.1.3 EO standard dilutions for solvent methods [10), 11)]

For increased safety and accuracy, it is recommended that EO and ECH standards of known and certified concentrations be purchased from a commercial source. If this is not possible, then stock EO standards can be prepared from the pure compound as described below.

Set up an EO standard gas cylinder as described in J.1.1 with the volumetric flask, previously purged as described, placed in a dry ice/isopropanol bath, or equivalent, to condense the EO gas into a liquid. Only the polyvinyl chloride tubing and attached hypodermic needle supplying EO from the gas cylinder are connected to the vial. There is no need to vent the vial with a second hypodermic needle, since EO is collected as a liquid.

Fill the vial with an adequate volume of liquid EO, close the valve on the gas cylinder and remove the hypodermic needle attached to the polyvinyl chloride tubing. Remove the vial from the ice bath.

Weigh a sealed 100 ml volumetric flask (with a PTFE-sealed valve) containing about 60 ml of solvent to the nearest 0,1 mg. Add five drops of liquid ethylene oxide to the flask and reweigh the flask. Fill the flask with solvent to the 100 ml line, invert and shake intermittently [12)].

Prepare dilutions of the solution by diluting aliquots with an appropriate volume of solvent. If, for example, exactly 100 mg of EO were added to 100 ml of solvent, the resulting concentration would be 1 mg/ml. Diluting 1 ml of this solution to 10 ml yields a 100 µg/ml EO standard. Prepare standard solutions of higher or lower EO concentrations in a similar manner. Prepare standards to maximize the GC detection while bracketing the EO level expected in the test sample.

Inject duplicate 1 µl to 5 µl aliquots of each standard on to the column of the gas chromatograph to obtain responses for peak area or peak height.

Store stock standard solutions in a refrigerator when not in use or if purchased, at the conditions specified by the manufacturer (see Annex F). Prepare calibration standards fresh daily. Discard after use.

In the practice of GC, experience has shown that as samples are injected on to the GC column, the precision of the injection improves as the volume of the injection increases. The constant error associated with the inaccuracies of the syringe calibration becomes a smaller fraction of the draw volume as the draw volume increases. For accuracy, do not choose a syringe having a draw volume less than 10 % of the syringe volume.

In attempting to increase draw volume accuracy by increasing the actual draw volume, caution should be taken to not overload the GC column. With current autosampler technology, the issue of injection volume accuracy and precision are not a concern.

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10) A previously cooled syringe will aid in transferring liquid EO. Care should be taken to make sure that the syringe needle does not touch the solvent.

11) Experience has shown that the measurement errors associated with the preparation of the stock solutions are constant, irrespective of the volume being prepared. The percentage error will be reduced if large volumes are prepared and then used as needed.

12) If it is necessary to store the volumetric flask temporarily, it has been found that the standard solutions are most stable when the volumetric flask is stored inverted.

J.2 Preparation of ECH standards

Accurately weigh a 100 ml volumetric flask containing about 60 ml water to the nearest 0,1 mg. Add ECH (about 100 mg) drop-wise to the flask. Re-weigh the flask and calculate the difference between the two masses; then dilute to volume with water and shake. Store stock standard solutions in a refrigerator when not in use (see Annex F). Establish storage stability and shelf life for ECH stock. Prepare working standards daily and discard after use.

Equilibrate the ECH standards to room temperature. Prepare working standards at a minimum of three concentrations. Test the linearity of the GC responses at these concentration ranges prior to their use as a standard curve. Prepare the standards to maximize the GC detection while bracketing the ECH levels expected in the test sample. Inject duplicate 1 µl to 5 µl aliquots of each standard on to the column of the gas chromatograph to obtain responses for peak area or peak height.

NOTE This procedure can also be used for the preparation of EG standards.

Annex K
(informative)

Ethylene oxide residue measuring methods

K.1 Results of interlaboratory evaluation of methods

K.1.1 EO methods

An interlaboratory evaluation was conducted at 13 laboratories using several EO methods (see References [112], [113] and [114]) on a series of samples with analytical values distributed from about 40 ppm to about 350 ppm. The estimated total coefficient of variation of the methods is given in Table K.1.

Table K.1 — Comparison of intra- and interlaboratory variations

EO method	Intralaboratory	Interlaboratory
Headspace method	3,7 %	21,3 %
Acetone method	4,1 %	16,3 %
DMF method	2,9 %	8,3 %
Aqueous method	2,7 %	17,0 %

Another interlaboratory evaluation was made, in which each laboratory used the same EO method (see Reference [89]). Linear regression data were obtained by comparing results obtained in two laboratories for a series of samples with analytical values distributed from 3,6 ppm to 26 ppm.

The regression equation calculated was: $y = 0{,}04 + 0{,}904x$; correlation coefficient $r = 0{,}974$ ($p < 0{,}000\ 01$). The intralaboratory coefficient of variation of the method was estimated as 4,0 % at 14 ppm EO or 8,3 % at 30 ppm EO in the matrix tested (unpublished data provided by A. Nakamura, H. Kikuchi and K. Tsuji).

Analytical data from samples of three different EO levels were obtained using both the solvent extraction followed by a headspace gas analysis procedure (see Reference [136]) and the bromination method (see Reference [89]) in two laboratories. Results were compared using linear regression analysis, which gave the following regression data $y = -0{,}03 + 1{,}07x$; correlation coefficient $r = 0{,}999$. The interlaboratory coefficient of variation of the K.4.4 procedure was estimated as 4,7 %, 1,8 % and 2,7 % at 12 ppm, 25 ppm and 56 ppm EO respectively in the matrix tested (see Reference [132]).

K.1.2 ECH methods

An interlaboratory evaluation was conducted for ECH (see Reference [14]). The estimated total coefficient of variation of the methods was as follows:

— Intralaboratory 7,46 %

— Interlaboratory 10,99 %

These data were obtained for ECH concentrations of about 3,0 ppm to 100 ppm.

K.2 Apparatus and reagents

K.2.1 Apparatus

K.2.1.1 **Gas chromatograph**, equipped with a flame ionization detector (FID) or an electron capture detector (ECD) and chart recorder.

NOTE 1 The ECD will not detect EO unless it is first derivatized with hydrogen bromide.

NOTE 2 An electronic integrator is valuable in obtaining reproducible results.

K.2.1.2 **Hypodermic needles** and **polyvinyl chloride tubing**, as required for preparing standards.

K.2.1.3 **Volumetric glassware**, equipped with PTFE-lined septa or PTFE-sealed valves for preparing standards.

Care should be taken in selecting glassware of an appropriate volume in order to minimize headspace over the extraction solution or standard solution. When preparing liquid standards or extracts, headspace should not exceed 10 % of the standard or extractant volume.

K.2.1.4 **Micro-syringe**, (5 µl or 10 µl capacity) for injecting aliquots of the extract into the gas chromatograph.

K.2.1.5 **Fume hood**, to provide adequate ventilation while preparing standards and samples.

K.2.1.6 **Analytical balance**, capable of measuring to 0,1 mg.

K.2.1.7 **Gas regulator**, for lecture bottle containing EO.

K.2.1.8 **Gas-tight syringes**, of 10 µl, 5 µl, 100 µl and 1 000 µl capacities for use in preparing standards and for injecting headspace gas on to the column of the gas chromatograph.

K.2.1.9 **Laboratory oven**, capable of heating samples to (100 ± 2) °C.

K.2.1.10 **Laboratory oven**, capable of heating samples to (37 ± 1) °C.

K.2.1.11 **Water bath**, capable of maintaining samples at (70 ± 2) °C.

K.2.1.12 **Mechanical shaker**.

K.2.1.13 **Glass headspace vials with PTFE-lined septa**, of nominal 20 ml capacity for preparation of calibration standards.

K.2.1.14 **Flat-bottom screw-cap vial**, of a size that will accommodate the sample and extraction fluid, equipped with a PTFE-lined silicone septum and thin PTFE film, used for extraction of EO and reaction of EO with bromohydrin if applicable.

K.2.1.15 **Injection needle**, of dimensions 0,65 mm × 25 mm for addition of hydrobromic acid.

K.2.1.16 **Millipore filter**, of 0,45 µm pore size for filtration of the reaction mixture before chromatography.

K.2.1.17 **Refrigerator**, capable of maintaining samples between 2 °C and 8 °C.

K.2.2 Reagents

K.2.2.1 **Epoxyethane (ethylene oxide)**, in suitable gas bottle, 99,7 % pure.

K.2.2.2 **2-chloroethanol (ethylene chlorohydrin)**, 99 % assay.

K.2.2.3 **1,2-epoxypropane (propylene oxide)**, reagent grade.

K.2.2.4 **Freshly double-distilled hydrobromic acid**, prepared as follows:

Distil 100 ml of 47 % hydrobromic acid in the presence of 100 mg tin (II) chloride. Discard the first 25 ml of distillate and collect the next 50 ml of distillate. Re-distill 50 ml of the distillate in the presence of 50 mg tin (II) chloride, discard the first 15 ml of distillate and collect the next 20 ml of colourless liquid (bp 125 °C to 126 °C). Store in a glass-stoppered glass container and use within 1 week.

K.2.2.5 **Tin (II) chloride (stannous chloride)**, reagent grade.

K.2.2.6 **Water**, of purity suitable for GC.

K.2.2.7 **Ethanol**, of purity suitable for GC.

K.2.2.8 **Propanone (acetone)**, of purity suitable for GC.

K.2.2.9 **Dimethylformamide (DMF)**, of purity suitable for GC.

K.3 Standard preparation

K.3.1 Preparation of ethylene oxide standards

Where required, prepare appropriate standards as described in J.1.

K.3.2 Preparation of ethylene chlorohydrin standards

Where required, prepare ethylene chlorohydrin standards as described in J.2.

K.3.3 Preparation of propylene oxide (PO) standards

Prepare a PO standard by diluting PO in ethanol to provide a solution containing PO at a concentration of 0,5 µg/ml.

K.4 Product extraction

K.4.1 General

Prepare extracts according to the principles described in 4.4.

K.4.2 Extraction to simulate product use

Use water to simulate product use. Perform simulated-use extraction under conditions that provide the greatest challenge to the intended use.

For example, extract blood-contacting and parenteral devices with water by filling completely or flushing the blood path or fluid path (whichever is appropriate).

NOTE When filling completely, ensure that no voids remain.

Where it is not possible to fill components of the device that come into contact with the patient, place all, or a critical and representative portion, of the device in a suitable container and add water to achieve an appropriate sample/extraction fluid ratio. Exercise caution; take several representative portions of the device as necessary to ensure confidence in the data derived from small samples of larger devices.