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การประเมินทางชีวภาพของเครื่องมือแพทย์

เล่ม 7 : สารตกค้างจากการทำให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์

BIOLOGICAL EVALUATION OF MEDICAL DEVICES -

PART 7 : ETHYLENE OXIDE STERILIZATION RESIDUALS

สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

กระทรวงอุตสาหกรรม

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ปัจจุบันมีการทำเครื่องมือแพทย์ให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์ และมีปริมาณเอทิลีนออกไซด์และเอทิลีนคลอโรไฮดรินตกค้าง ซึ่งเป็นอันตรายต่อผู้ใช้จึงต้องกำหนดปริมาณที่ยอมให้มีได้ของเอทิลีนออกไซด์และเอทิลีนคลอโรไฮดริน เพื่อให้มีความปลอดภัยต่อผู้ใช้ จึงกำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม การประเมินทางชีวภาพของเครื่องมือแพทย์ เล่ม 7 : สารตกค้างจากการทำให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์ ขึ้น

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดขึ้นโดยรับ ISO 10993-7 : 1995 Biological evaluation of medical devices-Part 7 : Ethylene oxide sterilization residuals มาใช้ในระดับเหมือนกันทุกประการ (identical) โดยใช้ ISO ฉบับภาษาอังกฤษเป็นหลัก

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดขึ้นเพื่อให้ทันกับความต้องการของผู้ใช้ และจักได้แปลเป็นภาษาไทยในโอกาสอันควร หากมีข้อสงสัยโปรดติดต่อสอบถามที่สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

คณะกรรมการมาตรฐานผลิตภัณฑ์อุตสาหกรรมได้พิจารณามาตรฐานนี้แล้ว เห็นสมควรเสนอรัฐมนตรีประกาศตามมาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ. 2511

ประกาศกระทรวงอุตสาหกรรม

ฉบับที่ 3894 (พ.ศ. 2551)

ออกตามความในพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม
พ.ศ. 2511
เรื่อง กำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม
การประเมินทางชีวภาพของเครื่องมือแพทย์
เล่ม 7 : สารตกค้างจากการทำให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์

อาศัยอำนาจตามความในมาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ.2511 รัฐมนตรีว่าการกระทรวงอุตสาหกรรมออกประกาศกำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม การประเมินทางชีวภาพของเครื่องมือแพทย์ เล่ม 7 : สารตกค้างจากการทำให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์ มาตรฐานเลขที่ มอก. 2395 เล่ม 7-2551 ไว้ ดังมีรายการละเอียดต่อท้ายประกาศนี้

ประกาศ ณ วันที่ 23 กรกฎาคม พ.ศ. 2551
สุวิทย์ คุณกิตติ
รัฐมนตรีว่าการกระทรวงอุตสาหกรรม

มาตรฐานผลิตภัณฑ์อุตสาหกรรม

การประเมินทางชีวภาพของเครื่องมือแพทย์

เล่ม 7 : สารตกค้างจากการทำให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดขึ้นโดยรับ ISO 10993-7 : 1995 Biological evaluation of medical devices-Part 7 : Ethylene oxide sterilization residuals มาใช้ในระดับเหมือนกันทุกประการ (identical) โดยใช้ ISO ฉบับภาษาอังกฤษเป็นหลัก

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดปริมาณที่ยอมรับได้ของเอทิลีนออกไซด์ (EO) และเอทิลีนคลอโรไฮดริน (ECH) บนเครื่องมือแพทย์ที่ทำให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์ วิธีหาปริมาณเอทิลีนออกไซด์และเอทิลีนคลอโรไฮดริน และวิธีการพิจารณาเพื่อปล่อยผลิตภัณฑ์เครื่องมือแพทย์ที่ผ่านการทำให้ปราศจากเชื้อด้วยก๊าซเอทิลีนออกไซด์สู่ตลาด

รายละเอียดให้เป็นไปตาม ISO 10993-7 : 1995

Introduction

Requirements for the quality system for validation and routine monitoring of sterilization of medical products with gaseous ethylene oxide 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 under development by ISO/TC 194. The specific requirements 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.

When determining the suitability of ethylene oxide (EO) for sterilization of medical devices, it is important to ensure that the levels of residual EO and ethylene chlorohydrin (ECH) pose a minimal risk to the patient in normal product use. 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 ethylene glycol (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.

Product development and design should have considered the use of alternative materials and sterilization processes with the aim of minimizing exposure to residuals. Requirements herein are in addition to the biological testing requirements for each individually designed medical device as indicated in ISO 10993-1. The biological testing requirements, combined with the EO-sterilization process residue limits, form the justification that an EO-sterilized device is acceptable for use.

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 and guidance also is included in informative annexes.

EO-sterilized devices that have no patient contact (e.g. *in vitro* diagnostic devices) are not covered by this International Standard.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 10993. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

ISO 10993-3:1992, *Biological evaluation of medical devices — Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity.*

ISO 10993-10:1995, *Biological evaluation of medical devices — Part 10: Tests for irritation and sensitization.*

3 Definitions

For the purposes of this part of ISO 10993, the definitions given in ISO 10993-1 and the following definitions 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 using an extraction method using water that simulates product use.

NOTE 1 The burden of validation on the analytical laboratory is 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 the device is assigned to the most stringent category probable for duration of exposure and should take into consideration both tissue(s) exposed and temperature of exposure.

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

4 Requirements

NOTE 3 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 part of ISO 10993 are contained in informative annexes.

4.1 General

This clause specifies maximum allowable residues for ethylene oxide (EO) for each individual medical device sterilized with EO. Maximum allowable residues for ethylene chlorohydrin (ECH) when ECH has been found to be present in medical devices sterilized with EO also are specified.

No exposure limits are set for ethylene glycol (EG) because risk assessment indicates that when EO residues are controlled as required by this part of ISO 10993, it is unlikely that biologically significant residues of EG would be present (see E.1).

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 by 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 EO residual level at 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. For example, irritation effects shall be considered for all devices, particularly small devices (see E.2). This International Standard does not take account of the possibility of acute localized effects, for which insufficient data are available. Particularly for small devices, attention should be paid to the potential for such effects and the concentration of EO per unit of surface area.

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 duration of contact.

Devices shall be placed into one of three exposure categories in accordance with ISO 10993-1:1992, subclause 5.2:

a) limited exposure: devices whose single or multiple use or contact is likely to be up to 24 h;

b) prolonged exposure: devices whose single, multiple or long-term use or contact is likely to exceed 24 h but not 30 days;

c) permanent contact: devices whose single, multiple or long-term use or contact exceeds 30 days.

NOTES

4 If a material or device may be placed in more than one duration category, the more rigorous testing requirements 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.

5 As it is applied in this part of ISO 10993, "multiple use" is defined to mean repeated use of the same device.

4.3 Allowable limits

For each medical device, the maximum allowable doses of EO and ECH that are 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.

NOTE 6 The limits for permanent contact and prolonged contact devices are expressed as maximum average daily doses. These limits also 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. The procedure that was used to establish the allowable limits is described in E.2.

4.3.1 Permanent contact devices

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

20 mg in the first 24 h;

60 mg in the first 30 days;

2,5 g in a lifetime.

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

12 mg in the first 24 h;

60 mg in the first 30 days;

50 g in a lifetime.

4.3.2 Prolonged exposure devices

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

20 mg in the first 24 h;

60 mg in the first 30 days.

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

12 mg in the first 24 h;

60 mg in the first 30 days.

4.3.3 Limited exposure devices

The average daily dose of EO to patient shall not exceed 20 mg.

The average daily dose of ECH to patient shall not exceed 12 mg.

NOTE 7 The simultaneous use of more than one device or the use of devices in the treatment of neonates may result in additional exposure as described in E.2.1.1.

4.3.4 Special situations

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

Residue of EO in intraocular lenses shall not exceed 0,5 µg EO per lens per day, nor 1,25 µg per lens.

For blood oxygenators and blood separators, the average daily dose of EO to patient shall not exceed 60 mg.

For extracorporeal blood purification set-ups, the EO and ECH limits specified above for the prolonged and limited duration category apply, but the allowable EO dose for a lifetime may be exceeded.

NOTE 8 The rationale for specifying EO limits for certain devices that are at variance with the general requirements appears in E.2.1.3.

4.4 Determination of EO and ECH residuals

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

4.4.1 Safety considerations

DANGER — Analysts and others obtaining samples should perform all work involving the use of the chemicals and solvents required for these methods under the fume hood with appropriate protective clothing, and should review the Material Safety Data information for each chemical prior to such use.

4.4.1.1 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 affect 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 exposed workers. However, the results or several recent studies in workers have not been consistent with this finding.

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

NOTE 9 If ECH is not detected based on the results of analyses performed using the methods given in B.5.2 and B.5.7, no further monitoring for ECH is required.

Validated methods that meet this requirement are described in annex B. However, any method which has been shown to be analytically sound may be used provided it has been validated by demonstrating that the system meets the requirements set out in annex A, and has been evaluated against the referee methods contained in annex B.

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 dose to the patient in order to show compliance with 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 further to 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 reviewed in the literature (see annex F). Those methods that have been compared and evaluated in interlaboratory studies conducted by knowledgeable individuals in well-equipped laboratories are described in annex B. 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 in annex B.

Therefore, any method which 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, and the annex B methods can be used as referee methods against which to evaluate alternative methods.

4.4.3 Product sampling

4.4.3.1 Representative samples

Samples intended 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 C. 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.

4.4.3.2 Handling samples

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 also C.1.5). In addition, operator and analyst safety shall be ensured.

Samples should remain with the product load until the day of analysis. 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 in dry ice by overnight delivery service. Dry ice shall remain in the shipping container throughout the shipment and be present when the package is opened in the laboratory. As an alternative, test samples may be taken directly from the product load at the desired aeration interval and immediately placed into an appropriate extraction fluid or head space vial, which is sealed and then shipped to the laboratory for analysis.

Samples shall be prepared according to any applicable pre-use instructions in the product labelling.

Samples to be analysed should be placed in a fume hood and removed from the packaging. Extractions should be started as soon as possible after the device has been removed from the packaging, or pre-use preparations have been completed.

4.4.3.3 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 gas chromatography 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. Sample/extraction fluid ratios for various devices typically range from 1:2 to 1:10 (i.e. 1 g in 2 ml to 1 g in 10 ml). 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, and on a daily, monthly and up to a lifetime basis for permanent contact items. As indicated in annex E, exhaustive extraction as described below can be a useful alternative for permanent contact devices, given that shorter-term constraints are ensured.

4.4.6 Product extraction

There are two basic extraction methods employed for the determination of EO-sterilization residuals in medical devices: simulated-use extraction, which is the reference method, and 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 D.

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.

4.4.6.1 Simulated use extraction (reference method)

4.4.6.1.1 Simulated-use aqueous extraction is the reference method in that it is the only method which produces results directly comparable to 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 during their routine use, extraction methods which simulate use are required. Simulated-use extraction shall be carried out under conditions which provide the greatest challenge to the intended use.

For example, many blood-contacting and parenteral devices can be extracted with water or other aqueous fluids 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 (or that ensures total extraction), and at temperatures that provide the greatest realistic simulated challenge. An alternative is to prepare a series of extracts (a minimum of three is suggested) representing various shorter periods of time from which extraction rates can be used to calculate effects of longer or daily repeated exposure.

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 employed. A simulated-use extraction procedure shall be validated to demonstrate the actual exposure level to patients.

NOTE 10 The amounts of EO (or ECH) extracted by simulating normal product use are not necessarily similar to the total product residual content.

Water or other aqueous systems (Kroes *et al.*, 1985) are commonly used as extraction fluids for the recovery of residual EO and ECH in simulated-use extractions. These aqueous fluids are 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. 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 may be stored in the refrigerator for several days (see annex E) but, where water extraction is used, caution shall be taken, as EO may convert to ethylene glycol (EG) or ethylene chlorohydrin (ECH) (or both) during storage of the extract (Chesler *et al.*, 1985). It is incumbent upon the analyst to evaluate the possibility of conversion on storage at the analysis site.

4.4.6.1.2 Exhaustive extraction represents an acceptable alternative and can provide useful information. It produces results which 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 further to 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 days in 4.3.

4.4.6.2 Exhaustive extraction (acceptable alternative method)

4.4.6.2.1 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.

a) Residual ethylene oxide

A variety of extraction fluids have been used for the exhaustive recovery of residual EO. Thermal desorption followed by headspace gas analysis, as described in B.5.3, 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 B.5.4 and such procedures may be able to separate EO from co-extracted interfering chemicals from the sample matrix. The extraction fluids described in B.3.2 were evaluated through interlaboratory comparison testing (Marlowe, 1983; Marlowe *et al.*, 1986a; Marlowe *et al.*, 1986b). The extraction efficiency of other fluids shall be evaluated against one or more of the methods described in this part of ISO 10993 in order to establish their suitability in exhaustive extraction procedures.

Prudent analytical procedure dictates that, in the initial analysis of a given material, more than one 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.

b) Residual ethylene chlorohydrin

Water is typically used to extract residual ECH from medical devices.

4.4.6.2.2 Small devices shall be placed in a vial and subjected to extraction in their entirety, whereas for larger devices representative portions of the component materials may be selected when it is necessary to determine EO residues in part of the device. Caution shall be exercised in the latter case. It may be necessary to take several representative portions of the device in order to ensure confidence in the data derived from the small samples of larger devices.

These representative portions may be selected in one of two ways. If several varied materials are employed, 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.

4.4.7 Data analysis and interpretation

4.4.7.1 Calculation of amount of residue extracted

The concentration of residue observed in the extracts, AE, is converted to amount, in milligrams, as follows:

$$AE = \sum_{0}^{n} ER \times EV$$

Residue extracted by simulated use may be calculated as follows:

$$AR = \frac{ER \times m}{\varrho}$$

Residue extracted by exhaustive extraction may be calculated as follows:

$$AE = \frac{R_S \times m_D}{m_S}$$

where

AE is the extract residue, in milligrams;

n is the number of extractions;

ER is the milligrams of EO per millilitre of extract as derived from the standard curve;

EV is the extract volume, in millilitres;

AR is the mass of residue recovered, in milligrams;

m is the mass of extract, in grams;

ϱ is the density of water, in grams per millilitre;

R_S is the residue extracted from the sample, in milligrams;

m_D is the entire device mass, in grams;

m_S is the mass of sample, in grams.

4.4.7.2 Calculation of average delivered dose (*ADD*) for comparison to allowable limits in 4.3

For permanent contact devices the average delivered dose, *ADD*, in milligrams per day, is as follows:

$$ADD = \frac{AE}{25\,000}$$

where

25 000 is the days per lifetime;

AE is as above.

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

For prolonged exposure devices,

$$ADD = \frac{AE}{30}$$

where

30 is the days per month;

AE is as above.

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

For limited exposure devices,

$$ADD = AE$$

where AE is as above.

5 Product release

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 C).

For release of batches of sterilized product, one of the two methods in 5.1 and 5.2 shall be used.

5.1 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 B and meet the requirements for EO and, if applicable, ECH set out in 4.3.

5.2 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 market-place 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 C are to be considered by pooling data from sterilization loads taken from aeration of quarantine storage at different times of the year if aeration temperatures differ. 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 or EN 550 ([1] and [2]), 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.

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, *PL*, 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 prediction limit, *PL*:

$$x_0 = \frac{y_0 - a}{b}$$

$$PL = x_0 + t_\alpha \times \sqrt{\frac{(s_\alpha)^2}{b^2} \times \left[1 + \frac{1}{n} + \frac{(y_0 - y_\mu)^2}{b^2 \times \Sigma(x_i - x_\mu)^2}\right]}$$

where

x_0	is the calculated average value of the release time corresponding to the EO limit;
y_0	is the log value of EO limit;
a	is the intercept of the linear regression line;
b	is the slope of the regression line;
PL	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 log 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 C 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.

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.

A.2 Background

These requirements are discussed in reference books on gas chromatography (USP, 1989) 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 (Ball, 1984; Chesler *et al.*, 1985, Hubaux and Gilbert, 1970).

A.3 Symbols

For the purposes of this annex, the following symbols apply (see figures A.1 and A.2).

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

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, shall be greater than or equal to 1,2:

$$R = 2\frac{(t_2 - t_1)}{(W_2 + W_1)}$$

for peak area or peak height quantitation.

Alternatively, the following equation may be useful to calculate capacity factor, k', which shall be greater than or equal to 1,5:

$$k' = \frac{t}{t_a} - 1$$

Tailing, T, given by the following equation, shall be less than or equal to 1,5:

$$T = \frac{W_{0,05}}{2f}$$

For quantitation at low concentrations of EO and ECH, the signal-to-noise ratio should be at least 10:1 (it may be necessary to set the GC attenuation to 1 × 1 to determine the signal-to-noise ratio).

For precise calculations of resolution and tailing, the chart recorder speed should be at least 10 cm/min and peak height should be at least 75 % of full scale deflection.

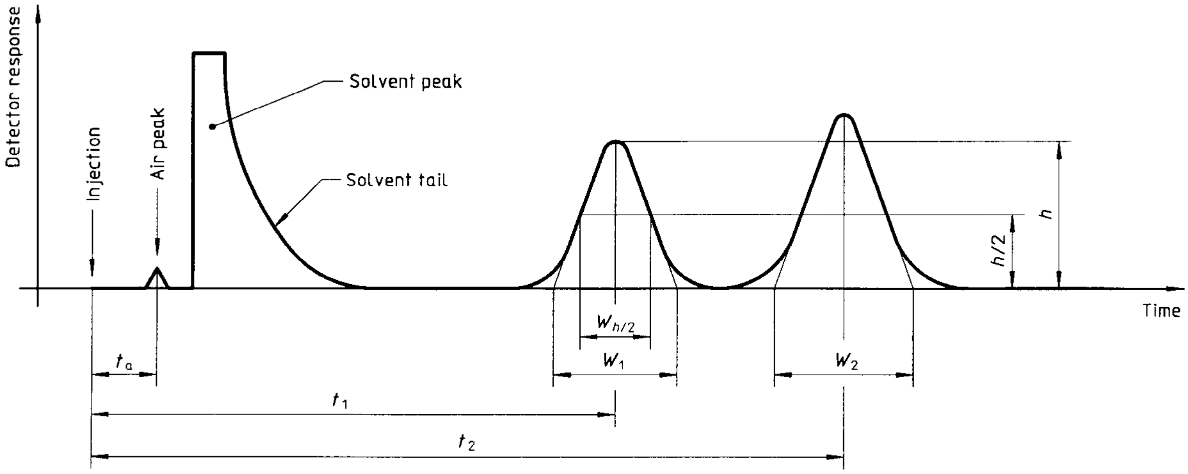

Figure A.1 — Chromatographic separation of two substances

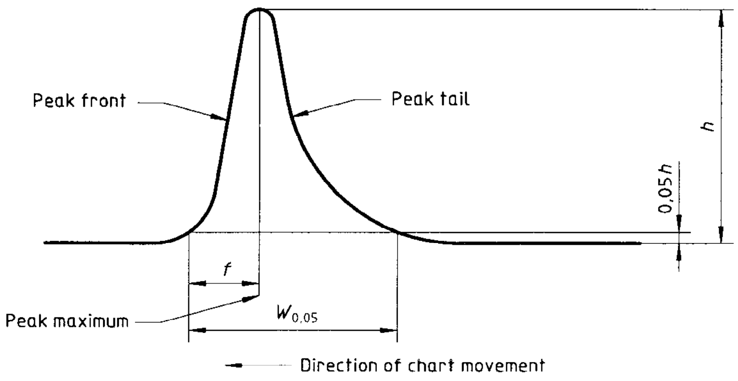

Figure A.2 — Asymmetrical chromatographic peak

A.4.2 Relative deviation of standard curve (*RSD*) should not exceed 5 % for EO and ECH for the range of standards used (AAMI, 1988 and AAMI, 1989):

$$RSD = \left(\frac{\sigma}{\lambda} \right) \times 100$$

$$\sigma^2 = \frac{\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n} \right] - S \times \left[\Sigma xy - \frac{(\Sigma x \Sigma y)}{n} \right]}{n - 2}$$

$$\lambda = \frac{y}{n}$$

where

n	is the total number of peaks;
y	is the chromatographic peak area or peak height;
x	is the concentration of the standard;
S	is the slope of the least squares regression line for the standard curve;
λ	is the mean;
σ	is the standard deviation;
σ^2	is the variance.

These criteria are calculated for duplicate 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, and the various textbooks on gas chromatography.

Annex B
(normative)

Gas chromatographic determination for EO and ECH

B.1 Chromatographic procedures

B.1.1 Ethylene oxide residue measuring methods

Many methods are suitable for quantitatively analysing extracts for ethylene oxide. A number of procedures for exhaustive extraction followed by gas chromatography (GC) for the determination of EO have been described. Reference to several published methods as well as several review articles are given in annex F. There are probably just as many unpublished methods for determining residual ethylene oxide levels, and because of the diversity in medical devices, published methods may not be suitable for all devices. Therefore, any method which has been shown to be analytically sound and evaluated against the validated reference methods described in this document can be used.

"Analytically sound" means the method demonstrates sufficient precision, selectivity, linearity 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.

The methods described in this annex are proposed as referee methods against which an alternative method shall be evaluated. These methods are explained in the annex so that the analyst may choose the most applicable one. For a more detailed discussion of each method the original literature should be consulted. 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 expiration point.

B.1.2 Preparation of EO standards

B.1.2.1 General

The following paragraphs outline the procedure for preparation of GC standards.

NOTE 11 An alternative is to purchase standards that have been prepared under Good Manufacturing Practices control and which are known to be stable.

Prepare 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.

Connect the EO standard gas cylinder to a serum vial (approximately 30 ml capacity) as shown in figure B.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 vent 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 (see 4.4.1).

Place another length of tubing onto the EO cylinder regulator and connect to a hypodermic needle. Insert the second, 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 mmHg[1] and 20 °C.

The concentration of ethylene oxide, in micrograms per millilitre, according to the ideal gas law may be calculated for any given temperature, t, in degrees Celsius, and pressure, p, in millimetres of mercury, using the following equation:

$$\rho_{\mathrm{EO}} = 0{,}706 \frac{p}{273 + t}$$

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[1] 1 mmHg = 133,322 Pa, or 760 mmHg = 101,325 kPa

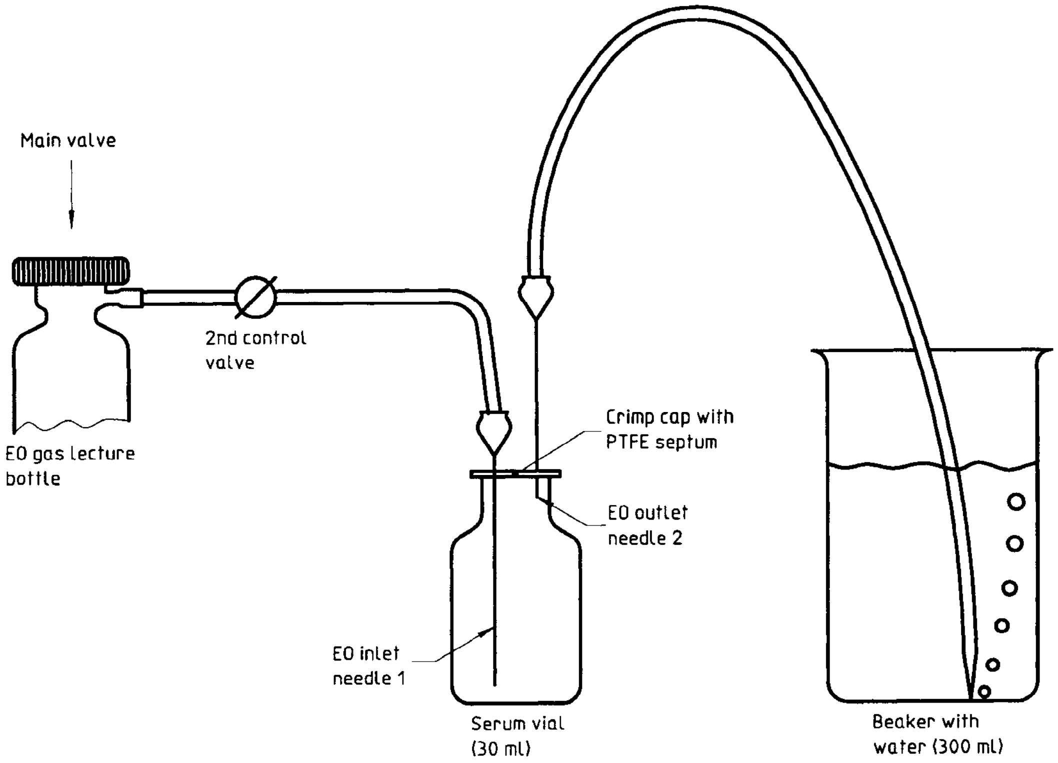

Figure B.1 — Apparatus for preparation of EO standards

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

B.1.2.2 EO standard dilutions for headspace methods

Dilute the standard from B.1.2.1 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 pointed upward.

Place the nitrogen-flushed vial onto the upward-pointing syringe needle and inject the 10 µl of EO into the vial. Do not flush the syringe and 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 as described in B.1.2.1.

Inject duplicate 100 µl aliquots of the gas from the second standard vial onto 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.

B.1.2.3 EO standard dilutions for solvent methods

NOTES

12 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.

13 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.

14 This procedure is also used to prepare aqueous EO standards.

Set up an EO standard gas cylinder as described in B.1.2.1 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 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.[2]

Prepare dilutions of the solution by diluting aliquots with an appropriate volume of solvent. If, for example, exactly 100 ml 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 onto the column of the gas chromatograph to obtain responses for peak area or peak height.

In the practice of gas chromatography, experience has shown that as samples are injected onto 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.

B.1.3 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) dropwise to the flask. Reweigh 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 E). Discard in a proper manner after 14 days.

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 onto the column of the gas chromatograph to obtain responses for peak area or peak height.

B.2 Precision of methods

B.2.1 EO methods

An interlaboratory evaluation was conducted at 13 laboratories using several of the EO methods described in annex B (Marlowe *et al.*, 1986a; Marlowe *et al.*, 1986b; Marlowe, 1983) 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 B.1.

Table B.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

Another interlaboratory evaluation was made of the EO method described in B.5.6 (Kikuchi *et al.*, 1988). 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

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[2] 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.

followed by headspace gas analysis procedure described in B.5.4 (Oba *et al.*, 1982) and the bromination method described in B.5.6 (Kikuchi *et al.*, 1988) 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 B.5.4 procedure was estimated as 4,7 %; 1,8 % and 2,7 % at 12 ppm, 25 ppm and 56 ppm EO in the matrix tested (Nakamura *et al.*, 1989).

B.2.2 ECH methods

An interlaboratory evaluation was conducted using the ECH methods described in B.5.7 (AAMI, 1989). 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 µg/ml to 100 µg/ml.

B.3 Apparatus and reagents

B.3.1 Apparatus

B.3.1.1 Gas chromatograph, equipped with a flame ionization detector (FID) or an electron capture detector (ECD).

NOTE 15 An electronic integrator is valuable in obtaining reproducible results.

B.3.1.2 Hypodermic needles and **polyvinyl chloride tubing** as required for preparing standards.

B.3.1.3 Volumetric glassware equipped with **PTFE-lined septa** or **PTFE-sealed valves** for preparing standards.

NOTES

16 Crimp-cap glassware also requires a crimping tool.

17 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.

B.3.1.4 Micro-syringe (5 µl or 10 µl capacity) for injecting aliquots of the extract into the gas chromatograph.

B.3.1.5 Fume hood to provide adequate ventilation while preparing standards and samples.

B.3.1.6 Analytical balance capable of measuring to 0,1 mg.

B.3.1.7 Gas regulator for lecture bottle containing EO.

B.3.1.8 Gas-tight syringes, of 10 µl, 50 µl, 100 µl and 1 000 µl capacities for use in preparing standards and for injecting headspace gas onto the column of the gas chromatograph.

B.3.1.9 Laboratory oven, capable of heating samples to 100 °C ± 2 °C.

B.3.1.10 Laboratory oven, capable of heating samples to 37 °C ± 1 °C.

B.3.1.11 Water bath, capable of maintaining samples at 70 °C ± 2 °C.

B.3.1.12 Mechanical shaker.

B.3.1.13 Glass headspace vials with PTFE-lined septa and crimp-cap, of nominal 20 ml capacity for preparation of calibration standards.

NOTE 18 Crimp-cap glassware also requires a crimping tool.

B.3.1.14 Flat-bottom screw cap vial, of 4 ml capacity (≈ 15 mm o.d.) equipped with a PTFE-lined silicone septum and thin PTFE film, used for extraction and reaction of EO.

B.3.1.15 Injection needle, of dimensions 0,65 mm × 25 mm for addition of hydrobromic acid.

B.3.1.16 Millipore[3] filter, of 45 µm pore size for filtration of the reaction mixture before chromatography.

B.3.1.17 Refrigerator, capable of maintaining samples between 2 °C and 8 °C.

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3) "Millipore" is the trade-name of a product. This information is given for the convenience of users of this part of ISO 10993 and does not constitute an endorsement of the product by ISO. Equivalent products may be used if they can be shown to lead to the same results.

B.3.2 Reagents

B.3.2.1 Epoxyethane (ethylene oxide), in suitable gas bottle, 99,7 % pure.

B.3.2.2 2-chloroethanol (ethylene chlorohydrin), ⩾ 99 % assay.

B.3.2.3 1,2-epoxypropane (propylene oxide), reagent grade.

B.3.2.4 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. Redistil 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.

B.3.2.5 Tin(II) chloride (stannous chloride), reagent grade.

B.3.2.6 Water, of purity suitable for gas chromatography.

B.3.2.7 Ethanol, of purity suitable for gas chromatography.

B.3.2.8 Propanone (acetone), of purity suitable for gas chromatography.

B.3.2.9 Dimethylformamide (DMF), of purity suitable for gas chromatography.

B.4 Standard preparation

B.4.1 Preparation of ethylene oxide standards

When required, prepare appropriate standards as described in B.1.2.

B.4.2 Preparation of ethylene chlorohydrin standards

Where required, prepare ethylene chlorohydrin standards as described in B.1.3.

B.4.3 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.

B.5 Product extraction

B.5.1 General

Prepare extracts according to the principles described in 4.4.6.

B.5.2 Extraction to simulate product use

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

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

NOTE 19 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 or user, place all, or a critical and representative portion, of the device in a suitable container with an appropriate sample/extraction fluid ratio. Take several representative portions of the device as necessary to ensure confidence in the data derived from small samples or larger devices.

Extract samples for a time equivalent to or exceeding the maximum time for single use (or that ensures total extraction), and at temperatures that provide the greatest simulated challenge, as described in 4.4.6. Alternatively, prepare a series of extracts (minimum of three is suggested) representing various shorter periods of time and use these extraction rates to calculate the effects of longer or daily repeated exposure.

If the assay is not performed immediately, decant the extract from the sample and seal in a 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 up to 4 days. Take care when using water extraction to assay EO as EO may convert to EG or ECH, or both during the storage of the aqueous extract (Chesler *et al.*, 1989).

B.5.3 Exhaustive procedure using thermal extraction

Weigh a 1 g sample to the nearest 0,1 mg and place into a capped, 15 ml septum vial. Place the sealed vial in a 100 °C oven and heat for 60 min. Remove the vial from the oven, equilibrate to room temperature, and shake vigorously prior to sampling. Inject duplicate 100 µl samples of the headspace gas onto the column of the gas chromatograph, and determine the areas or heights of the EO peaks. Calculate the mean for the duplicate samples.

Remove the cap from the vial under a hood, and purge the vial for 30 s with dry nitrogen. Replace the cap using a new septum and repeat the heating and injection to exhaustion. Exhaustion is achieved when an amount of EO is extracted less than 10 % of first extraction. Calculate the EO in the sample with reference to the standard curve by summing the EO values obtained for the mean peak area or peak height measurements made in each of the several sample heatings.

NOTES

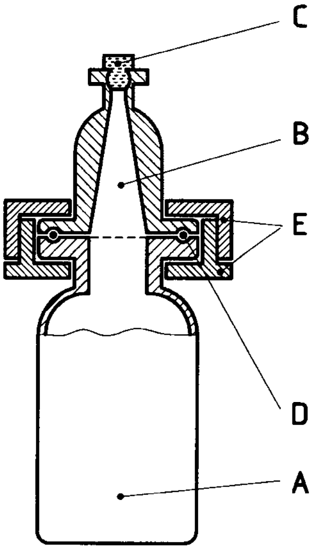
20 The time/temperature regimen described in this clause is relatively arbitrary. Varying the time to achieve an equilibrium headspace partial pressure of EO is better experimental technique. Take care that column packing material is not picked up on the needle during injection. Experience has shown that testing the hot sample immediately after it has been removed from the oven will result in an error often greater than 20 % because of loss of material from the syringe as it is removed from the vial and its pressure equilibrates to the room pressure. Some materials resorb EO as the temperature equilibrates to room temperature. Some materials also appear to resorb the EO completely in the vial if allowed to cool. In the analysis of these materials, samples and standards may need to be injected onto the column while they are still hot or warm and then purged (as described above) without further cooling.

21 Automated procedures for headspace gas analysis are being investigated by ISO/TC 194/WG 11 with a view to their inclusion in future editions of this part of ISO 10993.

B.5.4 Exhaustive extraction with ethanol followed by headspace gas analysis of ethanol extract

B.5.4.1 Calibration standards

Prepare EO standards by diluting EO in ethanol to provide solutions containing EO at concentrations of 0,4 µg/ml; 0,8 µg/ml; 1,2 µg/ml; 1,6 µg/ml and 2 µg/ml. Prepare a standard containing propylene oxide (PO) in ethanol at a concentration of 0,5 µg/ml as described in B.4.3. Cool these standard solutions and appropriate numbers of the special headspace bottles (figure B.2) in a dry ice/isopropanol bath, or equivalent. Transfer appropriate aliquots of each EO standard solution and the same volume of the PO standard solution to the headspace bottles. Heat the headspace bottles at 70 °C for 30 min and inject duplicate 100 µl to 1 ml aliquots of the headspace gas from each bottle onto the column of the gas chromatograph. Measure the height or area of the EO and PO peaks, and plot the peak height or peak area ratio against the EO concentration to give a calibration line.



Key

A	Liquid
B	Headspace
C	Septum
D	O-ring
E	Clamp

Figure B.2 — Special headspace bottle

B.5.4.2 Analysis procedure

Weigh a 5 g (or 0,5 g) sample, cut into small pieces (5 mm long for tubing, 10 mm square for sheet), to the nearest 0,1 mg and place into a headspace bottle of 100 ml (or 10 ml) capacity. Add 50 ml (or 5 ml) of PO standard solution (0,25 µg/ml) to the bottle. Cap the bottle, crimp the cap and heat the sealed bottle at 70 °C for 3 h with gentle shaking. Inject duplicate 100 µl to 1 ml samples of the headspace gas onto the column of the gas chromatograph and determine the EO/PO peak ratios. Calculate the mean EO content for the duplicate samples by reference to the calibration line.

B.5.5 Exhaustive extraction with solvent

Accurately weigh an approximately 1 g product sample and place it in capped volumetric glassware of the appropriate volume to minimize the headspace. Transfer 10 ml of the chosen solvent by pipette into the volumetric flask. Cap the volumetric flask and allow to stand for 24 h at room temperature.

NOTE 22 These temperatures and times were those used in the referee evaluation. Other validated temperatures and times can be substituted.

Inject duplicate 1 µl to 5 µl aliquots onto the column of the gas chromatograph. Calculate the EO in the samples by reference to the standard curve and calculate the mean for the duplicate samples.

B.5.6 Exhaustive extraction with ethanol followed by preparation of bromohydrin derivative and chromatography using gas chromatograph equipped with ECD

B.5.6.1 Calibration standards

Prepare EO standards by diluting EO in ethanol to provide solutions containing EO at concentrations of 0,4 µg/ml; 0,8 µg/ml; 1,2 µg/ml; 1,6 µg/ml and 2 µg/ml. Prepare a standard containing PO in ethanol at a concentration of 0,5 µg/ml as described in B.4.3. Prepare standard mixtures by mixing equal volumes of each EO standard solution and the PO standard solution.

Transfer 1 ml of each standard mixture to a screw-cap vial. Add two drops (≈ 0,015 g) of hydrobromic acid to the mixture through the septum with an injection needle. Allow the vial to stand for 1 h at room temperature. Heat the vial for 1 h at 50 °C in a water bath with gentle shaking then cool to room temperature.

Add 0,02 g sodium bicarbonate to the vial and shake the vial longitudinally for 30 min. Allow the vial to stand for 10 min. Shake the vial again horizontally for 30 min. Allow the vial to stand for 10 min and centrifuge at 3 000 r/min for 5 min. Filter the mixture through a small Millipore filter.[3)4)]

Inject duplicate 1 µl aliquots of each filtrate onto the column of the gas chromatograph to obtain responses for peak height ratios of ethylene bromohydrin (EBH) vs. propylene bromohydrin (PBH). Prepare a calibration line by plotting EBH/PBH peak height ratios vs. amounts of EO, in micrograms.

B.5.6.2 Analysis procedure

Use this procedure with standards prepared as described in B.5.6.1.

Cool the PO standard solution (0,25 µg/ml) and a screw-cap vial in a dry ice/isopropanol bath or equivalent. Transfer 1 ml of the PO standard solution to the vial.

Weigh a 10 mg to 30 mg portion of the sample to the nearest 0,1 mg and place it into the vial.

Add two drops (≈ 0,015 g) of hydrobromic acid to the vial through the septum with an injection needle. Let the vial stand for 1 h at room temperature then heat the vial for 8 h at 50 °C in a water bath with gentle shaking. Heat the vial for an additional 16 h at 50 °C in a laboratory oven, then cool to room temperature.

Add 0,02 g sodium bicarbonate to the vial and shake the vial longitudinally for 30 min. Allow the vial to stand for 10 min. Shake the vial again horizontally for 30 min. Allow the vial to stand for 10 min and centrifuge at 3 000 r/min for 5 min. Filter the mixture through a small Millipore filter.[3)4)]

Inject duplicate 1 µl aliquots of each filtrate onto the column of the gas chromatograph to obtain responses for peak height ratios of ethylene bromohydrin (EBH) vs. propylene bromohydrin (PBH).

Calculate the mean of the duplicate samples and determine the EO in the sample by reference to the calibration line.

B.5.7 Exhaustive extraction of ethylene chlorohydrin using water

Accurately weigh a portion (or the entire sample) of approximately 1 g to 50 g into capped glassware of appropriate volume to minimize headspace. Transfer water at a ratio of between 1 to 2 and 1 to 10 (sample mass, in grams to water volume, in millilitres) into the container and cap. Allow to stand for 24 h at room temperature. Agitate the container and contents vig-

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4) Use of vials with U- or V- shaped bottoms occasionally causes incomplete neutralization, giving poor chromatograms.

orously on a mechanical shaker for approximately 10 min.[5]

Inject duplicate 1 μl to 5 μl aliquots onto the column of the gas chromatograph. Calculate the concentration of ECH in the sample from either the relative peak area or peak height of the chromatogram when referenced to the previously generated standard response curve.

B.6 Gas chromatography

B.6.1 General

Select the most appropriate methods from B.5.2 to B.5.7. Use the appropriate chromatographic procedure from those listed in table B.2.

NOTE 23 Optimization of conditions may be required.

B.6.2 Extraction to simulate product use

For EO, use condition number I with column oven temperature about 60 °C to 75 °C; for ECH, use condition number I (see table B.2) with column oven temperature about 150 °C to 170 °C or use condition number II. Inject 1 μl to 5 μl aliquots of the aqueous extract.

B.6.3 Exhaustive procedure using thermal extraction

Use condition number I with oven temperature about 125 °C. Inject 100 μl aliquots of the headspace gas.

B.6.4 Exhaustive extraction with ethanol followed by headspace gas analysis of ethanol extract

Use condition number IV.

B.6.5 Exhaustive extraction with ethanol followed by preparation of bromohydrin derivative and chromatography using gas chromatograph equipped with ECD

Use condition number VI.

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[5] These temperatures and times were those used in the referee evaluation (AAMI, 1989). Other appropriate temperatures and times can be substituted. If required, it may be more appropriate to agitate for the entire time. Some materials may not require agitation.

Table B.2 — Recommended gas chromatographic conditions

Condition No.	Column			Carrier gas		Temperature °C			Injection volume	Solvent
	Size length (m) x i.d. (mm)	Material	Packing		Flowrate ml/min	Oven	Injector	Detector	µl	
I	2 x 2	glass	3 % Carbowax 20M on Chromosorb 101 1) (80 to 100 mesh)	Nitrogen or helium	20 to 40	60 to 75 (EO) 150 to 170 (ECH)	200 to 210	220 to 250	1,0 to 5,0	water
II	2 x 2	glass	5 % Igepal CO-990 on Chromosorb T 1) (40 to 60 mesh)	Nitrogen or helium	20 to 40	140 to 160	200 to 250	240 to 280	1,0 to 5,0	water
III	3 x 3,2	stainless steel	20 % Tricyanoethoxy propane on Chromosorb W AW DMCS 1) (100 to 120 mesh)	Nitrogen or helium	20	60	100	200	1 000	headspace gas (over water extract)
IV	2 x 3	glass	25 % Flexol 8N8 on Chromosorb W AW 1) (80 to 100 mesh)	Nitrogen	40	50	120	120	100 to 1 000	headspace gas (over ethanol extract)
V	2 x 2	glass	Chromosorb 102 1) (80 to 100 mesh)	Nitrogen or helium	20 to 40	60 to 170	200 to 210	220 to 250	1,0 to 5,0	propanone or DMF
VI	2 x 3	glass	10 % Carbowax 20 M on Chromosorb W AW 1) (80 to 100 mesh) 2)	Nitrogen	60	120	250	250	1,0	ethanol

1) These are trade-names. This information is given for the convenience of users of this part of ISO 10993 and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

2) Column conditioning at 190 °C for 7 days is necessary before use.

Annex C
(informative)

Factors influencing product residuals

C.1 Sterilization process parameters

Sterilization process parameters are defined in ISO 11135 or EN 550. However, properly to analyse residues in EO-exposed devices, it is necessary to recognize those parameters which 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.

C.1.1 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.

C.1.2 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.

C.1.3 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.

C.1.4 Aeration

Residual EO in devices is also 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.

NOTES

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

25 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.

C.1.5 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 product samples or an extract thereof are 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.

C.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 need to be controlled. Lack of control may yield data about residue levels that are applicable only to the samples analysed.

Annex D
(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 D.1 represents suggested extraction conditions which could facilitate laboratory operations. Specific definitions for simulated use and exhaustive extraction are given in 4.4.6.

The guiding principle in selecting appropriate extraction methods for the determination of EO is the evaluation of dose to the patient in order to show compliance with the requirements set out in the body of 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 D.1 — Suggested extraction conditions

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

Annex E
(informative)

Rationale

E.1 Scope [clause 1]

Clause 1 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) and ethylene chlorohydrin (ECH). No maximum allowable residue limits are required for ethylene glycol (EG). When EO residues are controlled to the limits specified herein it is unlikely that a biologically significant amount of EG would remain on a device (Danielson *et al.*, 1990; Muzeni, 1985; Spitz and Weinberger, 1971).

For certain devices where the current state-of-the-art precludes meeting these limits, a higher dose is permissible due to the benefit provided to the patient. These devices include extracorporeal blood purification set-ups, where the maximum daily EO dose shall not exceed 20 mg, the maximum monthly EO dose shall not exceed 60 mg, but the maximum lifetime dose could exceed 2,5 g, and blood oxygenators and blood separators where the maximum daily EO dose and maximum monthly EO dose shall not exceed 60 mg and the maximum lifetime EO dose shall not exceed 2,5 g. (See 4.3.)

E.2 Allowable limits [4.3]

E.2.1 Setting residue limits for EO

E.2.1.1 Background

The residue limits for EO in medical devices were established by applying methods proposed by the US Pharmaceutical Manufacturer's Association (PMA, 1989) for setting residue limits for organic volatile impurities in chronically administered pharmaceuticals. Emphasis was placed on parenteral and oral data since these data more closely match potential systemic exposure to EO from the use of medical devices than do inhalation data. The procedure was modified to address systemic effects from limited exposure (< 24 h) and systemic effects from prolonged exposure (> 24 h to 30 days) (Conine *et al.*, 1992). The approach required that all relevant data be evaluated in the limit-setting process. The approach was also based on the concept that acute data should be the basis for acute limits, that subchronic and reproductive effects data should be the basis for prolonged exposure limits and that chronic and carcinogenicity data should be the basis of permanent exposure limits. In the event that acute data did not provide usable dose-response information apart from median lethal dosages, subchronic/reproductive toxicity data were used to substantiate the appropriateness of the residue limit derived from the acute data.

To set the systemic limits, the safety factors shown in table E.1, altered for duration of exposure, were used. Included in the consideration of the safety margin are the extrapolation of animal data to humans, the quality of the study from which the limits are derived, the application of these limits to persons of low body mass and the simultaneous use of several devices on a single individual. No specific values are attributed to any of these factors individually.

NOTE 26 These factors are established for this part of ISO 10993 at the time it is approved. The Technical Committee recognizes that these may be altered by the addition of data at the time of the next revision.

The general formula for calculating the limit, L, in milligrams per day, using safety factors was as follows:

$$L = \frac{D \times BW}{SM}$$

where

dosage, D, in milligrams per kilogram per day, may be one of the following:

NOEL	is the no-observed-effect-level;
LOEL	is the low-observed-effect-level;
NOAEL	is the no-observed-adverse-effect-level;
LOAEL	is the low-observed-adverse-effect-level;

LD_{50} is the median lethal dosage;

LDLo is the low lethal dosage;

TDLo is the low toxic dosage;

BW is the human body mass, in kilograms;

SM is the safety margin, equal to safety factor times modifying factor.

Since EO is genotoxic and has produced tumours in several animal studies and is considered by regulatory agencies and consensus groups throughout the world to be a human carcinogen, statistical quantitative risk assessment of the data to establish residue limits for permanent exposure also was used. Since cancer risk estimates have been performed for EO by many groups, these estimates were used to provide a residue limit that would represent the worst-case lifetime daily dose of EO associated with a 1 in 10 000 excess cancer risk as proposed by the Pharmaceutical Manufacturer's Association for EO as an organic volatile impurity in chronically administered pharmaceuticals (PMA, 1990). The 10^{-4} risk level is intermediate among risk levels recommended or used by various regulatory agencies. It reflects a risk-benefit consideration for sterile medical products essential to human well-being. Indeed, somewhat greater risks are generally deemed appropriate by society when health benefits are to be gained from product use. Without sterile medical devices, many life-saving procedures and equipment would be unavailable and nosocomial infections would return as a major health risk.

In summary, the limits for EO in medical devices were established based upon evaluation of many literature reports and upon consideration of several reviews (Bruch, 1973; Cyr *et al.*, 1989; Environ, 1987; EPA, 1985; Glaser, 1979; PMA, 1990). Since the potential irritancy of a medical device sterilized with EO is evaluated by biological testing, acute toxicity data, target organ effects data, animal carcinogenicity data and human tolerance data were deemed the most appropriate for the derivation of product residue limits for protection against potential adverse effects from EO exposure. In addition, in evaluating the potential toxicity of EO, as discussed below, consideration should be given to the simultaneous use of more than one device and the use of devices in the treatment of neonates [Environ, 1987; ISO 10993-1:1992, subclause 6.1 b) 5)].

Table E.1 — List of safety factors used to set systemic limits for EO

Systemic residue limit	Type of study	Dosage	Safety factor1)
Permanent exposure	Chronic toxicity (> 12 months treatment/exposure)	NOEL or NOAEL	10
		LOEL or LOAEL	⩾ 10
	Carcinogenicity	NOEL or NOAEL	100
		LOEL or LOAEL	⩾ 100
Prolonged exposure	Subchronic toxicity (⩽ 6 months treatment/exposure)	NOEL or NOAEL	100
		LOEL or LOAEL	⩾ 100
	Reproductive/developmental toxicity	NOEL or NOAEL	100
		LOEL or LOAEL	⩾ 100
Limited exposure	Acute toxicity	LD_{50} animal	> 100
		LDLo human or animal	⩾ 10 or ⩾100
		TDLo human or animal	> 1 or > 10

1) The actual safety factor used may be modified on the basis of the data under evaluation and professional judgement. In each case the additional modifying factor may range between 1 and 10. The actual safety margin represents a product of the safety factor and the modifying factor.

E.2.1.2 General considerations

The acute toxicity data and repeated dose data demonstrate that EO is readily accessible to the systemic circulation once it has been introduced into the body. Inspection of median lethal dosages ($LD_{50}s$) and no-observed-effect levels (NOELs) also suggest that the potency of EO at specific time intervals, limited exposure, etc., is comparable by oral and parenteral and even inhalation routes of exposure. Adverse effects have been observed at lower dosages, as the duration of exposure is increased. The specific target organ effects can be different, however. The allowable daily dose limits that are discussed in the clauses that follow reflect these general observations.

E.2.1.2.1 Permanent exposure limit

The limit for exposure of 30 days or more to life is 0,1 mg/day, not to exceed 20 mg in any given day or 60 mg in a month or 2 500 mg in a lifetime. This limit was based upon chronic toxicity and carcinogenicity data that have been reported by many investigators (Dunkelberg, 1982; Snellings *et al.*, 1984b; Lynch *et al.*, 1983, 1984; NTP, 1987). All of the studies, except for that reported by Dunkelberg (Dunkelberg, 1982) were inhalation studies. No acceptable parenteral data were found.

In the Dunkelberg study, animals were treated orally by means of disposable syringes equipped with tubes to deliver the material into the stomach, i.e. by gavage. Dosages ranged upwards from 2,1 (mg/kg)/day. In these studies, adverse target organ effects from chronic administration included decreased sperm function, skeletal muscle atrophy and precancerous lesions to the stomach, while several kinds of cancer including mononuclear cell leukaemia, primary brain tumours, peritoneal mesotheliomas, subcutaneous fibromas, lung adenomas/carcinomas, Harderian gland papillary cystadenomas, lymphomas, uterine/mammary gland adenocarcinomas and squamous cell carcinomas to the forestomach were found. In the oral study, only the stomach tumours were found, while the other changes were found only in the inhalation studies. These data were evaluated using both safety factor and statistical quantitative risk assessment techniques. While EO was considered to be a genotoxic carcinogen based upon its mutagenic potential and produced some tumour types in animals relevant to man, the lack of biodisposition data regarding EO in animals and humans and the lack of a clear epidemiology link of EO exposure and cancer in man precluded statistical quantitative risk assessment techniques as the sole means of calculation of the limit for permanent exposure to EO. Thus, both the safety factor approach and quantitative risk assessments were used to determine prospective permanent exposure limits. The comparison of results from both approaches then served as the basis for the permanent exposure limit (PMA, 1990; Conine *et al.*, 1992).

The key data that became the basis for the calculation of a prospective permanent exposure limit using safety factors are summarized in table E.2.

Inspection of these data reveals that LDLo dosages for EO for permanent exposure periods, i.e. 30 days to life, are comparable regardless of routes or effects although no acceptable data from which to access effects from parenteral exposure are available.

Table E.2 — Summary of data used to establish permanent exposure limit for EO

Data type	Oral LOEL (mg/kg)/day [Reference]	Inhalation LOEL (mg/kg)/day [Reference]
Chronic toxicity	2,1 — Prorated from 7,5 mg/kg twice weekly [Dunkelberg, 1982]	9,2 1) [Lynch *et al.*, 1983]
Carcinogenicity	2,1 — Prorated from 7,5 mg/kg twice weekly [Dunkelberg, 1982]	2,1 2) [Snellings *et al.*, 1984b]

1) Calculated from a LOEL value of 50 ppm in a 2-year study in Cynomolgus monkeys to assess sperm function. EO administered 7 h per day for 5 days a week. Presumed ventilation rate and body mass of 1,2 m^3 per day and 2,7 kg, respectively.

2) Calculated from a LOEL value of 10 ppm in a carcinogenicity study in rats administered EO for 6 h per day for 5 days a week. Presumed a ventilation rate of 290 l per day and a body mass of 0,5 kg.

The lowest LOEL, expressed in milligrams per kilogram per day, with cancer as the tissue response, a prorated dose of 2,1 mg/kg orally to rats for 3 years was used as the basis for calculation of a prospective permanent exposure limit, L_p, using the safety factor approach as follows:

$$L_p = \frac{D \times BW}{SM} = \frac{2{,}1 \times 70}{1\ 000} = 0{,}15 \text{ mg/day}$$

where

D is the lowest low-observed-effect-level in chronic toxicity or carcinogenicity studies;

BW is the adult body mass of 70 kg;

SM is the safety margin of 1 000 for translation of low effect level data in cancer bioassays to man. The safety margin takes account of the possibility of interspecies differences, the inherent variability within the human population, the nature, localization and incidence of the observed responses, the lack of parenteral data, the lack of an established no-effect level in the relevant studies and the benefit gained from the use of sterile medical devices.

Quantitative risk assessments were obtained from the literature. These cancer risk estimates have been calculated for EO by numerous groups as cited by Environ (Environ, 1987). These groups, including the FDA, California DHS, OSHA and USEPA, have employed linearized multistage models or Gaylor-Kodell linear proportional methods to generate unit cancer risk estimates from leukaemia, brain tumour, stomach tumour and mesothelioma data reported in animal studies. These unit cancer risk estimates range between 0,016 $[(\text{mg/kg})/\text{day}]^{-1}$ and 0,35 $[(\text{mg/kg})/\text{day}]^{-1}$. Translating these values to average, lifetime daily doses for a 70 kg adult with a worst-case 1 in 10 000 excess cancer risk yields a range of 0,02 mg/day to 0,44 mg/day with a mean of 0,12 mg/day. An example of these calculations for average dose, AD, using a unit cancer risk of 0,016 $[(\text{mg/kg})/\text{day}]^{-1}$ is as follows:

$$AD = \frac{Risk \times BW}{UCR} = \frac{0{,}000\ 1 \times 70}{0{,}016} = 0{,}44 \text{ mg/day}$$

where

$Risk$ is the excess cancer risk of 1/10 000;

BW is the adult body mass of 70 kg;

UCR is the unit cancer risk in units of $[(\text{mg/kg})/\text{day}]^{-1}$.

Upon evaluation of the prospective limit of 0,15 mg/day and the mean worst-case 1 in 10 000 excess cancer risk dose of 0,12 mg/day, it was determined that 0,1 mg/day would be adequately protective of the adverse effects of EO resulting from permanent exposure. The permanent limit covers potential exposure for a very wide period of time, from 30 days to 25 000 days in a 70-year lifetime. Thus, the actual, worst-case cancer risk resulting from exposure to EO at this limit could be much less than 1 in 10 000 in many cases since the limit presumes daily exposure to EO for 70 years. A study of the use of medical devices sterilized by EO has resulted in the estimate that the actual probability of cancer from exposure to EO from medical devices is low, in the vicinity of 7 in a million (Environ, 1987).

E.2.1.2.2 Prolonged exposure limit

The limit for exposure for 24 h to 30 days is 2 mg/day, not to exceed 20 mg in any given day or 60 mg in a month. This limit was based upon subchronic toxicity and reproductive effects data (teratogenicity, reproductive performance, fetotoxicity, etc.) generated in several species. These data have been reported by many investigators (Hollingsworth *et al.*, 1956; Woodard and Woodard, 1971; Balazs, 1976; Northup *et al.*, 1981; Snellings *et al.*, 1984a; NTP, 1987; Jacobson *et al.*, 1956; Jones-Price *et al.*, 1982; LaBorde and Kimmel, 1980; Hackett *et al.*, 1982; Snellings *et al.*, 1982a, 1982b). In oral, parenteral and inhalation studies lasting for varying time periods up to 226 days, EO has produced a wide variety of adverse effects including vomiting, tremors, respiratory irritation, injury to lungs, kidneys, testes, adrenals, thymus gland, liver and gastrointestinal tract, decreasing growth and body mass, impairment of nervous system function, paralysis and muscular atrophy (hind limb), and anaemia. Dosages ranged from 1 mg/kg to 100 mg/kg and more. Reproductive studies included exposure of animals for up to 12 weeks prior to mating, exposure throughout all or part of gestation, and exposure for up to 21 days after parturition. Dosages ranged from 5 mg/kg to 150 mg/kg or more. In these studies, EO produced maternal toxicity, embryotoxicity, fetotoxicity, delays in fetal development and cervical/thoracic skeletal malformations. This latter effect has been observed only in the offspring of mice given EO intravenously at a dosage of 150 mg/kg, about two-thirds of the LD_{50} of EO in female mice of 260 mg/kg. The key data that became the basis for the calculation of the limit for prolonged exposure are summarized in table E.3.

Inspection of these oral and parenteral data suggests that no-observed-effects-levels for EO for prolonged exposure periods, i.e. 1 day to 30 days, are comparable regardless of the route or type of effect, target organ or reproductive effect. Data from the inhalation studies show a similar pattern although the estimated subchronic NOEL value appears to be less than the NOEL values derived from the oral and parenteral data. The NOEL in the subchronic inhalation study appears low in part because of the concentration used in the study. The next larger concentration was 50 ppm, a concentration at which reduced locomotor activity, hunched posture during gait and reduced testicular mass were the only adverse effects reported by the investigators. Because the oral and parenteral data were most appropriate to medical devices, the lowest NOEL from parenteral administration, 9 mg/kg from an intravenous, teratology study in rabbits, was used as the basis of the calculation of the limit for prolonged exposure as follows:

$$L = \frac{D \times BW}{SM} = \frac{9 \times 58}{250} = 2 \text{ mg/day}$$

where

D is the lowest no-observed-effect-level, in milligrams per kilogram per day, in subchronic or reproductive effect studies by parenteral administration;

BW is the female body mass of 58 kg since the data selected was a teratology study in pregnant animals;

SM is the safety margin of 250 (safety factor of 100 times a modifying factor of 2,5) for translation of no-effect data in animals to reflect variation in species responses.

The limit thus provides an acceptable safety margin for a 58-kg adult from the potential adverse effects of EO resulting from prolonged exposure based on animal data.

E.2.1.2.3 Limited exposure limit

The limit for exposure for less than 24 h is 20 mg. This limit was based upon acute toxicity data generated in several animal species. These data have been reported by many investigators (Carpenter *et al.*, 1949; Smith *et al.*, 1941; Bruch, 1973; Jacobson *et al.*, 1956; Woodard and Woodard, 1971; RTECS, 1987). Although a limited amount of LDLo or TDLo data exists (PMA, 1990), LD_{50} data were used because they were the only appropriate data available for the assessment. The non-LD_{50} data included three LDLo values in the range of 100 mg/kg to 200 mg/kg. The only dose-response data were found in the acute inhalation study in mice (NTP, 1987). In this study, 9 of 10 mice died after exposure to EO at a concentration of 800 ppm (V/V) for 4 h while 0 of 10 mice died after exposure to EO at a concentration of 400 ppm (V/V). Thus, in the limited dose effect data which do exist, the dose response curves for these acute biological effects and the lethal and non-lethal dosages are quite close to each other and differ by a factor of less than 2. The LD_{50} data are summarized in table E.4.

Table E.3 — Summary of data used to establish prolonged exposure limit for EO

Study type	**Oral NOEL** (mg/kg)/day [Reference]	**Parenteral NOEL** (mg/kg)/day [Reference]	**Inhalation NOEL** (mg/kg)/day [Reference]
Subchronic toxicity	30 [Hollingsworth *et al.*, 1956]	25 [Northup *et al.*, 1981]	5 1) [Snellings *et al.*, 1984a]
Reproductive toxicity	No data	9 [Jones-Price *et al.*, 1982]	13 2) [Snellings *et al.*, 1982a]

1) Calculated from a NOEL value of 10 ppm in a 10 to 11 week study in mice administered EO for 6 h per day for 5 days a week. Presumed a ventilation rate of 43 l per day and a body mass of 30 g.

2) Calculated from a NOEL value of 33 ppm in a teratology study in pregnant rats administered EO for 6 h per day during gestation days 6 to 15. Presumed a ventilation rate of 290 l per day and a body mass of 0,35 kg.

Table E.4 — Summary of data used to establish limited exposure limit for EO

Milligrams per kilogram

Oral LD_{50}	Intravenous LD_{50}	Intraperitoneal LD_{50}	Subcutaneous LD_{50}	Inhalation LD_{50} 1)
rat: 72 rat: 240 guinea pig: 270 rat: 280 mouse: 280 rat: 330 mouse: 360 rabbit: 631	rabbit: 175 rabbit: 178 rabbit: 180 mouse: 260 mouse: 290 rat: 350 rat: 355 rat: 380	rat: 150 mouse: 175 mouse: 178 rat: 178 rat: 180 mouse: 180 rabbit: 251	mouse: 130 rat: 140 rat: 187 mouse: 190 rabbit: 200 mouse: 260	155 to 773 (estimated)

1) Calculated from 4 h LD_{50} values of 800 ppm to 4 000 ppm in rats (with intermediate values for other species) using a body mass (*BW*) of 250 g and a ventilation rate of 290 l/24 h.

Inspection of these data suggests that the toxicity of EO for limited exposure periods, i.e. less than 24 h, is comparable within a factor of three or so, regardless of route of exposure. Since the data reflect median lethal dosages and not low lethal or low toxic dosages, the lowest of the LD_{50} values, 72 mg/kg in rats, was used, rather than an intermediate value, as the basis of the calculation of the limit for limited exposure as follows:

$$L = \frac{D \times BW}{SM} = \frac{72 \times 70}{250} = 20 \text{ mg}$$

where

D is the lowest median lethal dosage in milligrams per kilogram;

BW is the adult body mass of 70 kg;

SM is the safety margin of 250 for translation of acute data from animals to one-time exposure in man. This takes account of the possibility of interspecies differences, the inherent variability within the human population, the fact that median lethal dosage (LD_{50}) data rather than no-effect level data were used, the quality of the data available and the benefit gained from the use of sterile medical devices.

The limit thus provides at least a 250-fold safety margin for a 70-kg adult from the potential adverse effects of EO resulting from limited exposure based on animal data. Other acute effects such as haemolysis of blood cells do not appear to be a problem even if the entire maximum daily dose of 20 mg were to be delivered in a few minutes (Tanaka *et al.*, 1982; Ohba, 1986). Also, the limit is acceptable in the context of the no-observed-effect level (NOEL) derived from subchronic/reproductive toxicity data based on the low, parenteral NOEL of 9 (mg/kg)/day or 522 mg/day if prorated to a 58-kg woman for repeated administration.

E.2.1.3 Special situations

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. In consequence, there is scope for relaxation of limits in life-threatening situations where it is not possible to meet the specified limits.

During the development of this part of ISO 10993, three special situations were recognized in which the limits of 4.3 would not be practical due to limitations of the devices themselves, or human data existed which indicated that the dose levels shown in 4.3 are not applicable. Human data are available from patient exposure to intraocular lenses which should be addressed by revision of the residue requirements for such devices. During treatment of blood with oxygenators or blood separators 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.

E.2.1.3.1 Intraocular lens limit

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 (Shimizu *et al.*, 1986; McDonald *et al.*, 1973; McDonald *et al.*, 1977; Edelhauser *et al.*, 1983 and Patel, 1993).

E.2.1.3.2 Blood oxygenators and blood separators

The limited exposure limit for such devices is 60 mg in a 24 h period. These devices are used in severe operations such as open heart surgery. This limit takes into consideration the acute need of the patient during such procedures while still allowing over an 80-fold safety factor. Under such circumstances this relaxation is warranted.

E.2.1.3.3 Extracorporeal blood purification set-ups

The maximum allowable EO dose of 2,5 g for a lifetime may be exceeded, provided that both the maximum daily EO dose of 20 mg and the maximum monthly EO dose of 60 mg are met. To exceed the 2,5 g lifetime dose of EO, a patient undergoing blood purification would need to be exposed to 2 mg EO three times every week and such exposure would need to continue for eight years. If this exposure were to continue for 70 years — and no one has undergone such treatment nearly that long — the cancer risk would increase from 1 in 10 000 to about 1 in 1 000. This added cancer risk is balanced out by the benefit of lifetime blood purification.

E.2.2 Setting residue limits for ECH

E.2.2.1 Background

The residue limits for ECH in medical devices were established using the methodology outlined in E.2.1 for EO except that statistical quantitative risk assessment methodology to establish a residue limit for permanent exposure that would represent a 1 in 10 000 excess cancer risk was not applied. ECH has exhibited no potential to produce cancer in bioassays in animals and is not considered even a possible human carcinogen by regulatory agencies or consensus groups. 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 E.2.2.2.

E.2.2.2 General considerations

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-effect levels (NOELs) also suggest that the potency of ECH at specific time intervals, limited exposure, etc., 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.

E.2.2.2.1 Permanent exposure limit

The limit for exposure of 30 days or more to life is 2 mg/day, not to exceed 12 mg in any given day or 60 mg in a month or 50 000 mg in a lifetime. This limit was based upon chronic toxicity and carcinogenicity data that has been reported by Johnson (1967b), Mason *et al.* (1971) and NTP (1985). 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 recieved ECH by dermal application for 103 to 104 weeks. Dosages ranged from 0,086 (mg/kg)/day to 71 (mg/kg)/day or more. In these studies, no increases in tumour incidence related to ECH administration or evidence of chronic toxicity [apart from a possible reduction in survival rates (Johnson, 1967b)] were found. The key data that became the basis for the calculation of prospective permanent exposure limits are summarized in table E.5.

Inspection of these data suggests that no-observed-effect levels for ECH for permanent exposure periods, i.e., 30 days to life, by oral and parenteral routes are comparable and are 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.

The lowest no-observed-effect level for chronic toxicity, 2,9 (mg/kg)/day administered subcutaneously to rats for at least a year, and for tumour production, 16 (mg/kg)/day orally to rats until 24 months of age, were used on the basis for calculations of a prospective permanent exposure limit, $L_{\text{p,chronic}}$, as follows:

$$L_{\text{p,chronic}} = \frac{D \times BW}{SM} = \frac{2{,}9 \times 70}{100} = 2 \text{ mg/day}$$

Table E.5 — Summary of data used to establish permanent exposure limit for ECH

Study type	Oral NOEL (mg/kg)/day [Reference]	Parenteral NOEL (mg/kg)/day [Reference]	Dermal NOEL (mg/kg)/day [Reference]
Chronic	4 LOEL [Johnson, 1976b]	2,9 Prorated from 10 twice weekly [Mason *et al.*, 1971]	No data
Carcinogenicity	16 1) [Johnson, 1976b]	No data	71 Prorated from 100 five times weekly1) [NTP, 1985]
1) Ethylene chlorohydrin produced no increases in tumour incidence at the highest dosage tested.			

where

D (dosage) is the lowest no-observed-effect level, in milligrams per kilogram per day, for chronic effects;

BW is the adult body mass of 70 kg;

SM is the safety margin of 100 (safety factor of 10 times a modifying factor of 10) reflecting a conservative translation of animal data to humans.

$$L_{\text{p,cancer}} = \frac{D \times BW}{SM} = \frac{16 \times 70}{100} = 11 \text{ mg/day}$$

where

D (dosage) is the lowest no-observed-effect level, in milligrams per kilogram per day, for tumour production (in fact no increase in tumour incidence occurred);

BW is the adult body mass of 70 kg;

SM is the safety margin of 100 (safety factor of 100 times a modifying factor of 1) reflecting the lack of tumour production in animal bioassays.

Upon examination of these prospective limits, 2 mg/day and 11 mg/day, it was determined that 2 mg/day 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.

E.2.2.2.2 Prolonged exposure limit

The limit for exposure for 24 h to 30 days is 2 mg/day, not to exceed 12 mg in any given day or 60 mg in a month. This limit was based upon subchronic toxicity and reproductive effects data (teratogenicity) generated in several species. These data have been reported by many investigators (Ambrose, 1950; Oser *et al.*, 1975; Balazs, 1976; Alleva cited in Balazs, 1976; Woodard and Woodard, 1971; Courtney *et al.*, 1982; Jones-Price *et al.*, 1985a and 1985b).

In repeated-dose, oral and parenteral studies lasting for varying time periods up to 403 days, ECH has 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), 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)/day to 93 (mg/kg)/day 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, fetal toxicity and, in one study, an increase in fetal malformations. This latter effect was observed only in the offspring of mice given ECH intravenously at a dosage of 120 (mg/kg)/day, a dosage well into the acutely lethal range (Jones-Price *et al.*, 1985b). The key data that became the basis for the calculation of the limit for prolonged exposure are summarized in table E.6.

Table E.6 — Summary of data used to establish prolonged exposure limit for ECH

Study type	**Oral NOEL** (mg/kg)/day [Reference]	**Parenteral NOEL** (mg/kg)/day [Reference]
Subchronic	13 [Oser *et al.*, 1975]	2,7 Prorated from 6,4 three times weekly [Lawrence *et al.*, 1971b]
Reproductive	50 [Courtney *et al.*, 1982]	9 [Jones-Price *et al.*, 1985a]

Inspection of these data suggests that no-observed-effects levels of ECH for prolonged exposure periods, i.e., 1 to 30 days 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. The lowest NOEL (no-observed-effect level) for parenteral administration of 2,7 mg/kg from an intraperitoneal study in rats was used as the basis of the calculation of the limit, L, for prolonged exposure as follows:

$$L = \frac{D \times BW}{SM} = \frac{2{,}7 \times 70}{100} = 1{,}9 \text{ mg/day}$$

where

D (dosage) is the lowest no-observed-effect level, in milligrams per kilogram per day, in subchronic and reproductive effects studies by parenteral administration;

BW is the adult body mass of 70 kg;

SM is the safety margin of 100 (safety factor of 100 times a modifying factor of 1).

While the calculated limit is slightly less than the actual limit itself (1,9 mg/day vs. 2 mg/day), the latter limit is considered to be adequately protective in light of the observation that ECH does not increase in toxicity after chronic vs. prolonged exposure. The limit thus provides almost a 100-fold safety margin for a 70-kg adult from the potential adverse effects of ECH resulting from prolonged exposure based on animal data.

E.2.2.3 Limited exposure limit

The limit for less than 24 h is 12 mg. This limit was based upon acute toxicity data generated in several animal species. These data have been reported by several investigators (Rowe and McCollister, 1982; Woodard and Woodard, 1971; Lawrence *et al.*, 1971a and 1972; RTECS, 1990; Mason *et al.*, 1971; Weil, 1972). Although a limited amount of acute data, other then medium lethal dosages, were available and evaluated, they were not appropriate for this assessment. The median lethal dosage data are summarized in table E.7.

Inspection of the data in table E.7 suggests that the toxicity of ECH for limited exposure, i.e., less than 24 h, is nearly identical regardless of the route of exposure. Since the data reflect median lethal dosages and not low lethal or low toxic dosages, the lowest of the LD_{50} values, 44 mg/kg in rats by intraperitoneal administration, was used rather than an intermediate value, as the basis of the calculation of the limit for limited exposure, L, as follows:

$$L = \frac{D \times BW}{SM} = \frac{44 \times 70}{250} = 12 \text{ mg/day}$$

where

D (dosage) is the lowest median lethal dosage in milligrams per kilogram;

BW is the adult body mass of 70 kg;

SM is the safety margin of 250 for translation of acute data from animals to one-time exposure in man. This takes account of the possibility of interspecies differences, the inherent variability within the human population, the fact that median lethal dosage (LD_{50}) data rather than no-effect level data were used, the quality of the data available and the benefit gained from the use of sterile medical devices.

Table E.7 — Summary of data used to establish limited exposure limit for ECH

Milligrams per kilogram

Oral LD_{50}	Intravenous LD_{50}	Intraperitoneal LD_{50}	Subcutaneous LD_{50}	Other LD_{50}
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

The limit this provides is at least a 250-fold safety margin for a 70-kg adult from the potential adverse effects of ECH resulting from limited exposure based on animal data. Also, the limit is acceptable in the context of the no-observed-effect levels (NOELs) derived from the subchronic/reproductive toxicity data based on the low NOEL of 2,7 (mg/kg)/day or 189 mg if prorated to a 70-kg adult for repeated administration.

E.2.3 Setting residue limits for EG

A risk assessment of ethylene glycol (EG), performed using the same method that was used for EO and ECH, was discussed at length. The assessment indicated limited exposures of 435 mg/day to 588 mg/day would be acceptable based upon acute exposures to animals (Rowe and Wolf, 1982; Woodard and Woodard, 1971; Latven and Molitor, 1939; Yin *et al.*, 1986; Karel *et al.*, 1947; Mason *et al.*, 1971; RTECS, 1990), and humans (Rowe and Wolf, 1982); prolonged exposures of 30 mg/day or 900 mg/month would be acceptable based upon subchronic and reproductive effects data in animals (Gaunt *et al.*, 1971; Woodard and Woodard, 1971; Tyl, 1988); and permanent exposures of 30 mg/day or 750 g/lifetime would be acceptable based upon chronic toxicity and negative carcinogenicity data (Blood, 1965; DePass *et al.*, 1986; Mason *et al.*, 1971; Morris *et al.*, 1942). No maximum allowable residue limits are required for ethylene glycol (EG). When EO residues are controlled to the limits specified herein it is unlikely that a biologically significant amount of EG would remain on a device (Danielson *et al.*, 1990; Muzeni, 1985; Spitz and Weinberger, 1971).

E.3 Determination of EO and ECH residuals [4.4]

E.3.1 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 recognized 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 that 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.

E.3.2 Analytical methods

E.3.2.1 Stability of EO in ethanol

During the interlaboratory comparison study of the EO method described in B.6.4 (Oba *et al.*, 1982), 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 days.

E.3.2.2 Stability of ECH

Prior to the interlaboratory comparison study of ECH (and EG), 11 laboratories participated in a study of the stability of ECH standards. Aqueous solutions of ECH were prepared by one lab 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 columns. 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 days.

E.3.2.3 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. However, during the ILC studies carried out on these procedures, the linear range of EO tested was 2 µg/ml to 50 µg/ml and the linear ranges of ECH tested were 3 µg/ml to 15 µg/ml. On the basis of the personal experiences of the participants in these ILCs, the linear range of these analytical systems can safely be extended to 100 µg/ml for EO and ECH. There are currently no data available to determine if the linear ranges can be extended to lower standard concentrations.

E.3.3 Data analysis and interpretation [4.4.7]

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 upon conformance with the requirements listed in 4.3.

Annex F
(informative)

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