International Standard

ISO 10993-7

Third edition
2026-04

Biological evaluation of medical devices —

Part 7: Ethylene oxide sterilization residuals

Évaluation biologique des dispositifs médicaux —
Partie 7: Résidus de stérilisation à l'oxyde d'éthylène

Reference number
ISO 10993-7:2026(en)

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Published in Switzerland

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Foreword

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

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This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 206, *Biological and clinical evaluation of medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 10993-7:2008), which has been technically revised. It also incorporates the Amendment ISO 10993-7:2008/Amd 1:2019 and the Technical Corrigendum ISO 10993-7:2008/Cor 1:2009.

The main changes are as follows:

— allowable limits and extraction conditions have been derived based on the patient population and the duration of use;

— the use of a risk assessment to establish allowable limits has been permitted;

— additional guidance on product release has been provided;

— additional guidance on determining residuals and the factors that affect residual has been provided.

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

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

As noted in the introduction to ISO 11135, 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 intended product use. Therefore, it is important that the use of alternative materials and sterilization processes are considered during product design and development. EO is known to exhibit a number of biological effects. In the development of this document, consideration was given to these effects, which include irritation, organ damage, mutagenicity, carcinogenicity, and reproductive effects in humans and animals. Similar consideration was given to the harmful effects of ECH and ethylene glycol (EG). ECH can be formed when EO comes into contact with free chloride ions, whereas EG is a hydrolytic reaction product of EO and water. In practice, for most devices, exposure to EO and ECH is considerably lower than the maximum allowable limits established according to this document. No allowable limits are set for EG because risk assessment indicated that when EO residuals are controlled, it is unlikely that biologically significant residuals of EG would be present.

Requirements herein are in addition to the biological evaluation requirements as indicated in ISO 10993-1. The biological evaluation, combined with the EO-sterilization process residual limits, form the justification that an EO-sterilized device is safe for its anticipated contact duration. Maximum allowable residuals for ECH, when ECH has been found to be present in medical devices sterilized with EO, are also specified. Local effects (e.g. irritation) have been considered and are incorporated in the TCL as given in 4.3.6.2 and Annex D for EO, and in 4.3.6.3 and Annex E for ECH.

In this edition of this document (i.e. ISO 10993-7:2026), an uncertainty factor approach is used to derive EO and ECH exposure duration-specific tolerable intake (TI) values (expressed in µg/kg/d). Furthermore, this edition of this document (i.e. ISO 10993-7:2026) introduces the conversion of each EO and ECH TI value into subpopulation-specific cumulative exposure-allowable limit values (expressed in milligrams per device), which are used to determine the extent that EO and ECH, extracted under clinically relevant conditions and time-periods, needs to be reduced post-sterilization.

This edition of this document (i.e. ISO 10993-7:2026) applies a different approach as compared to ISO 10993-17:2023 to establishing allowable limits to make it useful for development, validation, and routine control of ethylene oxide sterilization in the manufacture of finished medical devices with focus on the risk assessments associated with three chemical constituents that are potentially left in medical devices sterilized with ethylene oxide. This document extends this knowledge further by calculating the largest amount of EO, ECH or EG that can be present in a medical device such that it would always meet the requirements of ISO 10993-17 when that device has been exposed to the validated sterilization cycle parameters. This maximum amount or allowable limit is expressed in milligrams per device deemed acceptable when taken into the body through exposure to that medical device. These allowable limits will help determine the appropriate sterilization parameters such as sterilant gas concentration and dwell, as well as aeration temperature and hold time when validating the sterilization process to be used for a product or group of products. Furthermore, the allowable limits can be used by regulatory bodies, manufacturers, and processors to optimize processes and aid in the selection and qualification of alternative materials in order to protect patient health.

Biological evaluation of medical devices —

Part 7:
Ethylene oxide sterilization residuals

1 Scope

This document specifies allowable limits (AL) for residual ethylene oxide (EO) and ethylene chlorohydrin (ECH) in EO-sterilized medical devices, procedures for the measurement of EO and ECH, and methods for determining conformity so that devices can be released. Additional background, including guidance and a flowchart showing how this document is applied, are also included in Annexes A, B, C, D, E, F, G, H, I, J and K.

EO-sterilized devices or components that have neither direct nor indirect body or user contact (e.g. in vitro diagnostic devices) are out of scope of this document. This document does not apply to devices that have been demonstrated to not absorb or retain EO or its degradation product ECH, such as medical devices made exclusively of metal alloys and glass, see Clause C.5[228].

NOTE This document does not specify limits for ethylene glycol (EG). No device limits are specified for EG because the risk assessment in Annex F indicates that calculated allowable levels are higher than those likely to occur in a medical device.

2 Normative references

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

ISO 10993-1:2025, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

ISO 10993-23:2021, *Biological evaluation of medical devices — Part 23: Tests for irritation*

3 Terms and definitions

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

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

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

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

3.1
aeration
part of the sterilization *cycle* (3.5) during which the sterilizing agent and/or its reaction products desorb from the health care product until predetermined levels are reached

Note 1 to entry: Aeration can be performed within the sterilization chamber or in a separate chamber or room.

[SOURCE: ISO 11139:2018, 3.7, modified — Note 1 to entry has been added.]

3.2
allowable limit
AL
amount of *residual* (3.19) ethylene oxide or ethylene chlorohydrin on a single device that is permitted as a condition of release for patient use

Note 1 to entry: Allowable limits are expressed in milligrams per device for each applicable exposure period. These limits represent acceptable biological risks for medical devices under the circumstances of their anticipated exposure duration.

3.3
cumulative exposure
total quantity of ethylene oxide and ethylene chlorohydrin that contacts the body for a specified period of time

Note 1 to entry: Cumulative exposure can apply when consecutive uses of the same device or of new devices of the same type for the same patient or user applies (e.g. when one device is used repeatedly over a specified period of time).

3.4
concomitant exposure factor
CEF
numerical *safety* (3.20) factor that accounts for patient exposure to the simultaneous use of other ethylene oxide sterilized medical devices different from the subject medical device

Note 1 to entry: CEF is calculated from the reciprocal of the number of devices (one divided by the number of devices) used during a procedure. The *default value* (3.6) of 0,2 assumes four other devices are used during a procedure, see 4.4.5 and Clause D.6 for further details.

3.5
cycle
set of sterilization process parameters

3.6
default value
value or factor used in the derivation of a *tolerable contact level* (3.22) or *tolerable intake* (3.24), in the absence of specific data [e.g. an *uncertainty factor* (3.26)]

[SOURCE: ISO 10993-17:2023, 3.5, modified — the "worst-case exposure dose" has been removed from the definition.]

3.7
dose-response
relationship of dosage to observable harm

Note 1 to entry: In general, there are two types of dose-response relationships. The first type is the change in response of an individual to a range of doses. The second type is the distribution of the response among individuals to a range of doses.

[SOURCE: ISO 10993-17:2023, 3.6]

3.8
exhaustive extraction
multi-step extraction conducted until the amount of material extracted in a subsequent extraction step is less than 10 % of that determined in the initial extraction step

Note 1 to entry: Based upon the boiling point of ethylene oxide (EO) (10,7 °C) and the knowledge that substances, other than EO and ethylene chlorohydrin, can be extracted from the device under evaluation, gravimetric analysis is not appropriate for determining the exhaustivity level.

[SOURCE: ISO 10993-18:2020, 3.15, modified — "by gravimetric analysis (or achieved by other means)" has been removed from the definition and Note 1 to entry has been added.]

3.9
harm to health
adverse reaction, such as altered morphology, physiology, growth, development, reproduction or lifespan that

a) impairs function of an organ or system, organism or (sub)population,

b) reduces capacity to tolerate an impaired function, or

c) increases susceptibility to other influences that impair function

Note 1 to entry: Examples of (sub)population include, but are not limited to: male, female, preterm neonates, adults.

[SOURCE: ISO 10993-17:2023, 3.8]

3.10
load
sterilization batch
sterilization load
product, equipment or materials to be processed together within an operating *cycle* (3.5)

[SOURCE: ISO 11139:2018, 3.155, modified — the admitted terms "sterilization batch" and "sterilization load" have been added.]

3.11
implant
medical device, or component thereof, that is intended to be introduced into the human body or to replace an epithelial surface or the surface of the eye, by means of surgical intervention and that is intended to remain in place after the procedure

Note 1 to entry: The duration of an implant remaining in the body is dependent on the clinical need.

[SOURCE: ISO 10993-1:2025, 3.21]

3.12
irritation
localized non-specific inflammatory response to single, repeated or continuous application of a substance/material

Note 1 to entry: Skin irritation is a reversible reaction and is mainly characterized by local erythema (redness) and swelling (oedema) of the skin.

[SOURCE: ISO 10993-23:2021, 3.7]

3.13
lowest observed adverse effect level
LOAEL
lowest concentration or amount of an identified constituent found by experiment or observation which causes detectable *harm to health* (3.9) to the target organism under defined conditions of exposure

[SOURCE: ISO 10993-17:2023, 3.13, modified — Note 1 to entry has been deleted.]

3.14
minimally irritating level
MIL
lowest amount per surface area of an identified constituent that is irritating to the tissue at the contact site as determined by valid experimental or observational evidence

Note 1 to entry: The minimally irritating level is expressed in microgram per centimetre squared ($\mu g/cm^2$).

[SOURCE: ISO 10993-17:2023, 3.15]

3.15
modifying factor
MF
mathematical product of *uncertainty factors* (3.26)

[SOURCE: ISO 10993-17:2023, 3.16]

3.16
non-irritating level
NIL
greatest amount per surface area of an identified constituent that does not elicit *irritation* (3.12) to the tissue at the contact site as determined by valid experimental or observational evidence

Note 1 to entry: The non-irritating level is usually expressed as milligram per centimetre squared per centimetre squared (mg/cm^2) or microgram per centimetre squared ($\mu g/cm^2$).

[SOURCE: ISO 10993-17:2023, 3.17, modified — 'usually' and 'milligram per centimetre squared per centimetre squared (mg/cm^2) or' have been added to Note 1 to entry.]

3.17
no observed adverse effect level
NOAEL
greatest concentration or amount of an identified constituent found by experiment or observation which causes no detectable *harm to health* (3.9) to the target organism under defined conditions of exposure

Note 1 to entry: No observed adverse effect level is expressed in microgram per kilogram of body mass per day (µg/kg/d).

[SOURCE: ISO 10993-17:2023, 3.18]

3.18
physiologically based pharmacokinetic modelling
PBPK modelling
system of modelling biological effects taking into account metabolic and pharmacokinetic differences among species of animals

Note 1 to entry: Such data should be utilized whenever available and applicable to medical device anticipated exposure duration.

3.19
residual
quantity of ethylene oxide, ethylene chlorohydrin or ethylene glycol that remains in or on the product after ethylene oxide sterilization

3.20
safety
freedom from unacceptable risk

[SOURCE: ISO 14971:2019, 3.26]

3.21
simulated-use extraction
extraction using a method that simulates clinical use

Note 1 to entry: A simulated-use extraction is performed to estimate the type and amount of substances that are expected to be released from a medical device during its clinical use. A simulated-use extraction is designed to produce an extractables profile that represents the worst-case leachables profile, meaning that all leachables are also extractables and the levels of all individual extractables are at least equal to the level of all individual leachables.

[SOURCE: ISO 10993-18:2020, 3.35]

3.22
tolerable contact level
TCL
estimate of the surface-contact exposure to an identified constituent that is without appreciable *irritation* (3.12)

Note 1 to entry: Tolerable contact level is expressed in microgram per centimetre squared (μg/cm^2) of tissue at the contact site.

[SOURCE: ISO 10993-17:2023, 3.25]

3.23
tolerable exposure
TE
product of the *tolerable intake* (3.24), the body mass and the *concomitant exposure factor* (3.4)

Note 1 to entry: Tolerable exposure is normally expressed in milligrams per day to the patient.

3.24
tolerable intake
TI
estimate of the daily exposure of an identified constituent over a specified time period (e.g. acute, subacute, sub-chronic or chronic), on the basis of body mass, that is considered to be without appreciable *harm to health* (3.9)

Note 1 to entry: Tolerable intake is normally expressed in microgram per kilogram of body mass per day (μg/kg/d). It is derived to establish an *allowable limit* (3.2) for a medical device constituent.

[SOURCE: ISO 10993-17:2023, 3.26, modified — 'normally' has been added and 'toxicological exposure limit' has been replaced with 'an *allowable limit* (3.2)' in Note 1 to entry.]

3.25
toxicological risk assessment
determination of whether an exposure dose to a constituent can or cannot elicit appreciable *harm to health* (3.9)

[SOURCE: ISO 10993-17:2023, 3.29]

3.26
uncertainty factor
UF
numerical value that accounts for uncertainties when extrapolating a point of departure to individuals who can be exposed to a constituent of toxicological concern

EXAMPLE Extrapolation types include, but are not limited to: intraspecies, interspecies, dose route and study duration.

[SOURCE: ISO 10993-17:2023, 3.31]

4 Requirements

4.1 General

This clause specifies maximum ALs for residuals of ethylene oxide (EO) and ethylene chlorohydrin (ECH) for each individual medical device sterilized with EO. Local (acute) effects (e.g. irritation) have been considered and are incorporated in the TCL.

The requirements in this document shall be applied in addition to the requirements set out in ISO 10993-1. All applicable requirements of ISO 10993-1 shall take into account the EO residual level at the time of release for each individually designed medical device. The results of the biological assessment of the device may

lead to other limits than those specified in 4.3, which are designed to protect against local irritation and systemic effects.

Medical devices already on the market and tested to the previous edition of this document (i.e. ISO 10993-7:2008/Cor 1:2009 with ISO 10993-7:2008/Amd 1:2019) do not have to undergo the testing of this edition of this document (i.e. ISO 10993-7:2026). A review and confirmation that none of the issues identified in ISO 10993-1:2025, Clause 10 have occurred shall be carried out. If there are any changes, then a new biological risk assessment shall be carried out to demonstrate conformity to allowable limits. This may include re-testing to this edition of this document (i.e. ISO 10993-7:2026). The previous editions of this document reported a limit of 4 mg for EO and 9 mg for ECH for adults (70 kg body mass) with limited exposure [with the concomitant exposure factor (CEF) equal to 0,2 and an uncertainty factor 1 (UF1) of 10 for intra-species variability]. From a toxicological point of view, these values are not significantly different from the values calculated in this edition of this document (i.e. ISO 10993-7:2026) and thus, these changes in ALs do not warrant re-evaluating a product that met the limits of the previous edition of this document (i.e. ISO 10993-7:2008/Cor 1:2009 with ISO 10993-7:2008/Amd 1:2019).

A flowchart providing guidance for the application of this document to the determination of EO residuals in medical devices is given in Annex A.

NOTE 1 Information on the derivation of the limits in this document as well as other background information and guidance relevant to the use of this document is contained in Annexes C, D and E.

NOTE 2 Throughout this document, numbers calculated from formulae were rounded following the rules provided in NIST Special Publication 811[248]. Thereby, the number of (typically two) significant digits from the source literature were retained, and only increased (to typically three significant digits), where the calculated result was evaluated to provide the adequate amount of relevant information only with an increased number of digits. This evaluation is in line with NIST Special Publication 811:2008, B.7.2[248].

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, the medical device shall be categorized in accordance with the duration of body contact in accordance with ISO 10993-1:

a) limited exposure;

b) prolonged exposure;

c) long-term exposure.

If a device can be placed in more than one duration category, the more rigorous testing or evaluation considerations shall apply. If a device is intended for repeated or multiple usages, the decision into which duration category a device is placed shall take into account the potential cumulative effect, bearing in mind the period of time over which the cumulative exposure occurs. For example, a dialyzer cartridge is used for less than 24 h per treatment, but repeated usages of the same or a replacement cartridge for more than 30 d would categorize the cartridge as long-term exposure.

For medical devices that have very brief contact with the body, typically for less than one minute (e.g. lancets, hypodermic needles, capillary tubes), it can be possible to prepare a written justification that there is no potential for biological harm. For products with repeated use, the total exposure period shall be considered. However, for medical devices that can leave materials in contact with tissues after the medical device is removed (e.g. coatings, lubricants), a more detailed biological evaluation is required (see ISO 10993-1 for further guidance).

4.3 Allowable limits

4.3.1 General

For each medical device, the maximum exposure of EO and ECH to patients shall not exceed the AL using Formulae (1) and (2) based on the defaults in Table 1 for any of the applicable exposure categories (see 4.2). Alternative limits may be calculated based on risk assessment that accounts for device usage and patient

population. The procedure that was used to establish the tolerable intake (TI) is described in Annex D for EO and in Annex E for ECH.

Prolonged exposure devices carry additional limits for the first 24 h exposure period and, in the case of the long-term exposure devices, for the first 24 h period and the first 30 d period. These constraints set limits on the amount of EO and ECH that can be delivered to the patient during these early time periods.

The CEF uses a default value of 0,2 based on five devices used simultaneously. If data are available on the number of devices used at one time, for example, in multi-device systems, convenience kits, long-term exposure devices, then the default CEF of 0,2 may be modified (see 4.4.6, A.8 and D.6.1 for further justification).

The tolerable exposure (TE) shall be calculated based on the TI multiplied by body mass (m_b) and CEF.

$$TE = TI \times m_b \times CEF \quad (1)$$

where

TE is the tolerable exposure in mg/d;

TI is the tolerable intake in mg/kg/d;

m_b is the body mass in kg;

CEF is the concomitant exposure factor.

The AL shall be determined based on tolerable exposure multiplied by the days in the category unless an alternative limit can be justified. The lowest patient population mass, based on the anticipated exposure duration for the device, shall be used, see Clause C.4. The application of higher body masses associated for these sub population age groups, or lower body masses associated with younger sub population age groups, shall be justified and documented. See example in Clause K.11.

$$AL = TE \times \left(\frac{D}{N} \right) \quad (2)$$

where

AL is the allowable limit in milligrams per device;

D is the number of days of the medical device exposure duration in d;

N is the number of devices used during this duration.

When the number of medical devices does not apply (e.g. the largest surface area of device which can be in contact with the body is not the same as the surface area of the device which is tested, or powder or liquid device), the “number of devices used during this duration” in Formula (2) shall be replaced by “the quantity of devices used during this duration”. The quantity should be expressed in cm^2, g or ml as appropriate considering the form of the device and the AL shall be expressed in mg/cm^2, mg/g or mg/ml respectively. The definition of AL shall be modified accordingly.

NOTE The number of devices (N) considered in Formula (2) is not related to the CEF, but number subject devices during the exposure duration used for a treatment or therapy, which can be 1 or more.

The intended population shall be documented.

Table 1 — TE and AL default values for Formulae (1) and (2)

Exposure category	TI for EO mg/kg/d	TI for ECH mg/kg/d	CEF default	Anticipated exposure duration, *D* d
limited	0,3	0,64	0,2	≤ 1
prolonged	0,3	0,27		≤ 30
long-term	0,02	0,029		≤ 25 000 d for adults (>16 y) OR ≤ 5 840 d for paediatric (0 to 16 y) AND ≤ 19 160 d for adult (> 16 y)
For medical devices used in a paediatric sub population age group, the factors to consider in the selection of fewer days (i.e. < 5 840 d) are described in Clause C.4 and D.6.8.				

4.3.2 Limited exposure devices

The AL for limited exposure devices are based on the TI value of 0,3 mg/kg/d for EO, 0,64 mg/kg/d for ECH, with *CEF* = 0,2 (default) as established in Clause D.4 and Clause E.5 respectively. The total EO and ECH cumulative exposure estimate obtained by simulated-use or exhaustive extraction shall be compared to the limited exposure AL, unless otherwise justified. Alternatively, an assessment targeted to evaluate toxicological risk associated with exposure to EO or ECH from the actual anticipated exposure duration of a specific device as well as more clinically relevant details for the device and indication can be used to determine appropriate allowable limits using Formulae (3) to (5).

$$AL = TI \times m_b \times CEF \times \left(\frac{D}{N}\right) \tag{3}$$

EXAMPLE

$$AL_{EO} = 0{,}3 \times m_b \times 0{,}2 \times \left(\frac{D}{N}\right) \tag{4}$$

where

0,3 is the limited exposure tolerable intake value in mg/kg/d;

m_b is the body mass of anticipated patient population in kg;

0,2 is the default concomitant exposure factor;

D is the anticipated exposure duration up to 1 d;

N is the number of devices used during this duration.

$$AL_{ECH} = 0{,}64 \times m_b \times 0{,}2 \times \left(\frac{D}{N}\right) \tag{5}$$

where

0,64 is the limited exposure tolerable intake value in mg/kg/d;

m_b is the body mass of anticipated patient population in kg;

0,2 is the default concomitant exposure factor;

D is the anticipated exposure duration up to 1 d;

N is the number of devices used during this duration.

Examples of AL calculations can be found in Annex K.

4.3.3 Prolonged exposure devices

The AL for prolonged exposure devices are based on TI value of 0,3 mg/kg/d for EO, 0,27 mg/kg/d for ECH, body mass m_b, and with CEF equal to 0,2 (default) as established in Clause D.4 and Clause E.5. The cumulative exposure estimate obtained by simulated use extraction or exhaustive extraction shall be compared to the applicable prolonged duration limit for up to 30 d, unless otherwise justified. Alternatively, an assessment targeted to evaluate toxicological risk associated with exposure to EO or ECH from the actual anticipated exposure duration of a specific device as well as more clinically relevant details for the device and indication can be used to determine appropriate allowable limits using Formulae (6) and (7).

$$AL_{EO} = 0,3 \times m_b \times 0,2 \times \left(\frac{D}{N}\right) \quad (6)$$

where

0,3 is the prolonged exposure tolerable intake value in mg/kg/d;

m_b is the body mass of anticipated patient population in kg;

0,2 is the default concomitant exposure factor;

D is the anticipated exposure duration up to 30 d;

N is the number of devices used during this duration.

$$AL_{ECH} = 0,27 \times m_b \times 0,2 \times \left(\frac{D}{N}\right) \quad (7)$$

where

0,27 is the prolonged exposure tolerable intake value in mg/kg/d;

m_b is the body mass of anticipated patient population in kg;

0,2 is the default concomitant exposure factor;

D is the anticipated exposure duration up to 30 d;

N is the number of devices used during this duration.

Prolonged exposure devices shall meet limited exposure AL in 4.3.2 during the first 24 h period. Examples of these calculations can be found in Annex K.

4.3.4 Long-term exposure devices

4.3.4.1 Ethylene oxide

For EO, the tolerable exposure for long-term use devices is based on TI value of 0,02 mg/kg/d. The AL is determined by multiplying the TE with the number of days of anticipated exposure from a single or repeated use of a device for general populations (adults). Alternatively, an assessment targeted to evaluate toxicological risk associated with exposure to EO or ECH from the actual anticipated exposure duration of a specific device as well as more clinically relevant details for the device and indication can be used to determine appropriate allowable limits using Formulae (8) and (9).

$$TE_{\text{Adult EO}} = TI \times m_b \times CEF = 0,02 \times 60 \times 0,2 = 0,24 \text{ mg/d} \quad (8)$$

$$AL_{\text{Adult EO}} = 0{,}24 \times \left(\frac{D}{N} \right) \quad (9)$$

where

0,02 is the long-term exposure tolerable intake value in mg/kg/d;

m_b is the body mass of anticipated patient population in kg;

0,2 is the default concomitant exposure factor;

D is the anticipated exposure duration up to 19 160 d;

N is the number of devices used during this duration.

For devices to be used for paediatric patients during a specific time frame (age), the TE value can be calculated based upon the values given in Table D.9. For a device intended to be used throughout paediatric time frame (age 0 to 16 y) and through adulthood, the TE value is 0,11 mg/d, see D.6.8. The cumulative exposure estimate shall be compared to the applicable AL, unless otherwise justified.

$$TE_{\text{Paediatric EO}} = TI \times m_b \times CEF \quad (10)$$

$$AL_{\text{Paediatric EO}} = TE \times \left(\frac{D}{N} \right) \quad (11)$$

Long-term exposure devices shall also meet limited and prolonged exposure AL in 4.3.2 and 4.3.3 during the first 24 h period and the first 30 d, respectively. Examples of AL calculations can be found in Annex K.

4.3.4.2 Ethylene chlorohydrin

For ECH, the tolerable exposure for long-term use devices is based on TI value of 0,029 mg/kg/d. The AL is determined by multiplying the TE with the number of days of anticipated exposure from a single or repeated use of a device for general populations (adults) based on the anticipated exposure duration. Alternatively, an assessment targeted to evaluate toxicological risk associated with exposure to EO or ECH from the actual anticipated exposure duration of a specific device as well as more clinically relevant details for the device and indication can be used to determine appropriate allowable limits using Formulae (12) and (13).

$$TE_{\text{Adult ECH}} = 0{,}029 \times 60 \times 0{,}2 = 0{,}35 \text{ mg/d} \quad (12)$$

where

0,029 is the long-term exposure tolerable intake value in mg/kg/d;

m_b is the body mass of anticipated patient population in kg;

0,2 is the default concomitant exposure factor;

D is the anticipated exposure duration up to 19 160 d;

N is the number of devices used during this duration.

$$AL_{\text{Adult ECH}} = TE \times \left(\frac{D}{N} \right) \quad (13)$$

For devices to be used for paediatric patients during a specific time frame, the TE value can be calculated based upon the values given in Table E.5. For a device intended to be used for throughout the paediatric time

frame (age 0 to 16 y) and through adulthood the TE value is 0,16 mg/d, see E.4.2.3. The cumulative exposure estimate shall be compared to the applicable AL, unless otherwise justified.

$$TE_{\text{Paediatric ECH}} = TI \times m_{\text{b}} \times CEF \quad (14)$$

$$AL_{\text{Paediatric ECH}} = TE \times \left(\frac{D}{N} \right) \quad (15)$$

Long-term exposure devices shall also meet limited and prolonged exposure AL in 4.3.2 and 4.3.3 during the first 24 h period and the first 30 d, respectively.

4.3.5 Special situations

4.3.5.1 General

For certain medical devices, the default AL for EO and ECH are not appropriate. The rationale for special situation EO and ECH limits that are at variance with the general requirements are in Annex C. When special situations apply to special populations, the limits presented in 4.3.5.2 through 4.3.5.8 shall be adjusted, see Clause C.4. Examples of limits for special populations are provided in Table K.6.

The previous versions of this standard included special situation exposure limits based on 70 kg adult population. From a toxicological point of view these values are not significantly different from the values calculated in the current edition of this document (for unisex adults) and thus, these changes in ALs shall not trigger re-evaluating product that met the limits of the previous edition of this document, see 4.1 for additional requirements.

4.3.5.2 Ophthalmic implants with long-term (>30 d) tissue contact shall not exceed EO default specifications of 0,5 µg/device/d, or 1,25 µg/device and ECH default specifications of 2 µg/device/d or 5 µg/device. The EO and ECH limits and specifications for devices with limited and prolonged exposure to ophthalmic tissues shall be justified and documented, see C.2.2.

4.3.5.3 For blood cell separators used in patient and donor blood collection, the limited exposure limit for adults is 9 mg of EO and 19 mg of ECH, see C.2.3. Prolonged and long-term EO and ECH limits apply, see 4.3.3 and 4.3.4.

4.3.5.4 For extracorporeal blood purification devices, the limited exposure EO and ECH limits are 4,6 milligrams per device. The EO and ECH limits for prolonged and long-term may be exceeded, see C.2.6.

4.3.5.5 For peritoneal dialysis blood purification devices, the dose of EO shall not exceed 3,6 milligrams per device and ECH shall not exceed 3,2 milligrams per device during any 24 h period or 90 mg for 30 d. The EO limits for a long-term may be exceeded, see C.2.7.

4.3.5.6 For blood oxygenators and blood reservoirs, the limited exposure dose to the patient shall not exceed 54 mg of EO and 38 mg of ECH. The prolonged exposure dose of residuals shall not exceed 108 mg of EO and 97 mg of ECH, see C.2.4. The default long-term exposure limits apply, see 4.3.4.

4.3.5.7 For devices used in cardiopulmonary bypass procedures (other than blood oxygenators and blood reservoirs), the limited exposure limits shall be 18 mg for EO and 38 mg for ECH, see C.2.5. Prolonged and long-term EO and ECH limits apply, see 4.3.3 and 4.3.4.

4.3.5.8 For any device, such as drapes, that are intended to have loose contact with only intact skin, only the TCL limits apply or the device shall be non-irritating as specified in ISO 10993-23, see C.2.8 and 4.3.6.

4.3.6 Tolerable contact level

4.3.6.1 Overview

The TCL is designed to ensure that direct tissue-contacting devices (excluding circulating blood) do not cause local irritation. The TCL is based on the amount of EO and ECH per unit surface area of a device. Except in special situations (4.3.5), these limits shall be met in addition to the limits in 4.3.2. The TCL is expressed in units of micrograms per square centimetre for EO and milligrams per square centimetre for ECH. The unit of square centimetre represents the surface area of the device. Additional details on the TCL can be found in Annex D for EO and in Annex E for ECH. The TCL is based on the amount of EO and ECH measured during the limited exposure period per unit surface area of a device.

NOTE As TCL is based on amount of EO or ECH per unit surface area, adjustments for special populations (e.g. paediatrics) are not required.

4.3.6.2 Tolerable contact level for EO

The TCL_{EO} value shall not exceed 10 µg/cm^2 for the duration of use up to 24 h, or the device shall be non-irritating or negligible irritation as specified in ISO 10993-23, see A.3.10.

4.3.6.3 Tolerable contact level for ECH

The TCL_{ECH} value shall not exceed 5 mg/cm^2 for the duration of use up to 24 h, or the device shall be non-irritating or negligible irritation as specified in ISO 10993-23, see A.3.10.

4.4 Determination of EO and ECH residuals

4.4.1 Procedure

The procedure to determine conformity with AL (see 4.3) consists of extracting the residual from samples, determining the amount of residual, determining the contact surface area of the device, and analysing and interpreting the data. Additional guidance on evaluating residuals is provided in Annex A, Annex B, Annex C and Annex J.

4.4.2 Test method validation

A valid method of extraction and measurement shall be used to determine the amount of EO and, where necessary, ECH delivered to the patient. EO and ECH residuals analysis is normally conducted by gas chromatography (GC), see Annex H and Annex I. If EO and ECH residuals analysis is done using GC, the requirements of Annex G shall be met. Minimum requirements for such validation include system suitability, demonstration of accuracy, precision (repeatability, intermediate precision and reproducibility), specificity (sensitivity and selectivity), detection and quantification limits, linearity and range. Validation in accordance with the ICH Harmonized Tripartite Guideline[209] is sufficient to meet these minimum requirements.

The guiding principle in selecting appropriate extraction methods (see Annex A) for the quantitative determination of EO and ECH is the evaluation of the cumulative dose to the patient in order to show conformity with the requirements set out in 4.3. Where residuals are shown to be within the requirements for products tested by exhaustive extraction, there is no need to further challenge the device by simulated-use extraction, provided that all applicable limits in 4.3 are met. If ECH is not detected based on the results of analyses performed using the methods described in either J.5.7 or J.5.8, then no further testing for ECH is required.

When exhaustive extraction is used for prolonged or long-term exposure to a medical device, particular attention shall be paid to the limits expressed for the limited and prolonged exposure periods.

4.4.3 Product sampling

Samples to be used for residual analysis shall be representative of the finished product as presented to the sterilization process and consideration shall be given to worst-case sterilization conditions (e.g. allowed

double or triple sterilization, minimum aeration time). When selecting samples, consideration shall be given to the many factors described in Annex A and Annex B. Since these factors influence the initial levels of residuals in device components and the rate of residual dissipation, they shall also be considered when samples are retrieved from a processed load and sent to the laboratory for analysis.

4.4.4 Sample/fluid ratios

The volume of fluid used to extract residuals from devices, or representative sections of them, shall be sufficient to maximize extraction efficiency while maintaining detection sensitivity, see Annex A and Annex J for more information on product extraction.

4.4.5 Product extraction

The aim of product extraction is to determine the worst-case amount that can be delivered to the patient in actual use of the device: on a daily basis for limited exposure items; on a daily and up to monthly basis for prolonged exposure items; on a daily, monthly, and cumulatively up to a lifetime basis for long-term exposure items. When possible, sample preparation shall be performed in accordance with any applicable instructions for use. The worst-case extraction time shall be justified and documented.

EO-sterilization residuals in medical devices can be determined by simulated-use extraction or exhaustive extraction. Only the components of the finished product with direct or indirect contact are relevant for testing. The choice of extraction method shall be based on the anticipated exposure duration of the device and the following criteria.

— The extraction method chosen shall represent the worst-case anticipated exposure duration of the product to the patient and shall not be solely based on the desire for 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 exposure to the device as described in 4.2 and 4.3.

Guidance on product extraction can be found in Annex A.

4.4.6 Multi-device systems

A multi-device system is a set of components in a packaging system that are assembled as a product and used together in clinical practice. Due to the potentially complex nature of a multi-device system, an assessment can be appropriate to determine if products should be tested individually or assembled. Additional guidance is provided in Clause A.8.

A convenience kit is comprised of procedure-related health care products in which each health care product is used independently in clinical practice. For convenience kits, the limits for the products may be applied independently using the appropriate CEF or the AL calculated for the entire kit. If the number of devices used is different from the default of five devices, then the CEF shall be adjusted to the number of devices in the kit. See Clause A.8.

5 Product release

5.1 General

Product is in conformity with this document when it meets the requirements for EO and ECH. For batch release of EO-sterilized product, data shall be documented to demonstrate conformity to the requirement. Subclauses 5.2, 5.3 and 5.4 provide methods for demonstrating conformity. Other methods may also be appropriate. If sufficient experimental data on residual diffusion kinetics are available, it may be possible to group devices into families based on similarity of materials, manufacturing processes, sterilization process or clinical use. The rationale or data that justifies this decision shall be documented.

It is also feasible to establish a worst-case master product representing different products, see Clause A.5. The selection of a worst-case master product device shall be documented and justified. The amount of EO

and ECH as well as the rate of dissipation should be considered when completing this comparison if the products are above the release limits. The product aeration concerns documented in Annex B should be considered.

5.2 Batch release of products

Product may be released based on a batch-by-batch basis if the results conform to this document and the data are obtained from testing carried out according to appropriate procedures delineated in Annex J and meet the requirements for EO and, if applicable, ECH set out in 4.3.

5.3 Release of products at specified minimum aeration time

Data collected at the same time point (aeration time) can be used to establish the minimum aeration time. Subsequent product may be released when data from three separate sterilization batches tested at the same time point (aeration time) were obtained from testing carried out according to appropriate procedures with a 95 % upper confidence limit which meets the requirements for EO and, if applicable, ECH set out in 4.3. Annex K provides worked examples of product release calculations.

5.4 Procedure for product release using residual dissipation curves

Dissipation curves are used to estimate the post-sterilization time required for products or families of products. Products shall be released to the marketplace in accordance with predetermined post-sterilization times and conditions established by experimental dissipation curves so that the target EO residual levels for the device, as set out in 4.3, are ensured. Annex K provides worked examples of product release calculations.

Dissipation of EO from most materials and devices follows first-order kinetics, i.e. (log [EO])∝(time after sterilization). A plot of the 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 residual. When the plot of the logarithm of the experimentally determined EO concentration against time after sterilization is determined not linear, or results are below detection limits, alternative methods may be applied, such as batch release testing (see 5.2), the specified minimum aeration time (see 5.3) or the application of non-linear regressions analysis. The use of non-linear regressions analysis shall be documented and justified.

Regression analysis of pooled data from sufficient time points (minimum of 2) from at least three sterilization batches of the same product aerated under the same conditions to establish the nature of the dissipation curve will enable product to be released at the calculated upper 95 % prediction limit, L_p, for the allowed residual limit for the product. Time-concentration curves for devices made from combinations of dissimilar materials do not always fit this simple pattern over the entire range and can require different handling.

Formulae (16) and (17) provide the calculations for the prediction limit, L_p.

$$x_o = \frac{y_o - a}{b} \tag{16}$$

$$L_p = x_o + t_\alpha \times \sqrt{\frac{(S_\alpha)^2}{b^2} \times \left[1 + \frac{1}{n} + \frac{(y_o - y_\mu)^2}{b^2 \times \sum (x_i - x_\mu)^2}\right]} \quad (17)$$

where

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

y_o is the logarithmic value of the EO limit;

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

b is the slope of the regression line;

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

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

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

y_μ is the average of logarithmic EO values;

n is the number of values;

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

x_μ is the average of the times after sterilization;

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

NOTE 1 The 95 % prediction limit does not always apply to ECH as at atmospheric pressure ECH remains in liquid phase between −67 °C and 129 °C.

NOTE 2 Log_{10} or natural log (ln) can be used with the same outcome. However, the chosen logarithm must be consistently used within a set of calculations.

6 Adoption of products into established aeration family

An aeration family is developed by establishing different products or product families that can be included in the same cycle with the minimum aeration conditions established by the most challenging device for removal of EO residuals. A product may be added to an aeration family if deemed equivalent to or a lesser challenge than an existing qualified product. A technical review shall be performed comparing the candidate product with the existing qualified product used to demonstrate conformity to the AL. This review may include evaluation of characteristics noted in 5.1 (residual data) and Clause A.6. The outcome of the technical review, including the rationale for decisions reached, shall be documented.

7 Change evaluation

Changes to product, packaging, anticipated exposure duration, patient population(s), number of devices used or sterilization process shall be evaluated for their potential impact on residuals and the results documented. When this assessment indicates a potential increase in the level of residual EO or ECH, data to confirm conformity shall be obtained to ensure continued product acceptability. When this assessment indicates a decrease in the level of residual EO or ECH, consideration can be given to qualifying a new minimum aeration time.

Annex A
(informative)

Guidance for the application of this document for the determination of EO and ECH residuals in medical devices

A.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for implementation of the requirements by providing explanations and acceptable methods for achieving conformity with the specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the requirements in this document.

A.2 Background

When EO and ECH residual limits cannot be met within a required timeframe, then the risk of harm should be estimated and evaluated according to ISO 10993-1 and ISO 14971. If the risk of harm is estimated to be at an unacceptable level, then the risk should be controlled, unless risk control is not practical. When risk control is not practical, benefit-risk analysis should be applied. If there are device-specific standards that address the risk analysis (e.g. ISO 11979-5), the recommendations in the device-specific guidance or standard should be followed.

This guidance represents a practical means of applying the document to different products based on factors such as nature of exposure, duration of exposure, frequency of use, special situations of use (e.g. as cited in 4.3.5) and product size.

Extraction conditions for the determination of residual EO are given in Annex A and the requirements for determining EO and, if applicable, ECH residuals, are found in 4.4. Analytical procedures are described in Annex G, Annex H, Annex I and Annex J and appropriate simulated-use extraction procedure is described in Clause A.4. This enables users to develop and document the rationale for an appropriate simulated-use extraction procedure for their EO-sterilized products.

The analytical laboratory should work with the device manufacturer to demonstrate that the simulated-use extraction is carried out under conditions that provide the greatest challenge based on the anticipated exposure duration (i.e. worst-case). Product use simulation should be carried out assuming that the device is assigned to the most stringent category probable for the cumulative duration of exposure and temperature of exposure.

A.3 Guidance

A.3.1 The use of alternative materials and sterilization methods should have been considered during product development and design with the aim of minimizing exposure to residuals. The rationale and basis for this decision should be documented. Figure A.1 assists with the process of evaluating a device.

A.3.2 If the device has no patient or user contact, such as in vitro diagnostic devices, back table covers, Mayo stand covers, device handles, etc., this document does not apply.

NOTE Employee exposure limitations can be required by local occupational health regulations.

A.3.3 The patient population and the limits based on the highest potential residual EO and ECH exposure as a ratio to the limits should be established and determined. Typically, the lowest body mass patient special population has the highest potential exposure. If the device is in a special category, use the limits in 4.3.5. Additional guidance can be found in Annex C.

In some situations, a device specific TI/TE/AL can be appropriate, see Clause A.9.

A.3.4 The decision to test batch by batch at a specified minimum aeration time or to test via dissipation curve is dependent on several factors detailed in A.3.4.1 to A.3.4.3.

A.3.4.1 If a specified minimum aeration time has not yet been established, the use of batch release allows for the release of product while the required aeration time is established.

A.3.4.2 If an established minimum aeration time is anticipated to be sufficient to meet the release limit, testing at the specified minimum aeration time testing can be appropriate. End point testing has the disadvantage that, if the ALs are exceeded, additional testing is required, the amount of aeration time is still unknown, or the minimum established aeration time can be greater than required for the product in question.

A.3.4.3 The use of a dissipation curve, with data collected from a minimum of three different sterilization batches with data at different time points (minimum of one sample per batch) is useful for new products/processes to specify the minimum aeration time or when the existing minimum aeration time is not sufficient, see Clause K.5.

A.3.5 For prolonged and long-term exposure devices, exhaustive extraction using repeated 24 h simulated-use extractions (see A.4.2 and J.5) can be used for estimating limited, prolonged and long-term exposures, but does not extract all residuals from the device – only the clinically relevant levels. Exhaustive extraction using thermal extraction generates a total amount of EO that represents a cumulative exposure dose, and if these results meet limited exposure limits, prolonged and long-term exposure limits are also confirmed. When the final extraction in a simulated use study is greater than the long-term exposure allowable daily limit, the potential maximum long-term exposure should be addressed, which can require additional exhaustive extraction.

A.3.6 When it has been confirmed that a particular limit will result in a worst-case aeration time, it is possible to reduce subsequent testing to focus on just the variable that results in the worst-case aeration time. For example, if the data from the first sterilization batch for a long-term exposure device confirms that the 24 h EO TCL results result in a worst-case aeration time in dissipation curve calculations, testing of samples from subsequent sterilization batches can be performed via a single 24-hour extraction in lieu of an exhaustive extraction.

A.3.7 The decision tree in A.3.8 through A.3.10 is based on headspace or thermal exhaustive extraction first, followed by simulated-use exhaustive 24 h extractions, for prolonged and long-term devices. Exhaustive thermal extraction is not required in all circumstances.

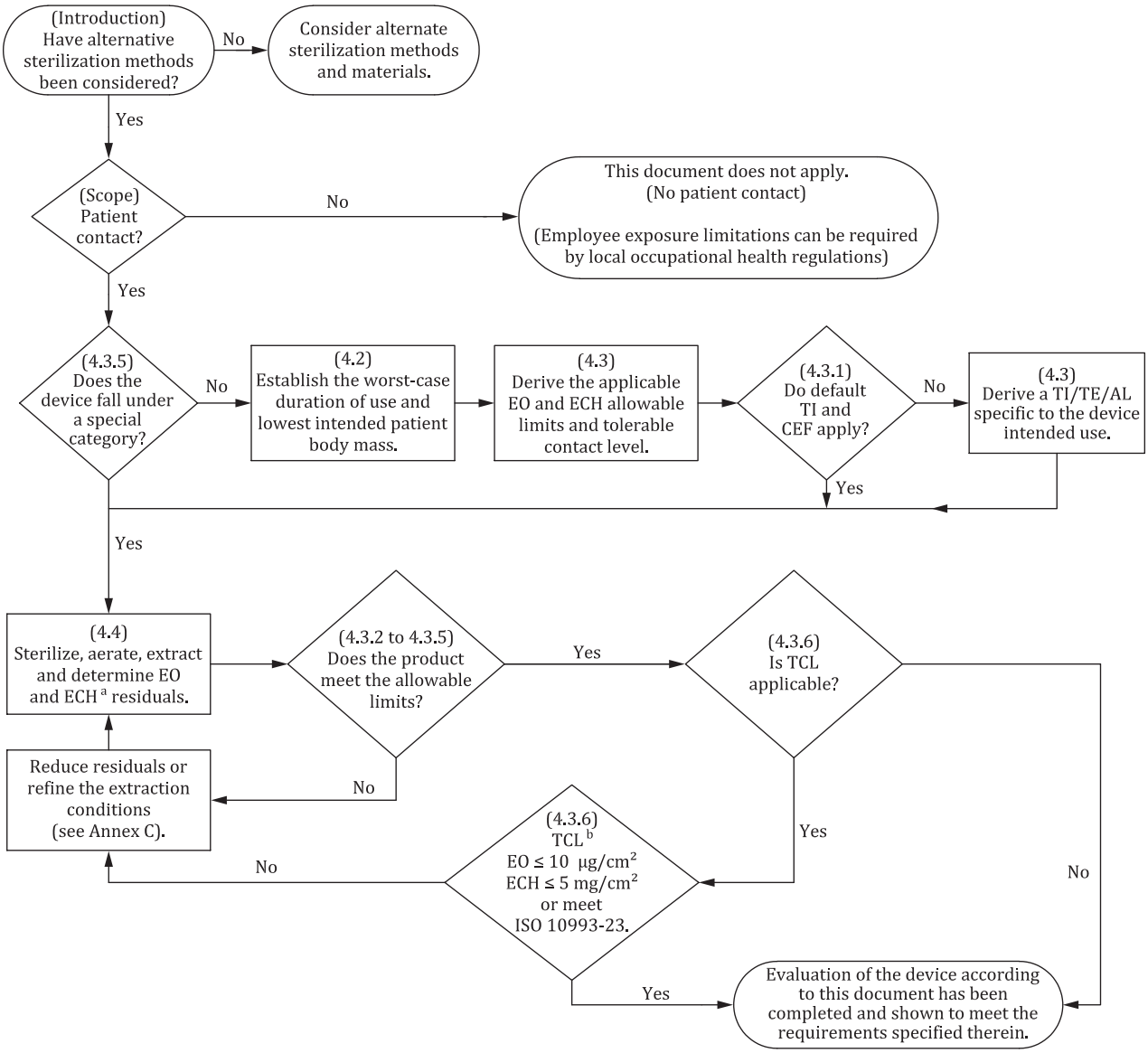
A.3.8 For long-term exposure devices (those contacting the patient for longer than 30 d to a lifetime), when headspace or thermal exhaustive extraction is used and the total measured EO and ECH are not more than the established limited exposure limit, the prolonged and long-term exposure limits are also confirmed and generating simulated-use extraction data is not needed (refer to A.3.10). When headspace or thermal exhaustive extraction is used and the total measured EO and ECH that represents the cumulative exposure dose exceeds either the established limited, prolonged or long-term exposure limits, then simulated-use extraction data should be generated (see A.4.2.2). Additional exhaustive extraction data at extended aeration time points can be generated. Exhaustive extraction is not always necessary for devices which are long-term exposure devices due to repeated use.

A.3.9 For prolonged exposure devices (those contacting the patient for more than 24 h up to 30 d), when exhaustive extraction is used and the total measured EO and ECH that represents the cumulative

exposure dose are not more than the established limited exposure limits, prolonged exposure limits are also confirmed and generating simulated-use extraction data is not needed (refer to A.3.10). When exhaustive extraction is used and the total measured EO and ECH exceeds either the established limited or prolonged exposure limits, then simulated-use extraction data should be generated (see Clause A.4) or additional exhaustive extraction data at extended aeration time points can be generated. Exhaustive extraction is not always necessary for devices which are long-term exposure devices due to repeated use.

A.3.10 For the irritation testing/tolerable contact level, when EO or ECH are released from the device at a concentration that exceeds the applicable limit in 4.3.6, a demonstration that the medical device does not elicit irritation at the time of release may be used to address this biological endpoint accordance with ISO 10993-23. Care should be taken to minimize the potential off-gassing of EO between the end of the specified aeration period and irritation testing, see A.4.1.2. When the risk of exposure to EO or ECH is

sufficiently high that an irritation response is expected, the risk can be managed according to ISO 10993-1 and ISO 14971.



a When chlorine is used as, or part of, a manufacturing process or material and it is demonstrated that residual ECH is not detectable on the surface of the medical device made of only non-absorbent materials (e.g. glass or metal), routine testing for residual ECH can be unnecessary, see Clause 1, NOTE 2.

b TCL does not apply to ocular devices, see 4.3.5.2.

Figure A.1 — Flowchart to assist in understanding steps necessary to apply this document

A.4 Determining EO and ECH residuals

A.4.1 Sample handing

A.4.1.1 Sample retrieval

Samples should be removed from the load at the time(s) specified in the protocol for establishing aeration hold time. The residual extraction should be initiated as soon as possible after removal of the sample from the load, or the sample may be kept frozen for extraction later. If the samples are shipped to the laboratory,

the samples should remain frozen during shipping. Samples shipped for lot release testing can be shipped using conditions equivalent or worse-case to those of the pending load awaiting release on such results. Caution should be exercised when product samples are removed from the sterilization load soon after the sterilization process is completed due to the risk of employee exposure to EO off-gassing from the product loads.

Delayed removal of the product samples from the processed load or lengthy shipment to a laboratory or extended 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 should be carried out and include seasonal variation if applicable.

A.4.1.2 Sample handling after processing

Samples should be stored frozen (≤ 0 °C) when analysis is delayed. Samples should remain frozen during shipment or shipped on dry ice. Dry ice should remain in the shipping container throughout the shipment and be present when the package is opened in the laboratory (if used), which can require overnight delivery. As an alternative, samples can be extracted after the designated aeration interval and immediately sealed in a container that is impervious to EO, e.g. a polytetrafluoroethylene (PTFE)-lined, septum-capped vial. The headspace in the container should be less than 10 % of the total volume, and the extraction fluid shipped to the analytical laboratory for analysis. If the extraction fluid is water, then shipment should be done such that the fluid is kept at less than 10 °C until arrival. Testing should be carried out to measure hydrolysis of EO to EG.

A.4.1.3 Sampling considerations at the lab

Precautions should 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 B.2.5). In addition, analyst safety should be considered. Samples should remain with the product load until the day of analysis or until test samples are retrieved and immediately frozen. Samples should be processed promptly after removal from the freezer to prevent unintended off-gassing. Samples should be prepared according to any applicable pre-use instructions in the product labelling. Extractions should be started as soon as possible after the device has been removed from the packaging or pre-use preparations have been completed.

A.4.1.4 Sample “blank”

In some instances, sample matrix components with the same retention time as the residuals being determined can be detected, resulting in higher EO or ECH results. If the results do not follow first order dissipation kinetics or results are above the detection limit, a “blank” sample may be evaluated for the possible presence of such interferences by the extraction of a non-sterilized sample using the identical procedure being applied to the EO-sterilized samples. In the event of materials being extracted from such a “blank” with conflicting or overlapping retention times in the GC analysis, chromatographic conditions can be modified to separate the interfering peak from the analyte peak or an alternative analytical procedure can be used.

A.4.1.5 Sample/fluid ratios

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

For example, worst-case anticipated exposure duration of many blood-contacting and parenteral devices can be simulated by filling or flushing the blood or fluid path components with water (whichever is appropriate) considering worst-case time and worst-case temperature.

When a device is too large to be extracted in its entirety, it can be necessary to extract several representative portions of the device components in order to ensure confidence in the data derived. These representative portions may be selected in one of two ways. If several varied materials are used, the proportion of each component, as compared with the total sample mass, should parallel the ratio of that component to the total mass of the device being tested. An alternative method would be to select one of the components for testing, subsequent to an evaluation demonstrating that it represented the worst-case with regard to residual content.

A.4.2 Extraction methods

A.4.2.1 General

The dose of residuals that the patient receives from the use of EO-sterilized devices is the critical parameter in the regulation of EO-sterilization residuals. In order to assess this patient or user dose, extraction procedures are required which simulate normal product use. In some cases, this can be achieved by simply filling the product with water, whereas in other cases more complicated simulations including continuous fluid flow can be required. It is recognised that there is no need to simulate product use if the requirements for EO residuals are already met by determination of the total amount of residuals in the product by exhaustive extraction.

The guiding principle in selecting appropriate extraction methods for the determination of EO is the evaluation of the dose to the patient in order to show conformity with the requirements set out in this document, using simulated-use wherever possible. For devices in the prolonged exposure category, it is important to note that the device should also meet the residual requirements of the limited exposure category, and that devices in the long-term exposure category should also meet the residual requirements of the prolonged exposure and limited exposure categories, whichever extraction condition is used. Where residuals 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, see Table A.1.

Table A.1 — Suggested extraction conditions

Device exposure duration (see 4.2)		
Long-term exposure (> 30 d)	**Prolonged exposure (24 h to 30 d)**	**Limited exposure (< 24 h)**
exhaustive extraction	exhaustive extraction	simulated-use extraction

For single use devices used for several days, where repeated use of new devices would result in categorization as prolonged or long-term exposure a simulated-use extraction may be an appropriate alternative. When an exhaustive extraction procedure is specified, it can be impractical for large or complex devices. In such cases, it can be necessary to extract representative portions of the device, then extrapolate the results to the entire device.

A.4.2.2 Simulated-use extraction

A.4.2.2.1 General

Simulated-use aqueous extraction is the reference method in that it is the only method that can produce a cumulative exposure dose that is directly comparable to the limits specified in 4.3. These limits are expressed in terms of delivered worst-case dose of EO and ECH to patients. Simulated-use extraction should be carried out under conditions that represent worst-case medical device anticipated exposure duration. When blood is returned from the device to the patient, it should be assumed that any EO residuals will remain in the body.

Because of its polarity and its ability to extract EO and ECH[92], water is commonly used for the recovery of residual EO and ECH in simulated-use extractions. Water is used for elution of EO residuals from the sample rather than to dissolve the sample material itself. If the intent is to simulate product use by filling the device, the device should be filled so as to eliminate any air pockets. Purified water is typically used for recovery of residuals (e.g. by distillation, reverse osmosis, deionization) and should not be chlorinated to prevent the conversion of EO to ECH after extraction.

For medical devices such as respirators, respirators filtration systems and ventilators, where contact from sterilization residuals can occur when gas or air is passed through the device and into the patient, a gaseous (air) extraction of the device may adequately simulate residual exposure if the gaseous extraction technique is first studied and sufficiently justified. It should be noted that ECH is not volatile in air at room temperature (ECH boiling point 129 °C).

A.4.2.2.2 Extraction time and temperature

Samples should be extracted for a time equivalent to or exceeding the maximum time for single use to provide the greatest realistic simulated challenge. The extraction time should not be less than 1 h. Devices that are used at body temperature should be extracted at 37 °C ± 2 °C (body temperature); extract devices used at ambient temperature (e.g. hypodermic syringes) at 20 °C to 25 °C (room temperature). Extraction temperature lower than 37 °C should be justified and documented. For prolonged and long-term use devices, exhaustive extraction or repeated simulated use extractions should be used to estimate the total amount of residuals, see Clause A.3 and J.5.2.

NOTE 1 Examples of heated medical devices include intravenous fluid warmers and atrial fibrillation ablation catheters.

NOTE 2 The amounts of EO or ECH extracted by simulating intended product use are not necessarily the same as the total product residual content.

A.4.2.2.3 Pre-treatment conditions

Where pre-treatment of the device is required prior to use, this pre-treatment may be conducted before the device is extracted. Where the device is filled for extraction, ensure that the process is done in a manner that eliminates entrained air pockets. Extract the device with water at the temperature and for the time established. Where the use of the device involves circulation of fluids (e.g. blood, dialyser fluid), extract the device using water to simulate the fluid's circulating in a manner consistent with product use. Document the rationale for the conditions established. Portions of the device that are not in contact with the body should be excluded, if possible. If portions of the device that do not contact the body cannot be excluded from testing, it can be appropriate to perform an assessment of the exposure.

If the assay is not performed immediately after extracting the medical device, the extract should be decanted from the sample and sealed in a vial that is impervious to EO, e.g. a PTFE-lined, septum-capped vial. The headspace in the vial of any standard solution or extract should be less than 10 % of the total volume. The extract can be stored in the refrigerator for several days (see Clause I.4). If high levels of ECH or EG are detected, the analyst can evaluate the possibility of EO conversion to EG or ECH when extracting the sample with water.

A.4.2.3 Exhaustive extraction

A.4.2.3.1 General

Exhaustive extraction is intended to obtain a total quantity of EO or ECH that represents the cumulative exposure dose for the specified time-period.

A.4.2.3.2 EO and ECH simulated use exhaustive extraction

Exhaustive extraction methods include the concept of repeated simulated-use extractions (generally 24 h each) that continue until the last extraction step performed produces a yield of the analyte which is less than 10 % of the yield of the analyte in the first extraction of the sample, see Annex J. The concept of repeated extractions can fail when the yield of the first extraction is very small, as in the case of a device with little residual 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. This method has the benefit of providing both the limited, prolonged and long-term residual data points.

When the medical device is long-term contacting and the simulated use exhaustive extraction duration is less than 30 days, relevance of the total measured EO/ECH to long-term cumulative exposure should be addressed (e.g. an elevated extraction temperature that accelerates release is used or evidence that late-bolus release is not expected to occur). Otherwise, the generation of a total measured EO/ECH that represents cumulative long-term exposure dose should be addressed according to A.4.2.3.3 and A.4.2.3.4.

NOTE Extraction at a clinically relevant temperature for a duration that is less than the entire duration that the medical device is in contact with the tissue does not necessarily generate a cumulative long-term exposure dose that represents the entire duration of medical device contact (e.g. the medical device is comprised of materials that retain EO/ECH and the rate of EO/ECH release increases after 30 d of contact with tissue).

A.4.2.3.3 EO headspace exhaustive extraction

Extraction procedures include thermal extraction followed by headspace gas analysis, non-aqueous solvent extraction procedures, with either headspace gas analysis of the solvent extract, chromatography of the solvent extract, or preparation of the bromohydrin derivative of EO which is determined using a more sensitive GC detector such as an electron capture detector (see Annex J).

When exhaustive extraction does not generate clinically relevant exposure data (e.g. for limited and prolonged exposure) and all applicable limits in 4.3 are met and residuals are demonstrated to be within the requirements for products tested by exhaustive extraction, there is no need to further challenge the device by simulated-use extraction.

For solvent extraction procedures, the selection of a suitable extraction vehicle 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 J.5.4 and such procedures can separate EO from co-extracted interfering chemicals from the sample matrix. Several extraction fluids have been evaluated through interlaboratory comparison testing[112][113][114].

When conducted as described, headspace methods are considered exhaustive since they are designed to generate a total quantity of residual EO that is present in the sample. However, headspace methods can be unfeasible or impractical for intact testing of large or complex devices. The analyst should exercise caution in the execution of exhaustive extraction methods when evaluating residual levels in high durometer polymer materials such as poly-(methylmethacrylate) to ensure total recovery of EO[216].

A.4.2.3.4 ECH exhaustive extraction

Water is typically used to extract residual ECH from medical devices using methods similar to those described for determining residual EO. Extract for 24 h at the appropriate temperature, repeating extractions until less than 10 % of the initial amount is recovered, see J.5.8.

A.5 Grouping of devices

Given sufficient experimental evidence on residual diffusion kinetics (e.g. the rate of EO gas dissipation from the packaging for the range of given devices), it can be possible to group devices based on similarities of materials and clinical use.

Devices of similar design but different sizes may be grouped and the worst-case may be selected for testing as representative of the group. The comparison of results from different device groups can establish a worst-case product device that can be used for reverification of conformity. Care should be taken when comparing EO residuals results to account for both EO levels and the rate of dissipation if results are above the established limits resulting in a device with lower initial levels requiring more aeration time to reach AL.

A.6 Worst-case product

It is feasible to establish a worst-case master test article representing different devices or families. Comparable EO residual test data including the dissipation kinetics should be considered, i.e. a medical

device with initial higher levels of residuals can dissipate faster based on the materials of construction or packaging resulting in a device with lower levels of residuals after sterilization to require more aeration to reach AL. Device surface area, device mass and device materials can be relevant considerations. A worst-case device can be a smaller device with a special population AL (i.e. lowest body mass patient) when considering the residual results as a percentage of the AL. Alternatively, it is also acceptable to test a product of a smaller size and extrapolate what the results would be for the product of the largest size (e.g. based on surface area, volume or mass).

A.7 Adoption of products into established aeration family

Products may be grouped into families (see ISO 11135:2014, D.12.5.11.1). For EO residuals, products can be grouped into families based on device characteristics that allow them to be aerated to safe residual levels using the same specified aeration conditions. Candidate product may be adopted into an existing aeration family in conformity with this document by a documented review of relevant characteristics or by qualification studies on samples retrieved after worst-case aeration (e.g. minimum time and temperature).

A.8 Multi-device systems (kits and trays) and convenience kits

For a multi-device system, an understanding of the different components, how they are used and how they are assembled into a product is important. The components that do not have patient or user contact, do not need to be tested. Due to the potentially complex nature of a multi-device system or convenience kit, a risk assessment can be useful to determine how to apply the limits. Multi-device systems or kits may be tested as a single assembled device or each device may be tested individually. Testing components individually would allow for a worst-case device to be established. Testing an assembled product can allow for the application of a single AL with adjustment to the CEF, if appropriate.

For a convenience kit, understanding the use of each product in clinical practice is important. If products can be placed in multiple exposure categories (i.e. limited, prolonged, long-term), then the products in the kit should be grouped according to their exposure category and limits should be applied to the each of those products for each category using the appropriate CEF.

A.9 Risk assessment

A toxicological risk assessment may be useful when the EO/ECH cumulative residual quantity exceeds the AL. ISO 10993-17 provides a process, requirements, criteria and guidance that device manufacturers can use for conducting a toxicological risk assessment.

Medical device anticipated exposure duration can indicate the need for medical device specific CEF values that differ from 0,2 if more or fewer devices are used.

EXAMPLE The default CEF is adjusted to 0,25 when the number of medical devices used concomitantly is 4.

The medical device anticipated exposure duration can also indicate the need for refinement of the scope and extraction conditions that are more representative of its anticipated exposure duration (i.e. in terms of nature of exposure and exposure duration).

For example, it can be deemed sufficient to extract only the inner surface of an administration set tubing which has no direct contact with the body where only the inner lumen of the tubing is in fluid/patient contact and not extracting the whole tubing with inner and outer surfaces which would most likely yield overestimated EO residuals regarding body contact.

In the case of a limited exposure device used for less than 24 h, the worst-case extraction time can be adjusted from the typical 24 h to the longest clinically relevant exposure time. The risk assessment should not assume that the risk will be acceptable, rather, risk assessment can indicate the need for further risk evaluation or risk control, which includes benefit-risk analysis if risk control is not practical.

A.10 Data analysis and interpretation

A.10.1 Calculation of amount of residual extracted

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

The residual extracted by simulated use can be calculated with Formula (A.1):

$$M_d = \sum_1^n (C_e \times V_e) \quad \text{(A.1)}$$

The residual extracted by exhaustive extraction of representative portion(s) of a device can be calculated with Formula (A.2):

$$M_d = \sum_1^n (C_e \times V_e) \times \frac{m_d}{m_s} \quad \text{(A.2)}$$

where

M_d is the extract residual, in mg;

n is the number of extractions;

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

V_e is the extract volume, in ml;

m_d is the entire device mass, in g;

m_s is the mass of sample, in g.

NOTE $\frac{m_d}{m_s}$ only applies if a portion of the device is extracted.

A.10.2 Calculation of average delivered dose, M_{add}

For long-term exposure devices, the average delivered dose, M_{add}, in milligrams per day, is calculated with Formula (A.3):

$$M_{add} = \frac{M_d}{D} \quad \text{(A.3)}$$

where D is the anticipated exposure duration, which is a maximum of 5 840 d for 0 y to 16 y and 19 160 d for adulthood, totalling 25 000 d per lifetime.

For prolonged exposure devices, the average delivered dose, M_{add}, in milligrams per day, is calculated with Formula (A.4):

$$M_{add} = \frac{M_d}{D} \quad \text{(A.4)}$$

where

M_d is the extract residual, in mg;

D is the anticipated exposure duration, with a maximum of 30 d.

Annex B
(informative)

Factors influencing product residuals

B.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with the specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the requirements in this document.

B.2 Sterilization process considerations

B.2.1 General overview

Sterilization process parameters are specified in ISO 11135. However, to properly analyse residuals in EO sterilized devices, it is necessary to recognise those parameters that have an effect on residual content. An understanding of EO kinetics can 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) can preclude the necessity of analysing each item of the product line. The parameters in B.2.2 to B.2.9 can affect residual content and can allow analysis of one or more "worst-case" representatives.

B.2.2 Material composition

Materials vary considerably in their ability to absorb, retain and release EO. When conversion of EO to ECH is possible, two similar devices made of different materials are likely to have very different residual 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 can require a representative sample of both materials to ensure accurate analysis. Composition and size can be particularly important when considering the simulation of normal product use.

B.2.3 Packaging

Packaging materials vary widely in their abilities to allow penetration and dissipation of both EO gas and the other possible residuals, which can in turn affect EO and ECH residual levels. Packing density and the density of the sterilization container or pallet are other sources of variability. The total number of layers of packaging should also be considered as each layer can act as a barrier to aeration of EO from devices.

B.2.4 Ethylene oxide sterilization cycle

Process conditions under which the device is exposed to EO will affect the residual levels. These conditions include humidity, gas concentration, dwell time, temperature, type of cycle (deep or shallow vacuum), and number of post exposure washes. For processes with sufficient controls, the nominal routine processing parameters can be used, and the 95 % upper confidence limit of the residual results can be used to establish the acceptable aeration time. Review the need for additional considerations when upper tolerances for exposure time and EO inject pressure rise is greater than 10 % of nominal value, or higher, if justified.

B.2.5 Aeration

Residuals in devices can vary as a function of aeration temperature, fresh air intake, load density and configuration, air flow, loading pattern, surface area of products being aerated and aeration time. If the aeration room/chamber has not demonstrated consistency in air flow and temperature, cold spots in the chamber should be addressed when determining where to place residual samples, see B.2.6. Placement of samples in the worst-case load, pallet and location in the aeration room is best practice when validating aeration time and temperature.

When aeration is carried out at ambient temperature, consideration of data from sterilization loads taken from aeration or quarantine storage at different times of the year can be required.

When using ambient aeration and samples that are not frozen until testing, consideration should be given to seasonal variations in aeration rates when samples are stored under conditions which differ from the routine ambient aeration conditions. Under certain circumstances, which can best be determined by experience, it can 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 ambient aeration, if used.

NOTE Degassing behaviour over the whole room, over the pallet, recorded with seasonal variations of aeration, when validating aeration time and temperature can be forecast under consideration of recorded warehouse conditions affecting aeration time taken into account the variation of load density and configuration, air flow, loading pattern, surface area of products being aerated in a justification for the appropriateness of the used sterilization process.

B.2.6 Aeration location considerations

Residual levels on the product can be impacted by various factors including the temperature, air flow, and location on the pallet, and configuration of the pallet, e.g. chimney in the centre, shrink wrap. A rationale can be made to determine the most appropriate location for product sample placement based on an assessment of these factors. EO concentrations in the aeration area can retard off-gassing, especially when low AL are required for certain patient subpopulations.

B.2.7 Load configuration

Consideration of the load configuration used for residual qualification should take into account the product, packaging and pallet configuration. This load can be the same as the most challenging load used for the sterilization validation or a different load configuration. Samples for EO residual testing should be placed in the load that is worst-case for EO residual dissipation. Loads that vary greatly in density or volume can be assessed to determine which presents a greater challenge to EO dissipation since the overall temperature of the load, EO absorption and airflow patterns can impact residual levels.

B.2.8 Repeat sterilization

Re-sterilization of product and the presence of other EO-sterilized medical devices in adjacent areas should be considered when evaluating EO residuals. It is common to qualify products for repeat sterilization cycles. Studies suggest that EO residuals are not significantly impacted by multiple sterilizations.[217]

If the product is qualified for multiple sterilizations, the time between sterilization cycles should be considered during the qualification. Testing of a limited number of samples from a single batch is typically sufficient to demonstrate that EO and ECH residual levels after multiple sterilization exposures are comparable to samples exposed to a single sterilization exposure. However, if EO and ECH residuals appear to be higher, it can be necessary to test three batches after double or triple sterilization according to 5.3 or 5.4.

This time between cycles can reflect what would typically happen during routine reprocessing or the samples can be frozen between runs to represent worst-case off-gassing between cycles. Where repeat sterilization has been demonstrated for a worst-case product to have negligible impact on EO residuals, the qualification of EO residuals may be performed after one cycle, where appropriately justified.

B.2.9 Multiple processes or equipment

If two or more different EO sterilization cycles are approved for a product or product family, the product samples from each cycle should be evaluated for testing and a release time determined for each process. When cycle parameters only differ by EO gas exposure dwell time or EO gas concentration, testing on the cycle with the longest gas exposure or the highest gas concentration is sufficient to determine the aeration time for the different cycles.

If more than one cycle is to be approved for a product or product family and the processes differ by more than just the exposure period, gas concentration, equipment, and facility, then a worst-case cycle/process can be used if data exists demonstrating which cycle/process is worst-case.

If more than one chamber/aeration area is to be approved for a product or product family, product samples from each chamber should be taken for testing and a release time determined for each chamber/aeration area except for the following conditions:

— process equivalence is established, at which point it can be acceptable to choose a single chamber/aeration area for product sampling and testing;

— worst-case equipment is defined, allowing for use of only the worst-case chamber/aeration area for sampling and testing instead of sampling from all chambers/areas under consideration.

Annex C
(informative)

Rationale for the provisions of this document

C.1 General

C.1.1 The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with the specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the data requirements in this document.

C.1.2 This annex specifies the rationale for establishing AL for ethylene oxide sterilization residuals in medical devices based on duration of contact. This annex includes the basis for establishing limits for EO and ECH.

C.2 Rationale for special situations

C.2.1 General

There are certain circumstances, for example major surgery, where the life-saving nature of the therapy significantly alters the risk-benefit analysis. The AL given per device are based on risks and benefits associated with less critical circumstances. Therefore, ISO 10993-1 and ISO 14971 allow for device benefit alterations in AL on a case-by-case basis for a specific device and its use. In consequence, there is room for the derivation of limits specific to a device used in life-threatening situations where it is not possible to meet the general limits derived in this document. Similarly, there can also be a need to tighten limits when warranted by risks in specific situations.

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

C.2.2 Intraocular devices

The residue limits for intraocular lens are 0,5 µg of EO and 2,0 µg of ECH per lens per day, or (and) 1,25 µg of EO per lens and 5,0 µg of ECH per lens. These limits are not based on the long-term exposure 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 of EO per lens per day. This is necessary to prevent corneal endothelial cell damage due to EO exposure.

When EO residuals are controlled as specified here for intraocular devices, it is unlikely that significant amounts of ECH will be present. This can be untrue for intraocular devices made from viscoelastic materials that contain chlorine. In such cases, studies indicate that the level of ECH that results in ocular toxicity is about four times greater than the corresponding EO level[44][117][118][119]. This should be taken into account when evaluating the acceptability of ECH levels associated with these devices.

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

For blood cell separators, the maximum AL for EO is 9,0 milligrams per device and the maximum AL for ECH should not exceed 19 milligrams per device. These devices are used for apheresis collection.

In this case the default assumption of five devices used simultaneously appears somewhat conservative. If one were to assume only two devices as a reasonable worst-case, the CEF would increase from 0,2 to 0,5. This would raise the AL to 9 mg for EO.

For EO, the AL is:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,3 \times 60 \times 0,5 = 9,0 \text{ milligrams per device} \quad \text{(C.1)}$$

where

TI is the tolerable intake of 0,3 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,5 based on 2 devices;

D is the anticipated exposure duration of 1 d;

N is the number of devices used during this duration, which is 1.

For ECH, the AL is:

$$TE = TI \times m_b \times CEF \times \frac{D}{N} = 0,64 \times 60 \times 0,5 = 19 \text{ milligrams per device} \quad \text{(C.2)}$$

where

TI is the tolerable intake of 0,64 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,5 based on 2 devices;

D is the anticipated exposure duration of 1 d;

N is the number of devices used during this duration, which is 1.

C.2.4 Blood oxygenators and blood reservoirs

The limits for blood oxygenators and blood reservoirs are 54 mg of EO per device and 38 mg of ECH per device in a 24 h period. These devices are used in life saving operations such as open-heart surgery. Such procedures are used on individual patients no more than once or twice in a lifetime. Since these devices are used for a day or less, the limits are relaxed. Under such life-saving circumstances, a further fifteen-fold relaxation of the EO limit and a five-fold relaxation of the ECH limit is warranted. For prolonged exposure, the EO limit is 108 milligrams per device and the ECH limit is 97 milligrams per device, see D.6.6 and E.4.2.2.

For limited exposure to EO, the AL is:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,3 \times 60 \times 0,2 \times \frac{1}{1} = 3,6 \times 15 = 54 \text{ milligrams per device} \quad \text{(C.3)}$$

where

TI is the tolerable intake of 0,3 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,2 based on 5 devices;

D is the anticipated exposure duration of 1 d;

N is the number of devices used during this duration, which is 1.

For limited exposure to ECH, the AL is:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,64 \times 60 \times 0,2 \times \frac{1}{1} = 7,7 \times 5 = 38 \text{ milligrams per device} \quad \text{(C.4)}$$

where

TI is the tolerable intake of 0,64 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,2 based on 5 devices;

D is the anticipated exposure duration of 1 d;

N is the number of devices used during this duration, which is 1.

For prolonged exposure to EO, the AL is:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,3 \times 60 \times 0,2 \times \frac{30}{1} = 108 \text{ milligrams per device} \quad \text{(C.5)}$$

where

TI is the tolerable intake of 0,3 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,2 based on 5 devices;

D is the anticipated exposure duration of 30 d;

N is the number of devices used during this duration, which is 1.

For prolonged exposure to ECH, the AL is:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,27 \times 60 \times 0,2 \times \frac{30}{1} = 97 \text{ milligrams per device} \quad \text{(C.6)}$$

where

TI is the tolerable intake of 0,27 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,2 based on 5 devices;

D is the anticipated exposure duration of 30 d;

N is the number of devices used during this duration, which is 1.

C.2.5 Devices used in cardiopulmonary bypass procedures, extracorporeal carbon dioxide removal (ECCO2R), continuous renal replacement therapy (CRRT) or therapeutic plasma exchange (TPE)

The limit for devices used in cardiopulmonary bypass procedures or ECCO2R is 18 mg of EO per device and 38 mg of ECH per device in a 24 h period. These devices are used in life-saving operations such as open-heart surgery or acute hypercapnic respiratory failure. Integrated CRRT or TPE sets are used for several days, generally less than 30 d. Such procedures are used on individual patients no more than once or twice in a lifetime and the default CEF of 0,2 appears overly conservative. A CEF of 1,0 appears more reasonable and the AL would be 18 mg of EO and 38 mg of ECH. The default prolonged exposure limit applies.

For limited exposure to EO, the AL is:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,3 \times 60 \times 1,0 \times \frac{1}{1} = 18 \text{ milligrams per device} \quad \text{(C.7)}$$

where

TI is the tolerable intake of 0,3 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 1,0 based on 5 devices;

D is the anticipated exposure duration of 1 d;

N is the number of devices used during this duration, which is 1.

For limited exposure to ECH, the AL is:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,64 \times 60 \times 1,0 \times \frac{1}{1} = 38,4 \text{ milligrams per device} \quad \text{(C.8)}$$

where

TI is the tolerable intake of 0,64 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 1,0 based on 5 devices;

D is the anticipated exposure duration of 1 d;

N is the number of devices used during this duration, which is 1.

C.2.6 Extracorporeal blood purification devices — Haemodialysis

Extracorporeal blood purification devices are used in patients multiple times, often over many years. When setting the AL for these devices, consideration is given to the benefit derived from blood purification. The maximum allowable EO dose of 4,6 g for a lifetime (19 160 d) may be exceeded, provided that the AL for EO of 4,6 mg for each use is met. In addition, the allowable ECH dose for a lifetime may be exceeded, provided that the AL for ECH of 7,7 mg for each use is met. To exceed the lifetime dose of EO, a patient undergoing blood purification would need to be exposed to 3,6 mg of EO 13 times every month and such exposure would need to continue for 6,4 y. Similarly, ECH lifetime exposure can be exceeded after 9,3 y of use by end-stage renal disease patients. The life-saving nature of these devices warrant a relaxation of the limits.

For EO

— AL for EO of 4,6 mg for each use applies,

— the lifetime dose of 4,6 g may be exceeded, and

— it takes approximately 6,4 y [4,6 g / (4,6 mg × 13 × 12 months)] to reach the maximum allowable lifetime EO dose for the use of such devices.

For ECH

— AL for ECH of 4,6 mg for each use applies,

— the lifetime dose of 6,7 g may be exceeded, and

— it takes approximately 9,3 y [6,7 g / (4,6 mg × 13 × 12 months)] to reach the maximum allowable lifetime ECH dose for the use of such devices.

C.2.7 Other blood purification devices

In addition to extracorporeal blood purification using a dialyzer, other dialysis approaches are known, one of which is peritoneal dialysis. Peritoneal dialysis is a therapy that infuses and stores dialysate into the abdominal cavity surrounded by the peritoneum that covers the viscera and removes unnecessary waste products and water in the blood through the peritoneum. Like extracorporeal haemodialysis, peritoneal dialysis has been used clinically for a long time. Since the benefits of peritoneal dialysis are equivalent to the extracorporeal haemodialysis described in C.2.6, acceptable risks of peritoneal dialysis are also considered to be equivalent to extracorporeal haemodialysis. However, unlike extracorporeal haemodialysis, peritoneal dialysis performs multiple dialysate changes during a day. There are various usage forms among the peritoneal dialysis related devices such as a long-term indwelling port in the body, a connecting tube from the port to bags that change every week, and tubing sets that change every day, etc. Therefore, the AL for EO of the peritoneal dialysis related devices should be set in consideration of their usage number in one month. For a 60-kg adult and assuming a CEF = 0,2, the value for EO is 108 mg per 30 d which is the maximum

allowable value for the extracorporeal haemodialysis device. The life-saving nature of these devices warrant a relaxation of the limits.

For example, automated peritoneal dialysis (APD) is a method of performing peritoneal dialysis by automatically refluxing a fluid using a dedicated device during sleep. The maximum AL for each APD tubing set used on a patient was set by considering

— 30 such devices over each month, and

— 3,6 mg/d for EO (108 mg for 30 d) and 3,2 mg/d for ECH (97 mg for 30 d) as the maximum AL.

The maximum allowable EO dose of 4,6 g for a lifetime may be exceeded, provided that the AL for EO of 3,6 mg for each use is met. In addition, the maximum allowable ECH dose of 6,7 g for a lifetime may be exceeded, provided that the AL for ECH of 3,2 mg for each use is met.

For a patient undergoing blood purification, exposure can be up to 3,6 mg of EO thirty times every month and such exposure would need to continue for 3,5 y to exceed the 4,6 g lifetime dose of EO.

Similarly, ECH lifetime exposure would be exceeded after about 5 y of use by end-stage renal disease patients.

For EO

— for prolonged exposure:

$$AL = TI \times m_b \times CEF \times \frac{D}{N} = 0,3 \times 60 \times 0,2 \times \frac{30}{30} = 3,6 \text{ milligrams per device} \quad \text{(C.9)}$$

where

TI is the tolerable intake of 0,3 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,2;

D is the anticipated exposure duration of 30 d;

N is the number of devices used during this duration, which is 30;

— the lifetime dose of 4,6 g may be exceeded;

— it takes 4,6 g per 108 mg per month which is equal to 43 months or approximately 3,5 y to reach the maximum allowable lifetime EO dose for the use of such devices.

For ECH

— for prolonged exposure:

$$AL = TI \times m_b \times CEF \times 30 \times \frac{D}{N} = 0,27 \times 60 \times 0,2 \times \frac{30}{30} = 3,2 \text{ milligrams per device} \quad \text{(C.10)}$$

where

TI is the tolerable intake of 0,27 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,2;

D is the anticipated exposure duration of 30 d;

N is the number of devices used during this duration, which is 30;

— the maximum allowable dose of ECH from use of 30 APD tubing sets per month is 97 mg;

— the lifetime dose of 6,7 g may be exceeded.;

— it takes 6,7 g per 97 mg per month which is equal to 62 months or approximately 5,1 y to reach the maximum allowable lifetime ECH dose for the use of such devices.

C.2.8 Drapes and similar devices

Drapes and similar devices with loose contact with intact skin provide benefits to patients with minimal risk. Surgical drapes are used to minimize the spread of infective agents to and from the patient, thereby contributing to a reduction in post-operative infections. For such devices contacting intact skin, no systemic toxicity is anticipated as they are loosely fitting, a significant portion does not contact the patient, and therefore, the AL derived based on systemic toxicity data for EO and ECH would not apply. Patient safety should be adequately protected by meeting the TCL or irritation test requirements. The TCL values for EO and ECH are based on local toxic effects. Thus, the TCL values of 10 $\mu g/cm^2$ for EO and 5 mg/cm^2 for ECH or the device having negligible irritation when tested according to ISO 10993-23 are the appropriate AL for drapes that contact intact skin.

C.3 Rationale for toxicological risk assessment

The default limits established in this document do not necessarily address all situations (e.g. a novel device or new technology that retains high levels of EO but is not one of the special situations described in this document or if a manufacture is unsure if the device qualifies as a special situation). A risk assessment following the guidance in this document or ISO 10993-17 can address special situations not already covered in this document while still providing a sufficient margin of safety.

C.4 Rationale for body masses for other patient demographic groups

Devices that can be used in paediatric patients, and are not contra-indicated for adult use only, should consider the lowest patient population. The choice of patient population should be documented and justified.

The default body masses as:

— 70 kg for adult male;

— 60 kg for adults (unisex);

— 10 kg for children (> 1 y to ≤ 16 y of age);

— 3,5 kg for infants (< 1 y);

— 1,5 kg for very low birthweight infants; or

— 0,5 kg for very low birthweight neonates (e.g. preterm neonates).

Where applicable to the anticipated exposure duration, body masses associated with any patient demographic age groups not covered by the default body mass values listed above require citation of data source and justification of a conservative approach (e.g. citation of CDC body mass demographic tables).

The intended patient population (i.e. the range of patients for which the device was designed to treat excluding off-label and emergency use care) can be determined by the manufacturer in consultation with health care provider/clinical evaluation. Guidance on patient masses for special populations can be found in References [225], [245], [246] and [247].

When the medical devices intended to use is for a wide range of general patients, the use of infant body mass (3,5 kg) is sufficiently conservative for the calculation of AL for infants and the general population. When the medical device intended to use is for low birthweight neonates, the use of lower body mass can be appropriate in the calculation of AL for this subpopulation.

C.5 Non-absorbent materials

The following residuals are expected after sterilization with EO:

— residual EO, due to the presence of EO as sterilant, see Annex D;

— EG, due to the presence of water as part of the required humidification, but not at clinically relevant levels, see Annex F;

— ECH, when chlorine is part of product or packaging materials in the load, see Annex E.

Solid, non-porous materials, such as metals, metal alloys and glass do not absorb EO and its related residuals[228][229][230]. Routine testing of sterilization residuals for these (device) materials is not necessary, because residuals on the surface of such (device) materials exhaustively dissipate during flushing conditions of sterilization cycles (i.e. elevated temperatures of typically above 35 °C and multiple pressures changes to down below 500 mbar[1)]).

1) 1 bar = 0,1 MPa = 10^5 Pa; 1 MPa = 1 N/mm^2.

Annex D
(informative)

Establishment of allowable limits for EO

D.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with the specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the requirements in this document.

D.2 Background

Ethylene oxide is a flammable gas that is irritating to body surfaces and highly reactive. It is mutagenic under many conditions, it has fetotoxic and teratogenic properties, it can adversely affect testicular function and it can produce injury to many organ systems in the body. EO has been investigated for cancers including lymphoma, leukaemia and breast cancer[241].

A long-term exposure AL was provided in the first edition of this document (i.e. ISO 10993-7:1995). The previous edition of this document (i.e. ISO 10993-7:2008) conducted an extensive toxicology review and provided long-term cancer and non-cancer ALs that were higher than the ISO 10993-7:1995 AL. Therefore, the ISO 10993-7:1995 AL was retained in the previous edition of this document (i.e. ISO 10993-7:2008). In this edition of this document (i.e. ISO 10993-7:2026), EO limits from published literature and those provided by government agencies were reviewed and compared to the existing EO AL derived in ISO 10993-7:1995 and ISO 10993-7:2008/Cor 1:2009. This document provides TI values as a means to calculate device-specific and duration-specific AL based on the device's anticipated contact population and actual use duration. A brief review of published EO limits is presented herein.

A long-term TI of 0,02 mg/kg/d was derived based on cancer effects and was derived using dose-response modelling of human epidemiological data.

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

D.3 Methods

D.3.1 General

Methods for calculating the EO limits are described in this annex.

D.3.2 Formulae used in limit derivation

D.3.2.1 General

The formulae for the derivation of the limited or prolonged exposure limits, and long-term exposure limits are presented in this subclause.

TI values for non-cancer effects of EO were derived by dividing the most relevant no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) values from critical studies by uncertainty factors to account for data on the variability in response to EO in human populations, potential species difference in potency and data deficiencies. Consistent with this philosophy, data on the variability in response to EO in human populations and on the potency of EO across species was used.

D.3.2.2 Step 1: Calculation of TI

Calculate the TI using Formula (D.1).

$$TI = \frac{NOAEL}{UF1 \times UF2 \times UF3} \text{ or } TI = \frac{LOAEL}{UF1 \times UF2 \times UF3} \quad \text{(D.1)}$$

where

TI is the tolerable intake, in mg/kg/d;

$UF1$ is the inter-individual variation among humans;

$UF2$ accounts for extrapolation from data derived in a species other than humans;

$UF3$ is the measure for the quality and relevance of the experimental data.

NOTE For common UF3 default recommendations, refer to ISO 10993-17:2023.

D.3.2.3 Step 2: Calculation of TE

Once the TIs have been developed, it is necessary to adjust the appropriate TI with a CEF. The CEF accounts for exposure to EO from multiple EO-sterilized medical devices. Given that half of all medical devices are sterilized by EO, the concurrent use of multiple EO sterilized devices is possible. While it is challenging to definitively enumerate the number of concurrent sources of EO exposure in any given user interaction, it is reasonable to assume that some concurrent EO exposure occurs. A default CEF of CEF = 0,2 is used which assumes concurrent exposure to up to five EO-sterilized medical devices used at the same time.

Calculate the TE using Formula (D.2).

$$TE = TI \times m_b \times CEF \quad \text{(D.2)}$$

where TE is the tolerable exposure, in mg/d.

D.3.2.4 Step 3: Calculation of AL

Calculate the AL using Formula (D.3).

$$AL = TE \times \left(\frac{D}{N} \right) \quad \text{(D.3)}$$

The potential exists for patients to be exposed to EO released from medical devices for short or long durations; as a result, it was necessary to derive limited or prolonged TI values, and long-term TI values. In addition, it is possible for patients to be exposed to EO via various routes of exposure. Although patients are typically exposed to EO via parenteral routes of exposure in clinical settings, very little toxicity data are available to derive TI values for these routes of exposure. In contrast, there is a large database of data available on the effects of EO in experimental animals and humans following inhalation exposure. To use this rich resource of inhalation toxicity data for setting parenteral TI values for EO, a method was developed for route-to-route extrapolation to derive estimates of internal dose following inhalation exposure.

D.3.3 Route-to-route extrapolation of dose

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

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

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

Exposure concentration parts per million	**Percent absorbed** %
10	94
33	74
50	68
100	61
1 000	36

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

D.3.4 Non-cancer risk assessment approach

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

D.3.5 Cancer risk assessment approach

The application of a weight-of-evidence test indicates that EO is a genotoxic carcinogen and that tumours seen in animals are relevant for humans. Accordingly, cancer-based TI values have been derived.

D.3.6 Effects not considered in deriving TI values for EO

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

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

D.4.1 Overview

The derivation of a non-cancer-based TI value for EO involves

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

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

These steps are described in D.5.2 and D.5.3, respectively.

D.4.2 Selection of critical studies

D.4.2.1 Limited or prolonged exposure category

A literature search for this edition of this document (i.e. ISO 10993-7:2026) was unable to identify any additional toxicity data relevant to limited or prolonged exposure durations. The basic approach is that acute data (e.g. from studies of 14 d or less) should be used to set limited exposure limits.

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

Table D.2 summarises the most relevant data for the derivation of the limited or prolonged exposure category TI for EO; however, it should be noted that many studies other than that listed in Tables D.2 to D.7 were reviewed in the process of setting TI values for EO.

Table D.2 — Studies used to derive limited or prolonged exposure category TI for EO

Species	Route	Exposure	NOAEL mg/kg/d	LOAEL mg/kg/d	Effect at LOAEL	Study
rabbit	IV	9 mg/kg, 18 mg/kg and 36 mg/kg daily on gestational day 4 to 16	9	18,0	decreased maternal mass gain	Reference [82]
rats	inhalation	10 mg/kg, 33 mg/kg or 100 mg/kg for 6 h/d on gestational day 6 to 15	9,1	27,6	depression of foetal body mass	Reference [169]

A similar NOAEL value for EO was derived from the inhalation study conducted by Snellings.[169] Decreased foetal body mass was observed following exposure of pregnant Fischer 344 rats to 100 ppm EO for 6 h/d on days 6 to 15 of gestation. No adverse effects were seen following exposure to 33 ppm EO. Using the absorbed dose data from the study[22], the absorbed dose equivalent to 33 ppm[169] is 9,1 mg/kg/d:

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

where

33 is the NOEL value in ppm;

1,8 is the conversion factor from ppm into m^3 for EO;

0,29 is the volume of air that is breathed in a day for infants;

6/24 is the dosage time-period in a day;

0,74/0,35 is the percentage of EO absorbed by the lungs of infants.

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

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

D.4.3.1 Overview

Uncertainty factors were retained from ISO 10993-7:2008/Cor 1:2009, see Table D.3.

Table D.3 — Uncertainty factors for TI derivation

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

D.4.3.2 Inter-individual variability (UF1)

D.4.3.2.1 General

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

Each of the factors that can play a role in contributing to variability in the human population to adverse effects associated with EO exposure is explored in D.4.3.2 for the purpose of identifying a value for UF1 that is appropriately protective for special subpopulations.

D.4.3.2.2 Polymorphism of EO detoxification enzymes

D.4.3.2.2.1 General considerations

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

EO, relative to the rest of the population. Since EO is detoxified by these enzymes, one would expect poor metabolizers of EO to be at increased risk of adverse effects, relative to the rest of the population. Considerable attention has been paid to the role that GSTT1 polymorphism can have in the variability in response seen in human populations to EO[50]. However, EO is primarily metabolized by EH in humans; consequently, it can be assumed that GSTT1 polymorphism is not expected to influence the variability in response of the human population to a significant degree. Despite the assumed principal role of EH to the metabolism of EO in humans, little attention has been paid to the potential role of EH polymorphism or inhibition on EO metabolism and, subsequently, on the risk posed by exposure to EO. The impact of polymorphisms of GSTT1 and EH on the response to EO in the human population is discussed in D.4.3.2.2.2 and D.4.3.2.2.3.

D.4.3.2.2.2 Role of GSTT1 polymorphism in the variability of the response of the human population to EO

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

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

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

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

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

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

NOTE The GSTT1 null genotype is associated with an increased risk of some cancers[48][207], but not necessarily with cancers associated with ethylene oxide exposure.

D.4.3.2.2.3 Role of EH polymorphism in the variability of the response of the human population to EO

Similarly to GSTT1, the expression of EH is polymorphically expressed in the human population[69][144], as a result, variability in EH activity in humans can be significant. For example, Mertes[123] found a 63-fold variability in the metabolism of EH substrates by human liver samples; however, 90 % of the samples deviated by less than a factor of three from the median. Kitteringham et al.[90] concluded that the impact that EH polymorphism has on EH activity in the human population would be covered by a factor of 10.

If the entire range of EH activity in the human population (including poor metabolizers and rapid metabolizers) can be encompassed by a factor of 10, the difference in activity between the mean of the

population and the most poor metabolizers can probably be described by a factor of five, depending on the shape of the distribution.

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

D.4.3.2.3 Inhibition of EO detoxification enzymes

D.4.3.2.3.1 Inhibition in disease states

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

D.4.3.2.3.2 Inhibition by drugs and other compounds

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

D.4.3.2.3.3 Significance of enzyme inhibition

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

D.4.3.2.4 Glutathione levels

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

D.4.3.2.5 Polymorphism of DNA repair capacity

Another pharmacodynamic factor, polymorphism in genes associated with DNA repair as well as carcinogen metabolism, can have an impact on cancer risk[73]. Presumably, individuals with inefficient DNA repair mechanisms can be at higher risk of EO-associated adverse effects than most individuals in the general

population. Some experimental data exist to support this assertion. Nivard[138] found up to 20-fold enhanced mutation rates in the absence of maternal nucleotide excision repair (NER) in Drosophila exposed to high concentrations of EO compared to repair proficient conditions. However, this increased mutation rate was not seen in Drosophila at lower doses. Therefore, although inefficient DNA repair can place some individuals at increased risk of EO-associated adverse effects, it is not possible to assess DNA repair polymorphism data in a quantitative manner to identify a value for UF1 for EO.

D.4.3.2.6 Aggregate variability

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

Knudsen[91] considered the impact of multiple factors that influence metabolic capacity on the magnitude of the UF to account for inter-individual variability of 10 would be sufficient.

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

D.4.3.3 Inter-species differences (UF2)

D.4.3.3.1 Overview

Before species differences in potency are considered when deriving a value for UF, it is important to ask whether the results seen in experimental animals exposed to EO are relevant for humans. As discussed in D.3.1, the critical endpoints for TI derivation for EO are reduced mass gain in rabbits and depression of foetal body mass in rats. Reduced mass gain in experimental animals is a general effect that is considered to be adverse and relevant for establishing TI values. Spermatogenesis in non-human primates is organizationally similar to the process that occurs in humans, with regard to length of the spermatogenesis cycle, the duration of spermatogenesis, and the number of mitotic divisions[124][195]. Consequently, non-human primates have been described as an appropriate model for experimental studies of human spermatogenesis. By analogy, it can be assumed that EO-induced effects on this process seen in cynomolgus monkeys would be applicable for humans. Since EO is thought to exert its carcinogenic effect as a direct acting genotoxic carcinogen, the effects seen in experimental animals are directly applicable for humans.

Based on allometric principles, humans are assumed to be more sensitive to the adverse effects of chemical compounds than experimental animals[127]. As a result, a default UF of 10 is used to account for the assumed difference in potency of a compound between experimental animals and humans. However, several lines of evidence suggest that the potency of ethylene oxide is equivalent among species. As described in more detail in D.4.3.2.3, the results of PBPK modelling suggest that an internal dose in mice, rats and humans is expected to be the same following inhalation exposure to a given concentration of EO. The results of the PBPK modelling exercise are supported by data on species equivalence in internal dose of EO and similar compounds (propylene oxide, styrene oxide) following inhalation exposure. These factors all support the selection of a value of one for UF2 for use in deriving TI values for EO.

D.4.3.3.2 Results of PBPK modelling

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

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

EO concentration ppm	AUC mg × h/l		
	mouse	rat	human
1	0,044	0,059	0,056
10	0,44	0,59	0,57

D.4.3.3.3 Species similarities in response

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

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

Dose metric	Species		
	mouse	rat	human
N-(2-hydroxyethyl)valine (HOEtVal) adduct increment (1 ppmh)	12	16	12
Dose in blood (μMh ppm^{-1})	0,5	0,35	0,3

D.4.3.3.4 Cross species comparison of potency for similar epoxides

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

D.4.3.3.5 Species differences in DNA repair rates

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

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

D.4.3.4 Quality and relevance of experimental data (UF3)

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

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

Justification is provided in Clause D.4 to support the selection of the following values for each of the uncertainty factors necessary to derive a non-cancer TI for EO:

— UF1 (inter-individual variability): 30;

— UF2 (inter-species extrapolation): 1;

— UF3 (data deficiency): 1.

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

The application of the selected MFs to the NOAEL values from the critical studies yields the non-cancer TI values for EO shown in Table D.7.

Table D.7 — Derivation of non-cancer TI Values

Exposure category	Study	NOAEL/LOAEL mg/kg/d	UF	TI mg/kg/d
limited/prolonged	Reference [169]	9 (NOAEL)	30	0,3
limited/prolonged	Reference [82]	9 (NOAEL)	30	0,3

D.5 Cancer-based TI values for EO

D.5.1 General overview

A cancer-based TI value for EO has been derived using dose-response modelling from human inhalation data.

D.5.2 Dose-response modelling of human data

Since the publication of the first edition of this document (i.e. ISO 10993-7:1995) and the second edition (i.e. ISO 10993-7:2008), new data has become available on the adverse cancer effects of EO in humans. When human data are available to assess the risk posed by exposure of patients to a carcinogenic compound, these data are preferred over animal data. A literature search was conducted to review the available cancer evaluations to compare to the cancer-based TI derived in ISO 10993-7:2008/Cor 1:2009. As mentioned previously, this cancer-based TI was not used in the derivation of the AL in the second edition of this document (i.e. ISO 10993-7:2008). Relevant publications since 2008 that evaluated EO exposure with respect to cancer risk are summarized in the following paragraphs of this subclause. When provided, the cancer unit risk has been converted to a TI and has been compared to the ISO 10993-7:2008 TI of 0,02 mg/kg/d.

In 2010, Valdez-Flores et al[241] published a quantitative cancer risk assessment based on the most updated epidemiology data at that time, the NIOSH and UCC cohorts. The risk of cancer death in these cohorts was not elevated, except for bone cancer, which was based on a small number of deaths, and no statistically significant relationship between cumulative EO exposure and cancer mortality was observed. Despite these negative findings, the excess cancer mortality risk for a continuous lifetime exposure was characterized based on US Environmental Protection Agency (EPA) guidelines[244] using several cohorts (e.g. NIOSH males) and cancer endpoints. The authors derived several effective concentrations corresponding to a one in a million excess environmental cancer risk for different worker groups, cancers and groups of cancers. Conversion of these concentrations to the corresponding exposures in mg/kg/d associated with a 10^{-4} cancer risk resulted in a range of cancer risk values correspond to TIs approximately 3-fold higher than the cancer-based TI derived in 2008. A subsequent publication by the same authors performed similar analyses to determine the occupational risk, which is a less appropriate comparison to medical device risk. This analysis was subsequently used by SCOEL in 2012 to recommend occupational limits[238].

Mikoczy et al.[236] reported a 16-year mortality and cancer incidence update of a cohort of Swedish sterilant workers. No statistically significant elevations in mortality and cancer incidence for lympho-hematopoietic cancers (LHCs) and some subtypes were observed. No evidence of increasing LHC risk with increasing cumulative EO exposure was observed. Breast cancer incidence and deaths were lower compared to the regional Swedish population, but incidence was increased to a statistically significant extent in the two highest cumulative exposure categories compared to the lowest. This lowest cumulative exposure group had much lower breast cancer incidence than the regional Swedish population. Furthermore, the higher exposed groups had cumulative exposures that are within the range of the lowest EO exposure category in the NIOSH cohort in which no such elevation in breast cancer incidence was observed. This study was limited by small numbers of observed cases and deaths, insufficient follow-up, and relatively low cumulative EO exposures.

In 2012, the International Agency for Research on Cancer (IARC)[232] reaffirmed the classification of EO as carcinogenic to humans (Group 1) based on limited evidence in humans, sufficient evidence in animals, and strong mechanistic evidence. IARC does not perform quantitative cancer risk assessments.

In 2016, the US EPA[244] conducted a quantitative cancer risk assessment based on some of the same data used by Valdez-Flores et al, specifically the NIOSH cohort. There were several methodological differences between the US EPA assessment and that of Valdez-Flores et al. The US EPA set a unit risk value of $3,0 \times 10^{-3}$ $(\mu g/m^3)^{-1}$ that at a 10^{-6} cancer risk level corresponds to a very low level of EO exposure, in the nanogram per kilogram per day range, which is within the range of estimates of endogenous EO exposure. The calculated risk value suggests that EO is one of the most potent known human carcinogens. US EPA also revised the EO carcinogenicity classification to "carcinogenic to humans" based on "strong, but less than conclusive" evidence in humans, "extensive evidence" in animals, and "clear evidence" of EO genotoxicity.

In 2017, Kirman and Hays[233] estimated endogenous EO exposure in air concentration equivalents using measured EO haemoglobin adducts in unexposed populations and correlations between these adducts and EO exposure concentrations in occupationally exposed workers. The estimates included adjustments for assumed inhalation rates and exposure frequencies between workers and the general population. These endogenous exposure equivalent estimates ranged from 0,37 ppb to 6,9 ppb (0,67 $\mu g/m^3$ to 12 $\mu g/m^3$) in air. Furthermore, Sheehan et al[239] estimated endogenous EO exposure based on EO haemoglobin adducts, based on the method of Kirman and Hays[233], but used a much larger database for the general population. Based on their calculations, endogenous EO exposure equivalents range from approximately 1,0 ppb to 5,3 ppb (1,8 $\mu g/m^3$ to 9,5 $\mu g/m^3$) in air for non-smokers.

In 2019, Marsh et al[235] published a meta-analysis of epidemiological studies on the association of occupational EO exposure and lympho-hematopoietic cancers (LHC) and breast cancer risk. The authors concluded that epidemiology studies published since 2000 provide the best information regarding breast cancer and LHC risks and EO exposure and that such studies do not support the conclusion that occupational exposure to EO is associated with an increased risk of LHC or breast cancer.

Also in 2019, Vincent et al[243] performed focused reviews on the epidemiological and toxicological evidence of EO carcinogenicity to validate the high carcinogenic potency of EO determined by the US EPA assessment. Consistent with Marsh et al., these authors concluded that EO epidemiology studies are "negative" and that therefore a risk assessment cannot be based on such studies. In addition, the authors concluded that the risk value calculated by US EPA appears not to be adequately justified.

In 2020, the Texas Commission on Environmental Quality (TCEQ)[240] published a carcinogenic dose-response assessment for EO to be used for air remediation and permitting. The TCEQ concluded that EO is likely to be carcinogenic to humans on the basis of insufficient human data, sufficient animal data, and a putative mutagenic mode of action. TCEQ also conducted a quantitative cancer risk assessment based on the same NIOSH cohort used by Valdez-Flores et al and US EPA, but TCEQ used a different methodology to calculate cancer risk. The assessment resulted in a cancer risk value that, assuming linearity between the 10^{-4} and 10^{-5} risk levels, corresponds approximately to a TI that is 1,6-fold lower than the current cancer-based TI. The unit risk value produced by TCEQ is $2,3 \times 10^{-6}$ $(\mu g/m^3)^{-1}$.

In 2021, the US National Toxicology Program (NTP), updated its classification of EO as "known to be a human carcinogen" based on "sufficient evidence" from studies in humans and "studies on mechanisms of carcinogenicity". NTP does not perform quantitative cancer risk assessments[237].

In 2022, Lynch et al[234] conducted a systematic review of the evidence for the carcinogenicity of EO. The authors concluded that relatively high exposures of EO are associated with increased incidence of several tumour types in animals and that a mutagenic or genotoxic mode of action is supported by available evidence, but that epidemiologic evidence does not show a clear or consistent increase in cancer associated with occupational EO exposure.

ATSDR published a Toxicological Profile for EO in 2022[231]. The profile provides summaries of the studies and conclusions from US EPA, IARC and others, but does not draw independent conclusions regarding EO carcinogenicity or cancer risk.

Overall, evaluations published since 2008 indicate that US agencies consider EO to be a human carcinogen, but that epidemiological evidence is not conclusive in itself, despite some studies with thousands of subjects and long follow up times. Other authors disagree that EO is a carcinogen based on many of the same epidemiological studies. The disagreements are, at least in part, based on differences in data analysis and, in particular, whether data on exposed workers should be compared to that from an external reference population or those from workers with the lowest exposures.

Only a small number of these publications – Valdez-Flores[241], US EPA[244] and TCEQ[240] – provide estimates of cancer risk for the general population who can be exposed to EO. Comparing cancer risk estimates across these references is not straight forward because the modelled cancer endpoints, the exposure-response models themselves and the target cancer risk levels vary between References [240], [241] and [244], and the cancer risk models are not necessarily linear with cancer risk levels. With these caveats, the tolerable intakes derived from the cancer risk levels from these publications range from $9,5 \times 10^{-6}$ to $5,7 \times 10^{-2}$ mg/kg/d at an excess cancer risk of 10^{-4} in Table D.8. An allowable cancer risk of 10^{-4} is used and takes into account risk benefit considerations in ISO 14971 arising from use of a medical device that has been shown to improve patient health outcomes. These values fall within a factor of three of the current cancer-based TI value of 0,02 mg/kg/d derived in ISO 10993-7:2008/Cor 1:2009 with the value from US EPA the exception, see Table D.8. Collectively, the more recent evaluations of EO carcinogenicity do not substantially change the cancer-based TI. Therefore, the cancer-based TI of 0,02 mg/kg/d is retained.

Table D.8 — Equivalent dose associated with 10^{-4} risk based on unit risk values compared to ISO 10993-7:2008/Cor 1:2009 cancer TI

Reference	Unit risk $(\mu g/m^3)^{-1}$	Equivalent dose associated with 10^{-4} risk mg/kg/d	Fold lower or higher than the ISO 10993-7:2008/Cor 1:2009 value
Reference [161] [a]	$5,8 \times 10^{-7}$	0,019	—
Reference [137] [a]	$5,1 \times 10^{-7}$	0,020	—
Reference [244] [b]	$3,0 \times 10^{-3}$	$9,5 \times 10^{-6}$	2 100 × lower
Reference [240] [b]	$2,3 \times 10^{-6}$	0,012	1,6 × lower
Reference [241] [b,c]	$9,3 \times 10^{-7}$	0,031	1,5 × higher
Reference [242] [b,d]	$5,0 \times 10^{-7}$	0,057	2,8 × higher

[a] Conversion to mg/kg/d based on an assumed ventilation rate of 10 m^3/d, a body mass of 70 kg and pro-rated from a 5-day work week, based on the exposure cohort of workers who were predominantly male. NIOSH[161] and UCC[137] designate the study population used to determine the unit risk. The NIOSH and UCC cohorts both consisted of EO exposure in healthy, North American adults, predominantly male, therefore, the conversion parameters of 70 kg body mass is retained. The cancer unit risk values in ISO 10993-7:2008/Cor 1:2009, Reference [161] are not publicly available.

[b] Conversion to mg/kg/d based on an assumed ventilation rate of 20 m^3/d and a body mass of 70 kg based on the dose modelling in References [240], [241] and [242] to determine cancer risk for continuous exposure of the general population.

[c] Unit risk derived from the lowest effective concentration corresponding to an extra risk of one in a million, 0,000 6 ppm, reported for any cohort and tumour type.

[d] Unit risk reported in Reference [242] corresponding to the low end of their recommended range of cancer potencies.

D.5.3 Cancer-based TI value

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

D.6 Calculation of TE levels and AL

D.6.1 TE

TI values are modified to account for the manner in which a particular device is used and to enable practical calculation of individual device limits. The TE is the product of the TI, body mass (m_b) and CEF, as shown in Formula (D.4):

$$TE = TI \times m_b \times CEF \quad \text{(D.4)}$$

The default mass adjustment assumes a 60 kg adult human body mass for either sex.

In the absence of specific information, the default value for the CEF corresponds to 0,2. In the event that the patient exposure period is less than the extraction duration, a toxicological risk assessment can be performed to account for the shorter exposure period.

The AL is the largest amount of EO deemed acceptable as a result of exposure from a medical device and is expressed in units of milligrams per device. The maximum amount of EO, expressed as mass EO from a medical device, is the product of the tolerable exposure limit, TE and the number of days a device can be used in the specific exposure duration categories, divided by the number of products used during this duration, as shown in Formula (D.5):

$$AL = TE \times \frac{D}{N} \tag{D.5}$$

D.6.2 Limited exposure TE

The limited tolerable exposure is the tolerable intake multiplied by the body mass multiplied by the CEF as shown in Formula (D.6):

$$TE = TI \times m_b \times CEF \tag{D.6}$$

$$TE = 0,3 \times 60 \times 0,2 = 3,6 \text{ mg/d}$$

where

TI is the tolerable intake of 0,3 mg/kg/d;

m_b is the body mass of anticipated patient population of 60 kg;

CEF is the concomitant exposure factor of 0,2 based on 5 devices.

D.6.3 Limited exposure AL

Medical devices whose cumulative exposure is less than or equal to 1 d:

$$AL_{\text{limited}} = 3,6 \times 1 = 3,6 \text{ milligrams per device for adults} \tag{D.7}$$

The calculated AL of 3,6 milligrams per device is not clinically significantly different than the existing established AL in ISO 10993-7:2008/Cor 1:2009 of 4 mg device.

D.6.4 Limited exposure AL for special populations

When the device is intended to be used in special populations, the appropriate patient body mass should be used for the derivation of the allowable residual limits. For example, if the device is intended to be used in premature neonates, neonates, children or adolescents, the allowable residual limits should be derived using the TI value of 0,3 mg/kg/d for EO and CEF of 0,2 with the appropriate body mass. Guidance on body masses for special populations is given in Clause C.4.

D.6.5 Prolonged exposure TE

The prolonged tolerable exposure is the tolerable intake multiplied by the body mass multiplied by the CEF as shown in Formula (D.8):

$$TE = TI \times m_b \times CEF \tag{D.8}$$

$$TE = 0,3 \times 60 \times 0,2 = 3,6 \text{ mg/d for adults}$$

D.6.6 Prolonged exposure AL

For medical devices whose cumulative exposure is more than 1 d but not more than 30 d, the prolonged exposure limit corresponds to an AL based on 3,6 mg/d multiplied by the anticipated exposure duration (up to 30 d) for a maximum of 108 mg for adults.

$AL_{\text{prolonged}} = 3,6 \times 30 = 108$ milligrams per device for adults

D.6.7 Long-term exposure TE

The long-term tolerable exposure is the tolerable intake multiplied by the body mass multiplied by the CEF as shown in Formula (D.9):

$$TE = TI \times m_{\text{b}} \times CEF \quad \text{(D.9)}$$

$TE = 0,02 \times 60 \times 0,2 = 0,24$ mg/d for adults

D.6.8 Long-term exposure AL

TE for EO at 0,24 mg/d appears adequately protective for adults.

For medical devices whose cumulative exposure is more than 30 d and where 19 160 d is assumed for a lifetime, the long-term exposure limit corresponds to: 0,24 mg/d multiplied by the anticipated exposure duration up to 19 160 d which equals to the maximum of 4 598 mg which equals to the maximum of 4,6 g in a lifetime for adults.

$AL_{\text{long-term}} = 0,24 \times 19\ 160 = 4\ 598$ milligrams per device for adults

Medical devices used in paediatric populations pose a special challenge in the derivation of an AL since the mass of the patient changes as the individual ages. A long-term exposure AL of 636 milligrams per device for individuals during the first 16 y (365 d/y multiplied by 16 y which is equal to 5 840 d) is protective for paediatric EO exposure. The 636 mg limit was calculated by summing the TE values for varying masses for each of the 16 y, see Table D.9. The values in Table D.9 were determined by using the mass for 10^{th} percentile female paediatric population (see Reference [245] and Table 1) as a conservative approach. The sum of paediatric cumulative exposure over 16 y, 636 mg, divided by the total of 5 840 d = 0,109 mg/d, rounds to 0,11 mg/d, when CEF is 0,2.

$$AL_{\text{long-term}} = TI \times m_{\text{b}} \times D \times CEF \quad \text{(D.10)}$$

where

TI is the tolerable intake (i.e. 0,02 mg/kg/d);

m_{b} is the body mass, in kg corresponding to the year;

D is the number of days in a year (i.e. 365 d), in d;

CEF is the concomitant exposure factor (e.g. 0,2 default).

Table D.9 — Derivation of paediatric long-term EO allowable limits

Age of patient	Mass of patient	One year cumulative exposure where	
		CEF = 0,2	*CEF* = 1,0
years	kg	mg	mg
<1	7,8	11	57
1	9,1	13	66
2	11	16	80
3	13	19	95
4	15	22	110
5	16	23	117
6	18	26	131
7	20	29	146
8	22	32	161
9	26	38	190
10	27	39	197
11	32	47	234
12	36	53	263
13	40	58	292
14	46	67	336
15	48	70	350
16	50	73	365
		AL is 636 milligrams per device	AL is 3 190 milligrams per device

The long-term exposure AL for paediatric is equal to 0,11 mg/d multiplied by the anticipated exposure duration, up to 5 840 d is equal to 642 mg for devices used exclusively during the entire first 16 years of life where 0,2 CEF is used as the default.

With a CEF equal to 1,0, the AL for paediatric is equal to 0,55 mg/d multiplied by the anticipated exposure duration, up to 5 840 d, which is equal to 3 212 mg for devices used exclusively during the entire first 16 y of life.

NOTE The difference in AL values presented here (i.e. 642 mg/d for CEF = 0,2) versus Table D.9 (i.e. 636 mg/d for CEF = 0,2) is due to rounding of significant digits when deriving the TE. Table D.9 shows AL values calculated with a TE = 0,109 mg/d for CEF of 0,2 and TE = 0,546 mg/d for CEF of 1,0, respectively, whereas rounded TE values (i.e. 0,11 for CEF of 0,2) are presented in the text.

Devices used in patients (individually or cumulatively) past 16 years should use the total of the AL (0,11 mg/d times the days of anticipated exposure duration with CEF of 0,2) after the paediatric exposure (up to 2 108 mg over remaining lifetime, assuming 19 160 d).

Alternatively, a toxicological risk assessment can be conducted for less-than-lifetime exposure.

In addition, the maximum dose of EO should not exceed limited (24 h) and prolonged (30 d) limits.

D.6.9 Calculation of tolerable contact level

D.6.9.1 Rationale

Given that EO can be irritating, a calculation of a TCL is relevant for direct tissue contacting EO-sterilized devices (excluding circulating blood).

D.6.9.2 Selection of critical studies

A number of studies[12][117][168][179] contain dose-response data that can be used to derive a TCL for EO.

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

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

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

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

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

The NIL values obtained from these studies are summarised in Table D.10.

Table D.10 — Comparison of studies of irritation effects of EO

Study	Device or material	NIL or MIL µg/cm^2
Reference [117]	cardiac catheter	103
Reference [168]	PVC block	321
Reference [179]	gauze patch	424
Reference [11]	cotton pellet	833

D.6.9.3 Selection of uncertainty factors for TCL derivation

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

D.6.9.4 Inter-individual variability (UF4)

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

D.6.9.5 Inter-species differences (UF5)

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

D.6.9.6 Data deficiencies

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

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

The critical concept in deriving either a TI or a TCL for a compound is "dose-to-patient" or bio-available dose. When local irritation studies are conducted by placing the material in contact with the skin or mucosa, a certain amount of the EO can remain in the device and a certain amount can be volatilized. Either of these processes result in less EO being available to produce the irritant effect at the target tissue site. Data are not available on the bio-available dose of EO[11][168][179] but it is assumed that 50 % of the dose reaches the target site. As a result, a factor of two is used to account for questions about bio-available dose in these studies.

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

The UF4, UF5 and UF6 values applied to each study, the resulting MF and the corresponding TCL values are presented in Table D.11.

Table D.11 — Uncertainty factors and modifying factors derived for studies of the irritation of EO and the TCL derived from these data

Study	Site	NIL or MIL µg/cm^2	UF4	UF5	UF6	MF	TCL µg/cm^2
Reference [117]	implantation	103	3	1	3	10	10,3
Reference [168]	dermal	321	5	1	12	60	5,4
Reference [179]	dermal	424	5	1	6	30	14,1
Reference [11]	mucosa	833	3	1	12	36	23,1

The TCL of 10 µg/cm^2 is adequately protective for EO-induced local effects in various tissues based on the values derived from these four diverse studies and considering the clinical relevance of the contacting tissues (mucosa and implantation).

D.6.9.7 Limit based on TCL value

Formula (D.11) gives the calculation of a mass limit based on the TCL:

$$m_{\text{dev, BSC}} = TCL \times A \qquad \text{(D.11)}$$

where

$m_{\text{dev, BSC}}$ is the mass of EO per device, i.e. the maximum dose to the patient, in mg from the first 24 h of exposure or the duration of use, whichever is less;

TCL is the tolerable contact level, in µg/cm^2;

A is the surface area of medical device sample extracted, in cm^2.

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

EXAMPLE 1 The device surface area in contact with the body is 75 cm^2 and the TCL limit is 10 µg/cm^2.

$m_{\text{dev, BSC}} = 10 \times 75 = 750$ micrograms per device

Alternatively, the amount of EO in a device divided by the surface area would provide the µg/cm^2 to compare against the TCL limit.

EXAMPLE 2 The device surface area in contact with the body is 75 cm^2 and 2,5 mg of EO extracted in the first 24 h would have 33 µg/cm^2 (2 500 micrograms per device divided by 75 cm^2), which does not pass the TCL limit of 10 µg/cm^2.

Annex E
(informative)

Establishment of allowable limits for ECH

E.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the requirements in this document.

E.2 Background

E.2.1 Ethylene chlorohydrin 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.

The acute toxicity data and repeated dose data demonstrate that ECH is readily accessible to systemic circulation following skin, oral and parenteral exposure. The inspection of median LD_{50} and NOAELs also suggests that the potency of ECH at specific time intervals, limited, prolonged and long-term exposure, is comparable by oral and parenteral routes of exposure. Based upon data generated in sub-chronic 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 Clauses E.2 to E.5 reflect these general observations. For the prolonged and long-term exposure categories, the existing limits from the first edition of this document (i.e. ISO 10993-7:1995) have been retained, although the derived TI values and corresponding device limits from the evaluation presented herein support higher levels. The rationale for retaining the current limits is the successful clinical history since adoption of the 1995 and 2008 editions, and the ability of manufacturers to conform with these limits. Concomitantly, there is no current clinical or manufacturing reason to raise the existing limits for the prolonged and long-term categories to the levels supported by the evaluation described herein.

NOTE When chlorine is used as, or part of, a manufacturing material during the manufacture of the medical device and it is demonstrated that residual levels of ECH, in addition to EO, are not detectable on the surface of the medical device made from non-absorbent materials, routine testing for these sterilization residuals is not unnecessary.

E.2.2 The residual limits for ECH in medical devices contained in this annex were established using the methodology outlined in this annex to establish the TI. The limits for ECH in medical devices were established based upon extensive literature evaluation. 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 Clause E.4.

E.3 Methods

E.3.1 Overview

The approach described in this annex was used to derive TI values for ECH for various durations of exposure.

The potential exists for patients to be exposed to ECH released from medical devices due to the extent of medical device exposure. As a result, it was necessary to derive separate limited, prolonged and long-term TI values for this compound.

E.3.2 Route-to-route extrapolation of dose

Patients are typically exposed to ECH via parenteral routes of exposure in clinical settings and very little toxicity data are available to derive TI values for these routes of exposure. In contrast, there is a database available on the effects of ECH in experimental animals. Similarly, limited data exist which reference the ECH exposure to patients via the inhalation route. Because ECH is rapidly absorbed and eliminated[220], oral and parenteral routes of exposure were considered comparable. ECH exposure results from the derivatization of EO to ECH with the addition of a chlorine molecule and therefore exists due to environmental factors. A route-to-route extrapolation of dose to inhalation exposure was not conducted as part of this risk assessment for ECH.

E.3.3 Non-cancer risk assessment approach

TI values for non-cancer effects of ECH were derived by dividing the most relevant NOAEL or LOAEL values from critical studies by uncertainty factors to account for data on the variability in response to EO in human populations (UF1), potential species difference in potency (UF2) and data deficiencies (UF3). Data on the variability in response to ECH in human populations and on the potency of ECH across species was used in deriving UF values.

E.3.4 Cancer risk assessment approach

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

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

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

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

E.4.1 Selection of uncertainty factors for non-cancer effects

Table E.1 lists the uncertainty factors used to derive the TI value.

Table E.1 — Uncertainty factors for TI derivation

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

E.4.2 Selection of critical studies

E.4.2.1 Limited exposure limit

The AL for the limited exposure limit of ECH for the duration of less than 24 h is 9 mg/d. This limit is based on the data collected from a sub-chronic intraperitoneal injection study in rats for 30 d of 6,4 mg/kg ECH as the NOAEL[103]. This dose was derived from a one-tenth dose level of a previously conducted study by the same investigators, which led to an LD_{50} value of 64 mg/kg[102]. Similar acute toxicity LD_{50} results were reported by several investigators[104][116][159][162][194][203] in several species by various routes of administration. Acute toxicity data, including median LD_{50}, were available and evaluated, although they were not appropriate for this assessment. The LD_{50} data are summarised in Table E.2.

The inspection of the data in Table E.2 suggests that the toxicity of ECH for limited exposure, i.e. less than 24 h, is nearly identical regardless of the route of exposure and is relatively similar across species.

Since the data reflect LD_{50} values in Table E.2, and not a NOAEL or a LOAEL, then the value determined as the NOAEL provided by Reference [103] was used as previously indicated. In that study, the dose of 6,4 mg/kg/d was chosen by the investigators to represent a one-tenth of the LD_{50} value in the original acute study. The sub-chronic study was successful in establishing a no-effect level and the value of 6,4 mg/kg/d was used to formulate the AL for ECH using the appropriate uncertainty factors and modifying factors:

$NOAEL$ = 6,4 mg/kg/d

Table E.2 — Median LD_{50} for limited exposure allowable limit for ECH

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

UFs used for the calculation of the TI value are:

— UF1 (inter-individual variation among humans): 10;

— UF2 (inter-species variation): 11;

— UF3 (quality/relevance of the data): 1.

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

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

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

a) MF is the multiplication of the UF values:

$$MF = UF1 \times UF2 \times UF3 \quad \text{(E.1)}$$

$$MF = 10 \times 1 \times 1 = 10$$

$$TI = \frac{NOAEL}{MF}, \text{ or } TI = 6,4 \div 10 = 0,64 \text{ mg/kg/d}$$

b) CEF:

$$CEF = 0,2$$

c) TE:

$$TE = TI \times m_b \times CEF \quad \text{(E.2)}$$

$TE = 0,64 \times 60 \times 0,2 = 7,7 \text{ mg/d}$ for adults

d) AL:

$$AL = TE \times \left(\frac{D}{N} \right) \quad \text{(E.3)}$$

AL = 7,7 × 1/1 = 7,7 mg/d for less than 24 h exposure for adults.

The limit is acceptable in the context of NOAELs derived from the sub-chronic and reproductive toxicity data based on the low NOAEL of 0,64 mg/kg/d.

E.4.2.2 Prolonged exposure limit

The AL for the exposure for cumulative exposure greater than 1 d up to 30 d is 7,7 mg/d in the first 24 h and 3,2/d for up to 30 d period (3,2 mg × 29 d = 96 mg in total). This limit was based upon sub-chronic toxicity and reproductive effects data (teratogenicity) generated in several species. These data have been reported by many investigators.[8][10][18][38][83][85][103][145][203][221] In repeated-dose, oral and parenteral studies lasting for varying time periods up to 403 d, ECH produced a variety of adverse effects including death (accompanied by increased relative organ masses, darkened mottled liver, haemorrhagic adrenals, haemorrhagic pituitary gland, haemorrhagic gastrointestinal tract, myocarditis, thyroid congestion and congestive pulmonary changes in one study). Additionally, ECH produced decreased body mass and growth, increased brain, adrenal, kidney, lung and thyroid mass, small testes or testicular injury, emesis, decreased haemoglobin, packed cell value and haematocrit, liver injury, ectopic haematopoiesis and bone marrow hypercellularity, and a shift in white blood cells towards lymphocytes. Dosages ranged from about 2,7 mg/kg/d to 93 mg/kg/d or more. Reproductive studies were solely teratology studies in which ECH was administered during various time periods of gestation. In these studies, ECH produced maternal toxicity, foetal toxicity and, in one study, an increase in foetal malformations. This latter effect was observed only in the offspring of mice given ECH intravenously at a dosage of 120 mg/kg/d, a dosage well into the acutely lethal range[80]. The data that became the basis for the calculation of the limit for prolonged exposure are summarized in Table E.3.

Table E.3 — Data used to establish prolonged exposure limit for ECH

Study type	Oral NOAEL mg/kg/d	Parenteral NOAEL mg/kg/d
sub-chronic	13[145]	2,7 prorated from 6,4 three times weekly[103]
reproductive	50[38]	9[83]
sub-chronic	45[221]	—

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

$NOAEL$ = 6,4 × 3 /7 = 2,7 mg/kg/d (E.4)

UFs used to calculate the TI value are:

— UF1 (inter-individual variation among humans): 10;

— UF2 (inter-species variation): 1;

— UF3 (quality/relevance of the data): 1.

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

a) MF is the multiplication of the UF values:

$$MF = UF1 \times UF2 \times UF3 \quad \text{(E.5)}$$

$$MF = 10 \times 1 \times 1 = 10$$

$$TI = \frac{NOAEL}{MF} \text{ or } TI = 2{,}7 \div 10 = 0{,}27 \text{ mg/kg/d}$$

b) $CEF = 0{,}2$

c) TE:

$$TE = TI \times m_b \times CEF \quad \text{(E.6)}$$

$TE = 0{,}27 \times 60 \times 0{,}2 = 3{,}2$ mg/d for adults for up to 30 d

d) Prolonged exposure AL:

$$AL = TE \times \left(\frac{D}{N} \right) \quad \text{(E.7)}$$

The prolonged exposure AL is the TE of 3,2 mg/d multiplied by the anticipated number of days (up to 30)) and divided by the number of devices used within this category for a maximum of 96 milligrams per device

In addition, the maximum dose of ECH should not exceed 7,7 mg for any one-day period for adults.

The limit is acceptable in the context of NOAELs derived from the sub-chronic and reproductive toxicity data based on the low NOAEL of 0,27 mg/kg/d for repeated administration.

E.4.2.3 Long-term exposure limit

The AL for long-term cumulative exposure of greater than 30 d is 8 750 mg, not to exceed 7,7 mg/d during the first 24 h or 96 mg in the first month. This limit was based upon chronic toxicity, genotoxicity and carcinogenicity data that has been reported in References [81], [116] and [133]. In these studies, rats received ECH in drinking water until 24 months of age, rats received ECH by subcutaneous injection twice weekly for at least a year and rats and mice received ECH by dermal application for 103 weeks to 104 weeks. Dosages ranged from 0,086 mg/kg/d to 71 mg/kg/d or more. In these studies, no increases in tumour incidence related to ECH administration or evidence of chronic toxicity with the exception of a possible reduction in survival rates were found[81]. The key data that became the basis for the calculation of prospective long-term exposure limits are summarised in Table E.4.

Inspection of these data suggests that the NOAEL for ECH for long-term exposure periods, i.e. 30 d to life, by oral and parenteral routes are comparable. These data are also comparable to those generated in sub-chronic and reproductive toxicity studies.

Table E.4 — Data used to establish long-term exposure limit for ECH

Study type	Oral	Parenteral	Dermal
chronic	4 mg/kg/d of LOAEL[81]	2,9 mg/kg/d of LOAEL (prorated from 10 mg/kg twice weekly)[116]	no data
chronic	45 mg/kg/d of NOAEL[221]	no data	no data
carcinogenicity	16 mg/kg/d of NOAEL[81]	no data	71 mg/kg/d of NOAEL (prorated from 100 mg/kg five times weekly)[133][a]

[a] Ethylene chlorohydrin produced no increases in tumour incidence at the highest dosage tested.

The LOAEL for chronic toxicity, 2,9 mg/kg/d, administered subcutaneously to rats for at least one year, and for tumour production, 16 mg/kg/d orally to rats until 24 months of age, was used for the calculation of a prospective long-term exposure AL of 8 750 mg as follows:

$LOAEL$ = 2,9 mg/kg/d

UFs used to calculate the TI value are:

— UF1 (inter-individual variation among humans): 10;

— UF2 (inter-species variation): 10;

— UF3 (quality/relevance of the data): 1.

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

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

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

a) The MF is the multiplication of the UF values:

$$MF = UF1 \times UF2 \times UF3 \quad \text{(E.8)}$$

MF = 10 × 10 × 1 = 100

$$TI = \frac{LOAEL}{MF}, \text{ or } TI = 2,9 \div 100 = 0,029 \text{ mg/kg/d}$$

b) The default CEF is based on five devices:

CEF = 0,2

c) The TE value is calculated from Formula (E.9):

$$TE = TI \times m_b \times CEF \quad \text{(E.9)}$$

TE = 0,029 × 60 × 0,2 = 0,35 mg/d for adults.

d) AL:

$$AL = TE \times \left(\frac{D}{N} \right) \quad \text{(E.10)}$$

The TE of 0,35 mg/d is multiplied by the anticipated exposure duration (up to 19 160 d) for an Al of up to 6 706 mg (6,7 g) in a lifetime for adults.

Medical devices used in paediatric populations pose a special challenge in the derivation of an AL since the mass of the patient changes as the individual ages. A long-term ECH AL of 925 milligrams per device for individuals during first 16 years (365 d/year × 16 years = 5 840 d) is protective for paediatric ECH exposure. The 925 mg limit was calculated by summing the TE values for the varying masses for each of the 16 years, see Table E.5. The values in Table E.5 were determined by using the mass for 10^{th} percentile female paediatric population (see Reference [245] and Table 1) as a conservative approach. The sum of paediatric cumulative exposure values over 16 years, 925 mg, divided by the total of 5 840 d = 0,16 mg/d, when *CEF* = 0,2.

$$AL = TI \times m_b \times D \times CEF \quad \text{(E.11)}$$

where

TI is the tolerable intake (i.e. 0,029 milligram per kilogram per day);

m_b is the body mass, in kg for each year;

D is the number of days in a year (i.e. 365 d), in d;

CEF is the concomitant exposure factor (i.e. 0,2).

Table E.5 — Derivation of paediatric long-term exposure AL

Patient age years	**Patient mass** kg	**One year cumulative exposure** milligrams per device
< 1	7,8	17
1	9,1	19
2	11	23
3	13	27
4	15	32
5	16	34
6	18	38
7	20	42
8	22	47
9	26	55
10	27	57
11	32	68
12	36	76
13	40	85
14	46	97
15	48	102
16	50	106
		AL is 925 milligrams per device

The long-term exposure paediatric AL is the TE of 0,16 mg/d multiplied by the number of day anticipated exposure duration, up to 5 840 d (maximum of 925 mg) for devices used exclusively during the entire first 16 years of life, where 0,2 CEF is used as the default.

Devices used in patients (individually or cumulatively) past 16 years should use the total of the AL (0,16 mg/d multiplied by the days of anticipated exposure duration with a CEF of 0,2) after the paediatric exposure (up to 3 066 mg over the remaining lifetime, assuming 19 160 d).

Alternatively, a toxicological risk assessment can be conducted for less than a lifetime exposure.

In addition, the maximum dose of ECH should not exceed limited (24 h) and prolonged (30 d) limits.

E.5 Calculation of ECH tolerable contact level

There are limited published data available for the irritation effects of ECH. The calculation of a TCL is relevant. It is assumed that a TCL derived limit is appropriate for direct tissue contacting devices.

Studies were conducted in which dermal administration of undiluted ECH led to an insignificant irritation response in a rabbit. Intradermal and intramuscular injection of ECH, however, led to strong irritation response at the site of injection. Dilution of the ECH solutions to intradermal tissue and penile mucosa resulted in a modulated response. At up to 80 % dilution, there was little to no irritation response found by the investigators[61][102]. Additional intradermal and Draize eye irritation tests by these investigators resulted in high irritation score for undiluted ECH. However, ECH solutions of 1 % (by volume) showed little to no irritation. These data indicate that ECH is highly irritating to intradermal tissues. As a result, a TCL and an intradermal TCL can be derived for both non-irritating level (N_{IL}) and minimally irritating level (M_{IL}) concentrations of ECH, respectively.

The TCL is derived for ECH in the following manner. A modifying factor approach is used to calculate the TCL. This approach incorporates the use of uncertainty factors (see Clause E.5) to provide an acceptable margin of safety against irritation. Formula (E.12) calculates the TCL, in milligrams per square centimetre, using the modifying-factor approach:

$$TCL = \frac{N_{IL}}{(M_F \times A)} \text{ or } TCL = \frac{M_{IL}}{(M_F \times A)} \tag{E.12}$$

where

M_F is the modifying factor ($UF4 \times UF5 \times UF6$);

N_{IL} is the non-irritating level, in mg;

M_{IL} is the minimally irritating level, in mg;

A is the body contact surface area, in cm^2.

Justification is provided in Clause E.4 to support the selection of the following values for each of the uncertainty factors necessary to derive a TCL:

— UF4 (inter-individual variability): 10;

— UF5 (inter-species extrapolation): 1;

— UF6 (data deficiency): 1.

Lawrence et al.[103] administered dermally to rabbits a maximum of 80 % solution of ECH in a volume of 0,2 ml per 3,27 cm^2 (0,5 in^2) surface area. The N_{IL} is calculated to 160 mg of ECH per 3,27 cm^2 (the N_{IL} of 5 % solution can be calculated by multiplying 0,2 ml by 80 g per 100 ml, which is equal to 1,6 g) and resulted in no observed irritation in the skin.

Therefore, the TCL of 4,89 mg/cm^2 is calculated by dividing 160 mg by a MF of 10 multiplied by 3,27 cm^2.

$TCL = 160 / (10 \times 3{,}27) = 4{,}89$ mg/cm^2

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

A secondary intradermal irritation study in the rabbit was conducted by Lawrence et al.[103] using several dilutions of ECH. In this study, all dilutions caused dramatic local dermal irritation leading to local tissue necrosis with minor exception. The dilutions of 1 % and 5 % ECH were both non-irritating and minimally irritating (doubtful), respectively, according to the standard scoring method used. This was suggested to be due to the retention of ECH in the local area, which was subsequently not bio-transformed and therefore

led to the toxic local tissue response. Not overlooking this, a secondary minimal irritation level (MIL) was calculated from these intradermal doses; M_{IL} of 0,5 % solution can be calculated by multiplying 0,2 ml by 5 g per 100 ml, which is equal to 10 mg of intradermal ECH.

This means that intradermal exposure is non-irritating at a dose of up to 10 mg. While an intradermal TCL cannot be calculated without a specific contact surface area, a 10 mg dose is greater than the AL established for limited exposure (7,7 mg), prolonged exposure (3,2 mg/d) and long-term exposure (0,4 mg/d). Therefore, the AL established are considered protective of potential irritation effects from intradermal exposure.

ECH above 1 % concentration in saline can elicit irreversible immediate mild eye irritation. The instillation of 0,1 ml of solution into rabbit's eye observed every 30 min for 3 h demonstrated that undiluted chlorohydrin and 20 % to 80 % dilutions in saline were highly irritant and that 2,5 % to 10 % dilutions were irritant. Dilutions containing less than 1 % chlorohydrin were non-irritating[102].

Annex F
(informative)

Ethylene glycol

F.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document nor is it intended to infer that this annex is the only means of achieving conformity. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with specified requirements. Methods other than those given in this the annex can be used, providing their performance achieves conformity with the requirements in this document.

F.2 Background

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

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

NOTE This information is included for completeness, as it is not considered necessary to set AL for EG when EO limits are controlled to the levels specified in this document.

F.3 General considerations

F.3.1 Overview

The acute toxicity data and repeated dose data demonstrate that, although EG is not very potent, it is accessible to the systemic circulation following oral and parenteral exposure. The inspection of the median LD_{50} and NOAELs also suggests that the potency of EG at specific time intervals, limited exposure, etc. is comparable by oral and parenteral routes. Based upon data generated in sub-chronic and chronic toxicity studies, EG does not appear to become more potent as the duration of exposure is increased. The kidneys represent the primary target organ for EG.

F.3.2 Limited exposure

EG does not represent a practical harm to health from exposure to medical devices for less than 24 h in duration. This conclusion is based upon acute toxicity data generated in several animal species and reports from the literature regarding poisoning following ingestion of EG or products containing EG in humans[85][101][116][160][162][203][204]. There are also numerous reports regarding human death resulting from the ingestion of EG. Based upon these data, the estimated lethal dosage in humans of EG is 1,4 ml/kg[160] or about 111 g to an adult. However, as it is known that the saturation limit (*SL*) of human metabolism of EG is

125 mg/kg[20][148] and that human data is always more persuasive in terms of setting safety levels, this dose was used as the basis for the calculations of the AL for limited exposure as follows.

SL = 125 mg/kg/d

UFs used to calculate the TI value are:

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

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

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

MF is the multiplication of the UF values:

$$MF = UF1 \times UF2 \times UF3 = 10 \times 1 \times 1 \text{ or } MF = 10 \quad \text{(F.1)}$$

$$TI = \frac{SL}{MF} = \frac{125}{10} = 12{,}5 \text{ mg/kg/d}$$

CEF:

CEF = 0,2 (default value)

TE:

$$TE = TI \times m_b \times CEF \text{ or } TE = 12{,}5 \times 60 \times 0{,}2 = 150 \text{ mg/d} \quad \text{(F.2)}$$

AL:

$$AL = TE \times \left(\frac{D}{N} \right)$$

AL = 150 × 1 = 150 mg/d

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

F.3.3 Prolonged exposure

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

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

Dosages ranged from 40 mg/kg to 5 000 mg/kg or more. In the teratology studies (all conducted by oral administration), EG produced maternal toxicity, embryo toxicity, foetal toxicity, and abnormalities to skeletal and visceral tissues at dosages above 150 mg/kg. In the multigenerational studies (also conducted only by oral administration), a dosage of 1 840 mg/kg (estimated from a study in which EG was administered

at a concentration of 0,5 % in drinking water[97] produced no adverse effects while a dosage greater than 1 000 mg/kg was required before indications of foetal toxicity (decreased pup mass), embryo toxicity (decreased litter size) and teratogenicity were produced.

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

$NOAEL$ = 50 mg/kg/d

UFs used to calculate the TI value are:

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

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

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

MF is the multiplication of the UF values:

$$MF = UF1 \times UF2 \times UF3 = 10 \times 5 \times 1 \text{ or } MF = 50 \tag{F.3}$$

$$TI = \frac{NOAEL}{MF} \text{ or } TI = \frac{50}{50} \text{ or } TI = 1{,}0 \text{ mg/kg/d} \tag{F.4}$$

CEF is the reciprocal of five devices (default value):

CEF = 0,2

TE is calculated from the TI, body mass and CEF:

$$TE = TI \times m_b \times CEF \tag{F.5}$$

TE = 1,0 × 60 × 0,2 = 12 mg/d

AL is the TE multiplied by the days of exposure:

$$AL = TE \times \left(\frac{D}{N} \right) \tag{F.6}$$

The AL is 12 mg/d multiplied by the anticipated exposure duration (up to 30 d) for a maximum of 360 mg for adults.

F.3.4 Long-term exposure

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

The inspection of these data revealed some route sensitivity in the no-observed-effect levels for EG for long-term exposure period, i.e. 30 d to life; however, they are comparable to those generated in sub-chronic and reproductive toxicity studies. To provide the greatest protection for the patient, the lowest NOEL for chronic toxicity, 40 mg/kg/d administered in the rats' diet for two years, was used as the basis for the calculation of the AL for long-term exposure as follows:

$NOAEL$ = 40 mg/kg/d

UFs are used to calculate the TI value:

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

— UF2 (inter-species variation): 5 (similar response);

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

MF is the multiplication of the UF values:

$$MF = UF1 \times UF2 \times UF3 = 10 \times 5 \times 1 \text{ or } MF = 50 \tag{F.7}$$

$$TI = \frac{NOAEL}{MF} \text{ or } TI = \frac{40}{50} \text{ or } TI = 0,8 \text{ mg/kg/d} \tag{F.8}$$

CEF is the reciprocal of five devices:

CEF = 0,2 (default value)

TE is calculated from the TI, body mass and CEF:

$$TE = TI \times m_b \times CEF \tag{F.9}$$

TE = 0,8 × 60 × 0,2 = 9,6 mg/d

AL is the TE multiplied by the days of exposure:

$$AL = TE \times \left(\frac{D}{N} \right)$$

The AL is 9,6 mg/d multiplied by the anticipated exposure duration (up to 19 160 d) for a maximum of 184 g for adults.

F.3.5 Tolerable contact level

Global tolerable contact levels were not developed for EG as the local concentration and specific routes of exposure play a key role in determining the potential for local irritancy. It is recommended that local irritation of EG be addressed by the application of ISO 10993-23. A review of the literature indicated that EG has an overall low potential for skin irritancy. A single exposure to 10 % EG was negative in a human patch test[96] while in another human study, repeated exposure indicated that EG is a minor skin irritant[168].

Annex G
(normative)

Evaluation of gas chromatograms

G.1 General

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

Many analytical methods for these EO-sterilization residuals have been described in References [28], [29], [34], [40], [47], [62], [65], [68], [70], [74], [76], [77], [86], [87], [89], [94], [105], [110] to [114], [125], [126], [131], [132], [140], [151], [155], [175], [196], [199], [200], [206] and [208] to [217]. However, the enormous diversity of materials and methods of construction of sterile medical devices can, in certain cases, still present problems in determining residual EO and ECH levels using the methods given in the Bibliography. Therefore, any method that has been shown to be analytically sound and validated (i.e. demonstrated accuracy, precision, specificity, detection and quantification limits, linearity, and range) can be used. Annex G to Annex J contain general validation requirements for gas chromatographic methods.

G.2 Background

General validation requirements are discussed in References [208], [209], [210], [211], [212] and [213] on GC and should be reviewed by analysts before their use of any of the procedures. Also recommended is a review of the articles concerning detection limits[16][36][75].

G.3 Minimum requirements

G.3.1 For these procedures, it is recommended that the minimum requirements are met for these parameters (see Figure G.1 and Figure G.2).

Resolution, R, is calculated as shown in Formula (G.1):

$$R = 2\frac{(t_2 - t_1)}{(W_2 + W_1)} \tag{G.1}$$

R shall be greater than or equal to 2,0 for peak area or peak height quantitation.

Alternatively, Formula (G.2) can be useful to calculate the capacity factor, k', which shall be greater than 1,5 for well-resolved peaks:

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

Tailing, T, given by Formula (G.3), shall be less than or equal to 1,8 for the EO and ECH peaks:

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

G.3.2 Relative deviation of the standard curve (RSD) should not exceed 5 % for EO and ECH for the range of standards used[13][14].

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)^2}{n-2}} \tag{G.4}$$

$$\%\,RSD = \frac{\sigma}{\lambda} \times 100 \tag{G.5}$$

$$\lambda = \frac{\sum y}{n} \tag{G.6}$$

where

n is the total number of observations;

y_i is the observed value of y;

$\hat{y}_i$ is the predicted value of y from the linear regression;

λ is the mean;

x is the concentration of the standard;

σ is the standard deviation of the linear regression;

$\sum y$ is the sum of y.

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

G.4 Chromatographic baseline

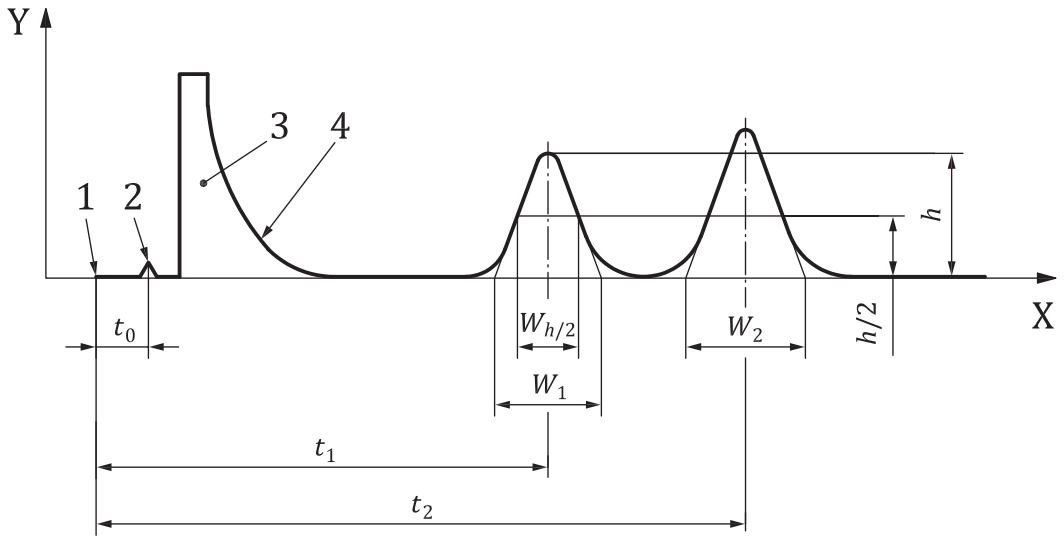
In addition, it is recommended that the chromatographic baseline returns to within 5 % of the initial baseline between chromatographic runs.

G.5 Resources

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

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

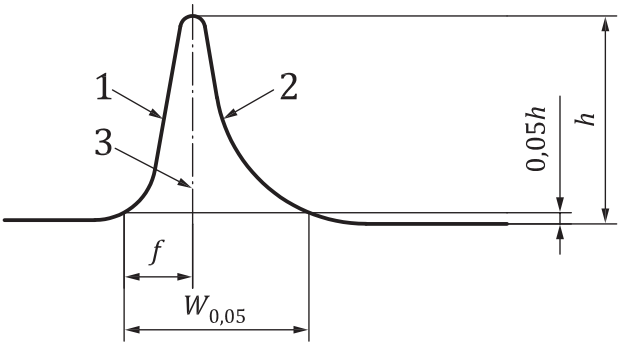
— the various textbooks on GC.



Key

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

Figure G.1 — Chromatographic separation of two substances



Key

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

Figure G.2 — Asymmetrical chromatographic peak

Annex H
(informative)

Gas chromatographic determination for EO and ECH

H.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document nor is it intended to infer that this guidance is the only means of achieving conformity. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the requirements in this document.

H.2 Chromatographic procedures

H.2.1 Preparation of standards

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

H.2.2 General

Two alternatives are commonly available for preparation of GC standards:

a) the use of commercially available standards;

b) the preparation of standards either volumetrically, by diluting known volumes of EO gas or gravimetrically, by diluting a known mass of liquid EO; in all cases, a standard curve of peak height or peak area response versus EO concentration shall be prepared.

NOTE 1 Peak area response compiled by the software of computer-controlled GC instrumentation is more precise than measuring peak height when determining EO concentrations.

NOTE 2 Many GC methods that use a capillary column instead of a packed column produce an EO and ECH result during a single sample run.

NOTE 3 Analytical methods utilizing internal standards help to reduce variation in results.

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

The way to determine these validation data is reported in numerous validation guidelines[208][210][211][212][213][214].

H.3 Criteria for validating gas chromatographic methods

H.3.1 General overview

Many methods are suitable for quantitatively analysing extracts for ethylene oxide. A number of procedures for exhaustive extraction followed by GC for the determination of EO have been described. There are probably just as many unpublished methods for determining residual ethylene oxide. Due to the diversity in medical devices, published methods can be unsuitable for all devices. Therefore, any method that has been shown to be analytically sound and meets the performance criteria described in this document can be used.

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

A number of analytical methods for assessing levels of EO and ECH residuals have been reviewed from References [13], [14], [15], [19], [28], [29], [35], [40], [47], [65], [68], [70], [74], [76], [77], [86], [87], [89], [92], [94], [112], [113], [131], [132], [140], [155], [156], [174], [175], [210], [211], [212], [213], [214], [215], [216] and [217].

Guidance on analytical method validation is available in numerous guidelines including ICH Q2[208].

H.3.2 Accuracy

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

Spiked samples as the means of determining accuracy can be reported as a percentage of recovery of a known added amount of analyte in the sample. However, for EO, this method for determining accuracy is extremely difficult to carry out because of the volatility of this compound. As an alternative, the use of commercially available certified standards is recommended. Thus, the measure of accuracy becomes the mean measured results divided by the accepted true value together with the confidence interval. In either case, the percent recovery can be calculated as shown in Formula (H.2):

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

where

R is the recovery in percent;

R_o is the result obtained;

a is the accepted value.

t_v is the true value.

If the standards are prepared in a solvent other than what is used to extract test articles, the matrix effects should be evaluated. This evaluation can be achieved by preparing the accuracy samples that are matrix-matched to the solvent used to extract the test articles.

For example, if the standards are prepared in methanol and the test articles are extracted in water, the accuracy evaluation should include a spike and recovery performed in both methanol and water across the method range.

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

H.3.3 Precision

H.3.3.1 Overview

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

H.3.3.2 Repeatability

Repeatability can be assessed using a minimum of nine determinations covering the specified range of standards used (i.e. three replicates each at three different concentrations). Data generated from method accuracy as in H.3.2 can be used for repeatability assessment.

Repeatability can be calculated as the relative standard deviation (coefficient of variation) of the peak area as specified in Formula (G.5).

The percentage of RSD for EO and ECH should not exceed 5 % for the range of the standards used. The percentage of RSD is calculated as described in G.3.2.

H.3.3.3 Intermediate precision

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

As a minimum, data generated as described in Clause H.3, for two separate events is recommended to indicate the intermediate precision of the test method. The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported.

H.3.3.4 Ruggedness/reproducibility

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

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

H.3.4 Linearity

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

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

H.3.5 Method detection limit

H.3.5.1 Overview

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

There are many ways to determine the method detection limit; approaches other than those given in H.3.5.2 and H.3.5.3 are also acceptable.

H.3.5.2 MDL based on signal-to-noise

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

H.3.5.3 MDL based on the standard deviation of the response

The method detection limit is determined by preparing a known standard of the analyte of interest near the estimated MDL and calculating the standard deviation for seven injections of the standard.

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

where

s is the standard deviation of injections;

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

H.3.6 Quantitation limit

H.3.6.1 Overview

The quantitation limit (QL) is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. The limit of quantitation should be at or below the applicable allowable limit. There are many ways to determine quantitation limit; approaches other than those given in H.3.6.2 and H.3.6.3 are also acceptable.

H.3.6.2 QL based on signal-to-noise

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

H.3.6.3 QL based on the standard deviation of the response

The quantitation limit can be expressed as shown in Formula (H.3):

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

Annex I
(informative)

Preparation of EO and ECH standards

I.1 General

The guidance given in this annex is neither intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This document is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the requirements in this document.

I.2 Preparation of EO standards

I.2.1 General

For increased safety and accuracy, it is recommended that commercially available EO and ECH standards of known and certified concentrations are used. If this is not possible, then stock EO standards can be prepared from the pure compound as described in this clause.

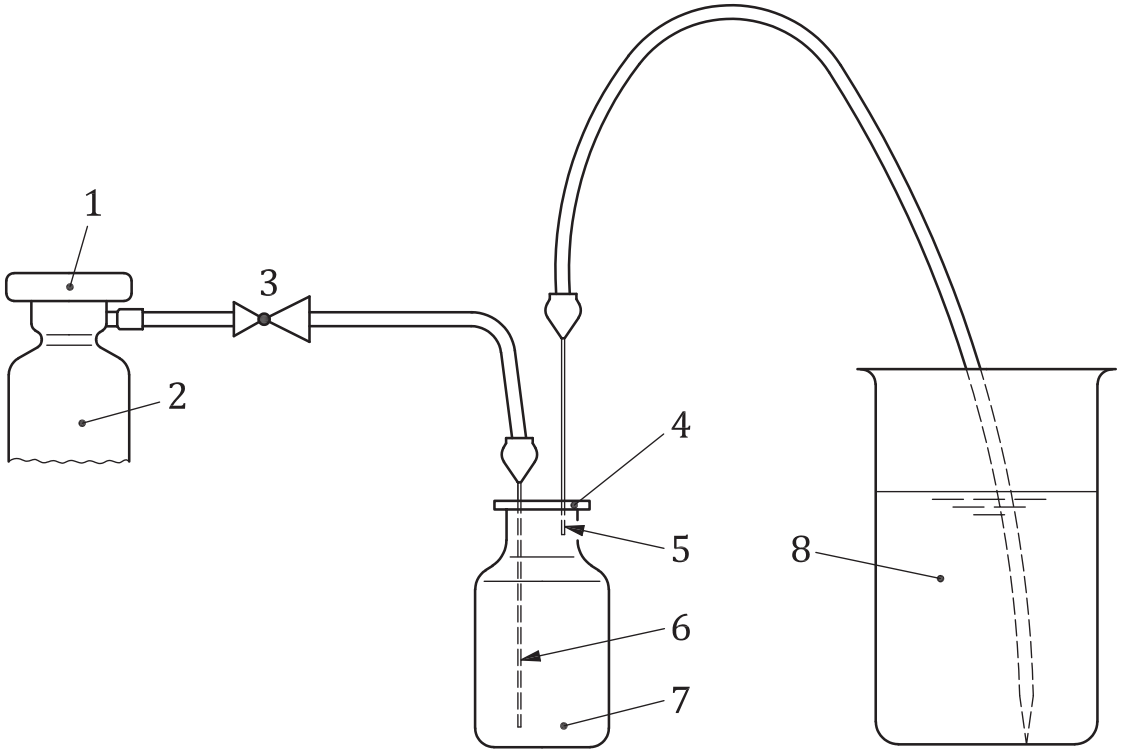
I.2.2 Collection of EO gas

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

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

Place another length of tubing on the EO cylinder regulator and connect to a hypodermic needle. Insert the second needle, or inlet needle 1, through the vial septum, and push the point down to the bottom. Start the EO flow through the system so that bubbles emerge from the vent tube at the rate of one per second. Purge the vial for about 15 min. Remove the inlet needle from the vial and allow the EO gas in the vial to equilibrate to atmospheric pressure by removing the vent needle from the vial as the last bubble emerges from the vent tube in the beaker. Using the ideal gas law, it can be shown that the concentration of EO in the vial is approximately 1,8 µg/µl at 760 mm Hg[2)] and 20 °C.

2) 1 mm Hg = 133,322 Pa or 760 mm Hg = 101,325 kPa.



Key

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

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

The concentration of EO, C_{EO}, in µg/µl, according to the ideal gas law, can be calculated for any given temperature, T, in °C, and pressure, p, in mmHg, using Formula (I.1):

$$C_{EO} = 0{,}706 \frac{p}{273+T} \quad \text{(I.1)}$$

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

I.2.3 EO standard dilutions for headspace methods

Dilute the standard from I.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 pointing upward.

Place the nitrogen-flushed vial on to the upward-pointing syringe needle and inject the 10 µl of EO into the vial. Do not flush the syringe; immediately remove it from the vial. The vial now contains 18 µg of EO at 20 °C and 760 mmHg. Adjust the concentration of EO for the ambient conditions described in I.2.1.

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

Store stock standard solutions in a refrigerator when not in use or if purchased, at the conditions specified by the manufacturer (see I.4.1). Establish storage stability and shelf life for EO stock. Prepare calibration standards fresh daily. Discard after use.

I.2.4 EO standard dilutions for non-aqueous solvent methods

Set up an EO standard gas cylinder as described in I.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. A previously cooled syringe aids the transfer of liquid EO. Care should be taken to make sure that the syringe needle does not touch the solvent.

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.

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.

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

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

NOTE 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.

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

Store stock standard solutions in a refrigerator when not in use or if purchased, at the conditions specified by the manufacturer (see I.4.1). Prepare calibration standards fresh daily. Discard after use.

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

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

I.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. Re-weigh the flask and calculate the difference between the two masses; then dilute to volume with water and shake. Store stock standard solutions in a refrigerator when not in use (see I.4.2). Establish storage stability and shelf life for ECH stock. Prepare working standards daily and discard after use.

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

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

I.4 Analytical methods

I.4.1 Stability of EO in solution

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

During the interlaboratory comparison study of the EO method described in J.5.4[140], a study was made of the stability of standard solutions of EO in ethanol. Solutions of EO at concentrations of 25 µg/ml, 50 µg/ml and 100 µg/ml were prepared and stored both at refrigerator temperature and at 40 °C. These solutions were analysed at different times over periods of up to six weeks. The study showed that, at 40 °C, the EO concentration was reduced to 70 % of the original concentration after two weeks for the 50 µg/ml and 100 µg/ml standards, whereas all the standards studied were stable to within 10 % of the original concentration after storage at refrigerator temperature (5 °C) for up to 60 d. Where water extraction is used, caution should be taken, as EO can convert to EG or ECH (or both) during the extraction period as well as during storage of the extract[35]). The analyst should evaluate the possibility of this conversion to EG or ECH at the analysis site when extracting the sample with water.

I.4.2 Stability of ECH in solution

Prior to the interlaboratory comparison study of ECH, 11 laboratories participated in a study of the stability of ECH standards. Aqueous solutions of ECH were prepared by one laboratory and shipped to all participants. The solutions were stored at refrigerator temperature upon arrival. These solutions were analysed at different periods of time, such as immediately after arrival, 1 week after, and 2, 3, 4, 8 and 12 weeks after arrival, by various types of column. The study showed that there is no significant difference in the concentration in the first two weeks. It was concluded that ECH standard solutions are stable when stored at refrigerator temperature for at least 14 d.

I.4.3 Linearity of standard curve

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

Annex J
(informative)

Ethylene oxide and ethylene chlorohydrin residual measuring methods

J.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This guidance is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for implementation of the requirements by providing explanations and acceptable methods for achieving conformity with specified requirements. Methods other than those given in this guidance can be used, providing their performance achieves conformity with the requirements in this document.

J.2 Results of interlaboratory evaluation of methods

J.2.1 EO methods

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

Table J.1 — Comparison of intra-laboratory and interlaboratory variations

EO method	Intra-laboratory	Interlaboratory
headspace method	3,7 %	21,3 %
acetone method	4,1 %	16,3 %
DMF method	2,9 %	8,3 %
aqueous method	2,7 %	17,0 %

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

The calculated regression formulae was $y = 0{,}04 + 0{,}904x$ with a correlation coefficient $r = 0{,}974$ ($p < 0{,}000\ 01$). The intra-laboratory 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.

Analytical data from samples of three different EO levels were obtained using both the solvent extraction followed by a headspace gas analysis procedure[136] and the bromination method[89] in two laboratories. Results were compared using linear regression analysis, which gave the regression data $y = -0{,}03 + 1{,}07x$; correlation coefficient $r = 0{,}999$. The interlaboratory coefficient of variation of the J.5.4 procedure was estimated as 4,7 %, 1,8 % and 2,7 % at 12 ppm, 25 ppm and 56 ppm EO respectively in the matrix tested[132].

J.2.2 ECH methods

An interlaboratory evaluation was conducted for ECH[114]. The estimated total coefficient of variation of the methods was as follows:

— intra-laboratory: 7,46 %;

— Interlaboratory: 10,99 %.

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

J.3 Apparatus and reagents

J.3.1 Apparatus

J.3.1.1 Gas chromatograph, equipped with a flame ionization detector (FID) mass spectrometer (MS) or an electron capture detector (ECD) and adequate signal recording system.

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

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

If mass spectrometer is used as the detector, the following conditions have shown to be useful.

— A single quadrupole MS instrument is recommended with ionisation by electronic impact at 70 eV.

— Calibration should be performed after each tune of the instrument and at least once a week.

— The source temperature is set in accordance with instrument manufacturer recommendations. The source temperature for GC instruments is typically at or above 200 °C.

— The quantification of EO, ECH and EG are performed using selected ion monitoring (SIM) mode whereas the identification is performed in full scan mode (*m/z* = 25 to 350).

— For EO quantification, *m/z* = 29 is used as quantifier ion whereas *m/z* = 44 and 15 are used as qualifier ions. For ECH, *m/z* = 31 is used as quantifier and *m/z* = 43 and 44 as qualifiers. At least, for EG, *m/z* = 31 is used as a quantifier ion and *m/z* = 33 and 43 as qualifiers.

J.3.1.2 Hypodermic needles and polyvinyl chloride tubing, as required for preparing standards.

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

Care should be taken in selecting glassware of an appropriate volume 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.

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

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

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

J.3.1.7 Gas regulator, for lecture bottle containing EO.

J.3.1.8 Gas-tight syringes, of 10 µl, 5 µl, 100 µl and 1 000 µl capacities for use in preparing standards and for injecting headspace gas on to the column of the gas chromatograph.

J.3.1.9 Laboratory oven, capable of heating samples to (100 ± 2) °C.

J.3.1.10 Laboratory oven, capable of heating samples to (37 ± 1) °C.

J.3.1.11 Water bath, capable of maintaining samples at (70 ± 2) °C.

J.3.1.12 Mechanical shaker.

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

J.3.1.14 Flat-bottom screw-cap vial, of a size that will accommodate the sample and extraction fluid, equipped with a PTFE-lined silicone septum and thin PTFE film, used for extraction of EO and reaction of EO with bromohydrin if applicable.

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

J.3.1.16 Millipore filter, of 0,45 µm pore size for filtration of the reaction mixture before chromatography.

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

J.3.2 Reagents

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

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

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

J.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. Re-distil 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 (boiling point between 125 °C to 126 °C). Store in a glass-stoppered glass container and use within one week.

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

J.3.2.6 Water, of purity suitable for GC.

J.3.2.7 Ethanol, of purity suitable for GC.

J.3.2.8 Propanone (acetone), of purity suitable for GC.

J.3.2.9 Dimethylformamide (DMF), of purity suitable for GC.

J.4 Standard preparation

J.4.1 Preparation of ethylene oxide standards

Where required, prepare appropriate standards as described in Clause I.3.

J.4.2 Preparation of ethylene chlorohydrin standards

Where required, prepare ethylene chlorohydrin standards as described in Clause I.4.

J.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.

J.5 Product extraction

J.5.1 General

Prepare extracts in accordance with the principles described in Annex A.

J.5.2 Extraction to simulate product use

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

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

Extract samples for a time equivalent to or exceeding the maximum time for single use, and at temperatures that provide the greatest simulated challenge, as described in Annex A. Alternatively, prepare a series of extracts (a 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 should be less than 10 % of the total volume. The extract can be stored in a refrigerator at (5 ± 3) °C. The analyst should establish the shelf life and storage time. Take care when using water extraction to assay EO, as EO can convert to EG or ECH or both during the storage of the aqueous extract[35].

J.5.3 Ethylene oxide exhaustive procedure using thermal extraction (headspace method)

Weigh a 1 g sample to the nearest 0,1 mg, place into a capped septum vial, and heat in an oven at an appropriate temperature for an appropriate amount of time. The time/temperature regimen is relatively arbitrary. Vary the time to achieve an equilibrium headspace partial pressure of EO.

The headspace GC conditions which have shown to be useful are:

— headspace oven temperature: 80 °C;

— transfer line and injector temperature: 110 °C;

— headspace equilibration time for samples: 60 min.

During linearity tests, a particular attention should be paid to a possible polymerization of EO during analysis which can lead to a detector signal plateau (not linear). This problem can be solved using a less concentrated standard solution.

Remove the vial from the oven. Inject duplicate 100 µl samples of the headspace gas on to the column of the gas chromatograph and determine the areas or heights of the EO peaks. Calculate the mean for the duplicate samples.

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 can need to be injected on to the column while they are still hot or warm and then purged without further cooling.

Automated headspace analysers are commercially available. However, the technique can be performed manually.

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 which is less than 10 % of the first extraction or until there is no analytically significant increase in the cumulative residual levels detected. The concept of repeated extractions fails when the yield of the first extraction is very small, as in the case of a device with little residual or a sample that releases the analyte at a very slow rate. In such cases, extraction should continue until either the relative standard deviation from the last three extraction cycles is below the precision of the analytical method used for exhaustive endpoint determination or the measurements from the last three extraction cycles are below the detection limit (method sensitivity) of the analytical method. 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 sample heatings.

J.5.4 Ethylene oxide exhaustive extraction with ethanol followed by headspace gas analysis of the ethanol extract

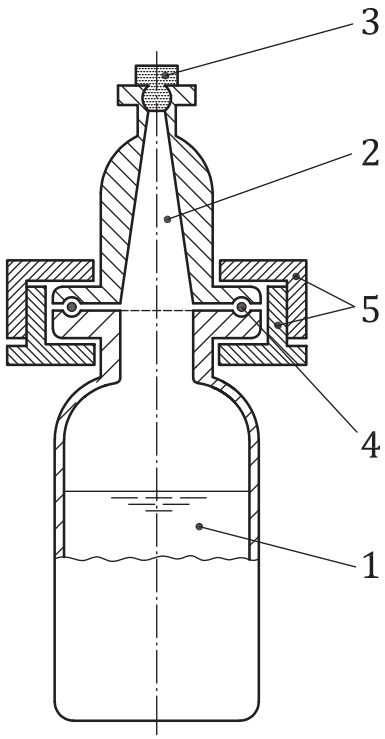
J.5.4.1 Calibration standards

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 2,0 µg/ml. Prepare a standard containing propylene oxide (PO) in ethanol at a concentration of 0,5 µg/ml as described in J.4.3. Cool these standard solutions and appropriate numbers of the special headspace bottles (Figure J.1) 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 on to 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 (X axis) against the EO concentration (Y axis) to create a calibration line.

The addition of PO to the EO standards is used as an internal standard to evaluate the accuracy in the preparation of the EO standards. The plot of the EO/PO ratio against the target EO concentrations of the standards would ideally result in a linear correlation coefficient, r, of 1,000 and a linear formula of $y = 0{,}5x + 0$. Parameters such as these indicate a perfectly straight calibration line that has a slope of 0,5 with a y-intercept of zero. A correlation coefficient of 0,999 or better can be used. A linear slope greater than 0,5 with a y-intercept of zero indicates that all the EO standards were lower than the target concentrations. A slope of less than 0,5 with a y-intercept of zero indicates that all the EO standards were higher than the target. Note that calibration lines with a y-intercept greater or less than zero will lead to EO sample results that are higher or lower than the actual concentration, respectively, especially at lower EO sample concentrations. The degree of inaccuracy depends on the distance that the y-intercept is from zero. Lastly, the height or area of the PO peak itself should remain relatively constant. Fluctuation in the PO peak height or area indicates variability in the injection volume of the sample into the GC. However, this should not be an issue with the current level of GC technology.

J.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 on to 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 described in J.5.4.1.



Key

1 liquid
2 headspace
3 septum
4 O-ring
5 clamp

Figure J.1 — Special headspace bottle

J.5.5 Ethylene oxide 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 (25 ± 2) °C.

These temperatures and times were those used in the study reported by Marlowe[112][113][114]. Other validated temperatures and times can be required to achieve an exhaustive extraction (see J.5.3).

Inject duplicate 1 µl to 5 µl aliquots on to 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.

J.5.6 Ethylene oxide exhaustive extraction with ethanol followed by bromohydrin derivative preparation and chromatography using a gas chromatograph equipped with an ECD

J.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 2,0 µg/ml. Prepare a standard containing PO in ethanol at a concentration of 0,5 µg/ml as described in J.4.3. Prepare standard mixtures by mixing equal volumes of each EO standard solution and the PO standard solution. This plot is done for the same reason as the plot of the EO/PO ratio versus EO standard concentrations as described in J.5.4.1.

Transfer 1 ml of each standard mixture to a screw-cap vial. Add two drops (ca. 0,015 g) of hydrobromic acid to the mixture through the septum using 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 (50 s^{-1}) for 5 min. Filter the mixture through a small millipore filter.

NOTE The use of vials with U-shaped or V-shaped bottoms occasionally causes incomplete neutralization, giving poor chromatograms.

Inject duplicate 1 µl to 5 µl aliquots of each filtrate on to the column of the gas chromatograph to obtain responses for peak height ratios of ethylene bromohydrin (EBH) versus propylene bromohydrin (PBH). Prepare a calibration line by plotting EBH/PBH peak height ratios versus amounts of EO (µg). This is done in a manner analogous to that described in J.5.4.1 for EO and PO peaks.

J.5.6.2 Analysis procedure

Use this procedure with standards prepared as described in J.5.4.1.

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 in the vial.

Add two drops (approximately 0,015 g) of hydrobromic acid to the vial through the septum using 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 (50 s^{-1}) for 5 min. Filter the mixture through a small millipore filter.

Inject duplicate 1 µl to 5 µl aliquots of each filtrate on to the column of the gas chromatograph to obtain responses for peak height ratios of ethylene bromohydrin (EBH) versus propylene bromohydrin (PBH).

Calculate the mean of the duplicate samples and determine the EO in the sample by reference to the calibration line described in J.5.4.1.

Since some medical device materials can contain bromide ions (e.g. butylated rubbers), there is the potential for the formation of EBH as a degradation product of the EO, similar to the formation of ECH from the presence of chloride ions. Therefore, a portion of the sample should be analysed for the presence of EBH as a sterilization residual prior to the preparation of the bromohydrin derivative.

J.5.7 Simulated-use extraction for ethylene chlorohydrin using water

Use the procedure described in J.5.2.

J.5.8 Exhaustive extraction for 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 sufficient water to cover the sample portion while ensuring that the container is filled and capped. Extract for 24 h at the appropriate temperature, repeating extractions until less than 10 % of the initial amount is recovered. Agitate the container and contents vigorously on a mechanical shaker for approximately 10 min before sampling.

NOTE These temperatures and times are those used in the referee evaluation[14]. Other appropriate temperatures and times can be required to achieve an exhaustive extraction (see J.5.3). If required, it can be more appropriate to agitate for the entire time. Some materials do not need to be agitated.

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.

J.6 Gas chromatography

J.6.1 Suitability of the GC analysis

Select the most appropriate methods and procedures for the chromatographic procedure:

— resolution between the peak of interest (EO or ECH) and the nearest one should be ≥ 2,0;

— for EO and ECH peaks, tailing should be less than or equal to 1,8;

— the calibration standards' calculated concentrations should be within ±15 % of their target concentrations.

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

NOTE In order to improve the accuracy of the measurement, and to detect problems with an injection, many chromatographers use internal standards in their method.

J.6.2 Extraction to simulate the product use for the determination of EO or ECH

Inject 1 µl to 5 µl aliquots of the aqueous extract from J.5.2 or J.5.7.

J.6.3 Exhaustive procedure using thermal extraction

Inject 100 µl to 1 ml aliquots of the headspace gas.

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

Inject 100 µl to 1 ml aliquots of the aqueous extract from J.5.4.

J.6.5 Exhaustive extraction with ethanol followed by bromohydrin derivative preparation and chromatography using a gas chromatograph equipped with an ECD

Inject 1 µl to 5 µl aliquots of the aqueous extract from J.5.6.

Annex K
(informative)

Examples of product release methods

K.1 General

The guidance given in this annex is not intended as a checklist for assessing conformity with this document nor is it intended to infer that this guidance is the only means of achieving conformity. This annex is intended to assist in obtaining a uniform understanding and implementation of this document. Guidance is provided to improve understanding for the implementation of the requirements by providing explanations and acceptable methods for achieving conformity with specified requirements. Methods other than those given in this annex can be used, providing their performance achieves conformity with the requirements in this document.

This annex provides examples of the calculations for meeting the AL. Guidance on the selection of the different methods outlined in Clause 5 can be found in A.3.4.

K.2 Examples of limited exposure AL

Table K.1 — Examples of limited exposure AL

Patient population	Patient mass kg	EO milligrams per device	ECH milligrams per device
adult – unisex (> 16 y)	60	3,6	7,7
adolescent (10 y to 16 y)	25	1,5	3,2
child (1 y to 9 y)	10	0,6	1,3
infant (< 1 y)	3,5	0,21	0,45
The AL values in this table assume the use of CEF of 0,2 and one subject device.			

K.3 Examples of prolonged exposure AL for 10 d of anticipated exposure

Table K.2 — Examples of prolonged exposure AL for 10 d of anticipated exposure

Patient population	Patient mass kg	EO milligrams per device	ECH milligrams per device
adult – unisex (> 16 y)	60	36	32
adolescent (10 y to 16 y)	25	15	14
child (1 y to 9 y)	10	6,0	5,4
infant (< 1 y)	3,5	2,1	1,9
The AL values in this table assume the use of CEF 0,2 and one subject device.			

K.4 Example of special situation limited exposure AL

Table K.3 — Example of special situation limited exposure AL

Patient population	Patient mass	Blood cell separators (EO) (see 4.3.5.3)	Blood cell separators (ECH) (see 4.3.5.3)	Cardio-pulmonary bypass device (EO) (see 4.3.5.6)	Cardio-pulmonary bypass device (ECH) (see 4.3.5.6)	Blood oxygenators and blood reservoirs (EO) (see 4.3.5.7)	Blood oxygenators and blood reservoirs (ECH) (see 4.3.5.7)
	kg	milligrams per device	milligrams per device	milligrams per device	milligrams per device	milligrams per device	milligrams per device
adult – unisex (> 16 y)	60	9,0	19	18	38	54	38
adolescent (10 y to 16 y)	25	3,8	8,0	8	16	23	16
child (1 y to 9 y)	10	1,5	3,2	3,2	6,4	9,0	6,4
infant (< 1 y)	3,5	0,53	1,1	1,1	2,2	3,2	2,2
The AL values in this table assume the use of CEF 0,2 and one subject device.							

K.5 Minimum aeration product release method

K.5.1 General

K.5.1.1 When a specified minimum aeration time is utilized for a sterilization process, demonstrating that new or existing products meet the AL at this time point can be preferred to a dissipation curve. This method can be used for both EO and ECH.

K.5.1.2 Data from three different sterilization batches are collected at the same time point. This data is averaged and a 95 % upper confidence limit is calculated to confirm that future products would also meet the AL.

K.5.2 Examples

K.5.2.1 Example 1: End point residual calculation

For a limited exposure device used in adults, three samples are tested in sterilizer batch A after 24 h aeration with results of 3,3 milligrams per device, 3,7 milligrams per device and 3,4 milligrams per device of EO. Products from sterilizer batch B were tested after 24 h aeration with results of 2,7 milligrams per device, 3,0 milligrams per device and 3,2 milligrams per device. Products from sterilizer batch C were tested after 24 h aeration with results of 2,5 milligrams per device, 3,0 milligrams per device and 3,5 milligrams per device.

The average of these results is 3,1 milligrams per device of EO with a standard deviation of 0,384 milligrams per device.

Using Formula (K.1), the 95 % upper confidence limit (UCL) is 3,4 milligrams per device:

$$I = \bar{x} + z \times \left(\frac{\sigma}{\sqrt{n}} \right) = 3,1 + 1,96 \times 0,384 / 3 = 3,4 \text{ milligrams per device} \quad \text{(K.1)}$$

where

I is the 95 % upper confidence limit;

$\bar{x}$ is the average of the results;

z is the Z-value of 1,96 based on a 95 % confidence interval (CI);

σ is the standard deviation;

n is the number of samples.

K.5.2.2 Example 2: End point residual calculation

Using the same data set as example 1 in K.5.2.1, the 95 % upper confidence limit can be estimated by adding two standard deviations to the mean.

3,1 + 2 × 0,384 = 3,9 milligrams per device

K.6 95 % prediction limit calculation

K.6.1 The 95 % prediction limit, given by Formulae (16) and (17), determines the minimum aeration time from the data from three sterilization batches collected at different time points. The prediction limit calculation can be used for limited, prolonged, long-term exposure and TCL calculations. The more data included in the calculation reduces the uncertainty and will generally improve (reduce) the prediction limit estimate of aeration time. A low r-value in the regression line (significant variability in data) is factored into the formulae and will result in a higher aeration time calculation. Collecting data across a wide range of aeration times including results that are below the limit(s) provides increased accuracy. The TCL values are derived from the 24 h extraction data. The example product in Table K.4 has long-term patient contact with 19 160 d anticipated exposure duration and a surface area of 175 cm^2. Based on this data, the product would require 27 h aeration to reach the limited exposure limit, see Table K.5. For prolonged and long-term exposure, the AL are met with no additional aeration, see Table K.6 and Table K.7. The tolerable contact level AL is achieved with no additional aeration, see Table K.6.

Table K.4 — Hypothetical EO residual results

Sterilization batch	Aeration time x_i h	1st 24 h extraction milligrams per device	1st 30-day extraction milligrams per device	Total extraction milligrams per device	TCL µg/cm^2
1	11,5	6,5	17,5	17,5	37
1	23	2,1	5,7	5,7	12
1	37	1,5	4,1	4,1	9
2	12	5,4	14,6	14,6	31
2	24	1,8	4,9	4,9	10
2	48	1,2	3,2	3,2	7
3	13	3,2	8,6	8,6	18
3	23,5	2,2	5,9	5,9	13
3	47	0,9	2,4	2,4	5

K.6.2 The variables in Table K.5 are input into Formulae (16) and (17) for each limit to generate the 95 % prediction limit (L_p) for the aeration time for the limited exposure data.

$$x_0 = \frac{y_0 - a}{b} \tag{K.2}$$

$$L_p = x_0 + t_\alpha \times \sqrt{\frac{(S_\alpha)^2}{b^2} \times \left[1 + \frac{1}{n} + \frac{(y_0 - y_\mu)^2}{b^2 \times \sum (x_i - x_\mu)^2}\right]} \tag{K.3}$$

Table K.5 — Limited exposure 95 % prediction limit

Variable	Description	Value
EO limit	EO limit, in mg	4
x_0	average value of the release time corresponding to the EO limit, $x_0 = (y_o - a)/b$	12,971 93
y_0	natural logarithmic (ln) value of the EO limit	1,386 29
a	intercept of the linear regression line	1,933 25
b	slope of the regression line	−0,042 16
t_α	student t-value at significance of 0,05 with (n − 2) degrees of freedom with a one-tail test	1,894 58
$(s_\alpha)^2$	residual variance of the regression line and is derived by taking the squared standard deviation (variance) of the residual values (this is calculated by Medstat as the mean square error)	0,079 79
y_μ	average of the natural log of the EO values	0,813 55
n	number of test samples per run	9
x_i	individual time after sterilization at which measurements are made	see Table K.4
x_μ	average of the times after sterilization, in h	26,555 555 56
$\sum=(x_i - x_\mu)^2$	sum of squares for x (time), in h	1 637,722 222
L_p	95 % prediction limit of required aeration time, in h	27,01

K.6.3 The variables in Table K.6 are input into Formulae (16) and (17) for each limit to generate the 95 % prediction limit (L_p) for aeration time for the prolonged exposure data.

Table K.6 — Prolonged exposure 95 % prediction limit

Variable	Description	Value
EO limit	EO limit, in mg	108
x_0	average value of the release time corresponding to the EO limit, $x_0 = (y_0 - a)/b$	−41,349 56
y_0	logarithmic (base 10) value of the EO limit	2,033 42
a	intercept of the linear regression line	1,272 68
b	slope of the regression line	−0,018 40
t_α	student t-value at significance of 0,05 with (n − 2) degrees of freedom with a one-tail test	1,894 58
$(s_\alpha)^2$	residual variance of the regression line and is derived by taking the squared standard deviation (variance) of the residual values (this is calculated by Medstat as the mean square error)	0,014 80
y_μ	average of the Log10 of the EO values	0,784 11
n	number of test samples per run	9
x_i	individual time after sterilization at which measurements are made	see Table K.4
x_μ	average of the times after sterilization, in h	26,555 555 56
$\sum=(x_i - x_\mu)^2$	sum of squares for x (time), in h	1 637,722 222
L_p	95 % prediction limit of required aeration time	−16,53

K.6.4 The variables in Table K.7 are input into Formulae (16) and (17) for each limit to generate the 95 % prediction limit (L_p) for the aeration time for the long-term exposure data. As all EO was extracted within the first 30 d, the same data was used for prolonged and long-term exposure.

Table K.7 — Long-term exposure 95 % prediction limit

Variable	Description	Value
EO limit	EO limit, in mg	6 000
x_0	average value of the release time corresponding to the EO limit, $x_0 = (y_0 - a)/b$	−136,182 08
y_0	natural logarithmic (ln) value of the EO limit	8,699 51
a	intercept of the linear regression line	2,930 44
b	slope of the regression line	−0,042 38
t_α	student t-value at significance of 0,05 with (n − 2) degrees of freedom with a one-tail test	1,894 58
$(s_\alpha)^2$	residual variance of the regression line and is derived by taking the squared standard deviation (variance) of the residual values (this is calculated by Medstat as the mean square error)	0,078 44
y_μ	average of the natural log of the EO values	1,805 47
n	number of test samples per run	9
x_i	individual time after sterilization at which measurements are made	see Table K.4
x_μ	average of the times after sterilization, in h	26,555 555 56
$\sum=(x_i - x_\mu)^2$	sum of squares for x (time), in h	1 637,722 222
L_p	95 % prediction limit of required aeration time, in h	−84,18

K.6.5 The variables in Table K.8 are input into Formulae (16) and (17) for each limit to generate the 95 % prediction limit (L_p) for the aeration time for TCL.

Table K.8 — Tolerable contact level 95 % prediction limit

Variable	Description	Value
EO limit	EO limit in µg/cm^2	10
x_0	average value of the release time corresponding to the EO limit, $x_0 = (y_0 - a)/b$	32,710 36
y_0	logarithmic (base 10) value of the EO limit	1,000 00
a	intercept of the linear regression line	1,594 21
b	slope of the regression line	−0,018 17
t_α	student t-value at significance of 0,05 with (n − 2) degrees of freedom with a one-tail test	1,894 58
$(s_\alpha)^2$	residual variance of the regression line and is derived by taking the squared standard deviation (variance) of the residual values (this is calculated by Medstat as the mean square error)	0,016 06
y_μ	average of the Log10 of the EO values	1,111 81
n	number of test samples per run	9
x_i	individual time after sterilization at which measurements are made	see Table K.4
x_μ	average of the times after sterilization, in h	26,555 555 56
$\sum=(x_i - x_\mu)^2$	sum of squares for x (time), in h	1 637,722 222
L_p	95 % prediction limit of required aeration time, in h	46,79

K.7 Example of tolerable contact level

K.7.1 General

Conformity to the TCL limits can be accomplished by, either

a) dividing the EO and ECH extracted in the first 24 h by the device surface area with the result being less than the EO and ECH limits, or

b) using Formula (D.10).

Care should be taken for medical devices which contain but are not limited to non-absorbent materials/components (see Clause C.5) with direct contact so as not to underestimate or overestimate the level of residuals per surface area. The entire surface area of the components extracted should be included in the TCL calculation.

K.7.2 TCL example 1

Three samples are tested in sterilizer from each batch A, B and C after 12 h, 24 h and 36 h of aeration. The device surface area is 60 cm^2.

The amount of EO per centimetre cube is calculated by dividing the micrograms per device by the surface area (cm^2).

Table K.9 and Table K.10 demonstrate the calculated TCL results.

Table K.9 — Example of EO TCL calculations

Batch and sample	Aeration time h	EO result micrograms per device	Device surface area cm^2	TCL result $\mu g/cm^2$
A1	12	3 300	60	55
A2	24	1 500	60	25
A3	36	300	60	5,0
B1	12	3 700	60	62
B2	24	1 800	60	30
B3	36	200	60	3,3
C1	12	3 400	60	57
C2	24	1 600	60	27
C3	36	100	60	1,7

Table K.10 — Example of ECH TCL calculations

Batch and sample	Aeration time h	ECH result milligrams per device	Device surface area cm^2	TCL result mg/cm^2
A1	12	1,2	60	0,02
A2	24	1,0	60	0,02
A3	36	1,3	60	0,02
B1	12	0,9	60	0,02
B2	24	< 0,8	60	< 0,01
B3	36	1,0	60	0,02
C1	12	< 0,8	60	< 0,01
C2	24	< 0,8	60	< 0,01
C3	36	< 0,8	60	< 0,01

The data set at 36 h aeration averages 3,3 µg/cm^2 with a 95 % UCL of 6,6 µg/cm^2 (95 % UCL is equal to the mean added to two standard deviations). Applying the 95 % prediction limit in 5.4 to this 9-sample data set indicates that 38,34 h of aeration is required for the device, approximating the 95 % UCL limit conclusion of the minimum aeration calculation. Note that to use the specified minimum aeration method, three batches of data are required at the minimum aeration time point.

ECH results are acceptable after 12 h, therefore additional testing at 24 h and 36 h is not required.

K.7.3 TCL example 2

Using the same data set as example 1 in K.7.2, the TCL dose is established using Formula (D.11).

$$m_{\text{dev, BSC}} = TCL \times A \qquad \text{(K.4)}$$

where

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

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

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

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

$$m_{\text{dev, BSC}} = 10 \times 60 = 600 \text{ µg}$$

Using this calculation, the 95 % UCL milligrams per device must be below 600 µg to meet the TCL limit. The average result at 36 h is 200 micrograms per device with a standard deviation of 100 micrograms per device resulting in a 95 % UCL of 400 milligrams per device indicating that at 36 h, the TCL limit is met.

For ECH,

$$m_{\text{dev, BSC}} = 5 \times 60 = 300 \text{ mg}$$

All results meet this requirement.

K.8 Application of allowable limits

When a device has repeated use (e.g. when a device is used and then replaced with a new device of the same kind after a specific duration of time), consideration should be given to the device's cumulative use duration when calculating the AL and designing the extraction study.

The cumulative exposure estimate obtained by simulated use extraction or exhaustive extraction should be compared to the applicable AL(s). If the extraction study was performed under exhaustive conditions, then the cumulative amount of EO or ECH may be considered a total quantity of exposure and compared against the applicable limits, noting that this result can be an overestimation of the limited and prolonged exposure. If the extraction study was performed under simulated-use conditions (i.e. repeated 24 h extractions, see A.4.2.3.2), then the amount of EO or ECH can be considered a daily (limited) exposure.

To calculate the AL, Formula (2) is used. The duration of the medical device exposure is determined by the three medical device exposure duration categories: limited (< 24 h), prolonged (1 d to 30 d) and long-term (> 30 d). The number of days of the medical device exposure duration category are listed in Table K.11.

Table K.11 — Duration of exposure to the medical device

Exposure category	Default d	Alternative d
limited	1 d	simulated use
prolonged	no more than 30 d	based on the anticipated exposure duration
long-term	default 25 000 d	
long-term (paediatric only)	no more than 5 840 d (0 to 16 y)	
long-term (adult only)	default 19 160 d (>16 y)	

To calculate the AL, the number of devices used during this duration is also determined, which is the number of devices that the manufacturer intends to be used cumulatively in each exposure category. For example, if a new device is used daily for 29 d, then the number of devices is 29. If three devices of the same kind are used simultaneously every day for four days, then the number of devices used in the limited duration category is three (three devices a day) and the number of devices used in the prolonged category is 12 (3 devices per day over 4 days is equal to 12 $\text{devices}_{\text{Prolonged}}$).

As EO sterilization is ubiquitous, the CEF of 0,2 is designed to be a safety factor that accounts for the unknowing simultaneous use of EO sterilized devices. The CEF may be modified if the manufacturer has knowledge of how the device is used simultaneously with other EO sterilized devices.

K.9 Example of a repeated use long-term exposure device (new device used every week)

K.9.1 Context

After 12 hours aeration, the device was extracted in water at 37 °C using consecutive 24 h extractions that reached exhaustion (less than 10 % of the initial extraction mass) after four extractions.

NOTE This example focused on a single data set. Two additional sets of data would be required for either

a) end point testing to confirm conformity, or

b) a dissipation curve to be constructed.

The results of the four extractions are:

— 1st extraction: 2 mg of EO, 0,5 mg of ECH;

— 2nd extraction: 1 mg of EO, < 0,1 mg of ECH;

— 3rd extraction: 0,6 mg of EO, < 0,1 mg of ECH;

— 4th extraction: 0,1 mg of EO, < 0,1 mg of ECH;

— cumulative EO: 3,7 milligrams per device;

— cumulative ECH: 0,5 milligrams per device.

The assumptions are:

— 18 000 d is the cumulative exposure duration;

— a new device is used every 7 d;

— the indicated patient population is adults only of a body mass of 60 kg.

The formulae are:

$$TE = TI \times m_{\text{b}} \times CEF$$

$$AL = TE \times \left(\frac{I_d}{N} \right)$$

where

TI is the tolerable intake;

m_b is the body mass, in kg;

CEF is the default 0,2 representative of five devices used simultaneously;

I_d is the number of days in exposure category, which is 1 for limited duration, 30 for prolonged duration and 18 000 for long-term exposure.

The reference TIs are:

— limited and prolonged EO TI: 0,30 mg/kg/d;

— long-term EO TI: 0,02 mg/kg/d;

— limited ECH TI: 0,64 mg/kg/d;

— prolonged ECH TI: 0,27 mg/kg/d;

— long-term ECH TI: 0,029 mg/kg/d.

K.9.2 AL calculations

K.9.2.1 General procedure for AL calculation

First, calculate the 24-h limited duration AL. Then, calculate the prolonged-duration (30 d) AL. Lastly, calculate the long-term exposure AL (18 000 d). Compare the calculated AL in milligrams per device to the extracted quantity in milligrams per device.

K.9.2.2 Limited exposure AL calculation

Calculate the EO limited exposure AL:

$$AL_{\text{EO,limited}} = 0,3 \times 60 \times 0,2 \times \frac{1}{1} = 3,6 \text{ milligrams per device}$$

Calculate the ECH limited exposure AL:

$$AL_{\text{ECH,limited}} = 0,64 \times 60 \times 0,2 \times \frac{1}{1} = 7,7 \text{ milligrams per device}$$

K.9.2.3 Interpretation of results for limited exposure

The first extraction of 2 milligrams per device of EO demonstrates conformity with the calculated AL for a limited exposure duration, i.e. 2 milligrams per device of EO extracted in the first 24 h is less than the calculated 3,6 milligrams per device of EO limit.

The first extraction was 0,5 milligrams per device ECH. The first extraction demonstrates conformity with the calculated AL for a limited exposure duration, i.e. 0,5 milligrams per device of ECH is less than the calculated 7,7 milligrams per device ECH limit.

K.9.2.4 Prolonged exposure AL calculation

Assuming that patient usage is every 7 d, 4,5 devices per month would be required (e.g. 30/7 which is approximately 4,5).

Calculate the EO prolonged exposure AL:

$$AL_{\text{EO,prolonged}} = 0,30 \times 60 \times 0,2 \times \left(\frac{30}{4,5} \right) = 24 \text{ milligrams per device}$$

Calculate the ECH prolonged exposure AL:

$$AL_{\text{ECH,prolonged}} = 0,27 \times 60 \times 0,2 \times \left(\frac{30}{4,5} \right) = 22 \text{ milligrams per device}$$

The interpretation of results for prolonged exposure is as follows.

— The total extracted quantity of EO is 3,7 milligrams per device of EO. A total exposure dose of 3,7 milligrams per device of EO is less than the calculated prolonged exposure limit of 24 milligrams per device.

— The total extracted quantity of ECH is 0,5 milligrams per device of ECH. A total exposure dose of 0,5 milligrams per device is less than the calculated prolonged exposure limit of 22 mg of ECH.

K.9.2.5 Long-term exposure AL calculation

Assuming that patient usage is every 7 d, 2 571 devices per lifetime would be required (e.g. 18 000 d/7 d = 2 571).

Calculate the long-term EO AL:

$$AL_{\text{EO,long-term}} = 0,02 \times 60 \times 0,2 \times \left(\frac{18\,000}{2\,571} \right) = 1,7 \text{ milligrams per device}$$

Calculate the long-term ECH AL:

$$AL_{\text{ECH,long-term}} = 0,029 \times 60 \times 0,2 \times \left(\frac{18\,000}{2\,571} \right) = 2,4 \text{ milligrams per device}$$

K.9.2.6 Interpretation of results for long-term exposure

The total extracted quantity of EO is 3,7 mg. Assuming patient usage every 7 d results in 2 571 devices per lifetime would be required (e.g. 18 000 d divided by 7 d is equal to 2 571). A total exposure dose of 3,7 milligrams of EO per device exceeds the calculated 1,7 milligrams per device EO. Further exposure refinement is needed.

For EO, the estimated exposure of 3,7 mg of EO per device multiplied by 2 571 devices per the lifetime results in an estimated exposure of 9 514 mg of EO over a lifetime. This exposure exceeds the calculated long-term exposure limit of 4 371 mg of EO (1,7 milligrams per device EO multiplied by 2 571 devices per lifetime is equal to 4 371 mg of EO over a lifetime). This product would require sterilization cycle modifications (e.g. change in gas concentration, additional aeration), re-evaluation of the exposure assumptions (e.g. refinement of the CEF, see K.9.2.7), or a risk assessment to determine the appropriate limits commensurate for the actual device usage and patient population.

The total extracted quantity of ECH is 0,5 milligrams per device. Assuming that patient usage is every 7 d, 2 571 devices per lifetime would be required (e.g. 18 000 d divided by 7 d equals to approximately 2 571). A total exposure dose of 0,5 milligrams per device of ECH is less than the calculated 2,4 milligrams per device of ECH.

K.9.2.7 Example of exposure assumption refinement

A risk assessment refining the exposure assumptions can be produced to address the EO limit for a long-term exposure device based on having a repeated use every 7 d.

This example of an insulin delivery system requires no other simultaneously applied EO sterilized devices in long-term exposure. Therefore, the CEF can be set to 1,0 in this specific case, where:

$$TE = 0,02 \times 60 \times 1 \times \left(\frac{18\,000}{2\,571} \right) = 8,4 \text{ milligrams per device}$$

K.9.2.8 Exposure assumption refinement interpretation

The total extracted quantity of EO is 3,7 milligrams per device. Assuming that patient usage is every 7 d, 2 571 devices per lifetime would be required (e.g. 18 000 d divided by 7 d is equal to 2 571). A total exposure dose of 3,7 milligrams per device of EO is less than the calculated 8,4 milligrams per device of EO. No further exposure refinement is needed.

K.10 Example of a repeated use catheter (two new devices used every 3 d for 14 d)

K.10.1 Context

After sterilization and a 24 h aeration period, the device was extracted in water at 37 °C using consecutives extractions for 24 h each, exhaustive extraction was completed using consecutive extractions of 24 h until less than 10 % of the initial extraction was recovered.

NOTE This example focused on a single data set. Two additional sets of data would be required for either

a) end point testing to confirm conformity or

b) a dissipation curve to be constructed.

The results of the four extractions are:

— 1st extraction: 0,2 mg of EO, 0,5 mg of ECH;

— 2nd extraction: 0,1 mg of EO, < 0,1 mg of ECH;

— 3rd extraction: 0,07 mg of EO, < 0,1 mg of ECH;

— 4th extraction: <0,01 mg of EO, < 0,1 mg of ECH;

— cumulative EO: 0,37 milligrams per device;

— cumulative ECH: 0,5 milligrams per device.

The assumptions are:

— 14 d is the cumulative exposure duration;

— two new devices are used every 3 d;

— two devices are used simultaneously;

— the indicated patient populations are:

  — adults of a body mass of 60 kg;

  — children of a body mass of 10 kg.

The formulae are:

$$TE = TI \times m_b \times CEF$$

$$AL = TE \times \left(\frac{D}{N} \right)$$

where

TI is the tolerable intake;

m_b is the body mass, in kg;

CEF is the default 0,2 representative of five devices used simultaneously;

N is the number of devices used during each exposure duration, which is 2;

D is the number of days in the exposure category, which is 1 for limited duration and 14 for prolonged duration.

The reference TIs are:

— limited and prolonged EO TI: 0,30 mg/kg/d;

— long-term EO TI: 0,02 mg/kg/d;

— limited ECH TI: 0,64 mg/kg/d;

— prolonged ECH TI: 0,27 mg/kg/d;

— long-term ECH TI: 0,029 mg/kg/d.

K.10.2 AL calculations

K.10.2.1 Repeated use catheter limited exposure AL calculation

Calculate the EO limited exposure AL:

$$AL_{\text{EO,limited, Adult}} = 0,3 \times 60 \times 0,2 \times \frac{1}{2} = 1,8 \text{ milligrams per device}$$

$$AL_{\text{EO,limited, Child}} = 0,3 \times 10 \times 0,2 \times \frac{1}{2} = 0,3 \text{ milligrams per device}$$

Calculate the ECH limited exposure AL:

$$AL_{\text{ECH,limited, Adult}} = 0,64 \times 60 \times 0,2 \times \frac{1}{2} = 3,8 \text{ milligrams per device}$$

$$AL_{\text{ECH,limited, Child}} = 0,64 \times 10 \times 0,2 \times \frac{1}{2} = 0,64 \text{ milligrams per device}$$

K.10.2.2 Interpretation of results for limited exposure

The first extraction of 0,2 milligrams per device of EO demonstrates conformity with the calculated AL for a limited exposure duration because 0,2 milligrams per device EO extracted in the first 24 h is less than the calculated 1,8 milligrams per device of EO limit for adults and 0,3 milligrams per device of EO limit for children.

The first extraction was 0,5 milligrams per device of ECH. The first extraction demonstrates conformance with the calculated AL for a limited exposure duration because 0,5 milligrams per device of ECH extracted is less than the calculated 3,8 milligrams per device of ECH limit for adults and 0,6 milligrams per device of ECH limit for children.

K.10.2.3 Repeated use catheter prolonged exposure AL calculation

The allowable limits are calculated assuming that patients use two devices simultaneously every 3 d for 14 d results. Cumulative duration is prolonged exposure based on 14 d.

Calculate the EO prolonged exposure AL:

$$AL_{\text{EO,prolonged, Adult}} = 0,30 \times 60 \times 0,2 \times \left(\frac{3}{2}\right) = 5,4 \text{ milligrams per device}$$

$$AL_{\text{EO,prolonged, Child}} = 0,30 \times 10 \times 0,2 \times \left(\frac{3}{2}\right) = 0,9 \text{ milligrams per device}$$

Calculate the ECH prolonged exposure AL:

$$AL_{\text{ECH,prolonged, Adult}} = 0,27 \times 60 \times 0,2 \times \left(\frac{3}{2}\right) = 4,9 \text{ milligrams per device}$$

$$AL_{\text{ECH,prolonged, Child}} = 0,27 \times 10 \times 0,2 \times \left(\frac{3}{2}\right) = 0,81 \text{ milligrams per device}$$

K.10.3 Interpretation of results for prolonged exposure

The total extracted quantity of EO is 0,37 milligrams per device of EO. A total exposure dose of 0,37 milligrams per device of EO is less than the calculated prolonged exposure limits of 5,4 milligrams per device EO for adults and 0,9 milligrams per device EO for children.

The total extracted quantity of ECH is 0,5 milligrams per device of ECH. A total exposure dose of 0,5 milligrams per device of ECH is less than the calculated prolonged exposure limits of 4,9 milligrams per device of ECH for adults and 0,81 milligrams per device of ECH for children.

K.10.4 Repeated use catheter long-term exposure AL calculation

No long-term exposure AL is required. The maximum duration of use is 14 d.

K.11 Example of a cochlear implant device (single use)

K.11.1 Context

After sterilization and a 12 h aeration period, the device was extracted in water at 37 °C using consecutives extractions of 24 h until less than 10 % of the initial extraction mass was recovered.

NOTE This example focused on a single data set. Two additional sets of data would be required for either end point testing (see 5.3) or a dissipation curve (see 5.4).

The results of the four extractions are:

— 1st extraction: 0,07 mg of EO, 0,2 mg of ECH;

— 2nd extraction: 0,02 mg of EO, < 0,1 mg of ECH;

— 3rd extraction: < 0,01 mg of EO, < 0,1 mg of ECH;

— cumulative EO: 0,09 milligrams per device;

— cumulative ECH: 0,2 milligrams per device.

The assumptions are:

— 25 000 d is the cumulative exposure duration where;

— 5 840 d (0 to 16 y);

— 19 160 d (>16 y);

— two devices are used;

— the indicated patient populations are:

  — adults of a body mass of 60 kg;

  — children (> 1 y to ≤ 16 y of age) of a body mass of 10 kg;

  — infants (< 1 y) of a body mass of 3,5 kg.

The formulae are:

$$TE = TI \times m_{\mathrm{b}} \times CEF$$

$$AL = TE \times \left(\frac{D}{N} \right)$$

where

TI	is the tolerable intake;
m_{b}	is the body mass, in kg;
CEF	is the default 0,2 representative of five devices used simultaneously for limited exposure during implantation and 1,0 for prolonged and long-term exposure because no other EO sterilized devices are used concomitantly;
N	is the maximum number of devices used during each duration, which is 2;
$I_{d,\mathrm{limited}}$	is the number of days in the limited duration exposure category, which is 1;
$D_{\mathrm{prolonged}}$	is the number of days in the prolonged duration exposure category, which is 30;
$D_{\mathrm{long\text{-}term,\ Paediatric}}$	is the number of days in the long-term duration exposure category, 0 to 16 y, which is 5 840;
$D_{\mathrm{long\text{-}term,\ Adult}}$	is the number of days in the long-term duration exposure category, which is 19 160 d (> 16 y).

The reference TIs are:

— limited and prolonged EO TI: 0,30 mg/kg/d;

— long-term EO TI: 0,02 mg/kg/d;

— limited ECH TI: 0,64 mg/kg/d;

— prolonged ECH TI: 0,27 mg/kg/d;

— long-term ECH TI: 0,029 mg/kg/d.

K.11.2 Cochlear implant device AL calculations

K.11.2.1 General procedure for AL calculation

First, calculate the 24-h limited duration AL. Then, calculate the prolonged-duration (30 d) AL. Lastly, calculate the long-term child ALs (5 840 d) and then the long-term adult AL (19 160 d). Compare the calculated AL in milligrams per device to the extracted quantity in milligrams per device.

K.11.2.2 Limited exposure AL calculation

Calculate the EO AL for the limited exposure for the lowest body mass population:

$$AL_{\text{EO,limited, Infant}} = 0,3 \times 3,5 \times 0,2 \times \left(\frac{1}{2}\right) = 0,11 \text{ milligrams per device}$$

Calculate the ECH AL for the limited exposure for the lowest body mass population:

$$AL_{\text{ECH,limited, Infant}} = 0,64 \times 3,5 \times 0,2 \times \left(\frac{1}{2}\right) = 0,22 \text{ milligrams per device}$$

K.11.2.3 Interpretation of results for limited exposure

The first extraction of 0,07 milligrams per device of EO demonstrates conformance with the calculated AL for a limited exposure duration because 0,07 milligrams per device of EO extracted in the first 24 h is less than the calculated 1,8 milligrams per device of EO limit for adults and 0,3 milligrams per device of EO limit for children and 0,11 milligrams per device of EO limit for infants.

The first extraction was 0,2 milligrams per device of ECH. The first extraction demonstrates conformity with the calculated AL for a limited exposure duration because 0,2 milligrams per device of ECH extracted in 24 h is less than the calculated 3,8 milligrams per device ECH limit for adults and 0,6 milligrams per device ECH limit for children and 0,22 milligrams per device ECH limit for infants.

K.11.2.4 Cochlear implant device prolonged exposure AL calculation

Assuming that patients use up to two devices once (e.g. no repeated use), the maximum number of devices used during this duration is two devices.

Calculate the EO AL for prolonged exposure for the lowest body mass population:

$$AL_{\text{EO,prolonged, Infant}} = 0,3 \times 3,5 \times 0,2 \times \left(\frac{30}{2}\right) = 3,2 \text{ milligrams per device}$$

Calculate the ECH AL for prolonged exposure for the lowest body mass population:

$$AL_{\text{ECH,prolonged, Infant}} = 0,27 \times 3,5 \times 0,2 \times \left(\frac{30}{2}\right) = 2,8 \text{ milligrams per device}$$

K.11.2.5 Interpretation of results for prolonged exposure

The total extracted quantity of EO is 0,09 milligrams per device EO. A total exposure of 0,09 milligrams per device of EO is less than the calculated prolonged exposure limit of 3,2 milligrams per device of EO for infants, children and adults as the infant limit is the lowest and applicable for all groups.

The total extracted quantity of ECH is 0,2 milligrams per device of ECH. A total exposure of 0,2 milligrams per device ECH is less than the calculated prolonged exposure limits of 2,8 milligrams per device of ECH for infants, children and adults as the infant limit is the lowest and applicable for all groups.

K.11.2.6 Cochlear implant device long-term exposure AL calculation

Medical devices used in paediatric populations pose a special challenge in the derivation of an AL since the mass of the patient changes as the individual ages. A long-term exposure AL of 636 milligrams per device of EO for individuals during the first 16 y (365 d/y multiplied by 16 y which is equal to 5 840 d) is protective for paediatric and adult EO exposure where 0,11 mg/d and includes the CEF safety factor of 0,2, see Table D.9. The AL was adjusted for the number of devices used simultaneously.

A long-term exposure AL of 925 milligrams per device of ECH for individuals during the first 16 y (365 d/y multiplied by 16 y which is equal to 5 840 d) is protective for paediatric ECH exposure where

0,16 mg/d for the first 16 y accounts for the changing body mass (925 mg divided by 5 840 d which is equal to 0,16 mg/d) and through adulthood includes the CEF safety factor of 0,2, see Table E.5.

Assuming that patients use up to two devices once (e.g. no repeated use), the maximum number of devices used during this duration is two devices.

Calculate the EO long-term exposure AL:

$$AL_{\text{EO,long-term}} = 0,11 \times \left(\frac{25\,000}{2} \right) = 1\,375 \text{ milligrams per device}$$

Calculate the ECH long-term exposure AL:

$$AL_{\text{ECH,long-term}} = 0,16 \times \left(\frac{25\,000}{2} \right) = 2\,000 \text{ milligrams per device}$$

K.11.2.7 Interpretation of results for long-term exposure

The total extracted quantity of EO is 0,09 milligrams per device. A total exposure dose of 0,09 milligrams per device of EO is less than all the calculated long-term EO paediatric limit for both the first 16 y and the remaining 19 160 d.

The total extracted quantity of ECH is 0,2 milligrams per device. A total exposure dose of 0,2 milligrams per device of ECH is less than all the calculated long-term ECH paediatric limit for both the first 16 y and the remaining 19 160 d.

K.12 Example of an insulin delivery system

K.12.1 Context

This device is used from birth through adulthood. A new device is used every 3 d. The duration of contact is 72 h per device. After sterilization and a 12 h aeration period, exhaustive extraction in water at 37 °C using consecutives extractions of 24 h was completed until less than 10 % of the initial extraction mass was recovered.

The results of batch 1 are:

— 1st extraction: 0,4 mg of EO, 0,2 mg of ECH;

— 2nd extraction: 0,2 mg of EO, < 0,1 mg of ECH;

— 3rd extraction: 0,03 mg of EO, < 0,1 mg of ECH;

— cumulative EO: 0,63 milligrams per device;

— cumulative ECH: 0,2 milligrams per device.

The results of batch 2 are:

— 1st extraction: 0,5 mg of EO, 0,2 mg of ECH;

— 2nd extraction: 0,3 mg of EO, < 0,1 mg of ECH;

— 3rd extraction: 0,04 mg of EO, < 0,1 mg of ECH;

— cumulative EO: 0,84 milligrams per device;

— cumulative ECH: 0,2 milligrams per device.

The results of batch 3 are:

— 1st extraction: 0,3 mg of EO, 0,2 mg of ECH;

— 2nd extraction: 0,15 mg of EO, < 0,1 mg of ECH;

— 3rd extraction: 0,03 mg of EO, < 0,1 mg of ECH;

— cumulative EO: 0,48 milligrams per device;

— cumulative ECH: 0,2 milligrams per device.

Estimation of 95 % UCL is as follows.

— The average of the first 24 h extractions is 0,40 milligrams per device with a standard deviation of 0,1 milligrams per device. The average +2 standard deviations estimates a 95 % UCL of 0,60 milligrams per device after 12 h aeration.

— The average of the total extractions is 0,65 milligrams per device with standard deviation of 0,18 milligrams per device. The average +2 standard deviations estimates a 95 % UCL of 1,0 milligrams per device after 12 h aeration.

The assumptions are:

— 25 000 d is the cumulative exposure duration where;

— 5 840 d (0 to 16 y);

— 19 160 d (> 16 y);

— one device used every 3 d;

— the indicated patient populations are:

  — adults of a body mass of 60 kg adult body mass;

  — children (> 1 y to ≤ 16 y of age) of a body mass of 10 kg;

  — infants (< 1 y) of a body mass of 3,5 kg.

The formulae are:

$$TE = TI \times m_{\mathrm{b}} \times CEF$$

$$AL = TE \times \left(\frac{I_d}{\mathrm{N}} \right)$$

where

TI	is the tolerable intake;
m_{b}	is the body mass, in kg;
CEF	is the default 0,2 representative of five devices used simultaneously for limited exposure and prolonged infant which reasonably anticipates additional health issues within the first 30-days of birth 1,0 for long-term exposure because no other EO sterilized devices are used concomitantly;
$I_{d,\mathrm{limited}}$	is the number of days in the limited duration exposure category, which is 1;
$I_{d,\mathrm{prolonged}}$	is the number of days in the prolonged duration exposure category, which is 30;
$I_{d,\mathrm{long\text{-}term,\ Paediatric}}$	is the number of days in the long-term duration exposure category, 0 to 16 y, which is 5 840;
$I_{d,\mathrm{long\text{-}term,\ Adult}}$	is the number of days in the long-term duration exposure category, which is 19 160 (> 16 y).

The reference TIs are:

— limited and prolonged EO TI: 0,30 mg/kg/d;

— long-term EO TI: 0,02 mg/kg/d;

— limited ECH TI: 0,64 mg/kg/d;

— prolonged ECH TI: 0,27 mg/kg/d;

— long-term ECH TI: 0,029 mg/kg/d.

K.12.2 Insulin disposables AL calculations

K.12.2.1 General procedure for AL calculation

First, calculate the 24-h limited exposure duration AL. As this device is intended for infants through adulthood, the lowest body mass of 3,5 kg is used in the calculations. Then, calculate the prolonged exposure duration (30 d) AL. Lastly, calculate the long-term exposure infant ALs (5 840 d) and then the long-term adult AL (19 160). Compare the calculated AL in milligrams per device to the extracted quantity in milligrams per device.

K.12.2.2 Limited exposure AL calculation

Calculate the EO AL for limited exposure for the lowest patient population body mass of 3,5 kg:

$$AL_{\text{EO,limited, Infant}} = 0,3 \times 3,5 \times 0,2 \times \left(\frac{1}{1}\right) = 0,21 \text{ milligrams per device}$$

Calculate the ECH AL for limited exposure for the lowest patient population body mass of 3,5 kg:

$$AL_{\text{ECH,limited, Infant}} = 0,64 \times 3,5 \times 0,2 \times \left(\frac{1}{1}\right) = 0,45 \text{ milligrams per device}$$

K.12.2.3 Interpretation of results for limited exposure

The 95 % UCL estimates 0,6 EO milligrams per device in the first 24 h of patient exposure. With the lowest patient population mass of 3,5 kg the AL of 0,21 milligrams per device is not met. EO residuals can be reduced by either modifying the EO cycle to reduce gas concentration, reduce exposure time, add addition air washes, or add additional aeration time. The CEF may be adjusted, if justified. To determine the required aeration time so that the product would meet the allowable limit can be established by constructing a 95 % dissipation curve instead of additional testing.

The first extraction of 0,2 milligrams per device of ECH demonstrates conformity with the calculated AL for a limited exposure duration because 0,2 milligrams per device of ECH extracted in the first 24 h is less than the lowest calculated (e.g. most sensitive patient population) limit of 0,45 milligrams per device ECH for infants.

K.12.2.4 Insulin disposable prolonged exposure AL calculation

Calculate the AL for prolonged exposure for the patient population with the lowest body mass:

$$AL_{\text{EO,prolonged, Infant}} = 0,3 \times 3,5 \times 0,2 \times \left(\frac{3}{1}\right) = 0,63 \text{ milligrams per device}$$

$$AL_{\text{ECH,prolonged, Infant}} = 0,27 \times 3,5 \times 0,2 \times \left(\frac{3}{1}\right) = 0,57 \text{ milligrams per device}$$

K.12.2.5 Interpretation of results for prolonged exposure

The 95 % UCL estimates a total of 1,0 EO milligrams per device from the device over 3 d. With the lowest patient population mass of 3,5 kg the AL of 0,63 milligrams per device is not met. EO residuals can be reduced by modifying the EO cycle to reduce gas concentration, to reduce exposure time, to add additional air washes or to add additional aeration time. The CEF may be adjusted, if justified. Constructing a 95 % dissipation curve instead of endpoint testing would determine the aeration time that the product would meet the AL.

The total extracted quantity of ECH is 0,2 milligrams per device of ECH. A total exposure of 0,2 milligrams per device of ECH is less than the calculated prolonged exposure limits of 0,57 milligrams per device of ECH for infants, children and adults as the infant limit is the lowest and applicable for all groups.

K.12.2.6 Insulin disposable long-term exposure AL calculation

Medical devices used in paediatric populations pose a special challenge in the derivation of an AL since the mass of the patient changes as the individual ages. A long-term exposure AL of 636 milligrams per device of EO for individuals during the first 16 y (365 d/y multiplied by 16 y which is equal to 5 840 d) is protective for paediatric and adult EO exposure where TE of 0,11 mg/day with a CEF of 0,2, see Table D.9.

A long-term exposure AL of 925 milligrams per device of ECH for individuals during the first 16 y (365 d/y multiplied by 16 y which is equal to 5 840 d) is protective for paediatric ECH exposure where TE of 0,16 mg/day for the first 16 y accounts for the changing body mass (925 mg divided by 5 840 d which is equal to 0,16 mg/day) and through adulthood includes the CEF of 0,2, see Table E.5.

Calculate the EO long-term exposure AL. The device is used from birth through adulthood with a new device used every 3 d:

$$AL_{\text{EO,long-term}} = 0,11 \times \left(\frac{3}{1} \right) = 0,33 \text{ milligrams per device}$$

$$AL_{\text{EO,long-term}} = 0,11 \times \left(\frac{25\,000}{8\,333} \right) = 0,33 \text{ milligrams per device}$$

Calculate the ECH long-term exposure AL.

A patient uses one device every 3 d for a lifetime (e.g. repeated use). The device is used from birth through adulthood with a new device used every 3 d:

$$AL_{\text{ECH,long-term}} = 0,16 \times \left(\frac{3}{1} \right) = 0,48 \times 8\,333 = 4\,000 \text{ mg}$$

K.12.2.7 Interpretation of results for long-term exposure

The 95 % UCL estimates a total 1,0 EO milligrams per device from the device over 3 d. With the lowest patient population mass of 3,5 kg the AL of 0,33 milligrams per device is not met. EO residuals can be reduced by modifying the EO cycle to reduce gas concentration, to reduce exposure time, to add additional air washes or to add additional aeration time. The CEF may be adjusted, if justified. Constructing a 95 % dissipation curve instead of endpoint testing would determine the aeration time that the product would meet the AL.

The total extracted quantity of ECH is 0,2 milligrams per device. A total exposure dose of 0,2 milligrams per device ECH is less than the calculated long-term ECH paediatric limit of 0,48 milligrams per device.

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