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Biological evaluation of medical devices — Requirements for interlaboratory studies to demonstrate the applicability of validated in vitro methods to assess the skin sensitization of medical devices

Évaluation biologique des dispositifs médicaux — Guide pour les études interlaboratoires visant à démontrer l'applicabilité des méthodes in-vitro validées pour évaluer la sensibilisation cutanée des dispositifs médicaux

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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*.

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

International Standards can be used to demonstrate the safety and compliance of medical devices. ISO 10993-10 specifies the procedure for the assessment of medical devices and their constituent materials with regard to their potential to induce skin sensitization (Type IV hypersensitivity reaction). The methods included in ISO 10993-10 are based on animal or human testing, with an annex on in vitro and in chemico tests for skin sensitization that have been validated for neat chemicals. The effort to reduce or replace the use of animals in toxicity testing has led to the development of many new non-animal methods. The test guidelines in References [56] and [57] include alternatives to animal testing methods for skin sensitization that have been previously validated to confirm their equivalence/superiority to the current in vivo methods. However, currently, none of the OECD test guideline methods are considered sufficient stand-alone replacements for in vivo tests that assess the skin sensitization potential of chemicals[1].

Current OECD test guideline methods are validated with neat chemicals and not with more complex mixtures such as medical devices or medical devices extracts. In order to use these methods in the specific context of medical devices, an evaluation is needed to verify their applicability for assessing skin sensitization of medical devices. Given the number of candidate test methods and the time that is required to assess them, it is important to ensure that the same science-based evaluation process and criteria are consistently applied to any new candidate test method. The purpose of this document is to provide a framework for the conduct of prevalidation and interlaboratory studies to assess the applicability of candidate test methods for assessing one or more key events related to OECD's adverse outcome pathway (AOP) for skin sensitization when evaluating medical devices[2].

Biological evaluation of medical devices — Requirements for interlaboratory studies to demonstrate the applicability of validated in vitro methods to assess the skin sensitization of medical devices

1 Scope

This document specifies the framework and the methodology to evaluate and demonstrate the applicability of a validated non-animal method from an OECD test guideline to assess the skin sensitizing potential of a medical device or a medical device material. This document addresses:

— the database of reference chemical skin sensitizers and non-skin sensitizers;

— reference materials;

— feasibility testing of candidate test methods, including any method optimization for use with extracts of medical devices;

— prevalidation of candidate test methods;

— the interlaboratory study:

  — sample preparation and coding;

  — spiking of the extracts from the negative control medical device material;

  — data collection;

  — statistical analysis to assess reliability and reproducibility.

The use of the approaches described in this document to assess the applicability of a candidate test method does not imply that the candidate test method can be used as a stand-alone test for evaluating the skin sensitization potential of medical devices. For certain candidate test methods, integrated approaches and/or defined approaches are needed.[1] The evaluation of skin sensitization potential of a medical device is described in ISO 10993-10.

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

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
allergen
sensitizer
substance or material that is capable of inducing a specific hypersensitivity reaction upon repeated contact with that substance or material

3.2
candidate test method
test method for in vitro skin sensitization testing of medical devices that is under evaluation

3.3
interlaboratory study
ILS
organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories according to predetermined conditions

3.4
interlaboratory reproducibility
between-laboratory reproducibility
measure of the extent to which different qualified laboratories, using the same protocol and testing the same substances, can produce qualitatively and quantitatively similar results

Note 1 to entry: Interlaboratory reproducibility is determined during the prevalidation and validation processes, and indicates the extent to which a test can be successfully transferred between laboratories.[29]

3.5
intralaboratory reproducibility
within-laboratory reproducibility
determination of the extent that qualified people within the same laboratory can successfully replicate results using a specific protocol at different times

3.6
prevalidation
PRE
initial phase of a *validation* (3.10) small-scale study intended to obtain preliminary information on the relevance and reliability of a *candidate test method* (3.2)

3.7
test article
material (e.g. a final finished device or a reference material) that is to be used to generate a *test sample* (3.8) (e.g. using extraction)

3.8
test sample
sample (e.g. a test article extract or spiked extract vehicle) that in its present form can be evaluated by a candidate test method

3.9
test system
system (e.g. in vivo animal model, in vitro cellular model and in-silico computational models) that is used for hazard identification as part of a test method

3.10
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.13, modified — Notes 1, 2 and 3 to entry have been deleted.]

4 Consideration on the process for demonstration of the applicability domain

The process for evaluating the applicability of a candidate test method for skin sensitization testing of medical devices shall include feasibility, prevalidation and interlaboratory studies (see Figure 1).

The required sample size was determined according to Reference [80]. The basis for the determination was that the candidate test methods for the application to medical device extracts or medical device material extracts, predict the spiked extracts (non-skin sensitizer versus skin sensitizer) as good as the original OECD test method with the neat chemicals used for spiking the extracts. To demonstrate concordance of results, it was assumed that at least 90 % of the test samples were predicted concordantly in the candidate test method as compared to the original OECD test method. It was also assumed that the concordance was not below 70 %. Considering a one-sided proportion of 0,05 and a power of 0,8, a sample size of 28 was calculated. The sample size was increased to 29 to account for potential drop-out of chemicals, e.g. due to commercial unavailability.

Prior to conducting a prevalidation study, a feasibility study may be needed to determine if any modification of the OECD TG protocols (e.g. dilution, solvents, incubation times, volume of test sample, stimulation index value) is necessary for the evaluation of medical devices.

Protocols for feasibility studies are not described in this document as the design of these studies should be specific to the OECD TG method.

If the candidate test method protocols planned for the prevalidation and interlaboratory studies deviate from the OECD TG protocol, the number and nature of the modifications as well as the data and documentation available (e.g. from a feasibility study) to support the modifications shall be provided. A scientific rationale for the impact of these changes on the acceptance of the method for assessing the skin sensitizing potential of sample tests should be provided to justify that the method used remains equivalent to the original OECD method.

The same candidate test method and protocols shall be used for both the prevalidation study and the interlaboratory study.

As the non-animal methods considered are already validated with single chemicals (but not with mixtures such as medical devices extracts) and integrated in OECD test guidelines (e.g. see Reference [91] and Reference [1] on defined approaches for skin sensitization) with historical data of chemicals assessment, the prevalidation step shall be conducted to:

a) prepare the standard operating procedures (SOPs), so that they can be readily used by other laboratories;

b) generate preliminary data on the reliability and relevance of the candidate test method for assessing skin sensitization of medical devices.

During the prevalidation and interlaboratory phases, evaluation of the performance of non-animal methods shall be performed with positive and negative control test samples (in accordance with Clause 5) that are representative of medical devices extracts. The concordance of results (compared to the original OECD test method) and reproducibility shall be calculated and compared to the targeted performance values in Clause 7 and Clause 8.

If the prevalidation study does not achieve the performance criteria in accordance with Clause 7, then additional feasibility testing can be needed to optimize the assay protocol for increased concordance and/or intralaboratory reproducibility prior to conducting a repeat prevalidation study. If the prevalidation study meets the performance criteria in accordance with Clause 7, then an interlaboratory study can be considered.

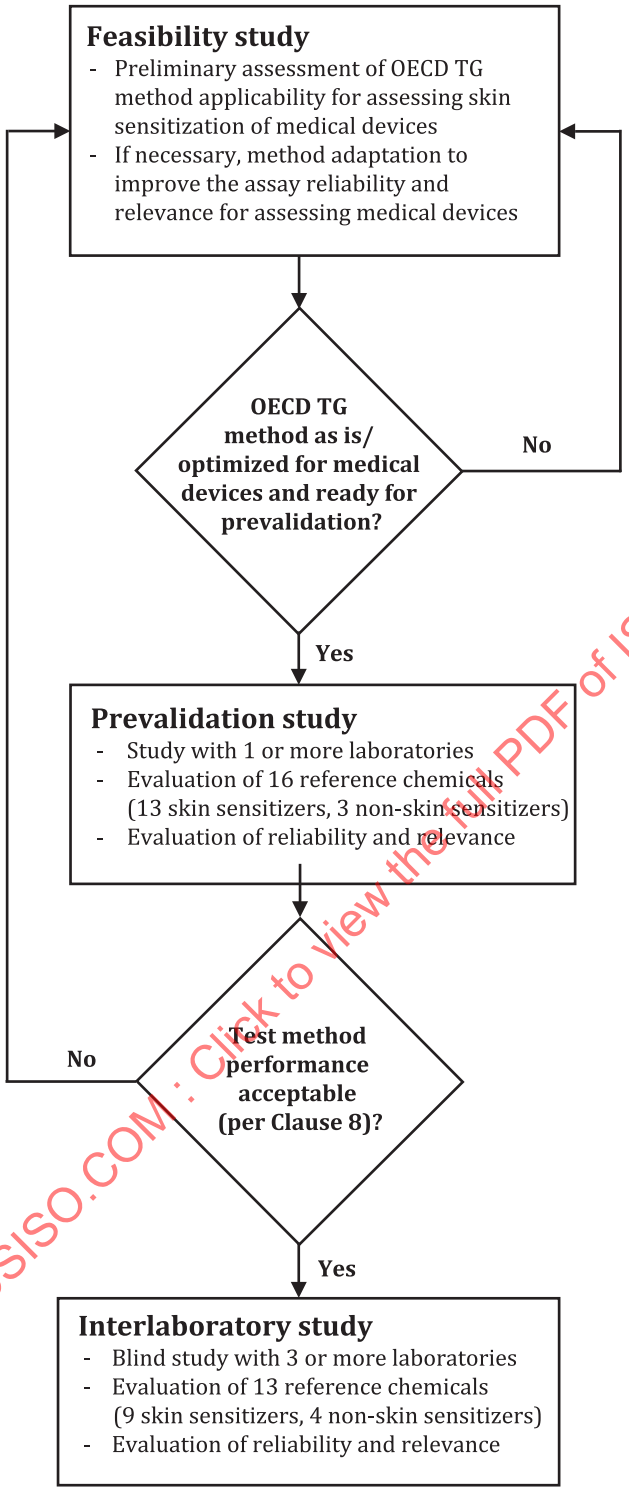

Figure 1 — General process for the evaluation of candidate test methods

5 Positive and negative control test samples

5.1 General

Due to the lack of existing positive reference test articles, samples for prevalidation and interlaboratory studies are comprised of negative reference material extracts spiked with a known concentration of a chemical skin sensitizer. By spiking an extract of an existing medical device material, the

final composition of test samples evaluated by in vitro test methods can better mimic the chemical complexity of a real extract containing a low concentration of one or more chemical skin sensitizers.

For this purpose, chemical skin sensitizers and non-skin sensitizers that can be identified in extracts of certain medical device materials have been selected. Annex A contains a database of reference chemicals with animal, and human data when available, that will be used in the prevalidation and interlaboratory studies.

5.2 Database of reference chemicals

A list of reference chemicals was developed including chemicals:

— representative of raw materials and/or leachables found in medical devices;

— representative of a balanced range of skin sensitizer potency (weak, moderate, strong and extreme);

— supported by robust reference data on potency and no-observed-adverse-effect-levels (NOAELs) from human and animal sources, including human repeat insult patch test (HRIPT), human maximization test (HMT), local lymph node assay (LLNA), closed-patch (Buehler) test and guinea pig maximization test (GPMT).

Additional criteria considered for selection of chemicals are:

— to be representative of the different mechanisms by which the compounds exert a skin sensitization effect;

— a range of physicochemical properties considered relevant to skin sensitization in Reference [1];

— commercially available compounds.

According the sample size considerations, a total of 29 chemicals are required. For each candidate test method evaluated, the results obtained for the original OECD test methods with neat chemicals will be collected or, when not available, generated experimentally.

NOTE The selection of chemicals already detected in medical devices or medical device materials was based on a review of References [3][4][5][6] and [7]. Particular attention was paid to the quality of the historical toxicological data for these chemicals. The estimated concentrations required to generate a threefold stimulation of proliferation in draining lymph node (EC3 values) are derived from curated databases such as SkinSenseDB,[8] the Cosmetics Europe database,[9] supplementary data from Reference [10], the integrated chemical environment (ICE) from the national toxicology program (NTP),[11] the National Toxicology Program Interagency Centre for the Evaluation of Alternative Toxicological Methods (NICEATM) LLNA Database[12] and the OECD curated database for OECD Guideline 497[13][14][15].

All chemicals are presented in Table 1 with name, CAS Registry Number®[1)], medical devices application examples and the spiking concentration to be used for prevalidation and interlaboratory studies. Additional information on their physicochemical and skin sensitizing properties in humans and animals is presented in Annex A (e.g. GPMT, LLNA EC3, human potency, reference publications and complementary data).

1) CAS Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Table 1 — Database of reference chemicals for PRE and ILS

S/NS	Set	Name	CAS	Application in the medical device industry	Spiking concentration *w/v* %
S	PRE	Glutaraldehyde	111-30-8	Disinfectant and crosslinker for animal derived tissue product	0,08
S	PRE	1,4-Phenylenediamine	106-50-3	—	0,11
S	PRE	Phthalic anhydride	85-44-9	—	0,16
S	PRE	Cobalt chloride	7646-79-9	Orthopaedic implants, stents, pacemakers	0,4
S	PRE	Phenylacetaldehyde	122-78-1	—	3
S	ILS	Hydratropic aldehyde	93-53-8	—	6,3
S	PRE	Alpha-hexylcinnamaldehyde	101-86-0	Ink, lubricants, etc.	10,8
S	PRE	Linolenic acid	463-40-1	Lubricants	9,9
S	PRE	Ethyl acrylate	140-88-5	Acrylates, methacrylates and monomers Found in adhesives, wearable devices, wound dressings	10
S	PRE	TPO (diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide)	75980-60-8	Photo-initiator for additive manufacturing in many light-cured acrylic polymers) such as composite materials, dental fillers, etc.	27
S	PRE	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	126-86-3	Plastic and rubber products	34,3
S	PRE	Isopropyl myristate	110-27-0	Plasticizer, lubricant	44
S	PRE	Tridecane	629-50-5	Solvent, rubber industry	48,4
S	PRE	Methyl methacrylate	80-62-6	Bone cement, dental materials, hearing aids	75
NS	PRE	Diethyl phthalate	84-66-2	Solvent, plasticizer, extractable associated with polyethylene and PET	1
NS	PRE	1,3-diphenylguanidine	102-06-7	Rubber accelerators Found in gloves	1
NS	PRE	Zinc oxide	1314-13-2	Anti-bacterial and anti-biofilm activity	1
S	ILS	2,4-Dinitrochlorobenzen (DNCB)	97-00-7	—	0,06
S	ILS	Formaldehyde (act. 37 %)	50-00-0	Sterilization-low temperature steam and formaldehyde (LTSF)	0,3
S	ILS	Isobornyl acrylate (IBOA)	5888-33-5	Plastic materials, valves, tubes lining, stoppers, sealants, coatings, inks, glues Found in adhesives, wearable devices such as glucose monitoring sensors, insulin patch pumps and some wound dressings	1
S	ILS	2-Mercaptobenzothiazole (MBT)	149-30-4	Rubber accelerators Gloves	1,35
S	ILS	2-hydroxyethyl acrylate	818-61-1	Acrylates, methacrylates and monomers Wound dressings, EKG electrodes, contact lenses	1,4
S	ILS	Nickel(II) sulfate hexahydrate ($NiSO_4$)	10101-97-0 7786-81-4	Nickel alloys and stainless steels in implantable medical devices	4,8
S	ILS	Abietic acid	514-10-3	Adhesives Wound dressing	15

Key

S skin sensitizer

NS non-skin sensitizer

w weight

v volume

The database of reference chemicals contains 29 chemicals, 7 non-skin sensitizers and 22 skin sensitizers. The selected chemicals are distributed in two sets. The first set of 16 chemicals (13 skin sensitizers and 3 non-skin sensitizers) which shall be used for the prevalidation phase, are labelled "PRE" in Table 1 and are included in Clause A.1. The second set of 13 chemicals (9 sensitizers and 4 non sensitizers) which shall be used for the interlaboratory study phase, are labelled "ILS" in Table 1 and included in Clause A.2. The size of this set, 13 chemicals, represents half the number of chemicals originally used for the validation of OECD test methods. It was considered appropriate from a statistical point of view because minor deviation of the protocols during the feasibility study are unlikely to affect the transferability and interlaboratory reproducibility.

Table 1 *(continued)*

S/NS	Set	Name	CAS	Application in the medical device industry	Spiking concentration *w/v* %
S	ILS	α-Methylstyrene	98-83-9	Intermediate used in the manufacture of plasticizers, resins and polymers	46
NS	ILS	Chlorobenzene	108-90-7	Intermediate in rubber, solvent in adhesives	1
NS	ILS	Octanoic acid	124-07-2	Antibacterial agent	1
NS	ILS	Glycerol	56-81-5	Lubricant	1
NS	ILS	Lactic acid	50-21-5	Monomer of polymer polylactic acid (PLA). PLA is commonly used in biodegradable polymers for drug delivery systems, tissue engineering, temporary and long-term implantable devices, etc.	1

Key

S skin sensitizer

NS non-skin sensitizer

w weight

v volume

The database of reference chemicals contains 29 chemicals, 7 non-skin sensitizers and 22 skin sensitizers. The selected chemicals are distributed in two sets. The first set of 16 chemicals (13 skin sensitizers and 3 non-skin sensitizers) which shall be used for the prevalidation phase, are labelled "PRE" in Table 1 and are included in Clause A.1. The second set of 13 chemicals (9 sensitizers and 4 non sensitizers) which shall be used for the interlaboratory study phase, are labelled "ILS" in Table 1 and included in Clause A.2. The size of this set, 13 chemicals, represents half the number of chemicals originally used for the validation of OECD test methods. It was considered appropriate from a statistical point of view because minor deviation of the protocols during the feasibility study are unlikely to affect the transferability and interlaboratory reproducibility.

The selected chemicals are representative of chemicals previously found in leaching studies of medical devices extracts (see Annex A). The skin sensitizers cover the different potency categories according to the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) classification[16] as represented in Figure 2: 9 weak, 8 moderate, 3 strong and 2 extreme skin sensitizers.

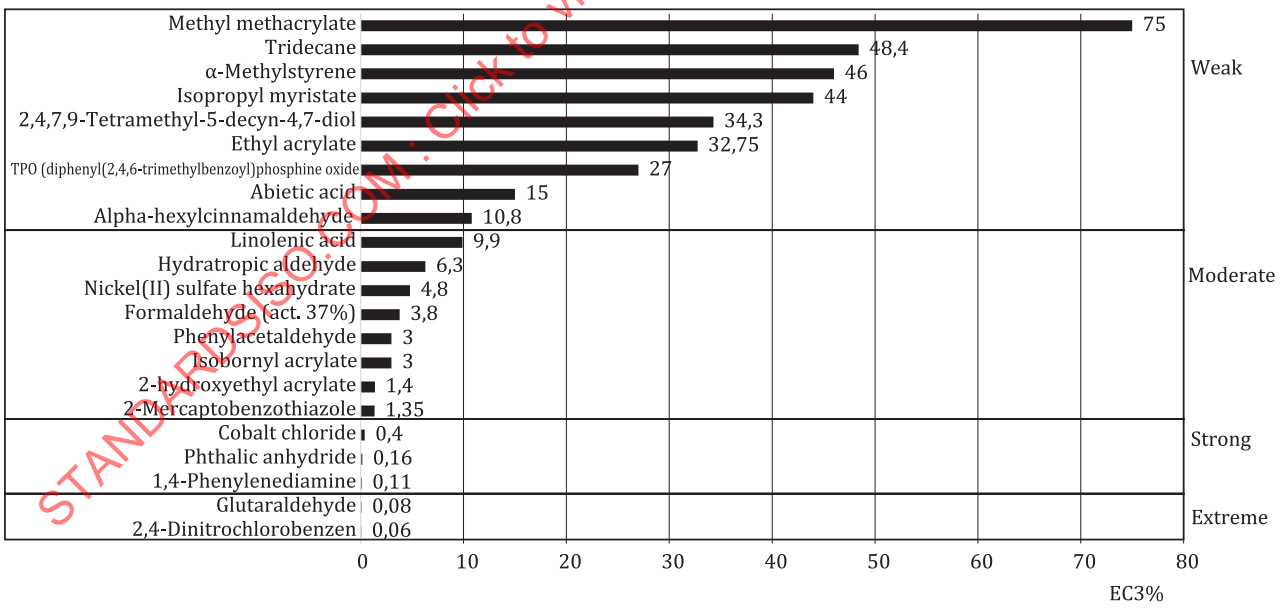

Figure 2 — Distribution of EC3 values of the reference chemical skin sensitizers

The physicochemical properties considered relevant to skin sensitization in the supporting document to Reference [1] are presented in Table 2 for the 29 chemicals selected compared to the 167 chemicals of the OECD database[13].

Table 2 — Physicochemical property ranges of the chemicals

Property	OECD Guideline no. 497[1] min. to max.	This document, i.e. ISO/TS 11796 min. to max.
MW (g/mol)	[30,0 to 512,6]	[30,0 to 348,4]
LogP	[–3,9 to 9,4]	[–1,8 to 7,5]
LogWS (mol/l)	[–7,6 to 1,2]	[–7,6 to 1,2]
MP (°C)	[–122,5 to 252,7]	[–105 to 411]
BP (°C)	[–19,1 to 445,3]	[–19,1 to 1 000]
LogVP	[–18,7 to 11,6]	[–8,5 to 2,916]

Key

MW molecular weight
BP boiling point
MP melting point
LogVP vapour pressure
LogP octanol-water partition coefficient
LogWS water solubility

5.3 Reference materials

5.3.1 General

In order to reproduce the real-life conditions of a medical device evaluation, a negative control medical device material shall be extracted under the usual extraction conditions used in an in vivo test. The negative control medical device material shall be a material commonly used in medical devices and known to release a complex mixture of non-skin sensitizing chemicals representative of a medical device extract. The polar and non-polar extracts of this negative control medical device material shall then be spiked with the skin sensitizing or non-skin sensitizing chemicals to perform the evaluation.

5.3.2 Negative control medical device material

The negative control medical device material shall be a silicone available commercially and known to release numerous compounds without eliciting a positive skin sensitization response. MED-2000 silicone supplied by Nusil Technology[2)] can be used as the negative control medical device material. Use of any alternative negative control medical device material shall be described and justified. After extraction in accordance with ISO 10993-12, the extracts shall be used as a negative control and for preparing the test samples by spiking these extracts with skin sensitizing and non-skin sensitizing chemicals.

5.3.3 Positive reference material

If a positive certified reference material supported by animal or human data is available, it can be used as a positive control according to ISO 10993-12:2021, 3.11.

NOTE The positive control is a well-characterized material and/or substance, which, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response in the test system.

2) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6 Preparation of the test samples

6.1 General

Test samples should reflect the chemical complexity of medical device extracts which can contain skin sensitizers at a concentration likely to induce a positive reaction in vivo.

6.2 Preparation of the extracts

Extraction of the negative control medical device material should be performed in polar and non-polar solvents according to ISO 10993-12.

EXAMPLE 1 Polar extraction vehicles can be water and physiological saline.

EXAMPLE 2 Non-polar extraction vehicles can be refined vegetable oil (e.g. cottonseed or sesame oil) of the quality defined in various pharmacopoeias recommended in ISO 10993-12, if the vehicle does not cause a positive response in the assay.

The use of extraction vehicles such as culture media with or without serum for any candidate cell-based test methods (in addition to the standard polar and non-polar vehicles) can be included in the method development to assess vehicle-related effects and extraction efficiency. However, use of any additional alternative extraction vehicles shall be described and justified with regards to comparative in vivo skin sensitization data as well as suitability for medical device extraction.

For the purpose of the interlaboratory study, the negative control medical device material will be received by the testing laboratories ready to use (e.g. adequate sample size or weight for extraction without need for additional laboratory work). An adequate volume of polar or non-polar solvent shall be added directly to the vials and extraction performed 50 °C for 72 h using an extraction ratio of 6 cm^2/ml for films or 0,2 g/ml for pellets in accordance with ISO 10993-12. After the extraction is completed, the extracts shall be collected and used within 24 h.

If an extraction solvent other than saline and oil is used for method development, the appropriate temperature for the negative reference material should be justified (e.g. extraction at 37 °C for 72 h is acceptable if cell medium with serum is used as the extraction vehicle).

6.3 Spiking of the extracts

To prepare the positive test samples, polar and non-polar extracts of the negative control medical device material (see 5.3.2) shall be spiked with a concentration of skin sensitizer known to induce a positive reaction in animals or humans. The final concentration of the chemical skin sensitizer in the extract shall be based on the recognized valid lowest concentration known to cause a positive skin sensitization response in an animal or a human. Table 1 (column “spiking concentration”) and Annex A give the recommended concentration that shall be used to spike the extracts.

In the absence of quantitative and confident data derived from human assays or from GPMT assays, the threshold EC3 value derived from the LLNA has generally been used as the lowest concentration known to cause a positive skin sensitization response in animal or human. As shown in Table 3, the EC3 value is commonly used for estimation of skin sensitizing potency and recommended by the World Health Organization in its guidance document “immunotoxicity for risk assessment of chemicals”[17] as a surrogate of human NOELs and benchmark doses (BMD)[18][19][20][21][22][23][24][25].

Table 3 — Derivation of a point of departure for risk assessment for skin sensitization induction

Type of data	Values used as a point of derivation[17]
Skin sensitization: Induction	
Human data	
HRIPT (or HMT)	NOEL (or BMD_5) (µg/cm^2 skin per day)
Laboratory animal data	
LLNA in mice	EC_3 (µg/cm^2 skin per day)[a]
GPMT or Buehler test in guinea pigs	Generally not suitable for derivation of a POD
Weight of evidence approach for grouping substance into a skin sensitizing potency category	Use of the lower boundary of category (expressed as µg/cm^2 skin per day)

[a] Skin sensitization potency in the murine LLNA is as reported as an EC3 in percentage of test chemical, in the vehicle, giving a threefold stimulation index (SI). For comparison with human repeat insult patch test data, the EC3 can be converted in units of micrograms per square centimetre by multiplying the value of EC3 in percent by 250.[25]

7 Prevalidation of candidate test methods

The goal of the prevalidation phase is to obtain preliminary information on the relevance and reliability of a candidate test method for assessing medical devices extracts.

The prevalidation stage will demonstrate the applicability of the assay to correctly classify test samples containing chemicals representative of the medical device domain in concordance with the original test method. For this purpose, a minimum of 16 chemicals from Table 1 shall be spiked into polar and non-polar extracts of the negative control medical device material to be evaluated by at least one laboratory in three independent runs. In cases where a chemical will not dissolve or produce a stable homogeneous suspension in a solvent, only the most appropriate solvent should be used. This shall be justified in the report. The skin concordance of the candidate test method with the original OECD test method in the prevalidation study shall reach 88,0 % based on the comparison between the chemical skin sensitization classification (i.e. skin sensitizer, non-skin sensitizer). The intralaboratory reproducibility shall also show a concordance of predictions (skin sensitizer versus non-skin sensitizer) equal or greater than 80,0 %.

This document does not provide guidance for prevalidation testing of candidate test methods for evaluation of applicability to non-extractable medical devices (e.g. gels, pastes, creams or particulates).

8 Interlaboratory study

8.1 General

After a successful prevalidation, an interlaboratory study can be considered. The main objectives of this step are to assess the robustness of the method and its transferability to other laboratories. Existing guidance documents and standards provide directions for conducting interlaboratory studies on the applicability of validated non-animal methods for determining the skin sensitization of medical devices[26][27][28][29].

8.2 Study organization

8.2.1 General

The organization and functions of the participants in the interlaboratory study follow the recommendations of the OECD Guidance no. 34[29] with a management team, participating laboratories and a lead laboratory.

8.2.2 Management team

The management team oversees the organization of the interlaboratory study, the collection of the resulting data and the preparation of reports.

8.2.3 Lead laboratory

A minimum of three independent laboratories shall participate in the interlaboratory study. For a candidate test method, a lead laboratory, preferentially the one that conducted the prevalidation phase, shall be responsible for providing the SOP for the method and the necessary assay datasheets and worksheets for the reports. The lead laboratory shall also be responsible for training the participating laboratories. It is necessary for each laboratory to demonstrate its competence in the use of the non-animal method by successfully classifying polar and non-polar control medical device material extracts spiked with 13 chemicals (9 skin sensitizers and 4 non-skin sensitizers) from the prevalidation set prior to participating in the study.

8.2.4 Sample management

Three runs of three independent batches of the experimental test system shall be conducted using blinded test chemicals. The chemicals used for test sample preparation shall be encoded and sent to the participating laboratory by a third-party laboratory not participating in the study.

Whenever possible, chemicals should be sent in appropriate containers in quantity sufficient to allow direct dilution by addition of a specified volume of the negative control medical device material extracts. This is to limit manipulation and sources of variability not directly related to the method being evaluated. In cases where a chemical will not dissolve or produce a stable homogeneous suspension in a solvent, only the most appropriate solvent can be used. This shall be justified in the report.

8.2.5 Independent biostatistician

The assessment of reproducibility, concordance and predictivity shall be performed at the end of the experimental step by an independent biostatistician.

8.3 Sample preparation

For the interlaboratory study, a sample size of 13 has been determined, assuming that the interlaboratory reproducibility in terms of concordant results (i.e. skin sensitizer, non-skin sensitizer) between three laboratories is at least 85 %, that it should not be below 50 % and considering a one-sided proportion of 0,05 and a power of 0,8. The set of 13 chemicals have been selected with 9 skin sensitizers (1 extreme, 1 strong, 5 moderate and 2 weak) and 4 non-skin sensitizers (see Clause A.2).

The third party laboratory shall prepare the samples and encode them before shipping to the participating laboratories. Samples shall be weighed or pipetted in sufficient quantity and packaged in suitable tubes to allow for the addition, whenever possible, of a fixed volume of extraction solvent from the negative control medical device material. After coding, the samples shall be sent to the participating laboratories.

Extraction of the negative control medical device material (see 5.3.2) by the participating laboratories shall be performed as described in 6.3. Spiking of the extracts shall be performed on the day the study is conducted. For this, the laboratory shall finalise the preparation of the samples by adding a fixed volume of the polar or non-polar extract of the negative control medical device material to the tube provided.

The final concentration of the chemicals classified as skin sensitizers in the test samples shall be the valid lowest skin sensitizing concentration (see 6.2) listed in Table 1 and in Annex A. For chemicals that are classified as non-skin sensitizer, the final concentration shall be just below the minimum irritating level, if applicable.

8.4 Performance review

Criteria for evaluating the applicability of an OECD in vitro method for medical devices will be based on the analysis of concordance of the candidate test method with the original OECD test method. The concordance shall reach 90 % combining the prevalidation and intralaboratory data (at least 26 of 29) based on the comparison of chemical skin sensitization classification (i.e. skin sensitizer, non-skin sensitizer). Reason for non-concordant results shall be discussed.

The interlaboratory reproducibility shall show a concordance of predictions (skin sensitizer versus non-skin sensitizer) between three laboratories equal or greater than 80,0 %. The intralaboratory reproducibility shall show a concordance of predictions (skin sensitizer versus non-skin sensitizer) within each participating laboratory equal or higher than 85,0 % This threshold is derived from the performance required by OECD to assess the predictive capacity of a proposed similar or modified test method[30][31][32].

9 Statistical analysis

For future interlaboratory studies the biostatistician should be familiar with the biological basis and the practical limitations of the proposed method and of the reference method. This knowledge will aid in the selection of appropriate statistical procedures, development of appropriate decision criteria and help with communicating the study results.

The biostatistician should be involved in the establishment of appropriate data management procedures for data collection and data submission. The interlaboratory study shall be designed with statistical support in order to allow a sound statistical evaluation of the reliability (repeatability and reproducibility) and relevance (accuracy) of the candidate test method. The method reliability shall include the qualitative and quantitative reproducibility of results within and between the participating laboratories. The candidate test method's predictive capacity (relevance) shall be assessed by comparing the classification predicted by the candidate test methods to data obtained with the reference test method currently accepted by regulatory agencies (e.g. LLNA, GPMT) and also, when possible, to human data or recognized human toxicity information. This shall be done by calculating performance parameters such as skin sensitivity, specificity, as well as positive and negative predictive values[29].

10 Test report

The test reports shall include at least the following details:

a) the test samples;

b) the International Standard used (i.e. ISO/TS 11796:2023);

c) a detailed description of the test method (including the relevant OECD test guideline used) and information on all modifications to the test method (see Clause 4);

d) a detailed description of the method employed in preparing the test samples, including the reference materials, extraction conditions and observations, chemical name and concentration of reference chemicals;

e) animal, human or other appropriate reference data for reference chemicals used to assess the test method's accuracy;

f) a description of the test acceptance criteria (e.g. standard deviation, negative and positive control performance); the acceptance criteria of the evaluated candidate test method shall be the same as those of the considered OECD method;

g) all individual raw data;

h) a description and reporting of statistical analysis;

i) an assessment of the results and classification of the test samples/devices (i.e. true positive, true negative, false positive, false negative) for the prevalidation and for the interlaboratory studies;

j) an assessment of the performance characteristics (i.e. accuracy, sensitivity, specificity, interlaboratory reproducibility, intralaboratory reproducibility);

k) any unusual features observed;

l) the date of the test.

Annex A
(normative)

Reference chemicals

A.1 Physicochemical properties

The physicochemical properties of the 29 reference chemicals to be used in the PRE studies corresponding to the requirements and recommendations in Clause 7, and in the ILS studies corresponding to the requirements and recommendations in Clause 8, are presented in Table A.1.

Table A.1 — Physicochemical properties

Set	Name	CAS	Molecular formula	MW g/mol	LogP	LogWS mol/l	MP °C	BP °C	LogVP Pa
PRE	Glutaraldehyde	111–30–8	$C_5H_8O_2$	100,12	−0,18	0,223	−11,9	129	−0,222
PRE	1,4-Phenylenediamine	106–50–3	$C_6H_4(NH_2)_2$	108,14	−0,3	−0,420	146	267	−2,893
PRE	Phthalic anhydride	85–44–9	C_8-H_4-O_3	148,12	1,6	−1,384	130,8	295	−3,286
PRE	Cobalt chloride	7646–79–9	$C_{l2}Co$	129,84	0,63		411	1 000	
PRE	Phenylacetaldehyde	122–78–1	C_8H_8O	120,14	1,44	0,250	33,5	195	−0,406
ILS	Hydratropic aldehyde	93–53–8	$C_{11}H_{16}O$	134,18	2,113	−1,659	14,92	203,5	−0,568
PRE	Alpha-hexylcinnamaldehyde	101-86-0	$C_{15}H_{20}O$	216,3	4,34	-4,9	39,9	303	−2,57
PRE	Linolenic acid	463–40–1	$C_{18}H_{30}O_2$	278,44	6,46	0,064	−13,8	392	−6,27
PRE	Ethyl acrylate	140–88–5	$C_5H_8O_2$	100,12	1,22	−0,780	−71,2	99,4	1,586
PRE	TPO (diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide)	75980–60–8	$C_{22}H_{21}O_2P$	348,37	3,1	−5,106	133	474	−8,49
PRE	2,4,7,9-tetramethyl-5-decyn-4,7-diol	126–86–3	$C_{14}H_{26}O_2$	226,35	3,61	0,243	44,8	255	−2,83
PRE	Isopropyl myristate	110–27–0	$C_{17}H_{34}O_2$	270,5	6,9	−7,289	3	292,4	−4,029
PRE	Tridecane	629–50–5	$C_{13}H_{28}$	184,37	6,73	−7,592	−5,08	235	−1,25
PRE	Methyl methacrylate	80–62–6	$C_5H_8O_2$	100,12	1,38	−0,812	−48	100,5	1,585
PRE	Diethyl phthalate	84–66–2	$C_{12}H_{14}O_4$	222,23	2,44	−2,331	−40,5	295	−2,678
PRE	1,3-diphenylguanidine	102–06–7	$C_{13}H_{13}N_3$	211,26	2,42	−2,322	149	170	−6,6
PRE	Zinc oxide	1314–13–2	ZnO	81,4	−0,12	−4,611			
ILS	2,4- DNCB	97–00–7	$C_6H_3ClN_2O_4$	202,55	2,17	−3,119	53	315	−4,071
ILS	Formaldehyde (act. 37 %)	50–00–0	CH_2O	30,03	0,35	1,173	−105	−19,1	2,916
ILS	IBOA	5888–33–5	$C_{13}H_{20}O_2$	208,3	3,9	0,544	97	242	−1,33
ILS	2-MBT	149–30–4	$C_7H_5NS_2$	167,3	2,42	−3,170	181	289	−5,366
ILS	2-hydroxyethyl acrylate	818–61–1	$C_5H_8O_3$	116,12	−0,21	0,935	−26,7	191	−0,934
ILS	Nickel(II) sulfate hexahydrate ($NiSO_4$)	10101–97–07786–81–4	$NiSO_4$	154,76	−0,84				

Key
MW molecular weight
BP boiling point
MP melting point
LogVP vapour pressure
LogP octanol-water partition coefficient
LogWS water solubility

Table A.1 *(continued)*

Set	Name	CAS	Molecular formula	MW g/mol	LogP	LogWS mol/l	MP °C	BP °C	LogVP Pa
ILS	Abietic acid	514–10–3	$C_{20}H_{30}O_2$	302,5	6,46		173,5	347	–7,933
ILS	α-Methylstyrene	98–83–9	C_9H_{10}	119,17	3,48	–3,009	–23,1	168	0,28
ILS	Chlorobenzene	108–90–7	C_6H_5Cl	112,56	2,34	–2,365	–45,2	131,7	1,078
ILS	Octanoic acid	124–07–2	$C_8H_{16}O_2$	144,2	3,05	–2,281	16,3	239	–2,431
ILS	Glycerol	56–81–5	C_3-H_8-O_3	92,09	–1,76	1,083	18,2	290	–3,775
ILS	Lactic acid	50–21–5	$C_3H_6O_3$	90,08	–0,72	1,045	40,8	208,7	–1,089

Key
MW molecular weight
BP boiling point
MP melting point
LogVP vapour pressure
LogP octanol-water partition coefficient
LogWS water solubility

A.2 Skin sensitizing properties in human and animal of the reference chemicals for prevalidation and interlaboratory studies

Skin sensitizing properties in human and animal properties of the 29 reference chemicals to be used in the PRE studies correspond to the requirements and recommendations in Clause 7 (see Tables A.2 to A.17), and in the ILS studies correspond to the requirements and recommendations in Clause 8 (see Tables A.18 to A.30).

The skin sensitizing properties in humans and animals are derived from the registration dossier of the chemicals available in the European Chemical Agency (ECHA) database[33] and from curated databases such as SkinSenseDB,[8] the Cosmetics Europe database,[9] supplementary data from Reference [10], the ICE from the National Toxicology Program (NTP),[11] the NICEATM LLNA Database[12] and the OECD curated database for OECD Guideline no. 497[13][14][15].

The identification of the mechanistic domains of chemical reaction was assessed with the Toxtree[3)] software version 3.1.0 (v3.1.0). Toxtree is a silico prediction software from Ideaconsult Ltd containing skin sensitization alerts based on the reaction mechanistic domains classification (opensource).

3) Toxtree is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Table A.2 — Glutaraldehyde (CAS 111-30-8)

Mechanistic domain of chemical reaction: Schiff base formation	
Human data	
Potency: extreme	One study with Bactron K-139 - pesticide: 61789-71-7: (10,0 %); 111-30-8: (5,0 %) One HRIPT study with glutaraldehyde: Concentration, 5 % incidence of positive responses: 1,071 4 %
LLNA (mouse) EC3 %: 0,08	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 0,08 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: 5 % in propylene glycol Challenge: 2,2 %
GPMT reference: Ballantyne et al., 1997[34]	
Medical device relevance: Present in disinfectant and crosslinker for animal derived tissue product.	
Observations: Used to clean med devices, used as a cold sterilant to disinfect a variety of heat-sensitive instruments, also used as a crosslinker for animal derived tissue product.	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: Y / CosEU: Y / NICEATM: Y Other references: de Groot, AC. 2018[35].	

Table A.3 — 1,4-Phenylenediamine (CAS 106-50-3)

Mechanistic domain of chemical reaction: Michael acceptor	
Human data	
Potency: strong	ICE database: 14 human studies: Concentration, one positive response: 0,02 % and 1,25 % Mean: 0,21 % Concentration, 5 % incidence of positive responses: 0,069 % and 1,25 % Mean: 0,24 % Reliability 1 to 2 From curated OECD human data: 7 HMT and 3 HRIPT studies. Induction concentration were from 0,001 % to 25 %
LLNA (mouse) EC3 %: 0,11	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 0,11 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: 1,0 % Challenge: 30 % and 3 %
GPMT reference: in ECHA dossier[33]	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: Y / CosEU: Y / NICEATM: N	

Table A.4 — Phthalic anhydride (CAS 85-44-9)

Mechanistic domain of chemical reaction: Acylating agent	
Human data	
Potency: strong	No human data in the ICE database
LLNA (mouse) EC3 %: 0,16	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 0,16 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: intradermal: 0,1 % Topical: 25 % Challenge: 10 %
GPMT reference: in ECHA dossier[33]: https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/15845/7/5/2	
Medical device relevance: in Jenke et al., 2013,[5] Jenke and Carlson, 2014[3] Present in the ELSIE database:Extractable and leachable compounds from materials used in container-closures for pharmaceuticals, biologics and medical devices (https://comptox.epa.gov/dashboard/chemical-lists/ELSIE)	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: Y / CosEU: Y / NICEATM: N Other references: OECD GD497:2021, Annex 3[15], Coleman et al., 2015[36], NIEHS report, 1999[37] Dearman, et al., 2000[38]	

Table A.5 — Cobalt chloride (CAS 7646-79-9)

Mechanistic domain of chemical reaction: specific toll-like receptor (TLR4) present in humans	
Human data	
Potency: strong	In NIH Publication no. 11–7709[39]: 2 HMT study with LOAEL of 1 148 µg/cm^2 and 3 620 µg/cm^2.
LLNA (mouse) EC3 %: 0,40	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 0,40 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	GPMT (modified) Induction: 3 % Challenge: 0,01 % – 3 %
GPMT reference: ECHA dossier,[33] NICNAS: report, 2014[40]	
Medical device relevance: orthopaedic implants, stents, pacemakers	
Observations: Cobalt sulfate (CAS 10124–43–3) and cobalt chloride (CAS 7646–79–9) have similar bioaccessibility and bioavailability in biological fluids to chemicals. They are used as analogue chemicals to assess the systemic hazards of chemicals in this group (cobalt dinitrate CAS 10141–05–6 and cobalt dinitrate hexahydrate CAS 10026–22–9) from NICNAS Australia, 2014[40]. No partition coefficient study because study technically not feasible in ECHA dossier,[33] but a value is provided in NIH Publication no. 11–7709[39]	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: N / CosEU: N / NICEATM: N	

Table A.6 — Phenylacetaldehyde (CAS 122-78-1)

Mechanistic domain of chemical reaction: Schiff base formation	
Human data	
Potency: moderate	Same data in ICE database and OECD GD497:2021, Annex 4[14] - positive in 4 HMT assays (induction dose per skin area: 1 296 µg/cm^2) negative in 2 HRIPT (induction dose per skin area: 531 µg/cm^2 and 1 550 µg/cm^2); it will influence of the design of the study
LLNA (mouse) EC3 %: 3,00	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 3,00 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Buehler test: Induction: 100 % Challenge: 100 %
GPMT reference: in ECHA dossier[33]	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: N / CosEU: N / NICEATM: N	

Table A.7 — Alpha-hexylcinnamaldehyde (CAS 101-86-0)

Mechanistic domain of chemical reaction: Michael acceptor (ToxTree v3.1.0)	
Human data	
Potency: weak	ICE database: four human studies: 2 HMT studies, negatives, induction dose per skin area: 7 776 µg/cm^2 2 HRIPT negatives with induction dose per skin area: 5 906 µg/cm^2 to 23 622 µg/cm^2
LLNA (mouse) EC3 %: 10,8	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 10,8 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	
GPMT reference: ICE database	
Medical device relevance:	
Observations: Used as a positive control (PC) in ISO 10993-10 and in OECD Guideline no. 406[64] and OECD Guideline 429[65].	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y / SkinSensDB: Y / CosEU: N / NICEATM: Y	

Table A.8 — Linolenic acid (CAS 463-40-1)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency: moderate	—
LLNA (mouse) EC3 %: 9,90	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 9,90 % based on the EC3 value
Non-skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: intradermal: 5 % / topical: 100 % Challenge: 50 %
GPMT reference: ECHA Dossier,[33] Basketter, 1992,[41] Kreiling et al., 2010[42]	
Medical device relevance: lubricants	
Observations: in OECD G497:2021, Annex 6 [43], linolenic acid is classified as false positive in LLNA compared to GPMT because of logP > 3,5. In ECHA dossier[33], this chemical is also suggested to be a false positive. Considered as a skin sensitzer based on SkinSense database classification.	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: Y / CosEU: N / NICEATM: Y	

Table A.9 — Ethyl acrylate (CAS 140-88-5)

Mechanistic domain of chemical reaction: Michael acceptor	
Human data	
Potency: weak	ICE: five human studies: 1 HMT study, positive, induction dose per skin area: 2 592 µg/cm^2 4 HRIPT positives with induction dose per skin area: 2 834 µg/cm^2 to 2 519 µg/cm^2 From curated OECD human data: 1 HMT and 4 HRIPT studies. Induction: 4 % (In two of the studies, the result was negative.)
LLNA (mouse) EC3 %: 32,75	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 10,00 % derived from induction dose per skin area in human
Non-skin sensitizer in GPMT and/or Buehler (guinea pig)	One study negative: Induction: 0,5 M / challenge: 0,3 M One study negative: Induction: 5 % / challenge 5 %
GPMT reference: ECHA dossier[33]	
Medical device relevance: acrylates, methacrylates and monomers. Found in adhesives, wearable devices, wound dressings.	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: Y / CosEU: Y / NICEATM: Y Other references: de Groot, AC. 2018,[35] Uter et al., 2015,[44] Spencer et al., 2016[45]	

Table A.10 — TPO (Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide) (CAS 75980-60-8)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency: weak	—
LLNA (mouse) EC3 %: 27,00	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 27,00 % based on the EC3 value
Data not available for GPMT and/or Buehler (guinea pig)	
Medical device relevance: used as photo-initiator for additive manufacturing in many light-cured acrylic polymers such as composite materials, dental fillers, etc.	
Observations: solubility to be confirmed	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: N / CosEU: N / NICEATM: N Other references: Jenke, 2014[3] SCCS opinion on the Trimethylbenzoyl diphenylphosphine oxide (TPO), 2014[46]	

Table A.11 — 2,4,7,9-Tetramethyl-5-decyn-4,7-diol (CAS 126-86-3)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Potency: weak	—
LLNA (mouse) EC3 %: 34,30	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 34,30 % based on the EC3 value
Data not available for GPMT and/or Buehler (guinea pig)	
Medical device relevance: plastic and rubber products.	
Other references: de Groot, AC. 2018,[35] Jenke et al., 2017[47]	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: N / CosEU: N / NICEATM: N	

Table A.12 — Isopropyl myristate (CAS 110-27-0)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency: weak	One HMT study negative in human (induction dose per skin area: 12 960 µg/cm^2 - equivalent to 50 %)
LLNA (mouse) EC3 %: 44,00	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 44,00 % based on the EC3 value
Non-skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: 5 % Challenge: 25 %
GPMT reference in ECHA dossier[33]	
Medical device relevance: plasticizer, lubricant	
Observations: classified non-skin sensitizer in the ECHA dossier[33]	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: N / CosEU: N / NICEATM: N Other references: OECD GD497:2021, Annex 3[15]	

Table A.13 — Tridecane (CAS 629-50-5)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency: weak	—
LLNA (mouse) EC3 %: 48,40	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 48,40 % based on the EC3 value
Data not available for GPMT and/or Buehler (guinea pig)	
Medical device relevance: solvent, rubber industry	
Observations: identified in extractable from polyethylene materials in materials used in pharmaceutical construct and their associated extractables[48]. Considered as false positive in Kern et al. 2010[49]: "...a number of chemicals may be regarded as false positive or false negative, a problem that may be expected with a predictive toxicology assay. A classic false positive, sodium lauryl sulfate, was noted in that first article. In the present article, a good example of the same phenomenon would be tridecane, which delivered an SI of 3.1 at a concentration of 50 %."	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: Y / CosEU: N / NICEATM: N	

Table A.14 — Methyl methacrylate (CAS 80-62-6)

Mechanistic domain of chemical reaction: Michael acceptor	
Human data	
Potency: weak	ICE: one human study (HRIPT) with no positive answers. An induction dose per skin area of 7 874 µg/cm^2 was used (equivalent to a 30 % concentration).
LLNA (mouse) EC3 %: 75,00	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 75,00 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: 100 % Challenge: 5 % NS in other studies ranging from 0,2 % to 10 %
GPMT reference: ECHA dossier[33]: — Human data: skin patch test data indicate that MMA is a contact skin sensitizer in humans with indication of a weak potency based on low prevalence in relevant cohort studies. — Animal data (guinea pig): inconsistent results from various test methods; mainly positive in studies with concentrations that indicate that MMA is a weak skin sensitizer.	
Medical device relevance: bone cement, dental materials, hearing aids, monomer of polymethyl methacrylate (PMMA) acrylic-based polymers	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: N/ SkinSensDB: Y / CosEU: Y / NICEATM: N Other references: de Groot, AC. 2018,[35] Gosavi et al., 2010[50]	

Table A.15 — Diethyl phthalate (CAS 84-66-2)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency:	Human patch test in ECHA dossier[33]: no allergic reactions were found among 309 persons tested from 1991 to 1996, but 2 irritant reactions were observed. Tested at 5 % in PET
Non-skin sensitizer in LLNA (mouse)	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 1,00 % based on GHS generic cut-off value for skin corrosion/irritation
Non-skin sensitizer in GPMT and/or Buehler (guinea pig)	Buehler test Topical induction: 50 % Challenge: 50 %
GPMT reference: in ECHA dossier[33]: dermal sensitization has been investigated using the Buehler method. There was no evidence of sensitization to diethyl phthalate. In a comparison of guinea pig skin sensitization tests by Klecak et.al. 1977[51], diethyl phthalate was not a skin sensitizer in the open epicutaneous test, Draize test, Maximization test and Freund's complete adjuvant test. Skin sensitization has also been investigated in the more recent LLNA and, again, diethyl phthalate was not skin sensitizing.	
Medical device relevance: solvent, plasticizer, extractable associated with polyethylene and PET Identified in Extractables and Leachables in Single-Use Bags for Biomanufacturing from Dorival-García, 2018[52]. Identified in Extractables and Leachables in Extractables and Leachables in Cosmetic Plastic Packaging, Murat, 2020[53].	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: N / CosEU: N / NICEATM: N	

Table A.16 — 1,3-Diphenylguanidine (CAS 102-06-7)

Mechanistic domain of chemical reaction: Acylating agent	
Human data	
Potency:	No data in ICE database
Non-skin sensitizer in LLNA (mouse)	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 1,00 % based on GHS generic cut-off value for skin corrosion/irritation
Non-skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: 1 % Challenge: 25 %
GPMT reference: in ECHA dossier[33]	
Medical device relevance: Rubber accelerators. Found in gloves.	
Observations: non-skin sensitizer in ECHA, skin sensitizer in human in Dejonckheere et al., 2019,[54]. No LLNA data.	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: N / CosEU: N / NICEATM: N	

Table A.17 — Zinc oxide (CAS 1314-13-2)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency: non-skin sensitizer	Negative in human from the SkinSenseDB database
Non-skin sensitizer in LLNA (mouse)	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 1,00 % based on GHS generic cut-off value for skin corrosion/irritation
Data not available for GPMT and/or Buehler (guinea pig)	
Medical device relevance: antibacterial and anti-biofilm activity	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: Y / CosEU: N / NICEATM: Y Other references: Jenke, 2013[5].	

Table A.18 — 2,4-Dinitrochlorobenzen (CAS 97-00-7)

Mechanistic domain of chemical reaction: SNAr (electrophile)	
Human data	
Potency: extreme	6 HRIPT studies: Concentration with one positive response: from 0,005 % to 0,125 %. The mean concentration is 0,032 %. Concentration, with 5 % incidence of positive responses: from 0,006 9 % to 0,037 5 %. The mean concentration is 0,024 %.
LLNA (mouse) EC3 %: 0,06	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 0,06 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Buehler test: First and second induction: 0,5 % Third induction: 0,1 % Challenge: 0,1 %
GPMT reference: ECHA dossier[33]	
Medical device relevance: —	
Observations: Tested in polar and non-polar solvent extracts[55] Used as PC in ISO 10993-10 Reference sample in the OECD 429 LLNA performance standard Belongs to the OECD 442D proficiency list[56] Used as PC in OECD 442E (4,0 µg/ml in DMSO/medium)[57]	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: Y / CosEU: Y / NICEATM: N	

Table A.19 — Formaldehyde (act. 37 %) (CAS 50-00-0)

Mechanistic domain of chemical reaction: Schiff base formation	
Human data	
Potency: moderate	ICE database - six human studies: 1 HMT positive with induction dose per skin: 1 148 µg/cm^2 5 HRIPT studies: 4 positives with induction dose per skin area: 287 µg/cm^2 to 2 857 µg/cm^2 1 negative with induction dose per skin area = 287 µg/cm^2 From curated OECD GD497 human data[14]: 1 HMT and 5 HRIPT studies Induction concentration (%) was from 0,37 % to 3,7 % 1 study (HRIPT) negative with 0,037 % induction
LLNA (mouse) EC3 %: 3,80	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 0,30 % based on the GPMT value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	GPMT Induction: 0,003 % - 3 % Challenge: 1 %
GPMT reference: in ECHA dossier,[33] in the NTP report of the Murine Local Lymph Node Assay[37] and in Frankild et al. 2000[58]	
Medical device relevance: sterilization-LTSF	
Observations: used as positive control in ISO 10993-10	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: Y / CosEU: Y / NICEATM: Y Other references: Aerts, 2020[59], OECD GD497:2021, Annex 4[14]	

Table A.20 — Sobornyl acrylate (CAS 5888-33-5)

Mechanistic domain of chemical reaction: Michael acceptor	
Human data not available	
Potency: moderate	—
LLNA (mouse) EC3 %: 3,00	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 1,00 % based on experts decision
No data available for GPMT and/or Buehler (guinea pig)	
Medical device relevance: plastic materials, valves, tubes lining, stoppers, sealants, coatings, inks, glues Found in adhesives, wearable devices such as glucose monitoring sensors, insulin patch pumps and some wound dressings	
Observations: numerous publications identifying IBOA as a skin sensitizer in different medical devices, including glucose monitoring devices. Rational for lowest concentration estimation: a) lowest concentration which has been tested in the animal model (LLNA) is 5 % and resulted in SI of 4,07; b) extrapolation with linear regression, quadratic regression and log linear extrapolation were performed and resulted in an extrapolated EC3 of 1,6 %, 2,2 % and 4,4 %; c) leverage RAC opinion and derived classification: based on human data IBOA fulfils HRIPT classification criterion for skin sensitization. 1A ≤ 500 µg/cm^2 threshold ≥ 2 %; d) predicted EC3 value from the GARDskin potency assay in vitro model is 0,848 %.	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: N / CosEU: N / NICEATM: N Other references: ECHA: Committee for Risk Assessment RAC,[60] Annex I to the CLH report,[61] Nath et al., 2020[62]	

Table A.21 — 2-Mercaptobenzothiazole (CAS 149-30-4)

Mechanistic domain of chemical reaction: Acylating agent	
Human data	
Potency: moderate	Two HMT studies in OECD GD497 Dose per skin area for one positive response (DSA_{01}) between 1 245 and 1 724 µg/cm^2 equivalent to an EC3 between 4,98 % and 6,9 % Two HMT studies in the ICE database: Concentration, one positive response (DSA_{01}): 2 % and 2,777 8 % Concentration, 5 % incidence of positive responses (DSA_{05}): 2,4 % and 3,33 %
LLNA (mouse) EC3 %: 1,35	Spiking concentration (w/v) to be used in the interlaboratory study: 1,35 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	GPMT Induction: intradermal 5 % / topical 25 % Challenge: 12 % –– Induction: intradermal: 1 % / topical: 25 % Challenge: 15 % –– Induction: intradermal: 0,4 % / topical: 10 % Challenge: 10 %
GPMT reference: in ECHA dossier,[33] DHHS (NIOSH) Publication no. 2014–142[63]	
Medical device relevance: rubber accelerators, gloves	
Observations: used as a PC in ISO 10993-10 and in OECD Guideline no. 406[64] and OECD Guideline no. 429[65]. Also in the proficiency list of the OECD TG442D[66]. In Jenke list of extractables (2014)[3]	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: N / CosEU: Y / NICEATM: Y Other references: Jenke, 2014,[3] OECD GD497:2021, Annex 4[14]	

Table A.22 — 2-Hydroxyethyl acrylate (CAS 818-61-1)

Mechanistic domain of chemical reaction: Michael acceptor	
Human data	
Potency: moderate	No human data in the ICE database nor in the OECD GD497:2021, Annex 4[14]
LLNA (mouse) EC3 %: 1,40	Spiking concentration (w/v) to be used in the interlaboratory study: 1,40 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: intradermal: 0,25 % / topical: 5,0 % Challenge: 1,0 %
GPMT reference: OECD, Screening Information Dataset Initial Assessment Report for hydroxyethyl acrylate, 2005[83]	
Medical device relevance: acrylates, methacrylates and monomers Wound dressings, EKG electrodes, contact lenses	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: N / CosEU: N / NICEATM: N Other references: in the NICEATM database, Peters et al., 1986,[67] Mestach et al., 2018[68], Basketter et al., 2014[69]	

Table A.23 — Nickel(II) sulfate hexahydrate (CAS 10101-97-0)

Mechanistic domain of chemical reaction: Specific toll-like receptor (TLR4) present in humans	
Human data	
Potency: moderate	—
LLNA (mouse) EC3 %: 4,80	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 4,80 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: intradermal: 0,1 % / topical: 5 % Challenge: 0,5 %
GPMT reference: in ECHA dossier[33]	
Medical device relevance: nickel alloys and stainless steels in implantable medical devices	
Observations: used as PC in OECD TG442E[70]. EC3 from supplementary material in Nishijo et al., 2019[71]	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: Y / CosEU: N / NICEATM: N Other references: Basketter et al., 2021[72]	

Table A.24 — Hydratropic aldehyde (CAS 93-53-8)

Mechanistic domain of chemical reaction: Schiff base formation	
Human data	
Potency: moderate	1 negative HMT in ECHA dossier[33] (tested at 2 % for induction and challenge). NOEL in human: 3 887 mg/cm^2
LLNA (mouse) EC3 %: 6,30	Spiking concentration (*w*/*v*) to be used in the prevalidation study: 6,30 % based on the EC3 value
Data not available for GPMT and/or Buehler (guinea pig)	
Medical device relevance:	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: N/ SkinSensDB: Y / CosEU: N / NICEATM: N Other references: de Groot, AC. 2018[35]	

Table A.25 — Abietic acid (CAS 514-10-3)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency: weak	—
LLNA (mouse) EC3 %: 15,00	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 15,00 % based on the EC3 value
Skin sensitizer in GPMT and/or Buehler (guinea pig)	Induction: intradermal: 4 % / topical: 25 % Challenge: 5 %
GPMT reference: in Abietic acid [MAK Value Documentation, 2013][73]	
Medical device relevance: adhesives, wound dressing	
Observations: non skin sensitizer when pure but, depending on storage, skin sensitization via autoxidation derived hydroperoxides; Roberts et al. 2007 (SkinSenseDB)[74]. Detected in medical device (MD) extracts from Jenke (2009)[48] and in extractables associated with PMMA materials.	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: N/ SkinSensDB: N / CosEU: N / NICEATM: N Other references: NICNAS, 2005[75], Omura et al., 2020[81]	

Table A.26 — α-Methylstyrene (CAS 98-83-9)

Mechanistic domain of chemical reaction: Michael acceptor	
Human data	
Potency: weak	—
LLNA (mouse) EC3 %: 46,00	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 46,00 % based on the EC3 value
Data not available for GPMT and/or Buehler (guinea pig)	
Medical device relevance: intermediate used in the manufacture of plasticizers, resins and polymers	
Observations: identified in extractable and leachable from some plastic packaging: Murat et al., 2020[76] Dorival-García et al., Non-volatile extractable analysis of prefilled syringes for parenteral administration of drug products, 2017[77]	
Chemical present in the following reference databases (Y/N): OECD G497: N / ICE: N/ SkinSensDB: N / CosEU: N / NICEATM: N	

Table A.27 — Chlorobenzene (CAS 108-90-7)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency:	—
LLNA (mouse) EC3 %: non-skin sensitizer	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 1,00 % based on GHS generic cut-off value for skin corrosion/irritation
Non-skin sensitizer in GPMT and/or Buehler (guinea pig)	
GPMT reference: Recommended Performance Standards from the Murine Local Lymph Node Assay Interagency Coordinating Committee on the Validation of Alternative Methods[78]	
Medical device relevance: intermediate in rubber, solvent in adhesives	
Observations: tested in polar and non-polar solvent extracts[55]	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: Y/ SkinSensDB: N / CosEU: N / NICEATM: N	

Table A.28 — Glycerol (CAS 56-81-5)

Mechanistic domain of chemical reaction: No skin sensitization reactivity domains alerts identified (ToxTree v3.1.0)	
Human data	
Potency:	—
Non-skin sensitizer in LLNA (mouse)	Spiking concentration (*w*/*v*) to be used in the interlaboratory study: 1,00 % based on GHS generic cut-off value for skin corrosion/irritation
EC3 %: non-skin sensitizer	
Data not available for GPMT and/or Buehler (guinea pig)	
Medical device relevance:	
Observations: In the proficiency list of the OECD TG442D[66] Detected in MD extracts from Jenke, 2014[3]	
Chemical present in the following reference databases (Y/N): OECD G497: Y / ICE: N/ SkinSensDB: Y / CosEU: Y / NICEATM: N	