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Cardiovascular biological evaluation of medical devices — Guidance for absorbable implants

Évaluation biologique cardiovasculaire des dispositifs médicaux — Directives pour les implants absorbables

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Contents

Page

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 documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2. www.iso.org/directives

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The committees responsible for this document are ISO/TC 194, *Biological evaluation of medical devices* and ISO/TC 150/SC 2, *Cardiovascular implants and extracorporeal systems*.

Cardiovascular biological evaluation of medical devices — Guidance for absorbable implants

1 Scope

The objective of this Technical Report is to provide interim Part-by-Part guidance on potential adjustments to various test methods within the 10993 series to account for the intentional release of soluble components or degradation products from absorbable medical devices. The content is intended to add clarity and present potentially acceptable approaches for reducing the possibility of erroneous or misleading results due to the nature of the absorbable material. All suggestions should be considered as preliminary and subject to change, with final dispositions implemented through direct modification to the respective parts of ISO 10993. Thus, interim adoption of any of the described adjustments requires an accompanying written justification.

2 Terms and definitions

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

2.1
absorb
<biomaterials> action of a non-endogenous (foreign) material or substance passing through or being assimilated by cells and/or tissue over time

2.2
degradation product
<byproduct> any intermediate or final result from the physical, metabolic, and/or chemical decomposition of a material or substance

2.3
degrade
to physically, metabolically, and/or chemically decompose a material or substance

2.4
leachable
substances that can be released from a medical device or material during clinical use

Note 1 to entry: In absorbable devices, leachables can be substances released from the as-manufactured product or substances generated and released as a consequence of its degradation (i.e degradation products).

[SOURCE: ISO 10993-12:2012, 3.10 modified — Note 1 to entry has been added.]

3 General considerations

Biological evaluation is the assessment of the ability of a device, device component, or a material to be present in the body without creating an adverse systemic impact and/or local effect on the surrounding cells and/or tissue. Biological evaluation of an absorbable material should be conducted in accordance with ISO 10993-1:2009 and other relevant parts (see ISO 10993-1:2009, Table A.1).

NOTE 1 General guidance regarding evaluation of devices in accordance with ISO 10993 series can be found in ISO/TR 15499.

By design, polymeric, ceramic, or metallic absorbable materials inherently produce relatively low molar mass degradation products when *in vivo*. The relatively elevated presence of these same products within the culture media can potentially impact the results of some biocompatibility tests. For example, in rare

cases if the degradation rate of an absorbable material is sufficiently rapid, elevated concentrations of one or more of the intended products could alter the pH and/or osmolality of an *in vitro* test system. Since the *in vivo* condition provides the combined presence of perfusion and carbonate equilibria, when evaluating intentionally degradable (i.e. absorbable) materials it can be considered acceptable, if necessary, to adjust the *in vitro* test solution pH and/or osmolality to bring the cell culture into a physiologic range – provided there is documented evidence this(these) factor(s) is(are) the potential source of an adverse result and post-adjustment testing within a physiologic range produces a successful result. Such adjustment of pH (using a buffer-appropriate acid or base) and/or osmolality (via dilution) to better approximate the *in vivo* environment helps to mitigate the presence of expected degradation products, functionally allowing the test solution to be evaluated for other causation.

Any pH or osmolality adjustment shall be justified. If under standard test conditions an adverse result is obtained, one should consider the cell type, cell media, culture conditions, and degradation products when determining the amount of osmolality adjustment to be used, if any. For example, with magnesium alloys evaluated with an human osteoblast cell type, it may not be appropriate to dilute the culture medium to less than 105 % of normal osmolality.

NOTE 2 Minimum value of 105 % derived from review of experimental results obtained from 10993 to 12 extraction of magnesium and magnesium alloy samples see Reference.[23]

To directly address these and other absorbable-specific method concerns, a list of relevant testing precautions for each relevant part of ISO 10993 has been compiled and is presented in Clause 6. All suggestions should be considered as preliminary and subject to change, with final dispositions implemented through direct modification to the respective parts of ISO 10993. Thus, interim adoption of any of the described adjustments requires an accompanying written justification. As a part of any justification, both local and systemic effects should be considered, as local pH and osmolality changes could result in toxicities that are clinically relevant.

Degradation products may be released into the media/tissue or reside in the degrading implant. Released degradation products that are generated either prior to product use (i.e. during processing or shelf-life) or during degradation should be characterized (e.g. chemical identity, quantity, and toxicity). Identification of the degradation products may be derived from chemical analyses of the implant or through a theoretical analysis. Literature data for implants manufactured from absorbable materials with an established history of safe clinical use (e.g. PGA) at the intended location may be helpful in identifying expected degradation products and potential toxicities - if one can demonstrate that equivalent manufacturing processes were used. A toxicological risk assessment using information from chemical analyses of degradation products over time, in conjunction with toxicity data from the literature may be sufficient to support an omission of biocompatibility testing from various stages of material degradation (either during device storage or in clinical use).

NOTE 3 Guidance regarding the identification and assessment of chemical degradation products and leachables can be found in ISO 10993-9 and ISO 10993-17.

Since absorbable materials are intended to degrade, potential exists for generation of transient particulate matter as the device breaks down. While an understanding of the potential clinical impact of such degradation is needed, a separate biocompatibility assessment of the absorbable particulates alone may not be necessary if the particles are both produced and absorbed at a rate that is similar to other materials of the same chemistry with a history of safe use in the intended clinical application. However, formulation chemistry as well as particle size could affect biological responses, so additional information and/or testing may be necessary to establish sufficient equivalency to support omission of full biocompatibility testing. Guidance regarding the determination of whether identification and/or quantification of particulates are needed can be found in ISO 10993-9:2009, Annex A.

4 Sterilization considerations

While biological evaluation can be conducted on any component at any stage in the manufacturing process, finished product evaluation needs to be conducted on sterilized finished devices or test samples that are representative of the final device. Evaluations should be conducted following terminal sterilization at a level that meets or exceeds anticipated commercial exposure. While higher sterilization

durations and intensities are generally considered as providing a more stringent evaluation, caution should be undertaken when sterilizing under harsher conditions (i.e. higher radiation dose) as more and different chemical by-products may be produced. If the final sterilized device is not used for testing, a rationale shall be provided that includes:

a) a description of all manufacturing differences between the test article and the final, sterilized device;

b) data that demonstrates that all differences between the test article and the final device do not impact their chemistry or degradation kinetics.

NOTE If potentially significant differences exist between the test article and final device (e.g. surface properties or device geometry when hemocompatibility testing), some test end points can be affected. In such situations, use of a test article cannot be representative of a final device.

5 Drug-device combination product considerations

For devices that include an active pharmaceutical ingredient (API), the presence of a pharmaceutical can affect the biological response. As such, separate testing of both the finished device including the API and devices constructed excluding any drug component should be considered. In addition, any potential for interaction between the pharmaceutical ingredient(s) and the as-manufactured or degrading absorbable component(s) should be both understood and assessed for its impact on device biocompatibility and the drug component itself.

Any pH or osmolality adjustment shall be justified. If under standard test conditions an adverse result is obtained, one should consider the cell type, cell media, culture conditions, and degradation products when determining the amount of osmolality adjustment to be used, if any. For example, with magnesium alloys evaluated with a human osteoblast cell type, it may not be appropriate to dilute the culture medium to less than 105 % of normal osmolality.

NOTE 1 Minimum value of 105 % derived from review of experimental results obtained from 10993 to 12 extraction of magnesium and magnesium alloy samples see Reference.[23]

NOTE 2 Additional guidance regarding evaluation of drug-device combination products can be obtained in ISO/TS 12417, which was developed for vascular medical devices.

Biological evaluation of identifiable and already previously well characterized chemical components, such as degradation products from some intentionally absorbable materials or APIs in drug-device combination products, may be optionally substituted with an appropriate toxicological evaluation. Such a justification might be generated through a chemical characterization of device extracts in conjunction with a biological risk assessment for the specifically identified chemicals, if the risk assessment considers:

a) How *in vitro* chemical extraction studies reasonably characterize the degradation and accumulation of chemicals *in vivo*;

b) Whether toxicity data are available from the literature to explore the biological response to multiple chemicals present from the same device;

c) Results from a subset of final product testing where surface properties and geometry may impact the toxicological profile of an absorbable device. For example, hemocompatibility testing might be needed if the surface properties and/or device geometry could impact the test results.

Devices that include API's can potentially impact the results with misleading positives when extracted at the recommended extraction ratio detailed in ISO 10993-12. Use of a direct dilution of the sample or a partition of the overall device evaluation may be considered.

6 Part listing and description of absorbable related issues in addition to the relevant parts of ISO 10993 series "Biological evaluation of medical devices"

6.1 ISO 10993-1:2009, Evaluation and testing within a risk management process

a) 5.3 c) and throughout ISO 10993 series

1) Supplemental Information:

i) Within the ISO 10993 series, the term PERMANENT is perceived as including CHRONIC or PERSISTENT implants that are physically present longer than 30 d. Since typically at least a limited amount of an absorbable material and/or its degradation byproducts can be expected to persist in the body past 30 d, such devices should be evaluated using the PERMANENT implant test criteria.

6.2 ISO 10993-2:2006, Animal welfare requirements

a) No identified adjustments/allowances/compensation for absorbable devices

6.3 ISO 10993-3:2003, Tests for genotoxicity, carcinogenicity and reproductive toxicity

a) Clause 4 – Genotoxicity Tests

1) 4.4 – Test Methods

i) Supplemental information to 4.4.1:

I) When evaluating absorbable materials using *in vitro* genotoxicity methods, the potential is present for artefactual positive results due to inadequately controlled pH, osmolality, or high levels of cytotoxicity within the culture media [see OECD Guideline 473(1997), Clauses 4, 14 and 38 and OECD Guideline 476(1997), Clauses 3, 14 and 36]. Since absorbable materials carry potential for at least partial dissolution during extraction, the test sample's mass may be monitored to ensure that sample concentration within the extract not does not exceed either the 5 mg/ml or 0.01M OECD limits for the chromosome aberration or mouse lymphoma test or the 5 mg/plate limit for the bacterial reverse mutation test. If extraction results in a higher concentration, dilution of the test liquid to no less than 80 % of the respective concentration limit is acceptable. Lower concentrations may be utilized if evaluated as part of a range of concentrations per OECD guidelines. The sample's cell culture may then be monitored for the presence of abnormal pH and/or osmolality for later consideration in the event of a positive result.

NOTE Significant discussion regarding the effects of abnormal pH and osmolality on genotoxocity testing is available in the ICPEMC report "Genotoxicity under extreme culture conditions," which provides a table showing the chemical-dependent nature of the impact of high osmolality on mammalian cell toxicity and chromosomal aberrations see Reference.[21] Additional guidance regarding appropriate follow-up to a positive *in vitro* genotoxicity test can be found in the internationally authored review article in Reference:[22]

6.4 ISO 10993-4:2002, Selection of tests for interactions with blood

a) Annex C.6 – Haemolysis Testing – General Considerations

1) Supplemental Information:

i) This subclause provides a brief description of direct and indirect haemolysis methods. In indirect methods, such as ASTM F756, extracts from absorbable materials can optionally be either diluted or partitioned into various stages of degradation to address byproduct

driven haemolysis. Thus, as a specific component of ISO 10993-12 and/or other more central 10993 Clauses/subclauses and/or parts, absorbable materials can be either:

Extracted once per the appropriate conditions for the test and the extract then adjusted to maintain test vehicle (i.e. solution) pH and/or osmolality

or

conduct separate sequential extracts representing different stages in the material's overall degradation that, without further adjustment, result in acceptable test vehicle (i.e. solution) pH and/or osmolality.

Any pH or osmolality adjustment shall be justified. If under standard test conditions an adverse result is obtained, one should consider the cell type, cell media, culture conditions, and degradation products when determining the amount of osmolality adjustment to be used, if any. For example, with magnesium alloys evaluated with a human osteoblast cell type, it may not be appropriate to dilute the culture medium to less than 105 % of normal osmolality.

NOTE Minimum value of 105 % derived from review of experimental results obtained from ISO 10993-12 extraction of magnesium and magnesium alloy samples, see Reference. [23]

If accelerated strategies are used to simulate real-time degradation, evidence should be provided to demonstrate that the resulting degradation products are compositionally representative of what occurs under physiologically relevant conditions.

6.5 ISO 10993-5:2009, Tests for *in vitro* cytotoxicity

a) Clause 4 – Sample and control preparation

1) 4.1 – General: "The test shall be performed on a) an extract of the test sample and/or b) the test sample itself. Sample preparation shall be in accordance with ISO 10993-12. Negative and positive controls shall be included in each assay."

i) Supplemental Information:

I) For absorbable devices, cytotoxic assessment can be conducted through *in vitro* evaluation the extract. Extracts from absorbable materials can be either diluted or partitioned into various stages of degradation to address degradation product driven cytotoxicity. Thus, as a specific component of ISO 10993-12 and/or other more central 10993 Clauses/subclauses and/or parts, absorbable materials can be either:

Extracted once per the appropriate conditions for the test and the extract then adjusted to maintain culture medium pH and/or osmolality

or

conduct separate sequential extracts representing different stages in the material's overall degradation that, without further adjustment, result in acceptable culture medium pH and/or osmolality.

Any pH or osmolality adjustment shall be justified. If under standard test conditions an adverse response is obtained, one should consider the cell type, cell media, culture conditions, and degradation products when determining the amount of osmolality adjustment to be used, if any. For example, with magnesium alloys evaluated with an human osteoblast cell type, it may not be appropriate to dilute the culture medium to less than 105 % of normal osmolality.

NOTE Minimum value of 105 % derived from review of experimental results obtained from ISO 10993-12 extraction of magnesium and magnesium alloy samples, see Reference [23].

II) For absorbable devices, it is allowed to limit temperature and duration of the extraction to deliver a controlled degradation that reflects an appropriately partitioned stage of degradation.

2) 4.2.3.2 – "For polymeric test samples, the extraction temperature should not exceed the glass transition temperature as the higher temperature can change the extractant composition." (in reference to a ISO 10993-12 extraction)

i) Supplemental Information:

I) For absorbable materials, extraction at temperatures above 37°C may lead to undesirable and/or non-representative changes in degradation mode and should, if possible, be avoided unless validated otherwise. Caution should be exercised for polymers extracted at temperatures that are near either a glass transition or melting temperature. Additionally, absorbable metals can potentially develop differing corrosion chemistry and/or modes (e.g. pitting, crevice, etc.) at elevated temperatures.

3) 4.2.3.3 – "Any pH adjustment of the extract shall be reported. Manipulation of the extract, such as by pH adjustment, should be avoided because it could influence the result.", and

and

4) Clause 6 – "The culture medium shall be maintained at a pH of between 7,2 and 7,4."

i) Supplemental Information:

I) The pH of the extract and culture are inherently linked and can lead to artefactual test failures (misleading positives) if inadequately controlled (i.e. pH outside the range of 7,2 to 7,4). Since the pH of the extract from an absorbable material is generally more easily monitored than is the culture media, adjustment of the extract can be considered as a means for practical control of culture pH. This approach would be considered as valid only if the product is specifically designed to degrade and its degradation byproducts are known to affect culture pH. **Maintenance of culture pH is perceived as compensation for the robust *in vivo* buffer capacity facilitated by the combined presence of perfusion and carbonate equilibria.**

Any pH or osmolality adjustment shall be justified. If under standard test conditions an adverse response is obtained, one should consider the cell type, cell media, culture conditions, and degradation products when determining the amount of osmolality adjustment to be used, if any. For example, with magnesium alloys evaluated with a human osteoblast cell type, it may not be appropriate to dilute the culture medium to less than 105 % of normal osmolality.

NOTE Minimum value of 105 % derived from review of experimental results obtained from ISO 10993-12 extraction of magnesium and magnesium alloy samples, see Reference [23].

6.6 ISO 10993-6:2007, Tests for local effects after implantation

a) General Note: ISO 10993-6 incorporates a large amount of information for assessment of absorbable devices

b) Clause 3 – Terms and definitions

1) 3.1 – degradation – decomposition of a material [ISO 10993-9:1999, definition 3.1]

and

2) 3.2 – degradation product – product of a material which is generated by the chemical breakdown or decomposition of the material. [ISO 10993-16:1997, definition 3.1]

i) Supplemental Information:

I) Since the above two definitions from ISO 10993-6 are somewhat limiting, the degrade definition provided within this document should be used when assessing the biocompatibility of an absorbable material or device.

c) Clause 5 – Test methods, general aspects

1) 5.1 – "Tissue and implantation site: For degradable/resorbable materials, the implantation site shall be marked in a manner suitable for identification of the site at the end of the designated time periods. The use of a non- invasive permanent skin marker and/or a template marking the placement of the specimen is recommended. In certain circumstances an appropriate negative control may be used as a marker for the location of the implant site. Exceptionally, a sham surgical procedure might be used to evaluate the impact of the procedure on the tissue involved; in these cases the specific justification shall be provided."

i) Supplemental Information:

I) Devices/subcomponents fabricated from suitably biocompatible permanent materials (e.g. gold band) may be utilized to provide an *in situ* mark of the location of *in vivo* placement.

2) 5.3 – Test periods: "In general, it is expected that experiments that go up to or beyond the point of absorption are needed for the evaluation of degradable materials."

i) Supplemental Information:

I) While gross and microscopic evaluation after complete device absorption is highly desirable, *in vivo* degradation profiling of the absorbable material and/or its byproducts to a state of limited visually-identifiable histological presence can also be considered acceptable. Thus, in the absence of complete degradation, absorption, and restoration to normal tissue structure and function, the overall data collected may be sufficient to allow characterization of local effects after implantation.

II) If present, an assessment needs to be made regarding the reversibility of any accompanying adverse pathology.

3) 5.5.3 – Implant retrieval and tissue sample collection

i) Supplemental Information:

I) Per ISO 10993-1, systemic effects should be evaluated based on the device and its components, and the toxicology data set already available. Such considerations should be emphasized if an implant's degradation products are not resolved within the histologically assessed area.

II) For absorbable materials, care should be taken to minimize artefacts due to histological processing and handling (further degradation of material, shrinkage, etc).

d) Clause 6 – Test report

1) c) "Retrieval and histological procedure…

i) Apply the following revised (changes underlined) sentence to paragraph c): "For degradable materials, the report shall include, but not be limited to, a description of the degree of degradation (when possible), including material characteristics at explantation (free particles, fibre formation, amorphous gel, crystallinity). Potentially relevant additional

observations, such as molecular weight changes and relative mass loss, should be considered."

e) Annex A – General considerations regarding implantation periods and tissue responses to degradable/resorbable materials

1) Supplemental Information:

i) Added consideration needs to be made toward common responses to various types of absorbable materials (e.g. local tissue responses, acidic byproducts from the alpha-hydroxyesters, and calcium and phosphorus degradation products from magnesium alloys, common metabolites, etc.).

ii) In the absence of complete degradation, absorption, and restoration to normal tissue structure and function, the overall data collected may be sufficient to allow characterization of local effects after implantation. While gross and microscopic evaluation after complete device absorption is highly desirable, *in vivo* degradation profiling of the absorbable material and/or its byproducts to a state of limited visually-identifiable histological presence can also be considered acceptable. Additionally, an assessment needs to be made regarding the reversibility of any accompanying adverse pathology.

6.7 ISO 10993-7:2008, Ethylene oxide sterilization residuals

a) 4.2 – Categorization of device

1) Supplemental Information:

i) ISO 10993-1 defines PERMANENT contact devices as those which have single or cumulative multiple use contact in excess of 30 d. Since typically at least a limited amount of an absorbable material and/or its degradation byproducts can be expected to persist in the body past 30 d, absorbable devices shall be evaluated using the PERMANENT implant end points unless justified otherwise. Functionally, this categorization ensures that the residual limits at 24 h, 30 d and overall are all considered.

6.8 ISO 10993-9:2009, Framework for identification and quantification of potential degradation products

a) Clause 1 – Scope

1) Supplemental Information:

i) This Part provides guidance regarding characterizing the composition of degradation products that are generated under *in vitro* test conditions. It does not provide for the biological evaluation or assessment of any identified degradation products, which are addressed in other parts of ISO 10993. Special attention should be drawn toward Annex A. where consideration of the need for such degradation testing is addressed.

b) Clause 3 – Terms and definitions

1) Supplemental Information:

i) During degradation pH needs to be controlled to a clinically relevant range, especially if pH can affect degradation product composition.

ii) The user needs to be aware that both the degradation rate and amount of generated byproducts can be affected if pH is not controlled to expected *in vivo* service conditions.

6.9 ISO 10993-10:2010, Tests for irritation and delayed-type hypersensitivity

a) Clause 6 – Irritation tests

1) Clause 6.2 – *in vivo* irritation tests — Factors to be considered in design and selection of *in vivo* tests (last two paragraphs)

"If the pH of the test sample is ≤ 2,0 or ≥11,5, the material shall be considered an irritant and no further testing is required. However, experimental evidence suggests that acidity and alkalinity of the test material are not the only factors to be considered in relation to the capacity of a material to produce severe injury. The concentration of the test material, its period of contact, and many other physical and chemical properties are also important.

"In exceptional cases where further risk characterization/assessment is needed, it might be necessary to test materials which are either an irritant or have a pH outside the range mentioned above. These cases shall be justified and documented."

i) Supplemental Information:

I) In any test sample, test extracts that have pH above 10 or below 2 carry potential for corrosive/damaging effects on the skin in single and/or repeated dose administrations. If these pH extremes do not reflect actual *in vivo* cell exposure conditions (i.e. physiological perfusion and/or buffering consistent with the intended application), testing with uncorrected pH could produce misleading and irrelevant failures. In such situations, adjustment of the extract pH to better reflect actual physiological conditions within the intended *in vivo* service condition can be considered acceptable, provided the justification and adjustment are both documented in the report.

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

a) Clause 4 – General considerations

1) Clause 4.1 – General (third paragraph)

"Physical and chemical properties of the test sample including, for example, pH, stability, viscosity, osmolality, buffering capacity, solubility and sterility, are some factors to consider when designing the study."

i) Supplemental information:

I) It is not unusual in repeated dose systemic toxicity studies to observe pH-related adverse effects that are not reflective of systemic toxicity (e.g. fore stomach tumours in rats repeatedly dosed by gavage with high/low pH test materials). Such repeated dosing may result in hyperplasia or even tumours at the site due to the repeated irritation and wound healing process. Similarly, repeated severe irritation and repair at the site of dose administration may result in hyperplasia and/or tumours due to inability of the cells to adequately replicate and repair errors in the rapidly dividing wound site – something that has been observed in chronic dermal studies with strong irritants and can be replicated by repeatedly physical wounding of the skin.

In any test sample, test extracts that have pH above 10 or below 2 carry potential for corrosive/damaging effects on the skin in single and/or repeated dose administrations. If these pH extremes do not reflect actual *in vivo* cell exposure conditions (i.e. physiological perfusion and/or buffering consistent with the intended application), testing with uncorrected pH could produce misleading and irrelevant results. In such situations, adjustment of the extract pH to better reflect actual physiological conditions within the intended *in vivo* service condition can be considered acceptable, provided the justification and adjustment are both documented in the report.

6.11 ISO 10993-12:2012, Sample preparation and reference materials

a) General discussion regarding Part 12 and standard extractions

and

b) Clause 7 – Test sample selection

1) 7.1 – "Testing shall be performed on the final product, or representative samples from the final products or materials processed in the same manner as the final product (see ISO 10993-1) or on appropriate extracts of any of these. The choice of test sample shall be justified."

NOTE Chemical characterization data may be useful in selecting extraction solvents and conditions.

i) Supplemental Information:

I) Regarding extraction solvent selection:

1. With absorbable materials, the general risk of invalid test results (i.e. variations from in vivo degradation rate and/or byproducts) increases as solvent characteristics and extract conditions depart from physiologic relevance.

2. Since solvent selection, presence of proteins, and electrolyte composition (if applicable) may affect (i.e. increase, decrease, eliminate) the rate of absorbable device degradation during extraction, the user should understand the relative impact the selected solvent system will have on both the degradation rate and the test result.

3. Since an absorbable sample's degradation mode/mechanism can be affected by the extraction solvent (including shifts in pH), the user should evaluate that the resulting degradation products are compositionally representative of what occurs under physiologically relevant conditions.

4. With absorbable materials, consideration should be made regarding the potential for solvent-induced physiologically irrelevant degradation.

II) Regarding extraction duration and/or temperature:

1. Extraction parameters can accelerate degradation of an absorbable material and can potentially generate overwhelming amounts of known byproducts that can affect the test result. In effect, extraction for 24 h concentrates degradation byproducts that in vivo would be both buffered and broadly perfused over that same duration.

2. With absorbable materials, extraction temperature(s) may need to be adjusted to reflect both physiological conditions and the specific thermal limitations of the material.

III) Regarding more detailed analysis/understanding:

1. Appropriate evaluation of absorbable devices can require scientific analysis of the extraction procedure, composition of extract, biological response, and how these relate to assessing safety of the device.

2. Absorbable material extracts can optionally be partitioned so as to independently assess various stages of degradation. Such partitioning is achieved by conducting separate sequential extracts representing different stages in the material's overall degradation that, without further adjustment, result in acceptable culture pH and/or osmolality.

3. Where absorbable degradation products are known to affect culture pH, it is considered acceptable to perform a direct titration adjustment of extract pH to facilitate subsequent maintenance of cell culture pH and/or to minimize potential

for physiologically irrelevant pH driven reactions with the extract(s). The accompanying test report should include acknowledgement of the pH adjustment, the initial extract pH, and the titrant type and volume.

c) Clause 8 – Test sample and RM preparation;

1) 8.1

i) Supplemental Information:

I) Add: d) test samples consisting of absorbable materials shall not be beyond their labelled expiration date as their degradation characteristics may be affected by shelf life.

d) Clause 10 – Preparation of extracts of samples

1) 10.3.2 – "For materials that dissolve or resorb under conditions of use, follow the extraction conditions described in 10.3.1. Perform extraction using the appropriate extraction vehicle and time/temperature conditions to simulate exaggerated exposure wherever possible. Complete dissolution may be appropriate."

i) Supplemental Information:

I) For polymeric absorbable materials, extraction above *in vivo* temperatures that are near or above the glass transition temperature may lead to changes in the polymer that are not representative of actual service conditions and should be avoided.

II) When evaluating absorbable devices, extraction of partially degraded materials and their related degradation products can be considered.

2) 10.3.8 – Statement: "Extract pH shall not be adjusted unless a rationale is provided."

i) Supplemental Information:

I) Since the *in vivo* condition provides the combined presence of perfusion and carbonate equilibria, when evaluating intentionally degradable materials it can be considered acceptable, if necessary, to adjust the *in vitro* test solution pH and/or osmolality to bring the cell culture into a physiologic range – provided there is documented evidence this(these) factor(s) is(are) the potential source of an adverse result and post-adjustment testing within a physiologic range produces a successful result. Such adjustment of pH (using a buffer-appropriate acid or base) and/or osmolality (via dilution) to better approximate the *in vivo* environment helps to mitigate the presence of expected degradation products, functionally allowing the test solution to be evaluated for other causation.

II) Extracts from absorbable materials can be either diluted or partitioned into various stages of degradation to address degradation product driven cytotoxicity. Thus, absorbable materials can be either:

Extracted once per the appropriate conditions for the test and the extract then adjusted to maintain acceptable culture medium pH and/or osmolality

or

conduct separate sequential extracts representing different stages in the material's overall degradation that, without further adjustment, result in acceptable culture medium pH and/or osmolality.

Any pH or osmolality adjustment shall be justified. If under standard test conditions an adverse result is obtained, one should consider the cell type, cell media, culture conditions, and degradation products when determining the amount of osmolality adjustment to be used, if any. For example, with magnesium alloys evaluated with an

human osteoblast cell type, it may not be appropriate to dilute the culture medium to less than 105 % of normal osmolality.

NOTE Minimum value of 105 % derived from review of experimental results obtained from ISO 10993-12 extraction of magnesium and magnesium alloy samples, see Reference [23].

3) 10.4.3 – "In the case of products that polymerize in situ, the samples to be tested shall represent the intended clinical conditions of use to provide information on the potential toxicity of the reacting components in the polymer during the curing process. Test extracts prepared at different times, if appropriate, shall be based on the kinetics of polymerization after mixing the components, including an extract prepared at the expected cure time. Testing of the material after curing shall be justified. Where extracts are used in the test methods, for evaluation of materials that cure in situ, initiation of the extraction shall occur from the point in the cure at which the material is placed in situ. For test methods that use these materials directly, e.g., direct contact or agar overlay cytotoxicity, implantation, some genotoxicity tests, and direct contact haemolysis, the material shall be used as in clinical use, with in situ cure in the test system."

i) Supplemental Information:

I) In the case of products intended to be absorbed, test extracts prepared to reflect different partitioned stages of degradation can be evaluated independently, provided they compositionally and concentratively include the relevant intermediate degradation products (if applicable).

e) Annex C – Principles of test sample extraction

1) "C.8 – For materials that dissolve or resorb in the body:

i) follow the conditions of Table 1;

ii) follow the temperature/times of 10.3.1;

iii) follow 10.3.9 regarding filtration or centrifugation."

iv) Supplemental Information:

I) Temperatures in 10.3.1 are likely not acceptable for most absorbable polymers (except 37°C). Additional consideration is needed for rapidly degrading polymers in regards to extraction time.

f) Absorbable metals specific – Sample preparation/extraction proposal/discussion

1) The type and concentration of metal ions developed in a standard extraction procedure are strongly dependent on a metal's bulk composition, surface composition, surface morphology, and extraction conditions (e.g. medium, pH, temperature, flow)

NOTE The composition and electrochemical potential of a starting surface may be different from the bulk composition/volume (e.g. special surface treatment, coating, heat treatment)

2) Hydrophobic/lipophilic (non-polar) extraction media may not be appropriate for assessment of an absorbable metallic implant that generates ionic degradation products *in vivo*.

3) If the composition of the *in vivo* degradation products for a particular absorbable material has already been established, then a toxicological evaluation can be made based on a metal implant's maximum expected rate of mass loss. Conversely, if the identify of *in vivo* degradation products from a particular composition or alloy have not been established, extraction into a material-appropriate physiologically-relevant solvent system and subsequent degradation product identification are both essential for a toxicological evaluation.

6.12 ISO 10993-13:2010, Identification and quantification of degradation products from polymeric medical devices

a) No identified adjustments/allowances/compensation for absorbable devices

6.13 ISO 10993-14:2001, Identification and quantification of degradation products from ceramics

a) No identified adjustments/allowances/compensation for absorbable devices

6.14 ISO 10993-15:2000, Identification and quantification of degradation products from metals and alloys

a) No identified adjustments/allowances/compensation for absorbable devices

6.15 ISO 10993-16:2010, Toxicokinetic study design for degradation products and leachables

a) No identified adjustments/allowances/compensation for absorbable devices

6.16 ISO 10993-17:2002, Establishment of allowable limits for leachable substances

a) 5.2.2 – Exposure duration considerations

   2) Supplemental Information:

      i) Since an absorbable material, its extracts, and/or its degradation byproducts can be expected to be absorbed and potentially excreted by the body over a definable period of time. The 30 d and at 25 000 d (68 years) "PERMANENT-contact devices" exposure normalization can be considered as inappropriate for implants that are intended to fully degrade over significantly shorter durations. Thus calculation of maximum allowable limit should be derived using duration of exposure equal to or less than the absorbable material's projected *in vivo* longevity.

6.17 ISO 10993-18:2005, Chemical characterization of materials

a) Scope

   1) "This part of ISO 10993 does not address the identification or quantification of degradation products, which is covered in ISO 10993-9, ISO 10993-13, ISO 10993-14 and ISO 10993-15."

and

b) B.6.1 – cites OECD 120:1996 and pH extraction pH 2 and 7 at 20°C and pH 9 at 37°C.

   1) This OECD method specifically states it is "…not applicable to liquid polymers and to polymers that react with water under the test conditions."

      i) Supplemental Information:

         I) The scope of this part states that this standard does not address the identification or quantification of degradation products. However, little to no guidance is provided to differentiate between a leachable and a degradation product – be the latter an inadvertent or intentional part of the implant design. Since none of these terms are defined within the standard, when assessing absorbable materials or devices utilize the following definitions as defined in Clause 2 of this document.

            1. Degradation product or byproduct