Biocompatibility Fundamentals for Medical Devices

Guidelines for planning, risk mitigation, testing, and compliance for the design and manufacture of medical devices

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Biocompatibility Fundamentals for Medical Devices

This ebook from Nelson Laboratories, LLC, provides a digest of pressing challenges and solutions for medical device design, development, and testing with regard to biocompatibility and toxicology planning, testing, and compliance. It is intended to serve as a guide for professionals and teams involved in QA/QC, quality engineering, R&D, regulatory affairs, manufacturing, and consulting in related areas.

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The New and Improved ISO 10993-12:2021

What Is Really Changing

While the updated standard features no major revisions, there is more discussion on what to consider for sample preparation.

By Helin Räägel, PhD
Every time there is an update to a standard, especially an overarching one like the ISO 10993-12 standard that covers sample preparation recommendations, it comes with a slight dose of anxiety. This is because it might mean there is now a gap between what is expected from the regulation and what was done when the testing or assessment for a medical device was originally performed. This time, however, I must say that the updated document was only slightly modified, without unexpected twists and turns, so everyone can breathe a little easier.

**Focus on Biological Testing**

The main focus for the update was to clearly divorce the sample preparation recommendations used for biological testing from those for chemical testing (with the latter being thoroughly outlined in ISO 10993-18:2020). Therefore, ISO 10993-12:2021 now clearly states that it defines requirements and gives guidance on the procedures in the preparation of samples and...
the selection of reference materials for medical device testing primarily in biological test systems in accordance with one or more parts of the ISO 10993 series.

The recommended sample preparation parameters captured in section 10.3.1 remained basically unchanged. The only addition was the emphasis of using the conditions for limited contact devices primarily in the cytotoxicity test [(37 ± 1) °C for (24 ± 2) hours]. Further clarification on extracting prolonged/long-term contacting devices was also provided. It indicates that for these types of medical devices, “extraction times of 72 h are recommended for cytotoxicity testing because extraction for 24 h may not be sufficient to obtain an extract that represents the chemicals released beyond 24 h of device use. However, if there are data available for the prolonged or long-term tissue-contacting devices which demonstrate that 24 h extraction is sufficient to release extractables/leachables from the device and extending the extraction time to 72 h does not result in release of additional chemicals from the device, the 24 h extraction is sufficient.”

While no major changes or revisions were included in the recommended sample preparation conditions, it does feel like there has been a shift in the intent of the guidance. This shift is to encourage manufacturers to think about the specific device being assessed, what makes sense in terms of the materials used, and the relevant clinical use conditions that should be considered during the biological testing setup. This comes from the fact that the medical devices themselves, as well as the materials they are made from, are becoming increasingly more complex, so standard evaluation methods might not be relevant or feasible. That being said, we should always keep in mind that the main purpose of the extractions typically used in biological testing is to obtain a test sample that is expected to be at least as aggressive as the conditions of clinical use.

For example, the new ISO 10993-12:2021 highlights that an extraction using both polar and non-polar extraction vehicles is typically recommended. However, “in some device specific circumstances, it may be appropriate to extract in only one extraction vehicle, either polar or non-polar,” such as in the case of a syringe pre-filled only with saline that will exclusively come into contact with saline/polar solvent and no other fluid. Furthermore, a statement of solvent compatibility was included to highlight that, “Selected solvents should not compromise (e.g., severe swelling, particulate generation and degradation) the medical material or devices.”

Complex Materials

Also, additional guidance regarding how to handle absorbable materials and medical devices containing such materials is now included in the updated standard. It provides some additional aspects to consider when testing these types of devices, like defining the appropriate temperature (based on thermal properties of the material and relevant clinical use conditions), potential impact to pH or osmolarity during extractions, etc.

The guidance clearly states that, “For
these materials, the extracts prepared based on 10.3 may have changes either in osmolarities or in the pH that may not be appropriate for the test system to be dosed. Any adjustment applied to the extracts prior to biocompatibility testing should be justified.” References to specific guidance documents, such as ISO 10993-3 (genotoxicity), ISO 10993-6 (implantation), ISO 10993-13 (degradable polymeric), ISO 10993-14 (degradable ceramics), ISO 10993-15 (degradable metals and alloys), ISO 10993-18 (chemical characterization), and ISO/TS 37137-1 (absorbable medical devices) are provided. It also recommends that “For absorbable materials that could potentially have toxic degradants and residuals, testing of intermediate products should be considered.” Or alternatively, a complete dissolution of the device may also be appropriate, if justified; however, “caution should be taken since complete device dissolution can create challenges for subsequent biological testing (e.g., difficulty in dosing animals with neat test extract if viscosity is increased, difficulty in interpreting in vitro cell-based test failure data in case of increased osmolality or pH change).”

The guidance also indicates that if an absorbable device is tested neat and “if the resulting solution contains all the constituents of the material, a second vehicle would not be needed.” Similarly, additional guidance was provided for in situ polymerizing materials for preparing a test sample that represents the intended clinical use conditions to provide information on the potential toxicity of the reacting components in the polymer during the curing process. Reference to nanoparticles and ISO 10993-22 was added into the guidance; however, due to their varied nature, not much more specific information was included in ISO 10993-12 other than a reference to the standard (ISO 10993-22) that should be consulted for more information.

**Sample Preparation**

Other critical components of the sample preparation step are the extraction ratio to be used as well as what specifically can...
and should be included in the surface area calculations of a test sample. If any way possible, only the patient/user-contacting parts should be included in the test sample. The guidance highlights that, “Non-patient contacting portions of the medical device should, if possible, be excluded either physically from test sample extracts or by exclusion of the surface area in the calculation of the extraction ratio.”

As seen in the previous revision of the standard, the surface area remains the preferred parameter overweight of the sample when determining the extraction ratio for sample preparation, unless the surface area cannot be defined, or the weight-based approach is demonstrated to be worse case. Also, another typical issue we face during sample preparation is when a device has various components of different thickness. In this case, is it appropriate to use the standard 3 cm²/mL or is the more stringent 6 cm²/mL more appropriate? Table 1 in ISO 10993-12 now recommends that if the medical device includes multiple tissue contacting components with different thicknesses, a way to address this is to base the ratio on the thinnest material layer of that component, which then would be the worst-case scenario.

In all, the changes imply that a more-thorough sample preparation thought process is now required and expected. However, I would also highlight that discussions with regulatory bodies prior to conducting the testing are always recommended (when diverging from the standard parameters) to ensure the thought process is also acceptable to the agencies reviewing your submissions.
Understanding the EU Medical Device Regulation for Device Companies

By Matthew R. Jorgensen, PhD, Audrey Turley, and Thor Rollins
The new European Union (EU) Medical Device Regulation (MDR) will impact medical device manufacturers’ product-development timelines. All companies intending to sell their devices in Europe must understand the new MDR and how it differs from the previous directives, especially in terms of new biocompatibility standard requirements. The regulation, which goes into effect in May 2020, mandates CE marking for some products that did not previously require it and also will require adherence to ISO 10993-1:2018 Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process, recently updated with more stricter guidelines in certain areas. This adherence to ISO 10993-1:2018 will most likely result in either more testing or documentation of justifications that could take a significant amount of time to perform. Device companies need to account for this extra time and expense to avoid delays in EU product approvals.

Recent issues regarding the safety of medical devices have led the European Union (EU) to make major changes in its requirements for product approvals. The result is the Medical Device Regulation (MDR), which was developed to ensure patient welfare, and to reflect recent technological and scientific developments in the medical device industry.

The Europe-wide regulation supersedes and replaces the previous Medical Device Directives (MDD) that have been in use for more than 25 years. The MDR was ratified in May 2017, and is being implemented until May 2020, which is the cutoff date for any new device to be approved. After May 2020, any devices being submitted for a CE mark must comply with the new MDR. By May 2025 all devices on the market in Europe must be MDR compliant.

Manufacturers must understand the new requirements because there may be a need to provide more biocompatibility information than before to prove that products and starting materials are safe. For example, compliance with the MDR may now require testing for additional biological endpoints such as genotoxicity or chronic toxicity. These tests may be performed either in vitro or by chemical analysis and some tests could take up to six months to complete. Therefore, companies should budget for both the appropriate time and cost needed to perform the testing, and the associated preparatory tasks, which may include making a representative device on which to conduct testing.
Key Changes in the MDR

Some sections of the MDR differ drastically from the MDD. A key point in the new regulation is that companies are no longer allowed to “grandfather” in any medical device to the EU and all products currently on the market need to be reaccredited, regardless of the length of time it has been on the market. Under the new MDR, which enforces stricter regulation of the Notified Bodies, all medical device manufacturers must prove that their devices are biocompatible without relying on a long history of safe use. This approach aligns with ISO 10993-1:2018.

Additionally, the MDR regulates the production facility environment and the additives used in the manufacturing and design of the product. Therefore, Notified Bodies will be more rigorous in ensuring that companies adhere to ISO 10993-1:2018, specifically, Table A.1, which includes six highlighted categories for determining biological endpoints.

Another factor manufacturers must consider, if they have not before, is that there is now an essential requirement or known in the MDR as General Safety and Performance Requirement (GSPR) that devices must be free from carcinogens, mutagens, and reproductive toxic substances (CMR). Per the regulation, companies must provide justification “regarding the presence of CMR and/or endocrine-disrupting substances in a concentration above 0.1 % weight by weight (w/w).”

Recommended First Steps for Manufacturers to Comply with the MDR

Manufacturers must first decide if they will market their devices in Europe. If that is the case, companies should ensure their device history files are up to date for each product. All materials used in each product should be specifically documented in order to streamline the approval process. Although a company may have been
in business for decades, personnel turnover may have resulted in knowledge of the material changes and processes being lost. Continually updating device history files can help avoid this situation.

Device history files should contain information on what is in the device, what it is made of, and how it originally came to market. They should state how the biocompatibility of the device was proved and what changes in materials, if any, have been made since that time. For example, a surgical tool may have originally been made with 316L stainless steel, changed to 316 stainless steel, and then had another material added or substituted. All those changes must be documented and evaluated.

The knowledge contained in a device history file is critical for the next step a company must perform: the gap analysis. A gap analysis requires the manufacturer to determine if the previous biocompatibility evaluation (documentation and testing) used for the device is 1) still applicable to the currently marketed device and 2) if that evaluation is compliant to ISO 10993-1:2018. It is likely that additional testing will be needed for compliance, especially when companies only submitted their devices in the EU, as common practice was to submit the list of device materials and state that they are commonly used in the medical device industry.

The gap analysis should be a written document with a plan to close the gaps for each product type or group. When it is necessary to perform additional biocompatibility testing, manufacturers must strategically consider which product might be the most representative of many devices, so that the company can minimize its overall testing burden. This can be achieved by categorizing similar products into a family grouping and testing them as such. In the gap analysis, manufacturers should prescribe options for the best path forward to address the gap(s) for each of the groups.

Another requirement is that an initial risk assessment (called a biological evaluation plan) must be performed. This plan contains device-specific information, including the device contact type and duration of use that is pertinent to evaluating the biocompatibility. Sometimes, this plan will recommend chemistry testing to gather more information about the chemicals a patient could be exposed to during use. The chemistry data is analyzed for patient safety through a toxicological risk assessment. In a toxicological risk assessment, a toxicologist uses a variety of resources to evaluate exposure data and the hazard of that exposure.

Possible Gaps that Manufacturers May Find

A key change in the MDR states that manufacturers must comply with ISO 10993-1:2018, a foundational document for device biocompatibility that is intended to evaluate the biological risks of devices with direct or indirect patient contact. Since ISO 10993-1 was recently updated in 2018, manufacturers may find gaps in their compliance with this current revision.

One of the requirements of ISO 10993-1:2018,
is that physical and/or chemical information is needed for all types of devices as a prerequisite for animal testing and evaluation. In some cases, if a manufacturer is able to obtain detailed information through supplier information (i.e. MSDS, CoA, tech sheets), this can be evaluated without having to perform chemistry testing. If physical and/or chemical information is not available, companies may need to consider testing to acquire the data needed to address this endpoint.

Another potential gap might be found in the requirements of Table A.1 of ISO 10993-1:2018. Six biological endpoints have been highlighted by being listed in their own column, where previously, they were discussed in the context of the standard. They are Physical and/or Chemical Information, Material-Mediated Pyrogenicity, Chronic Toxicity, Carcinogenicity, Reproductive/Developmental Toxicity, and Degradation. In the past, a manufacturer may have never considered these biological risks because they were not on the table, or not highlighted for a specific device, but it may be required under the new standard and thus more testing may be necessary.

Manufacturers with devices that are invasive to the body must also comply with the GSPR of the MDR regarding CMRs, as mentioned previously. This starts with information from the supplier which can take a significant amount of time as the details needed to meet the CMR requirement have not typically been required.

Conclusion

The MDR stringently advocates for patient safety, which requires more information from manufacturers to ensure that their products are biocompatible. Device manufacturers must be prepared for the additional testing that may be necessary to comply with the new regulation. Companies need to ensure that device history files are updated, and they must conduct thorough gap analyses using ISO 10993-1:2018 to determine if additional biocompatibility testing should be performed. The possible tests could add considerable time and cost to product-submission timelines, making it crucial that manufacturers understand the new requirements mandated under the MDR in order to get their products to market without delays.
FDA Draft Guidance for Biocompatibility of Certain Devices in Contact with Intact Skin:
The Naughty and Nice list

By Thor Rollins
In October 2020 FDA released a new draft biocompatibility guidance document which focuses on the biological evaluation of devices in contact with intact skin. As a draft document, it is therefore open for comments and possible changes. Release of this guidance signifies another step from FDA toward their 3R animal initiative (reduce, refine, and replace animal use in testing when feasible).

In the document FDA gives guidance on how to evaluate certain types of skin contacting medical devices. The reason behind the release of the draft seems due to a demand on FDA resources to evaluate these low-risk devices with subpar risk assessments. To help alleviate their resources, FDA developed this guidance to help medical device manufacturers know when a justification from testing is possible and insights on how it can be done.

The main substance of the document is to give examples of well-known, low-risk, skin-contacting materials that can be evaluated without testing (the nice list of materials), while also pointing out certain materials that have high enough risk that still require biocompatibility testing (the naughty list). Along with the naughty and nice list, FDA also gives considerations around supplier and process controls that must be evaluated in your submission when using this new avenue of no testing for skin contacting devices.

The guidance also gives instructions how to request a certain material to be added to the nice list or to be removed from the naughty list. FDA intends to review these suggestions and periodically assess whether any changes to guidance are warranted. If changes are warranted, FDA will issue an updated guidance.

The guidance is divided into a few sections, the first main section gives suggestions on how to use your supplier control and quality systems to address risks associated with suppliers and manufacturing residuals on these skin-contacting medical devices. They include:

- Purchasing controls (21 CFR 820.50) over material suppliers,
- Production and process controls for manufacturing (21 CFR 820.70) (residuals that could be toxic),
- Receiving, in-process, and finished device acceptance (21 CFR 820.80) for component and manufacturing materials,
- Analysis of quality data (21 CFR 820.100(a)(1)), including complaints, to detect quality problems, such as those that may reveal issues of cytotoxicity, irritation, or sensitization. These analyses should be done routinely (at least annually), and
- Complaints (21 CFR 820.198) should be received, reviewed, evaluated, and when necessary investigated. This should also be done routinely and timely looking for cytotoxicity, irritation or sensitization. Examples of this are:
  - redness (erythema),
  - swelling (edema),
  - irritation,
  - sensitization (delayed Type IV hypersensitivity),
  - allergy, and
  - immune response or other reactions on the skin where the device has contact.

It should be noted that this guidance is intended for devices that contact intact skin surfaces only, but includes all contact durations, and must be composed of materials included in the nice list shown below. FDA also recommends that devices that fall in these requirements go through a Q-Submission process to determine the following:

<table>
<thead>
<tr>
<th>NICE LIST</th>
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<tbody>
<tr>
<td><strong>Synthetic Polymers</strong></td>
</tr>
<tr>
<td>Acrylonitrile butadiene styrene (ABS)</td>
</tr>
<tr>
<td>Cured epoxy adhesives</td>
</tr>
<tr>
<td>Fluoropolymers including polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (ePTFE), polyvinylidene fluoride (PVDF), and fluorinated ethylene propylene (FEP);</td>
</tr>
<tr>
<td>High impact polystyrene (HIPS)</td>
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<tr>
<td>Polyamides, including nylon</td>
</tr>
<tr>
<td>Polybutylene terephthalate (PBT)</td>
</tr>
<tr>
<td>Polycarbonate (PC)</td>
</tr>
<tr>
<td>Polyetheretherketone (PEEK)</td>
</tr>
<tr>
<td>Polyether imide (PEI)</td>
</tr>
<tr>
<td>Polyethylenes, including low-density polyethylene (LDPE) and high-density polyethylene (HDPE)</td>
</tr>
<tr>
<td>Polyethylene terephthalate (PET)</td>
</tr>
<tr>
<td>Polymethylmethacrylate (PMMA)</td>
</tr>
<tr>
<td>Polycrylonitrile (POM)</td>
</tr>
<tr>
<td>Polyphenolsulfone (PPSU)</td>
</tr>
<tr>
<td>Polypropylene (PP)</td>
</tr>
<tr>
<td>Polyurethane (PU)</td>
</tr>
<tr>
<td>Silicone</td>
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| **Fabrics** |
| Polyurethane fabrics, including Lycra |
| Cotton fabrics |
| Polyamide fabrics, including nylon |
| Silk fabrics |
• If a legally US-marketed device made from the same material was found to be toxic in previous testing or resulted in adverse clinical findings after marketing that may be related to cytotoxicity, irritation, or sensitization.

• If the proposed device is indicated for use with neonates or pregnant women. As neonatal skin is more permeable, the risk that leachables may permeate the skin is higher. Similarly, chemicals that absorb through the skin may be transferred from a pregnant woman to her fetus.

• Or if it is a combination product or biologically derived material. Such products can cause adverse biological responses (e.g., cytotoxicity, irritation, or sensitization).

Discussion of the List

By far the most controversial and discussed part of this draft document is the inclusion of metals on the naughty list. It is true that many metals, including the ones that FDA specifically addresses in the draft, contain nickel. Nickel allergies are of concern with nickel sensitive populations and is most likely the reason that FDA specifically calls out titanium, stainless steel, and nitinol in the draft. Although most titanium is nickel free, some instances of nickel deposits in

<table>
<thead>
<tr>
<th>NAUGHTY LIST</th>
<th>Reason</th>
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<tr>
<td>Medical Device Characteristic</td>
<td>Reason</td>
</tr>
<tr>
<td>Materials not in the nice list</td>
<td>There are known risks, or FDA does not have adequate experience with these materials that may introduce toxicity risks.</td>
</tr>
<tr>
<td>Novel materials and bulk metals (e.g., titanium, stainless steel, nitinol, gold)</td>
<td>There is an increased risk that leachables can be transferred into the fluid or cream, and then absorbed through the skin.</td>
</tr>
<tr>
<td>Stored in or containing fluids or creams</td>
<td>There is a risk that polymerization or degradation products can change over time. The manufacturing process can impact the type and quantity of intermediate and final chemicals present in the device, which could introduce a toxicity risk.</td>
</tr>
<tr>
<td>Fabricated from in-situ polymerizing materials, absorbable materials, or hydrogels</td>
<td>There is an increased risk that leachables can be transferred through breached or compromised skin.</td>
</tr>
<tr>
<td>Contacts breached or compromised surfaces, such as abraded or shaved skin, or open or healing wounds</td>
<td>FDA is unaware of a history of safe use of single-use devices that are reused after reprocessing. Reprocessing of such devices can cause adverse biological responses (e.g., irritation).</td>
</tr>
<tr>
<td>Reprocessed single-use devices</td>
<td>Adhesives can cause adverse biological responses (e.g., irritation).</td>
</tr>
<tr>
<td>Includes adhesives to attach a device directly to the skin (e.g., electrode pads, on-body pump attachment systems)</td>
<td></td>
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biocompatible titanium implants may cause nickel allergy and subsequent allergic reactions.1,2

Don’t be devastated though, as this guidance does not automatically require biocompatibility testing for the naughty materials, it just requires a more detailed level of evaluation for the submission which was already required prior to the release of this guidance. FDA themselves state that, "Biocompatibility testing or detailed rationales for omission of this testing could address these concerns." Thus, if you have gold or titanium in your skin-contacting device, it is still valid to evaluate the biocompatibility risk of this device through a risk-assessment process; it just currently falls outside the guidance in this draft.

The last section of the guidance speaks to what information should be included in your premarket submission while following this guidance. The recommended information includes:

- A list of all materials used to fabricate the device with direct or indirect skin contact;
- A statement confirming (e.g., MDR analysis, literature search) that the listed materials have a documented history of safe use in legally US-marketed medical devices in contact with intact skin; and
- A statement confirming that none of the exclusions listed in this draft apply to the device.

For an IDE submission, FDA also recommends that you discuss any adverse biological responses from devices within the progress reports submitted pursuant to 21 CFR 812.150(b).

Specifically, describe any redness (erythema), swelling (edema), irritation, sensitization (delayed Type IV hypersensitivity), allergy, immune response, or other reactions observed by investigators during a clinical study with observations attributed to a specific device.

Lastly, FDA gives recommendations on what should be included in the Device Master Record (DMR). This recommendation clarifies that the sponsor should state that biocompatibility testing and a detailed rationale regarding manufacturing are not necessary, as biocompatibility risks
are addressed through reliance on relevant quality system requirements and post market controls related to:

- Purchasing controls (21 CFR 820.50) of device materials,
- Production and process controls (21 CFR 820.70) for manufacturing materials,
- Acceptance activities (21 CFR 820.80) for component and manufacturing materials,
- Corrective and preventative action (21 CFR 820.100),
- Complaint files (21 CFR 820.198), and
- Medical device reporting (MDR) (21 CFR 803).

They also recommend labeling information to help with devices used in patient populations that may not have the ability to recognize adverse biological responses (e.g. epilepsy and dementia). In this case FDA recommends the labeling verbiage below:

“Caretakers should assess patients for adverse reactions on the skin where the device has contact, such as redness (erythema), swelling (edema), irritation, sensitization (delayed Type IV hypersensitivity), allergy, immune response, or other reactions.”

Overall, the release of this draft guidance by FDA shows the transition from the “check-box” approach to more openly accepting the risk-based justifications when evaluating the biocompatibility of medical devices. And for that I put FDA on the nice list.


Choosing COLORANTS for Medical Devices

By Thor Rollins

You can download a more detailed discussion of this topic here.
Colorants are often added to the substrates and material components of medical devices with the primary intent of improving the usability of the devices. For instance, color coding can assist users in matching devices or sizes together when multiple options are available (Figure 1). Colorants can also be used to brand medical devices from a particular manufacturer, thereby ensuring that only compatible components and accessories are used with the company’s products (Figure 2).

And in some cases colorants can be used to improve the user appeal of a device, meeting the expectations of users and helping manufacturers to build and maintain customer loyalty (Figure 3).

Whatever the reasons behind the addition of a colorant in a medical device application, any such introduction or manipulation of the native materials of a medical device inherently raises the potential for biological risks to patients and users. Other types of additives typically improve the performance of a device in some significant and demonstrable manner, but colorants differ often do little to improve the risk:benefit ratio of a device.

In fact, medical device firms don’t always understand that even a seemingly innocuous
colorant can represent a potential toxicological risk. If there is no corresponding benefit from the use of the colorant, the risk:benefit ratio of the device becomes effectively zero, because the potential risks of the device outweigh its benefits.

Just like any other material, additive, or residue found in a medical device, colorants need to be evaluated for their risk under the intended use conditions for the device.

Regulated color additives

When used in the development and manufacture of medical devices intended for sale in the United States, colorants—more formally known as ‘color additives’—are broadly subject to requirements established under the authority of the Federal Food, Drug, and Cosmetic Act (FD&C Act). However, manufacturers searching for prescriptive guidance about the use of such colorants are likely to be frustrated by the dearth of information available from regulatory authorities; and the scattered nature of related guidance documents, practice recommendations, and sources that those who intend to use colorants in their medical devices should be familiar with.

Statutory authority: The FD&C Act defines a color additive as follows:

1. The term ‘color additive’ means a material which:
   A. Is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source, and
   B. When added or applied to a food, drug, or cosmetic, or to the human body or any part thereof, is capable (alone or through reaction with other substance) of imparting color thereto; except that such term does not include any material which the secretary [of the Department of Health and Human Services] by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring.

2. The term ‘color’ includes black, white, and intermediate grays.

According to the FD&C Act, medical devices containing a color additive are considered adulterated unless a regulation is in effect listing the color additive and the device for which it is intended to be used. Color additive violations are common reasons for FDA warning letters and import detentions.

Food and drug colorants: Products regulated
by FDA must comply with the agency’s color additive regulations. These have evolved from food additives to color additives with special uses and exemptions to the use of color additives in medical devices.

In 2016, FDA issued a final rule for the regulation of additives that are ‘generally recognized as safe’ (GRAS) when combined with foods. A compound accepted and placed on the GRAS list can be used in accordance with the terms of the listing without requiring further FDA review. Nevertheless, there is no GRAS provision for color additives in foods or any other FDA-regulated products, and all uses of color additives must be approved as safe by FDA prior to marketing.

Medical device colorants: In 1960 the FD&C Act was expanded to include the regulation of color additives used in cosmetics and medical devices. With regard to medical devices, the FD&C Act limits the applicability of these provisions to color additives that directly contact the body for ‘a significant period of time’—a term that has not yet been defined by FDA regulation.

It is a logical thought that additives approved for use in foods might also be suitable for use in medical products, however, there is no automatic exemption or permission granted for such substances to be used in applications other than those for which they have received FDA approval. Food-grade colorants are typically approved for very narrow and specific routes of administration and ingestion. But even those additives broadly approved for use in foods—and therefore expected to have contact with an individual’s gastrointestinal tract—do not automatically qualify for use in medical devices with a similar type of contact during use. And, of course, approval for contact with the gastrointestinal tract carries no weight when the colorant comes into contact with an intravenous environment or is exposed to the user via other routes of administration.

Consequently, medical device manufacturers hoping to take advantage of the previously approved colorants included among FDA’s GRAS listing of food additives to know that under the FD&C Act, a substance that imparts color is a color additive, subject to premarket approval requirements, unless it is used solely for a purpose other than coloring.

In order to determine whether a colorant is suitable for use in a particular medical device, the manufacturer must conduct studies specific to the device and its intended uses. Studies must be designed to reveal any biocompatibility issues that might be caused by using a colorant in the product, and to assess the potential risks to the patient that might come from use of the product with its colorant.

Code of Federal Regulations: FDA regulations regarding the use of color additives in medical devices are limited in both number and scope. Device manufacturers in search of definitive rules about the use of such additives frequently find themselves poring over the agency’s listings of color additives exempt from or subject to certification, which include separate sections for foods, drugs, cosmetics, and medical devices.
In the sections relating to medical devices, FDA has created two lists of color additives; one that identifies 22 additives that are exempt from the requirements for certification, and the other that describes nine additives that are subject to the requirements for certification. For each of the listed color additives, the regulations also define specific uses and restrictions.

Nearly all of the listed additives are permitted for use in one of just two broad product types: contact lenses and surgical sutures. One rule lists a color additive for use in bone cement.

In some cases, FDA’s color listing regulations permit the broad use of a color additive in a generic type of device, such as contact lenses. In other cases, the regulations place limitations on the use of an additive, for instance, permitting use of the additive in non-absorbable polypropylene sutures for general surgical use, but not in the equivalent sutures for ophthalmic surgical use.

Where FDA has listed a color additive for a specific device, manufacturers of those devices can use the additives with confidence. That’s good news for manufacturers of contact lenses and surgical sutures, but for the manufacturers of other types of medical devices, FDA’s color listing regulations are not particularly useful—and perhaps even a little confusing. (See full report cited at the top of this article for details.)

**Agency guidance:** Device manufacturers recognize that the complexity of technologies used in medical products is too great to expect that either statutes or regulations can incorporate all of the detailed requirements necessary for a regulatory assessment of any given product. Guidance documents and voluntary standards therefore play an important role in helping manufacturers understand the types of issues that FDA considers important, as well as the types of evidence needed to demonstrate that a product meets the agency’s expectations.

For most issues related to the assessment of color additives, FDA formerly relied on a 1995 Blue Book memorandum, “Use of International Standard ISO 10993, ‘Biological Evaluation of Medical Devices, Part 1: Evaluation and Testing.’” In February 2016, this guidance document and the related ISO standard provided the underpinnings of a major FDA webcast focused specifically on the topic of color additives in medical devices. FDA indicated a new Level 1 guidance document about color additives in medical devices was forthcoming, but this has not materialize.

Meanwhile, in June 2016, FDA withdrew and replaced its previous Blue Book memorandum with a final guidance document on “Use of International Standard ISO 10993-1, ‘Biological Evaluation of Medical Devices, Part 1: Evaluation and Testing within a Risk Management Process.’” While not specific to the topic of color additives in medical devices, the new guidance outlines the application of biocompatibility testing principles necessary to assess the suitability of color additives used in medical products.

All color additives carry a potential for toxicity, which is the main reason that FDA has recently been focusing its attention on such components. Biological risk assessments for colorants as with →
all of a device’s materials—including colorants and other additives, as well as any changes from previously marketed devices—should be an important part of a medical device manufacturers’ due diligence for ensuring patient safety.

When assessing the biological risks associated with the use of a particular color additive, manufacturers should review all of the uses for which the additive is already certified. But while it is useful to know that an additive is considered a food-grade GRAS substance, such information should be merely the starting point for assessing the biological risks associated with the additive. When reviewing the suitability of a particular color additive, certifications should be considered with all other risk assessment processes already in place.

Moreover, changing an approved medical device by incorporating new materials, changing a color additive, or altering a manufacturing process, should be considered a trigger for the manufacturer to perform a new round of biological risk assessment.

Assessing medical device contact

Not all uses of color additives result in risks that require significant evaluation and testing. Color additives can be incorporated in portions of a device that do not contact the patient or clinician and are therefore associated with very little or no risk (Figure 4). When it comes to assessing and documenting the biological risks that may arise from the use of color additives in medical devices, FDA’s Office of Device Evaluation (ODE) has adopted a hierarchical, risk-based approach that makes use of the type of contact and duration of contact categories outlined in ISO 10993-1.\(^5\)

FDA’s latest guidance document on biological risk evaluation incorporates consideration of device contacts with both patients and clinicians. For the purposes of its guidance, the agency writes, “the term ‘human body’ refers to either patient tissues or the clinical practitioner.”\(^{16}\) If a manufacturer intends to use a color additive in the handle of a medical device, for instance, it should be considered whether the handle is expected to be in contact with the skin of the clinician. And if so, will the clinician be gloved or otherwise protected with devices that are themselves biocompatible?\(^{16}\)

“Masks or gloves intended for protective purposes by clinical practitioners should be assessed for biocompatibility,” writes the agency. “Similarly, medical devices such as implants or skin electrodes also should be assessed for biocompatibility.”

Thus, the first step in the process of writing a biological risk assessment is to define the type of contact that the medical device will have with patients and others, whether in clinical settings or...
**Types of contact:** For the purposes of conducting biocompatibility evaluations, FDA mostly follows the definitions of contact types established in ISO 10993-1. But the agency also provides a route for assessing the biocompatibility of devices whose contact with patients or clinicians is extremely limited but potentially risky. The categories of contact used by FDA include direct contact, indirect contact; and transient contact.

**Duration of contact:** The color additive provisions of the FD&C Act apply to a medical device only when the device directly contacts the body for ‘a significant period of time.’ FDA has not defined this term, however, the Office of Device Evaluation (ODE) has indicated 30 days as the threshold for this period. Overall, color additive information is required for devices whose duration of contact is longer than 30 days, regardless of whether the type of contact is direct or indirect. There are specific considerations for Duration of Contact that include no contact, limited contact, prolonged contact, and permanent contact. (Again, see Table 1.)

Ultimately, when assessing the patient impact of a color additive, it is a good idea to consider worst-case scenarios. If there is no patient contact and there is no possibility of indirect contact—either with the patient or the clinician—and there is no bleeding effect, then the ISO 10993 requirement to perform a biological risk evaluation does not apply—especially in the case of a color additive.

For devices that are expected to have prolonged indirect contact or permanent contact, however, more time and effort is required to justify the addition or change of a color additive. Risk evaluation involves chemistry and toxicity assessment, and must be performed on a short-term contact device if particulates are found or color changes occur, and always for prolonged or permanent contact devices.

**Color additive risk evaluation**

To evaluate the risks associated with incorporating a particular color additive into a medical device, manufacturers must begin by gathering as much information as possible about the additive in question (Figure 5). Device manufacturers can obtain the hazard communication safety data sheets (HCSDSs) for →

---

<table>
<thead>
<tr>
<th>Type of Contact</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct contact</td>
<td>Device has physical contact with body tissue</td>
</tr>
<tr>
<td>Indirect contact</td>
<td>Device has contact with fluids or gasses on their way to having contact with body tissue</td>
</tr>
<tr>
<td>Transient contact</td>
<td>Device has contact with body tissue for less than a minute</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of Contact</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>No contact</td>
<td>Device has no direct, indirect, or transient contact with patients or clinicians</td>
</tr>
<tr>
<td>Limited contact</td>
<td>Device has unrepeated contact lasting less than 24 hours</td>
</tr>
<tr>
<td>Prolonged contact</td>
<td>Device has single or accumulated contact with the patient for periods lasting from 24 hours to 30 days</td>
</tr>
<tr>
<td>Permanent contact</td>
<td>Device has patient contact longer than 30 days</td>
</tr>
</tbody>
</table>

Table 1. Types and durations of medical device contact, as defined by FDA’s Office of Device Evaluation. For the purposes of conducting biocompatibility evaluations, FDA mostly follows the definitions of contact types established in ISO 10993-1.
Safeguarding Global Health®
- with every test we complete.

LAB TESTING
Biocompatibility
Extractables & Leachables
Packaging Validation
Lot Release
Sterilization Validation
Reusable Devices
Chemical Characterization

STERILIZATION
Terminal Sterilization
Including Inhalants & Injectables
Leading Sterilization Technologies
Cold Chain Management
Sterilization Validation

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a particular color additive.\textsuperscript{17} These are useful as a starting point, but not sufficient to be an endpoint for the manufacturer’s search.

Although the makers of color additives do not typically provide extensive information about their products directly to medical device manufacturers, some analytical laboratories are often able to obtain detailed additive information under the terms of the strict nondisclosure agreements. Such information enables laboratories, including Nelson Laboratories, to positively influence the outcome of a manufacturer’s studies in support of the adoption of a color additive.

Once the manufacturer has obtained as much information about the color additive as possible, the next step is to figure out what dose the patient may receive from use of the device, and may thereby be coming into contact with the patient.

Dose information is a vital element of a biological risk assessment for a color additive.

Another important element is the history of the additive’s use. Manufacturers should provide documentation about previous uses of the color additive, including whether it was used in similar device applications or with similar substrates. This is helpful when preparing a risk assessment to evaluate and mitigate the hazardous effects of using a particular color additive.

An adept strategy for eliminating unnecessary testing to determine the risks associated with the use of a color additive involves calculating what would happen if all of the additive in a device were to leach into the patient’s body at the same time. This approach begins with a toxicity assessment to determine the level at which the additive would become toxic to the patient or clinician, followed by calculations to determine the level at which the additive is considered toxic.

For example, if a device contained 1mg of the color additive, and that entire amount were to suddenly migrate out of the device and become bioavailable, would the device still be considered safe? If that question can be answered in the affirmative, additional testing for the colorant additive can be avoided.

In the event that the strategy produces a negative result—meaning either that the level of leached additive is considered unsafe, or that the full amount of the additive truly does become bioavailable all at once—FDA will require that additional chemistry testing be performed. Most additives are embedded and filled into the polymers of the device, so they cannot leach out all at the same time. But to determine how much of the additive actually does leach, the lab must perform extractability and leachability (E&L) testing.

Many color additives have a metal component that can be searched for in order to assess the leachability profile of the additive. If the
additive has copper in it, for instance, inductively coupled plasma mass spectrometry (ICP-MS) can be performed to look for copper, and extraction can be performed to look specifically for that distinct color additive. Alternatively, aggressive solvents can be used to remove as much of the color additive as possible during extraction. The extracts can then be analyzed for that one specific compound. The obtained leachability profile can then be applied to the same percentages of the additive to give an idea of how much of the additive is coming off the device. This so-called extractability profile can be useful in assessing the risk posed by a color additive in a given medical device with its intended contact type and duration.

**Toxicological risk assessment**

Once the color additive has been defined, and the amount of leaching and dose have been determined, the next step is to interpret the toxicological risk associated with use of the additive (Figure 5, see page 25). Methods for performing this type of assessment are described in ISO 10993-17.14

The first thing to determine is the additive’s threshold of toxicological concern (TTC), which is essentially a threshold or limit below which certain compounds are considered safe. This concept is part of the ICHM 7 guideline, and is considered a very conservative approach to defining safety limits for chemicals that are unknown or not well studied.18 If the amount of color additive shed by a device is below the TTC for that compound, the level of concern about that additive is significantly less than it would be if the level were above the TTC. The TTC has different limits depending on contact type, but the most conservative is 1.5 μg per day. Generically, that TTC can be applied to evaluate the risks associated with the use of a colorant.

A toxicological risk assessment also reveals the risk to the patient, as measured by performing a tolerable intake (TI) or tolerable exposure (TE) evaluation. TI is a dose, measured in mg/kg/day, below which adverse systemic effects are not likely to occur. TI is not intended to be protective for all adverse effects, but it is a useful measure as part of color additive assessments.15 Special attention should be paid to the target patient population. For instance, devices that are intended to be used for pediatric care or surgical procedures have different TI and TE values, which may be considerably lower than for adults. Other patient populations may have their own limits of exposure to specific additives.

To perform these tests, the lab uses the amount of compound coming off a device above the TTC, and applies safety factors to that amount. Those data are then compared to the level of the compound that has previously been considered safe. If the margin of safety is greater than one, there is no risk with that color additive; if it is less than one, there may be some potential risk of leaching associated with the additive. To summarize, the key steps involved in preparing a toxicology risk assessment include the following.
1. Determine the extractable/leachable results in mg per device.
2. Research the data available for the levels at which there are no observed adverse effects or a low number of observed adverse effects.
3. Apply the safety margins recommended in ISO 10993-17.
4. Calculate the margin of safety.

This process determines whether a device is safe in a case where all of its color leaches out of the device. If it is not, then chemistry is used to evaluate how much of the additive may be leaching under worst-case scenarios.

If the device is believed to be safe according to these assessments, the information can be submitted to FDA for an evaluation of the risk involved in using the proposed color additive. If the agency determines that the additive is not safe, the manufacturer may need to change the proposed additive, the amount being used, or the substrates used for the device.

**Changing colors**

Changing a colorant in an approved medical device may not seem like a significant alteration from a manufacturing viewpoint, especially if the base material remains the same. From a biocompatibility and safety viewpoint, however, color additives can have as many as 10 different compounds in their chemical makeup, any of which could have potentially toxic effects on a patient or end-user. Distinguishing whether a change in a color additive could be significant is the main purpose for an evaluation of risk from a biocompatibility perspective. There are existing resources for medical device manufacturers who choose to change a colorant on their device, but they may not be applicable to all device types. Knowledgeable biocompatibility experts can advise manufacturers about proposed changes to their medical devices, helping companies to get through the maze of required information on the way to FDA market approval.

**Conclusion**

Assessment of a product’s material and chemical makeup is an important first step in a risk evaluation for a medical device. Any material or additive that could contact the patient or end-user should be evaluated to ensure that it offers direct benefits that outweigh any risks associated with use of the device.

Colors or pigments added to the patient-contacting portions of a medical device should undergo the same process. Manufacturers should perform an evaluation of possible exposure to colorants, and an assessment of associated biological risks, based on the type and duration of contact. The evaluation should demonstrate that the biological risks related to use of the planned colorants are sufficiently low to permit the colorants to be present in or added to the medical device.
Medical Device Extractables and Leachables Testing

By Matthew R Jorgensen, PhD

You can download a more detailed discussion of this topic [here](#).
Over the past five years, the medical device community has swung from nearly full ignorance of the potential power of chemistry testing, to full adoration and acceptance, and now back – in a sense – to a state of scrutiny and skepticism. Regulators, in response to an influx of medical device submissions centered on supporting chemistry data, have increased their knowledge and finesse with the science and have been asking tough questions. Widely circulated studies have been critical of chemistry testing for toxicological evaluation of medical devices. Therefore, in response to this feedback, as medical device chemistry for toxicology (ChemTox) has matured, the overall strategy has changed dramatically on some points.

In this environment, with a new ISO 10993-18 requirement 10.4 of the MDR; the new ISO 21726; and story after story of rejections by the FDA on the basis of insufficient chemistry testing, it is common for us to hear “so, what are you guys doing for medical device chemistry testing?” This article provides a high-level answer to that question, which is, in short “everything we can.”

The primary goal of ChemTox is to provide data useful for an unambiguous toxicological risk assessment. To meet that requirement the study must be sufficiently broad in scope, sensitive enough to avoid missing potentially toxic compounds, and provide positive identifications. In addition to the foundational scientific requirements, the study must also meet regulatory expectations of completeness on points that labs might have a professional disagreement regarding scientific validity.

**Sufficient breadth in ChemTox**

Sufficient breadth in study design is essential to ensure that important classes of compounds that might migrate from a medical device aren’t missed in an extractables study. It is known that like dissolves like, and an extractable compound that isn’t soluble in the extraction solvent will not be discovered. Many medical devices contact heterogeneous fluids like blood or liquid in and around human tissues. Therefore, extraction solvents covering a range of polarities are required. The static dielectric constant is a good measure of solvent polarity, and is often used in pharmaceutical compounding to formulate liquids suitable to dissolve drugs.

In addition to ensuring breadth by guaranteeing a broad range of compounds are soluble in the extraction matrix, breadth
of analytical methods and instrumentation is also required. It is known that potential extractables span a wide range of molecular weights and volatility. At a minimum, a study should include a method for volatile organic compounds (VOCs, those compounds with a vapor pressure greater than water); semivolatiles (SVOCs, those compounds which are less volatile than water but still able to vaporize without decomposition or combustion); nonvolatiles (NVOCs, those compounds which would decompose before vaporization); and inorganic/elemental compounds. Within each of these categories, analytical methods and instrumentation parameters should be selected to provide maximum breadth. For example, both electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) in positive and negative mode have been requested by the FDA to ensure sufficient breadth. A typical extraction and analysis matrix providing sufficient breadth (and meeting current regulatory expectations) is shown below in Table 1.

As chemistry testing for medical devices grew in popularity, it made sense to adapt the ideas which had already matured in pharmaceuticals. One of the key ideas in pharmaceutical E&L is that there is always a two-step process: compound discovery and identification (extractables), followed by detailed quantification of potentially concerning players in the drug itself (leachables). The first step extracts the pharmaceutical material very aggressively to yield the greatest amount of extractables possible for identification; the second step targets compounds detected during the extractables study at concentrations above their toxicological threshold. By using real use conditions and targeted methods reported concentrations indicate the concentrations a patient truly is exposed to, allowing a toxicological assessment on realistic values.

For patient-contacting medical devices, a true leachables study is impossible because the device leaches into the body – not a drug. Therefore, we seek to conduct a single-phase study that both aggressively extract for

<table>
<thead>
<tr>
<th>Analytical Method</th>
<th>Polar (water)</th>
<th>Mid-Polar (IPA)</th>
<th>Non-Polar (hexane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elements</td>
<td>X</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ICP/MS and ICP/OES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOCs</td>
<td>X</td>
<td>X</td>
<td>N/A</td>
</tr>
<tr>
<td>Headspace GC/MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVOCs</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Direct Injection GC/MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>NVOCs</td>
<td>N/A</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>HRAM-UPLC/MS, APCI ±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVOCs</td>
<td>X</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>HRAM-UPLC/MS, APCI/ESI ±</td>
<td></td>
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</tr>
</tbody>
</table>

The question of extraction duration – is it exhaustive?

The term “extractables and leachables” (E&L) for medical devices is an adaptation or extension of the same term that has been applied to pharmaceutical container/closure systems.

Table 1: Typical Analytical Test Matrix
identification of hazards, and has an acceptable level of quantification. There has been worry that the conditions used for extraction are not aggressive enough to be adequately conservative in overestimating the amounts of compounds that a patient would be exposed to. Therefore, using a definition commonly applied in ISO 10993 for other tests, regulators have required that extractables studies are conducted exhaustively by serially extracting a device until the amount of material extracted is less than 10% of the initial extraction. The sum total of all is requested to be used as the daily exposure.

The execution of such a study has many issues. In sort, the historical use of non-volatile residual (NVR) measurements lack the sensitivity to be protective (there could be compounds which are concerning, and not exhaustively extracted, but below the sensitivity of the measurement), and they do not correlate well with mass spectral methods.

The current expectation is that for all devices, except those with clear limited contact, exhaustive extraction is performed. If NVR is pursued to provide evidence of exhaustiveness, then the submitter must be prepared for questions regarding why the amounts detected by mass spectroscopy do not align. A better, more complete, picture is given by serially extracting at 24-hour intervals and analyzing each extract with the full suite of analytical methods. This approach has been well accepted by the FDA, but greatly increases the cost and time of the study for little new information. We are working with the FDA on alternative methods to demonstrate that extractable studies are sufficiently protective.

**Sufficient sensitivity in ChemTox**

Sufficient sensitivity in ChemTox is required to ensure that chemicals that are not reported or identified are at such low concentrations.
that they would not be concerning from a toxicological perspective. Therefore, during the design phase of a study, toxicological information is needed to determine what that threshold is. ISO 21726 “Biological evaluation of medical devices - Application of the threshold of toxicological concern (TTC) for assessing biocompatibility of medical device constituents” provides baseline thresholds that can be used to understand the sensitivity required for identification and reporting (also called the analytical evaluation threshold or AET). The thought process of setting an appropriate AET is as follows:

1. Is it known if the device does not potentially cause genotoxicity? If yes (as evidenced by passing genotoxicity tests), then a Cramer Class based TTC is acceptable for use.
2. If genotoxicity potential is unknown, then a TTC protective against carcinogenicity should be used following ICH M7 considering the cumulative duration of use of the device.

The AET should be sufficiently low so that all compounds above the appropriate TTC are identified and reported, being sure to account for uncertainties related to estimating concentrations in a screening study. Special attention should go to the possible presence of compounds belonging to the cohort of concern as they should be looked at in concentrations below the AET.

After identifying the appropriate TTC for toxicological assessment, application of uncertainty factors in setting an AET can be tricky. Historically, labs have relied on two key papers which measured variance in response factors of compounds during screening\textsuperscript{4,5} to set their uncertainty between a factor of 2 and 4. More recently, regulators have been requesting lab specific data to support an appropriate uncertainty factor in screening and that the AET be clearly justified.

Providing an identification of sufficient quality

After ensuring the necessary breath in the analytical approach and applying a scientifically sound analytical evaluation threshold, the next step in ChemTox is to identify the detected compounds with a sufficient level of confidence. These identifications, together with the detected concentration of the compounds, define the starting point of the subsequent toxicological assessment.

While identification of inorganic compounds such as elements is straightforward because each element has a clear and unique atomic mass, identification of detected organic compounds is much more challenging. After chromatographic separation, these organic compounds are identified using mass spectrometry (MS). Basically, a MS fragments the detected compounds in a unique combination of mass fragments with specific abundances, resulting in a mass spectrum. One could say the mass spectrum is a compounds’ fingerprint. This fingerprint then can be used to trace back the identity of a compound.

Just like one needs sufficient proof to identify
the perpetrator of a crime, sufficient evidence is needed to conclude on a compound’s identity. Historically it was common practice to solely rely on mass spectral matching. However, mass spectral matching is only the first step to a confident identification and unreliable when used on its own.

Two problems are associated with mass spectral matching. The first one is the availability of libraries containing compounds which have good overlap with what can be expected to be present in and on medical devices. The second problem when solely relying on mass spectral matching is that even a high match factor can result in a wrong identification. (Fuller details on these problems are covered in the longer report.)

**Conclusion**

Medical device ChemTox has been a moving target while it has matured. While shifting expectations can be frustrating, studies conducted today provide much more thorough and protective data than just two to three years ago. It can be expected that things will continue to shift until regulatory bodies reach a consensus on their expectations of these studies and provide guidance on the same. Nelson Labs remains committed to frequent and transparent communication and guidance to both the FDA and the sponsors we help through this process.

3D Medical Devices and Biocompatibility: Testing Considerations

By Matthew R Jorgensen, PhD

You can download a more detailed discussion of this topic here.
The use of three-dimensional (3D) printing techniques to address challenging fabrication problems has become mainstream over the past decade. While this rich resource has extended fabrication of personalized medical devices to the limit of our imagination, the myriad materials and morphologies available present a unique concern from a toxicological perspective.

A range of standalone 3D printers are commercially available with compatible materials ranging from plastics to oxides and metals. Raw materials used in the fabrication process often have highly customized properties, achieved through the use of proprietary additives and specific microscale morphologies which can affect the overall biocompatibility of the finished device. Therefore, 3D printed medical devices require versatile approaches to the assessment of their biocompatibility that consider several factors: which will be addressed in this document:

1. Possible additives to raw materials which enhance workability
2. Details of the material curing process
3. Post-printing finishing and rinsing processes
4. Time allowed for aeration between device manufacture and use

Possible additives to raw materials which enhance workability

3D printed plastic materials can be grouped by the printing technology used; generally stereolithography, fused filament fabrication, and liquid-based extrusion. In each of these cases, one or more materials are printed in tandem with a sacrificial filler material that provides structural support during the printing process. Photolithographic methods like stereolithography and UV-cured liquid based extrusion use a mixture of polymer precursors called photoresist which polymerize into a durable solid on exposure to light. If the photoresist requires light with intensity above a certain threshold, extremely fine resolution on the order of hundreds of nanometers is possible by scanning tightly focused laser light through the photoresist. Direct writing involves the partial melting of raw materials through a heated nozzle into fine layers. The
structure and support material are deposited layer by layer, gradually building from the ground up. A compromise between photolithography and direct writing is also possible. In a process similar to inkjet printing, which produces thousands of colors by mixing three or four primary colors, different combinations of photoresists can be mixed and printed followed by exposure and polymerization with UV light.

Each technology for 3D printing of plastic materials involves materials with highly customized properties, enabled by their unique chemistries. Photolithography involves polymer precursors, photosensitizers, other additives, and solvents. Following exposure, precursors and reaction byproducts remain embedded in the structure raising concerns regarding their potential to leach out during clinical use. Thermoplastics used in direct writing processes include plasticizers and other additives essential for their workability but which may cause concern as some of these additives are not biocompatible. Following melting and drawing through the writing nozzle, the additives are redistributed through the material and the surface area is increased exponentially. These processes increase the availability of potential toxicants to their surrounding matrix in the body and potentially introduce a clinical exposure risk if not understood.

Evaluation of the biocompatibility of 3D printed devices should consider chemicals which are novel additives to otherwise well-known materials, as well as byproducts of the polymerization process. The availability of these chemicals for extraction into the matrix surrounding the device must be evaluated along with an assessment of their potential toxicological impact on a case-by-case basis. 3D printed medical devices present unique challenges from a biocompatibility assessment perspective due to their highly customized material properties.

Details of the material curing process

Additives to raw materials used in 3D printing are required to enhance workability. For 3D printed materials which are exposed to UV light and are cured, the details of the exposure and curing process can influence the chemical composition and other properties of the final material. An overview of the chemistry involved in the exposure and curing of photopolymers (also known as photoresists) is required to understand how these factors have the potential to impact the biocompatibility of the final structure.

Photopolymers are composed of several at least four critical components: monomers, oligomers, photo-initiator, and solvent (Figure 1).
UV light is the activator, setting off a cascade of reactions that result in the polymerization of monomers and oligomers into a durable solid. Monomers and oligomers contain chemical groups that allow them to bond to themselves and each other into long chains.

Photopolymerization is a complex process. Even under the most ideal printing conditions, each of the reaction steps in the polymerization process proceed at different rates and never to 100% completion. Therefore, the result of the photopolymerization reactions are, at best, a well-formed polymer with traces of precursors, intermediates, and byproducts as contaminants. As conditions drift from ideal, the proportion of contaminants increases.

Under clinical conditions it is possible for a wide range of compounds to leach from a 3D printed photopolymer device. In approaching the biocompatibility assessment of 3D printed photopolymer devices, these extractable/leachable compounds should be expected. Understanding the toxicological impact of these compounds requires expert review and evaluation on a case-by-case basis. Consideration should be given not only to the raw materials going into the printed photopolymer, but the details of the curing process which may be subject to process and environmental variability.

Post-printing finishing and rinsing processes

Additive manufacturing typically involves sacrificial support material, post-printing rinsing, and finishing processes which are secondary to the device material itself. While not necessarily
considered part of the final device from an engineering perspective, the impact of support material and finishing processes on the device must be considered.

3D printing creates devices by depositing the structure additively along with a selectively removable support material. Without sacrificial support materials, 3D printed designs would be severely limited. Because the support material is not intended to be part of the finished device, it may be overlooked as a possible source of biocompatibility issues. The sacrificial support material must be removed, however, each support material and removal method raises potential concerns from a biocompatibility perspective. (See Table):

Following removal of support materials, 3D printed devices may undergo subsequent finishing processes. Extruded thermoplastics may be smoothed through exposure to heated solvent vapors such as acetone or methylene chloride. The combination of heat and natural affinity of the solvent for the material creates ideal conditions for adsorption into the material surface. Metal parts may undergo passivation processes that introduce surface contaminants.

### Table: Support Material Type and Potential Concerns

<table>
<thead>
<tr>
<th>Support Material Type</th>
<th>Potential Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Soluble Thermoplastic</td>
<td>• Residual support material remaining in device crevices and on device surface</td>
</tr>
<tr>
<td></td>
<td>• pH of support material removal bath, generally &gt;12</td>
</tr>
<tr>
<td></td>
<td>• Contaminants from re-use of removal bath</td>
</tr>
<tr>
<td>Break-Away Material</td>
<td>• Incomplete removal of break-away parts</td>
</tr>
<tr>
<td>Particulate contamination from breakage</td>
<td>• Photopolymerized Gel</td>
</tr>
<tr>
<td></td>
<td>• Residual support material remaining in device crevices and on device surface</td>
</tr>
<tr>
<td></td>
<td>• Contamination of device material with support material solvents and additives</td>
</tr>
<tr>
<td></td>
<td>• Copolymerization of support material onto device surface</td>
</tr>
<tr>
<td>Metal/Metal Oxide Powder</td>
<td>• Particulates</td>
</tr>
</tbody>
</table>

**Time allowed for aeration between device manufacture and use**

Following printing, consideration must be given to the amount of time required for aeration of the device before use to allow additives near the surface to diffuse and volatize from the surface. This can be compared directly with aeration of ethylene oxide (EO) residuals, which is commonly used for in-package sterilization. Desorption of EO and its residual byproducts is a common concern for medical devices sterilized with this sterilization method. The amount of time required for aeration depends on the device materials, geometry, and concentrations of additives of concern. The need for adequate aeration is well illustrated by examining the law of diffusion governing the desorption of volatiles from a solid, and by comparing the diffusion coefficients of plastic additives to that of EO gas.

Desorption of EO or a plastic additive is limited by the ability of the chemical to diffuse to the surface of the device (Figure 3) and so is well...
described Fick’s laws of diffusion.

When a raw thermoplastic material goes through the 3D printing process any additives are redistributed evenly through the printed material. Likewise, unreacted photopolymer precursors and other components are distributed throughout 3D printed photopolymers. Even distribution results in a large concentration gradient of the additives at the material surface, where the concentration goes from the full formula concentration just inside the surface to zero outside. The large initial concentration gradient leads to an initial strong desorption, which tapers off slowly over time as the material develops an additive depleted surface.

In the longer discussion of this topic, which is linked to the top of this article, we consider EO for the plasticizer diethylhexyl phthalate (DEHP) in polypropylene (PP) in a typical printing application. This involves a fiber raw material that is well aerated prior to use, resulting in a thin additive depleted layer on the surface and a very low rate of desorption. Immediately upon being melted and drawn through the 3D printing nozzle, DEHP along with other volatiles start to desorb off of the material into the surroundings. The process slows exponentially as a depleted layer forms over the surface until – eventually – the material again resembles the raw material. In layman’s terms, data plots from this example explain “why that new car smell we all love, which is a cocktail of plasticizers and other additives, lasts long after the car leaves the factory.”

A typical EO sterilization process allows for 24-48 hours of heated aeration time, though many companies choose to follow this with additional days of ambient aeration. Based on a comparison of diffusion coefficients, the amount of time required for aeration of 3D printed medical devices could be significantly longer depending on the concentrations and types of additives. The diffusion coefficient and rate of desorption increase dramatically with temperature, so any post-printing sterilization steps at elevated temperatures may facilitate desorption. Decisions regarding the aeration time needed for 3D printed polymer devices should be based on observation, taking into account all post-printing steps and understanding that desorption will continue slowly over time.

As 3D printed medical devices continue to rise in popularity, so will the scrutiny on their biocompatibility. Assessment of these devices should consider not only the raw materials used, but the curing parameters, post-printing finishing processes, and aeration time.
How to Address Failed CYTOTOXICITY TESTING of Medical Devices

By Helin Räägel, PhD, Audrey Turley, Thor Rollins, Sarah Campbell, PhD and Matthew R. Jorgensen, PhD

You can download a more detailed discussion of this topic here. Additionally, Nelson Labs consulting experts contributed to a more exhaustive report in the peer-reviewed journal, Biomedical Instrumentation & Technology.¹
To ensure devices “first, do no harm,” medical device manufacturers are required by regulatory bodies to perform biocompatibility evaluations on their devices per standards, such as the ISO 10993-1:2018 (ISO 10993-1:2018). Regardless of the strategy towards biological evaluation, one test routinely used in initial screening is the cytotoxicity test, based on an in vitro cell culture system. While proven to be useful, cytotoxicity is a very sensitive test, prone to fail even when biological risk is not present. In this article, we will explain how to approach these failures to understand their impact on the safety of the medical device is important.

Cytotoxicity testing per AAMI/ISO 10993-5:2009/(R)2014 has historically been one of the most performed (and is considered the most reactive) of the biocompatibility tests as an efficient method to detect potential deleterious chemicals present in device extracts; in the medical industry, this test is used widely to screen for materials and/or processing residuals. However, these cell-based tests do not necessarily indicate in vivo toxicological effects or always relate to actual clinical patient safety. They often show a cytotoxic reaction when no clinical adverse effects are known or expected to occur to humans; for example, some soluble metal ions (such as silver and zinc) are intentionally added to devices for their antimicrobial properties and are known to exert toxic effects on cells in an in vitro setting, while their presence in bandages has demonstrated high patient tolerance and negligible effects upon clinical use.

Two common sources of cytotoxicity in medical devices are metals and plasticizers.

While some metal ions are shown to exert cytotoxic reactivity in the cell culture tests, it should be acknowledged that a variety of metals, such as copper, chromium, and zinc, are also present endogenously inside the human body. They are also present in our diet and a number of them are even consumed as dietary supplements. It should be noted though that, as always, the dose makes the poison, and while often beneficial at low doses, many elements at higher amounts may become toxic, causing adverse local and systemic effects in the body. Ionic chromium, cobalt, nickel, aluminum, and titanium have been shown to have potentially genotoxic actions and some are classified as carcinogenic depending on the dose and route of exposure. Therefore,
as a key point, the dose to which the end user will be exposed, rather than its presence, defines whether an element or compound is toxic on a whole-organism level (systemic toxicity).

The potential release of metal ions from a medical device is related to first the alloy but second also its processing. For instance, passivation and anodization surface treatments can affect potential metal ion release from medical device materials and thus alter the potential toxicological effects. This highlights the importance of using a final finished device for biological and chemical evaluation to ensure the results correlate to what the patient would be exposed to in a real life scenario.\textsuperscript{12,13}

The medical industry is flooded with plastic medical devices (such as soft catheters, blood bags, and syringes),\textsuperscript{14,15} which in many cases contain plasticizers (phthalates, bisphenol A) to modify physical properties like flexibility and elasticity of the plastic base resins.\textsuperscript{16} Phthalate esters, including bis(2-ethylhexyl) phthalate (DEHP) are common to medical device plastics. However, DEHP and its metabolite mono(2-ethylhexyl) phthalate (MEHP) have been linked to potential endocrine-disrupting properties,\textsuperscript{17} therefore, alternatives to DEHP have been developed, such as di(2-ethylhexyl) terephthalate (DEHT), trioctyl trimellitate (TOTM), and diisononyl phthalate (DINP). Material manufacturers have also developed some non-phthalate plasticizing compounds, for example, 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH), bis(2-ethylhexyl) adipate, and acetyl tributyl citrate.\textsuperscript{18} It has been demonstrated that phthalates in general have potential toxic effects therefore, patient exposure to phthalates through medical devices is rigorously assessed.\textsuperscript{19–21}

Plasticizers are not covalently bonded to the polymer backbone, but are instead embedded into plastic resin through mixing, and as a result, diffusion of these additives can occur more readily and result in their release and exposure to the patient. The rate at which each plasticizer migrates from materials is dependent on the molecular characteristics of both the plasticizer and the polymer within which it is embedded, and can vary greatly.\textsuperscript{26} Additional factors, such as the characteristics of the fluid that contacts the material can have an impact.\textsuperscript{27} Common routes of exposure include contact with patient tissues\textsuperscript{22} such as when a catheter is inserted into the body, through inhalation\textsuperscript{23}, or exposure to fluids (like blood) that have contacted DEHP-containing materials in for example blood storage bags. This may lead to negative health effects during the use of a medical device that incorporates these chemicals, especially among low body weight and developing neonatal patient populations.

Methods for analyzing cytotoxicity

The assessment of the cytotoxic potential of a medical device is considered a primary performance criterion under international standards, as it is the endpoint of concern listed for all patient-contacting devices regardless of
the clinical use of the device. The cytotoxicity assay, as indicated above, looks for any potential deleterious effects on the cells using an in vitro assay. If something potentially hazardous is being extracted from a material or a device, the cellular mechanisms are affected, resulting in decrease of cellular viability, changes in cellular shape or intracellular structures or even cell death. A severely cytotoxic device should be cautiously evaluated, or avoided, in an animal model to protect animal welfare against potential for major reactions. Alternatively, other in vitro methods, chemistry, and a written toxicological risk assessment could be considered.

Many medical device manufacturers, as well as regulatory agencies, have historically relied on the results of a minimum essential medium (MEM) elution cytotoxicity assay for evaluating cytotoxicity.

MEM Cytotoxicity Assay

The MEM elution assay involves exposing the mouse fibroblast cell line (L929) to device extracts in a controlled environment and examining the cells microscopically for overall cell health including the number of cells in viewing area and viability, with a focus on cell shape and cytoplasmic structures. Based on the criteria set forth in 10993-5, each sample is given a score (from 0 to 4) depending on the severity of the deformations brought about by the device or its extract (Figure 1).

The MEM assay is a relatively easy test to perform, but the accuracy of the results is not without bias, relying heavily on the level of experience of the assessor. Still, it remains a staple of biocompatibility testing for most U.S.-based medical device companies.

MTT Colorimetric Assay

Another common cytotoxicity method is the MTT assay, which measures cellular metabolic activity and provides a colorimetric quantitative result (Figure 2). It removes human bias from evaluations for a non-subjective quantitative measurement of absorbance from the colored solution produced by viable cells. When multiple dilutions are tested of the extract, the quantitative data provides a valuable basis for further analysis of the dose-response curve and can be used to define toxicity thresholds and dose curve midpoints for test samples, such as the half-maximal inhibitory concentration (IC50; often referred to as toxic concentration [TC50]), which is a concentration of a substance that yields a 50% decrease in cell metabolic activity. IC50 values often provide valuable toxicological information.
when evaluating the cytotoxic effects of a chemical solution in vitro.  

A large pool of MTT data is available through the use of this test method in academic research to assess the effects of substances on cells, however, caution should be used in interpreting some of the available data due to potential inconsistencies, such as the use of different cell line with different biological characteristics.

Additional methods, such as the XTT assay, NRU, or agar diffusion (often referred to as agar overlay), are discussed in 10993-5.2 In general, the XTT test is similar to the MTT test, with the main difference being the dye added to the cells to measure viability. Also, NRU uses a similar setup to the MTT test, where cell viability is quantitatively measured by the amount of a weak cationic dye (neutral red) that is taken up and bound inside lysosomes of living cells.

Evaluating Metals and Plasticizers in Cytotoxicity Failures

IC50 values for metals and plasticizers commonly used in manufacturing of medical devices can be found in a table in the longer version of this article (as referenced at the top of this article; references to these are found in footnotes 43 to 50.) Using data obtained from human or mouse fibroblast lineage cell lines using L929 cells as accepted by the FDA – plus additional data — research demonstrated the variability in the IC50 values that can occur, which is why 10993-5 has set forth more standardized methods and parameters to limit the impact on how the test is conducted in an effort to have more uniform (and comparable) outcomes.

Complications predicting a clinical response from in vitro data

As indicated above, the cytotoxicity test is a useful test to screen or assess the cytotoxic potentials of chemicals that may migrate from medical devices (either from materials or as residuals from processing) and is widely used as a tool to predict the potential clinical response. The main concern for medical device companies is how to translate the cytotoxicity data to a clinical response. As noted above, some chemical compounds and elements will demonstrate cytotoxicity in an in vitro setting that does not necessarily translate to the clinical use and in
vivo conditions (per Section 10 of 10993-52). Thus, the key question is: How should one proceed with identifying the actual clinical risks for the medical device when cytotoxic reactivity is observed?

One thing to note is that the in vitro cell culture system used for cytotoxicity assays is not a complex system, and it does not encompass most of the repair mechanisms that are available in vivo (when a device is placed in contact with tissue, for example). Thus, when a failed cytotoxicity result is encountered, it is critical to look to other data, especially in vivo data (if available), to gain more understanding of the clinical relevance for assessment of possible toxicological effects for the end user. Another key point is also to understand the contact type and duration for the given medical device, since the biological risks associated with a device depend heavily on how and for how long it is exposed to the patient during its intended use.

Altogether, to better understand the clinical risks associated with a potentially toxic substance, further assessment of how it might affect patient safety in the clinical setting is needed.

Below, an approach using a toxicological risk assessment is provided. It should be noted again that the rigor of evaluation and approach depends on contact type and duration; therefore, toxicological risk assessment may not be the most appropriate approach for all medical devices (e.g., for skin-contacting devices). However, for devices that are invasive and in longer-term contact, this approach can be successfully applied.

**Toxicological risk assessment**

To understand the framework of a toxicological risk assessment, an overall guideline on what to consider and how to approach the evaluation is given as background. ANSI/AAMI/ISO 10993-17:2002(R)2012 provides a standardized framework for the use of clinically relevant toxicity data, including guidance for the derivation of the toxicological threshold values, such as tolerable intake (TI; in mg/kg/day) and tolerable exposure (TE; in mg/day) levels. TI and TE levels represent the maximum dose at which an exposure to a substance does not produce adverse events or pose an unacceptable risk to human health. These threshold values are derived from experimental data, such as no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL), and also take into account several uncertainty factors (UFs), such as pharmacokinetic/toxicokinetic or metabolic differences among exposed people (UF1), extrapolation of effects between animals and people (UF2), and the quality and relevance of the experimental data (UF3). For medical devices, the TE also incorporates a utilization factor (UTF) that accounts for the variables affecting clinical exposure, such as frequency of device use or adjustments based on contact time (proportional exposure factor [PEF]) and potential exposure to similar chemicals or compounds from other
sources (concomitant exposure factor [CEF]), with a default recommendation value of 0.2 for CEF and 1.0 for PEF. The default UTF value of 0.2 accounts for possible concomitant exposure of five medical devices in a 24-hour period.

Data to support a proper toxicological risk assessment is gathered through a well-structured and planned extractables/leachables study. ISO 10993-18 provides guidance to ensure that the data is sensitive enough to be applied in a toxicological risk assessment; and results are then evaluated by a qualified toxicologist who is familiar with the device and an expert in performing toxicological risk assessments as outlined in ISO 10993-17.

Available resources for toxicologists to derive the applicable TI and TE levels include reference concentration values (inhalation exposures) and reference dose values from the Environmental Protection Agency (EPA); minimal risk limits (MRL) from the Agency for Toxic Substances and Disease Registry (ATSDR); and additional references are provided by sources including the European Chemicals Agency (ECHA) and World Health Organization (WHO). Reference dose values typically are calculated from NOAEL values divided by UFs and/or modifying factors. Reference doses can be derived from laboratory animal dosing studies in which a NOAEL, LOAEL, or benchmark dose can be obtained to provide of a daily oral exposure for a chronic duration (up to a lifetime) to the human population that is likely to be without an appreciable risk of deleterious effects during a human’s lifetime. ATSDR also uses the NOAEL/UF approach to derive MRL levels for substances. (Again, see the longer report for details).

The reference daily intake (RDI; also known as recommended daily intake) is the updated term for recommended daily allowance and are nutrient reference values developed by the Institute of Medicine (IOM). The established RDI is useful in a toxicological assessment, and may be used as the TI for dietary metals. In general, exposure values below a published allowable limit for an essential metal would be considered safe.

Another option to assess metals are the FDA guidance on permitted daily exposures (PDEs) for elemental impurities in finished drug products [Q3D(R1) Elemental Impurities: Guidance for Industry] which is useful for metals that do not have an RDI. This FDA guidance is broadly...
accepted globally. Additional resources for limits for metals not published in either of these sources can be drawn from the current EPA Table of Regulated Drinking Water Contaminates\textsuperscript{57} or the WHO’s Guidelines for Drinking-water Quality,\textsuperscript{58} as these are applicable due to their worst-case daily oral intake values.

Additional sources of information, including the use of in vitro or in silico (computational toxicology) alternative methods, such as analytical chemistry (i.e., the identification of extractables and leachable chemicals released from medical devices) and QSAR (quantitative structure-activity relationship) are seeing more and more application.\textsuperscript{60}

Comparing IC50 and systemically toxic concentrations

Currently, sparse data exist for relating cytotoxicity from medical devices to clinically obtained blood or tissue levels for metals/compounds.\textsuperscript{61,62} Furthermore, as indicated above, there may be discrepancies in the reported IC50 values arising from the specific cell line used, the quality of chemicals used, or experimental conditions. Therefore, as a conservative overestimation of the actual cytotoxic dosage, the lowest IC50 value reported should be considered for evaluation. Also, because many metals released during the extraction of a medical device for cytotoxicity testing play a vital role in a number of biochemical reactions in the body, their intake is necessary and therefore daily RDI (rather than restricted) levels apply.

Chromium is an interesting example, as is commonly found in many medical device alloys and has well-understood toxicity. For example, an oral intake of 11 mg/day Cr(III) is the permissible daily exposure per Q3D56; at the same time, 743 µmol/L Cr(NO3)3 was found to reduce the viability of 50% of the cell culture cells (IC50).\textsuperscript{44} To put this into a medical device perspective, mechanically polished Co-Cr alloys (e.g., orthopedic permanent implants) have been shown to release 300 to 600 ng/cm² of Co and less than 15 ng/cm² of Cr during the first week of exposure when placed in physiologically relevant media.\textsuperscript{63,64} An extensive review on the toxicological profile for Cr from the Agency for Toxic Substances and Disease Registry (ATSDR) discusses various exposure levels in relation to exposure routes and the potential adverse effects on human or animal health.\textsuperscript{65} It should also be highlighted that the valency of the Cr (e.g., Cr(III) versus Cr(VI) plays an important role and should be taken into consideration. An Occupational TE limit of 0.1 µg/m³ for Cr(VI) has been shown to be acceptable in terms of absolute excess risk (<4 per 10,000 according to the German Committee on Hazardous Substances).\textsuperscript{66} In a cell culture setup, however, Cr(VI) salts have been demonstrated to cause disturbances in cellular energy metabolism and cell cycle arrest already at 20 to 80 µmol/L, which makes it up to 500 times more cytotoxic than Cr(III) salts.\textsuperscript{67}

Zn is another metal that has been associated→...
with increased cytotoxic effects in an MTT assay at concentrations as low as 25.5 µmol/L, but ICH Q3D indicates that it has low inherent toxicity and, based on the FDA-recommended RDI up to 11 mg/day (11,000 µg/day for adults and children aged ≥4 years) or 3 mg/day (3,000 µg/day for infants) can be ingested without any clinical systemic effects. The above discussion highlights that the translation between IC50 values and clinical toxic concentrations can be difficult and requires thorough data analysis to define whether a medical device might have actual toxic adverse effects to the recipient.

Another often encountered scenario, where there might be a discrepancy between a cytotoxicity failure and its clinical relevance is where we have small metal devices; those are great example where the focus should be on the actual clinical dose rather than the concentration of a metal ion, as indicated by an IC50 value and manifested in a cytotoxicity test. For example, a minimally invasive fastener typically has a surface area of 19 mm², and ANSI/AAMI/ISO 10993-12:201269 (and recently updated ISO 10993-12:2021) stipulates that a device of this size (less than 0.5 mm thick) should be extracted at a ratio of 6 cm²/mL. Practical experimental conditions require a certain extraction volume (e.g., 30 mL MEM fluid) to allow sufficient coverage of L929 mouse fibroblast cells in the required number of replicates for the test. Therefore, 948 fasteners would need to be pooled in a 30-mL extraction to satisfy all testing requirements. If the fastener is made from nitinol and fails cytotoxicity, it would likely be due to leaching of Ni to produce a concentration on the order of the IC50 of 0.106 mmol/L (0.2 µg/fastener) —a situation that can be confirmed by subsequent chemical extraction and elemental analysis. Typical procedures involving stapling might have up to 10 fasteners; therefore, a clinical exposure from 10 fasteners would be 2 µg, which conservatively can be interpreted to mean 2 µg/day over time (recognizing that the true clinical exposure typically is less over time). The Q3D guidance provides an acceptable limit for chronic daily exposure to Ni through a parenteral route of 22 µg/day.56 Therefore, if the actual daily dose of Ni exposed to the patient from the staples is, at worst case, 2 µg/day, this is still more than ten times lower than the conservative limit specified by the Q3D.

Alternative options in case of MEM cytotoxicity testing failure

When determining the impact of a cytotoxic result to the patient, further information on device use, its components (e.g., composition), or other data may be beneficial. For instance, if there is an indication of a presence of a specific culprit that may induce the cytotoxic reactivity, then a thorough literature review highlighting the above references and acceptable levels for the specific compounds may be sufficient to mitigate the risks associated with the medical device.

For instance, in the case of a wound-healing product that incorporates silver as an anti-
bacterial component, the device most likely will fail an MEM cytotoxicity test; however, evaluating the amount of silver present in a given device and knowing its release and absorption kinetics will help in evaluating the actual potential toxicity to the patient during the intended use of the device.

Additionally, type of contact to the patient, may play a role in the assessment. Many medical tubings are manufactured from PVC which contains phthalates or other additives that may induce a cytotoxic response. However, it should be noted that the extractions used for testing may not accurately reflect the clinical use scenario. In the case where this tubing is only used for irrigation purposes, where contact to only polar solvent is applicable (as opposed to mixed polarity extraction solvent used for cytotoxicity testing), the cytotoxicity study may be adjusted to account for only polar extraction conditions to demonstrate whether clinically relevant conditions have an effect on the outcome of the test.

Additional in vivo testing data on the medical device or medical device extract with passing results also can help in strengthening the risk assessment and provide supplementary supportive information that no adverse effects are expected during clinical use. However, if multiple chemicals are playing a role and the material extractables profile is not fully understood, it is recommended to obtain specific data on the subject medical device through appropriate chemical characterization and its extractable and leachable profile, especially in cases where prolonged (>24-h) contact is expected in a clinical setting..70 The specific chemical (extractables/leachables) profile of the final finished medical device can thus be obtained and which can then be assessed for its potential to be harmful to the end user through a toxicological risk assessment performed using the framework described above.

Conclusion

Although the MEM elution assay or MTT assay are common and can provide valuable data about in vitro cytotoxic effects, potential adverse reactions in vivo may be overestimated and thus not always correspond to clinical outcomes. Analytical chemistry methods, such as chemical characterization, in combination with existing systemic toxicity data from scientific/government bodies (such as ATSDR, IRIS, ECHA) and published, peer-reviewed scientific articles, offer a framework for evaluating toxicological risk and clinical safety for the patient.

About Nelson Labs

Nelson Labs is a global leader in microbiological and analytical chemistry testing and advisory services for the medical device and pharmaceutical industries. Nelson Labs serves over 3,500 customers across 15 facilities in North America, Asia, and Europe. We have a comprehensive array of over 800 laboratory tests supporting our customers from initial product development and sterilization validation through regulatory approval and ongoing product testing for sterility, safety, and quality assurance. We are regarded as a best-in-class partner with a strong track record of collaborating with customers to solve complex issues. Learn more about Nelson Labs at www.nelsonlabs.com. Safeguarding Global Health® – with every test we complete.