# SurFACTS in Biomaterials

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## INSIDE THIS ISSUE



## From President Angela DiCiccio

As we embrace 2021 with determination for change, the SIBF board is **committed to listening** to the needs of our membership and being mindful to **evolve** in a direction that enables **growth of value**. This year will provide an incredible opportunity to reset, refresh, rebuild and reintroduce norms of interaction while incorporating new platforms and discoveries. Our **intention** is to *cultivate the principles* on which this Foundation was originally seeded and continue to **stimulate** an arena for non-traditional opportunities to discover, collaborate, mentor and explore across interdisciplinary stakeholders in the biomedical and surfaces fields.

Articles within this issue specifically touch upon our need as a society to reevaluate previous norms and challenge the generalized approach to solving certain problems like chemical analysis and sterilization. APS highlights the impacts of changes in regulations on our traditional approaches to Extractables and Leachables analysis, truly emphasizing that a generalized approach is sometimes inappropriate, and often insufficient to truly represent and understand realistic outcomes. Biocoat echoes the importance of situation specific methodologies and informed decision making, highlighting that while some sterilization methods may work great on some substrates, the outcomes on others could have wildly different impacts.

After reading this episode of SurFACTs, I challenge you to reflect on areas in your field where the “generalized” approach might be overused and a more mindful and informed method would drive value and more authentic insight. If you have a great example, I encourage you to submit for our next episode of SurFACTs or to tell us about it during this year’s Surfaces in Biomaterials Workshop and Symposium. Most importantly, think about leaders in this field and make sure to

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## From President Angela DiCiccio

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nominate them for this year's Surface in Excellence Award before the March 31 deadline!

Stay tuned for announcements about **new platforms** for collaboration, communication, and involvement. **Reach out** if you are motivated to jump in to accelerate our efforts, provide perspective, or have feedback in regards to your experiences! Most importantly, take a deep breath and enjoy learning and growing together.

# Sterilization Effects on Hydrophilic Coatings

*Bob Hergenrother, Ph.D. and Betsy Morgan, Biocoat, Inc.*

Almost all medical devices on the market go through the process of sterilization. The sterilization of medical devices can affect the materials and hydrophilic coatings of those devices due to the nature of methods that are used. The three known sterilization methods are Ethylene Oxide (ETO), Gamma Radiation and E-beam Radiation. With data, we are able to see how these three sterilization methods can affect hydrophilic coating performance measured by the testing of lubricity, durability and particulates.

ETO sterilization, the widest used method, has recently seen closures to their facilities due to ETO emissions, prompting the FDA to issue a statement of concern about the necessity of maintaining ETO processing capabilities. This in turn has prompted companies to consider qualifying other types of sterilization methods such as Gamma Radiation and E-beam Radiation, for their devices. However, gamma radiation poses its own availability concern due to the handling and security considerations of 60Co. As a hydrophilic coatings supplier, we need to understand how various sterilization methods affect the materials we use and allow for potential changes to the sterilization choices.

ETO, or Ethylene Oxide Sterilization, accounts for 50% of all medical device sterilizations in the US. To perform this method, parts are placed in a chamber filled with ETO gas with a certain amount of humidity. The parts are kept in the chamber for a certain amount of time at an elevated temperature. They must be packaged in something permeable to allow the gas and water vapor to permeate into the device and sterilize the surface. The parts are then vacuum-purged to reduce ETO levels after it is done. Microbial kill is accomplished by alkylation of amine groups of the DNA, as demonstrated by the use of Biological Indicators placed in the sterile load. One potential effect of ETO sterilization on medical devices is that the heat and humidity of the sterilization process can affect the materials. Another is that the ETO grafting onto the functional groups of the surface can change the properties of that surface.

Gamma sterilization accounts for about 40% of medical device sterilizations in the US. This process uses $^{60}Co$ as a radioactive isotype, which when undergoing nuclear decay produces gamma radiation to sterilize the device. The device is exposed to the radiation which affects the kill by creating free radicals that result in DNA scission. Instead of using biological indicators, microbial kill is demonstrated by the probability of Sterility Assurance of $10^{-6}$. The potential effect of using gamma sterilization is that it can have chain scission on the polymers that are used in the device. For example, polypropylene is susceptible to this degradation.

The e-beam method of sterilization only accounts for about 4% of medical device sterilizations in the US. In this process, electrons are accelerated in a 1 to 5 diameter beam and scanned over the device to be sterilized. Microbial kill is accomplished essentially the same way as gamma radiation, which is by forming DNA scissions from free radicals that are formed, demonstrated by the Sterility Assurance level of $10^{-6}$. This potential effect results again in having chain scission on the polymers. However, this tends to be less severe than gamma radiation because the depth of the electron penetration is less than the gamma particles.

At Biocoat, we evaluated our HYDAK® Thermal cure and UV cure hydrophilic coatings using these three different sterilization methods and measured post-

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## Sterilization Effects on Hydrophilic Coatings ...

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sterile performance via lubricity, durability, particulates and visualization of the coating. ETO sterilization was done at 50 degrees Celsius, 30% RH (relative humidity). Gamma and e-beam were both done at a nominal 45 kgy (kilogray sterilization dose).

For this study, we used five different coatings from our HYDAK Thermal cure process. These included:

1. HA (HA only)

2. HA-X (HA with Crosslinker)

3. HA-P1 (HA with Acrylic Polymer)

4. HA-P2 (HA with Acrylic Polymer: T-070) – this is our main coating

5. Syn (Hydrophilic Copolymer: T-018)

We also used two different coatings from our HYDAK UV cure process. These include:

1. UV-2/1: 2/1 ratio of Polymer A/ Polymer B

2. UV-4/1: 4/1 ratio of Polymer A/ Polymer B

(Both of these have a photoactive basecoat and topcoat)

The lubricity was evaluated using our frictional test pad, also known as a "pinch test", of either silicone or Delrin pads. During this process, 500 grams of force is applied to the pads while the device is pulled up through each side of the pad. We then cycle this process multiple times to measure the durability. For this study, we did 30 cycles using Pebax 55D, 2mm OD (outer diameter) tubing.

Visualization is accomplished by staining the hydrophilic coating blue. Prior to sterilization methods, the staining of the coating appears very dark blue.

The HYDAK Thermal cure results using each sterilization method are as follows:

For the pinch test, there is no change in friction for the HA only coating (#1) using ETO sterilization over 30 cycles. However, there is some change in reduction of friction when gamma and e-beam methods are applied. When crosslinkers are added (#2), this stabilizes the coating and again with the pre-sterile and ETO the friction stays about the same. With gamma, there is some degradation over time (although it is better overall than the HA only coating), whereas the e-beam method actually improves here. The HA coatings with acrylic polymers (#3&4) stabilizes the coating and again not much has changed. For one of them (#3), there is a slight increase in the friction using ETO, as well as pre-sterile for gamma and e-beam. For our main coating (#4) T-70, it looks good with the different sterilizations for both the lubricity and durability. Similarly, the synthetic polymer (#5), which is our T-18 coating, is designed to withstand radiation and has very stable results over the course of the 30 cycles for all three sterilization methods. (Shown in Figure 1)

The visualization results, which are obtained through staining the coatings, mostly match the friction results. The HA only coating (#1) stayed very dark blue through ETO, but then lightened considerably through gamma and e-beam, suggesting that the coating had been degraded from the radiation. The HA with crosslinkers (#2) improved on the e-beam with some reduction in color from the gamma.

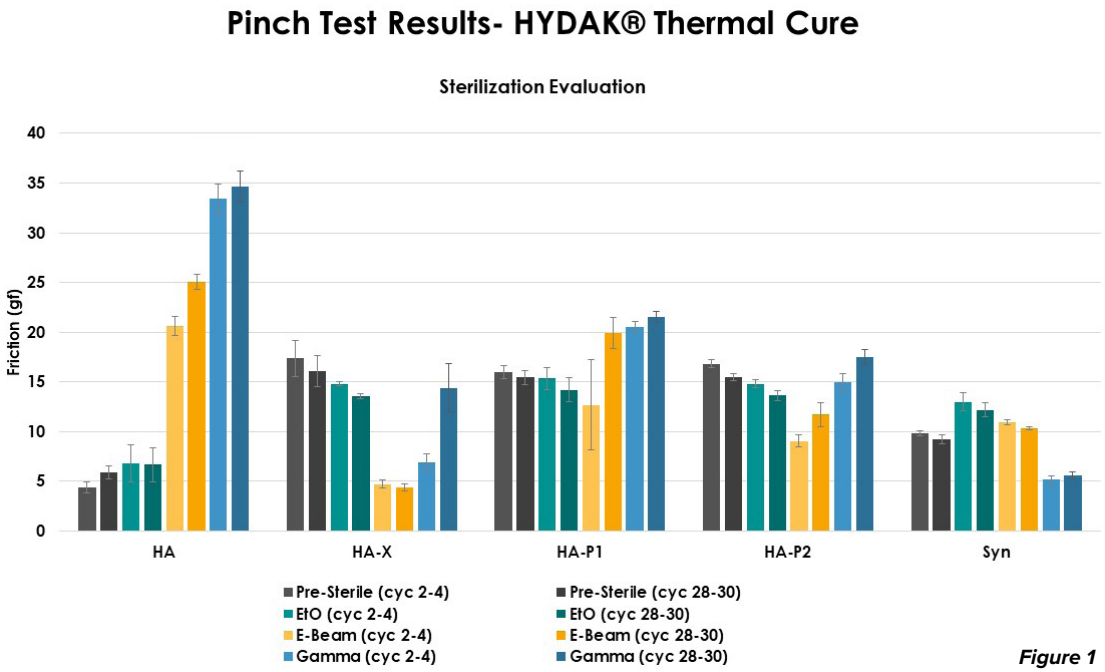


***Figure 1***

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## Sterilization Effects on Hydrophilic Coatings ...

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The HA with acrylic polymers (#3 & 4) look good through ETO with some changes in color from the gamma and e-beam, but more so with the gamma. The synthetic coating, T-18 (#5) has no change in color, which is consistent with the friction performance. (Shown in Figure 2)

The HYDAK UV cure results using each sterilization method are as follows:

During the development process of our new UV cure coating, looking at the two different ratios, we found that at the pre-sterile both of these coatings have about the same results for lubricity and durability. However, after ETO sterilization, one of them had worse friction results, but seemed to look good through the gamma and e-beam methods. For the 2:1 ratio coating (#1) there was a difference in the ETO friction compared to the 4:1 coating. We then compared this to the T-18 synthetic polymer. (Shown in Figure 3)

For the visualization measurement, compared to the synthetic T-18, the results are quite good in terms of the color after sterilization as compared to the Thermal cure coating counterparts.

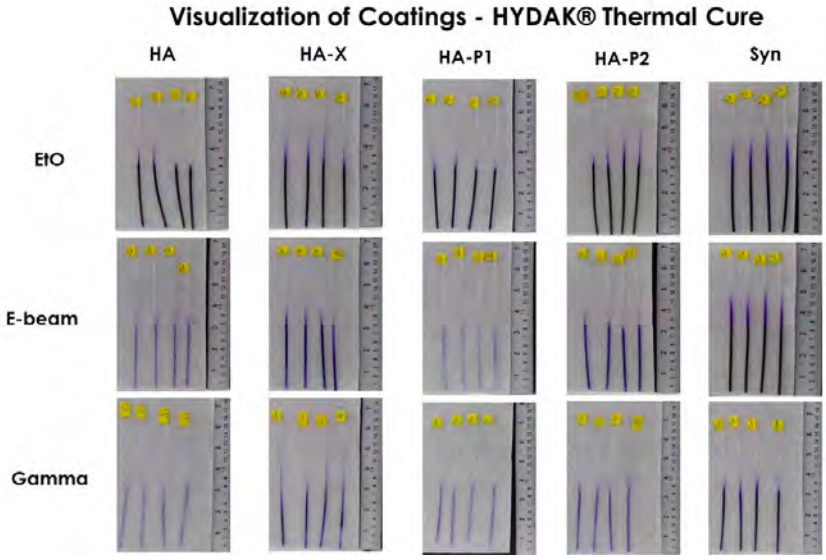


***Figure 2***

Finally, for the particulate measurements, we used the HYDAK UV cure coatings to see how they compare after the various sterilization methods. Nailing down the parameters and having good comparison data is critical for particulate measurements. For this particulate evaluation, we have a clinically relevant tortuous path test pathway. We were aggressive on the duration of testing for this study, performing 50 cycles back and forth in the pathway, flushing with high purity water after each 10 cycles and collecting the effluent in an HDPE container. To count the particles, we immediately tested the samples in a light obscuration chamber. The results presented include all particulates greater than 10 microns. Both UV cure coatings show similar behaviors with fairly low particulate counts. There are similar

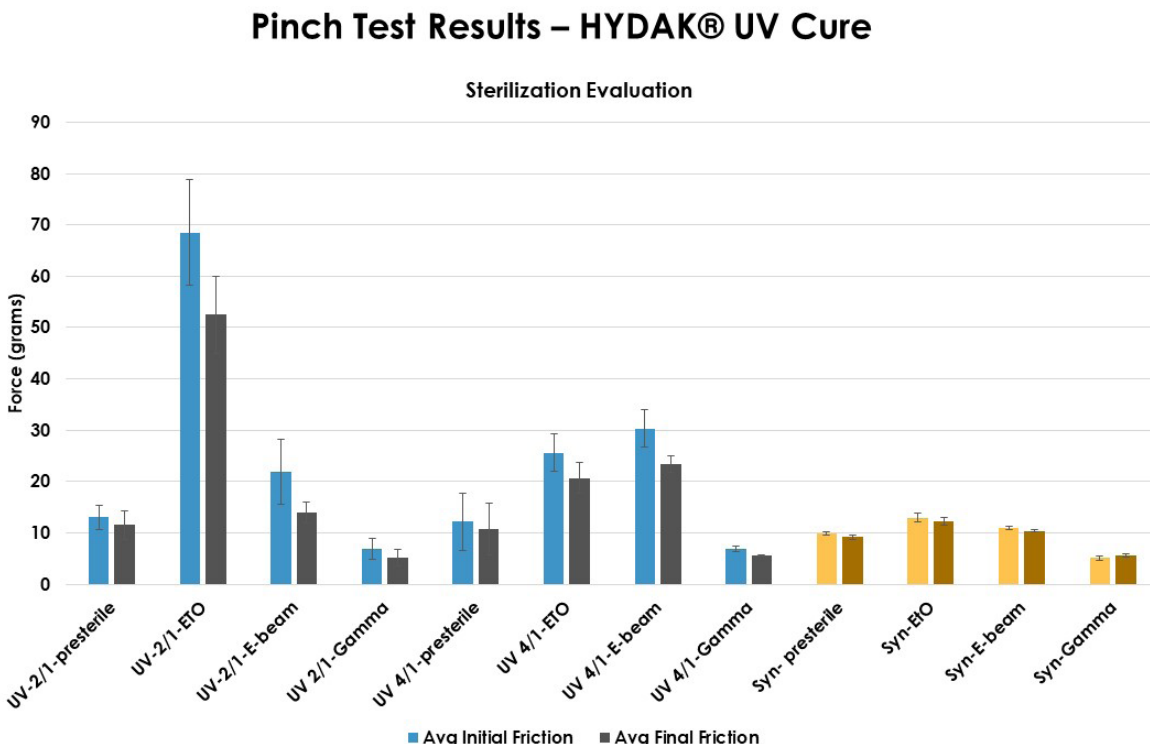


***Figure 3***

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## Sterilization Effects on Hydrophilic Coatings ...

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results with pre-sterile, ETO and e-beam with gamma having slightly higher particulate counts. (Shown in Figure 4)

In conclusion, sterilization processing does have an effect on the performance of hydrophilic coatings, however, it's possible for HA based coatings to withstand irradiation sterilization. Regardless of the cure system, Thermal cure or UV cure, as well as the type of coating, you should evaluate the sterilization effects. Testing should include looking at all aspects of a hydrophilic coating performance which includes lubricity, durability and particulate generation.

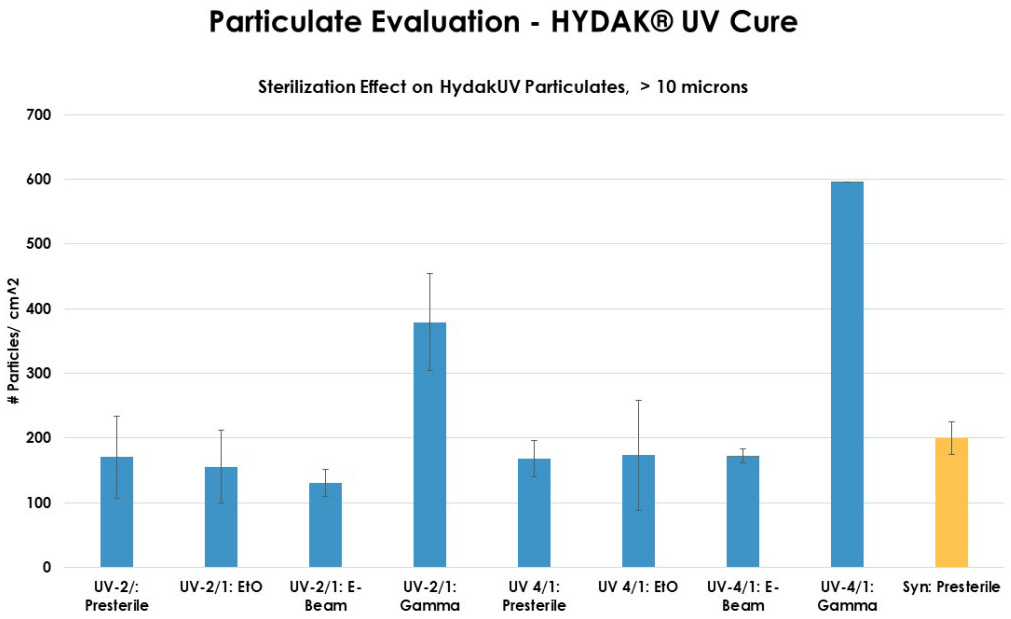


***Figure 4***

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# Agonies and Thrills of Es and Ls. Part 2: Solvent Effects

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## Abstract

Chemical characterization of complex medical devices presents enormous challenges even to the best in the field of analytical chemistry, chromatography and spectroscopy. Extractables and leachables (E&L) data collection, interpretation and toxicological risk assessment are major topics of ongoing forums, conferences and webinars. This article highlights some of the shortfalls of the process and provides guidance to improve data quality and ensure device safety.

## Introduction

The examples presented in previous webinars [1-2] were actual data as reported by various laboratories and excerpts from FDA feedback. Reviews of data showed rampant compound mis-identifications such as:

1. Anhydrides in protic solvent extracts
2. Significant levels of non-polar compounds in aqueous solvent extracts
3. Polar compounds in non-polar solvent and yet, no detection in corresponding polar extracts contrary to the principle of "like dissolves like."

Most laboratories performing E&L start with a pre-investigation to ensure proper selection of compatible solvents to maintain device integrity. Complex devices are constructed of a combination of polymers and components having different physico-chemical properties, and are often known to contain some level of reactive residuals. Isocynates and epoxide monomers constitute the building blocks of polyurethane and polyethers, and are widely used polymers in the construction of various medical devices. These monomers are very unstable in protic solvents, they react with a broad range of chemical compounds and yet, they are not classified as cohorts of concern [3] despite their potential production of DNA/RNA mutagenic adducts.

Solvent compatibility with extractables are rarely investigated and present a major challenge for detection and identification of reactive extractables and potential reactions with co-extractables such as epoxide [4], and isocyanates leading to identification of reaction products.[5]

In general, reaction end-products are thermodynamically more stable, less reactive and less toxic, thereby camouflaging the real risk to which the patient might be directly exposed.

## Results and Discussion

In order to understand the chemical compatibility of the solvent on chemical characterization, a heart valve constructed of gelatin and cross-linked with glycidyl ether 100 which failed the initial biocompatibility testing, was submitted for E&L evaluation following ISO-10993 part 12 and 18.[6,7]

The device was extracted with hexane, isopropyl alcohol and water following solvent biocompatibility testing to ensure device integrity. The extracts were analyzed by Gas Chromatography/Mass Spectrometry (GCMS), Ultra-Pressure-Liquid Chromatography Orbitrap High-Resolution Mass-Spectrometry UPLC/HRMS) and Inductively Coupled Plasma Spectroscopy (ICPMS). GCMS analysis showed no detection of any compound above the analytical threshold evaluation (AET). [8,9] However, LCMS operating in full scan in electrospray positive and negative modes, showed detection of small amount chloro products. The results prompted simulated-use extraction using saline for the determination of the leaching rate. LCMS analysis showed detection of various chloro-derivatives in high abundance.

To confirm the source of chloro-products, simulated-use extraction was repeated with control gelatin spiked with glycidylether 100 standard. LCMS analysis showed matching chromatographic profiles and perfect response ratio of isotopic chlorine peak areas for the different chloro-substituted compounds. The chloro-products showed no match using search library.

Hydroxy-alkyls, halide-alkyls, ethers including epoxides are poor candidates for detection by LCMS due to poor ionization. However, addition of halides to hydroxyl-compounds promote their ionization through inductive effect or electronegativity enhancing negative ionization of OH group, or through their lone donor electron pair increasing electron cloud density on oxygen for positive ionization. Otherwise, the hydrolysis

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products would go undetected, and identification of any residual epoxide completely overlooked.

The major advantage of HRMS is its selectivity allowing measurement of exact mass and distinction in minor changes in chemical structure. However, HRMS stops short of distinguishing between compounds with the same molecular formula leading to E&L search library illusion and confusion. A literature search as an example for molecular formula $C_{22}H_{20}O_9$, https://pubchem.ncbi.nlm.nih.gov/#query=$C_{22}H_{20}O_9$, shows over 200 different structural, binary and ternary adducts compounds exhibiting different substructures and various level of reactivity and toxicity.

Adduct formation in E&L presents a big challenge for LCMS identification and quantification and these reactions can range from simple to more complex. The mass spectra fragmentation pattern is also affected by instrument parameters, concentration, mobile phase composition [10] interference or contaminants, [11] rendering intra-laboratory reproducibility of E&L data enormously difficult. [12]

In method development/validation of analyte(s) in complex matrices, the sample extract is often "cleaned up" using various techniques to control matrix effect. However, E&L extracts are by design, analyzed "as is" without any substantial preparation. As a general rule, the more complex the device, the more complex the matrix effect, and it is directly related to volume reduction/concentration which is often performed to meet Analytical Evaluation Threshold (AET) requirements. Concentration generates a complex medium with the potential to affect structure of analytes through formation of adducts. Concentration also enhances reaction kinetics amongst potential co-extractants to yield by-product(s) that would eventually result in false positive identification of extractables.

## Conclusion

The search for the culprit molecules responsible for failure of biologically compatible extracts to pass the routine and common biocompatibility tests for cytotoxicity or irritation for similar devices may have been prematurely halted had the simulated-use extraction been performed using water or alcohol as extraction medium. The use of saline and subsequent reactions of Cl- with epoxides enhanced the detection and identification of the chemicals causing failure.

Another issue that might have emerged, had the simulated-use extracts been analyzed following target analysis as recommended by guidelines. Target analysis may have resulted in a release rate of chloro-byproducts causing no toxicological risk, and the device may have passed the approval process without any question asked. In such a case, identification of the chemical structures of the various chloro-byproducts is not as important as identification of the parent compound to which the patient is directly exposed.

In fairness to guidelines being ambiguous, it is not possible to be more specific given the variables that could easily affect the outcome. However, the guidelines 10993-18: 2020, page 29 states: ***"The successful completion of the chemical characterization outlined in this document can require expertise in material science or analytical chemistry to provide the necessary qualitative and quantitative data that a risk assessor can use to assess medical device safety."*** Yes, the emphasis is on expertise. In spirit, the guidelines are good but study conduct and application suffer from major flaws due to competition, cost control, business pressure, etc. The design of experiments as is generally being practiced has become so generic as applied throughout a large sector of the industry following protocol design limiting solvent selection to device integrity and identification based on search libraries. The results often fall short of expectations mainly due to lack of expertise in data interpretation and collaboration, basic knowledge of principles of chromatography and spectroscopy, chemical and physical properties of solvents and extractables. Search Libraries and exact molecular formula should be a tool but never the only unconfirmed tool for identification.

The responsibility of any laboratory conducting E&L is paramount because the ultimate safety assessment of the device depends on laboratory results. Toxicologists and reviewers often overlook how the identification of compounds are generated as long as a standard process is followed. At the end, it is possible that regulatory approval could be granted based on reporting reaction by-products, mis-identification or wrong data interpretation.

Finally, most laboratories' processes as well as reviewers put more emphasis on compliance to ensure the box is checked. We all believe that expertise pays off. Good E&L

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data provides guidance to medical device manufacturers to either revisit their manufacturing process to improve the quality of their product or face regulatory scrutiny, risk device failure and compromise patient safety. Finally, data quality provides unique standing to any organization in line with: You get what you pay for, and the data is as good as the one behind the machine.

N.B: In the next article: Part 3. Solvents selection and analytical techniques for characterization of reactive extractables and covalently bonded toxic un-extractables.

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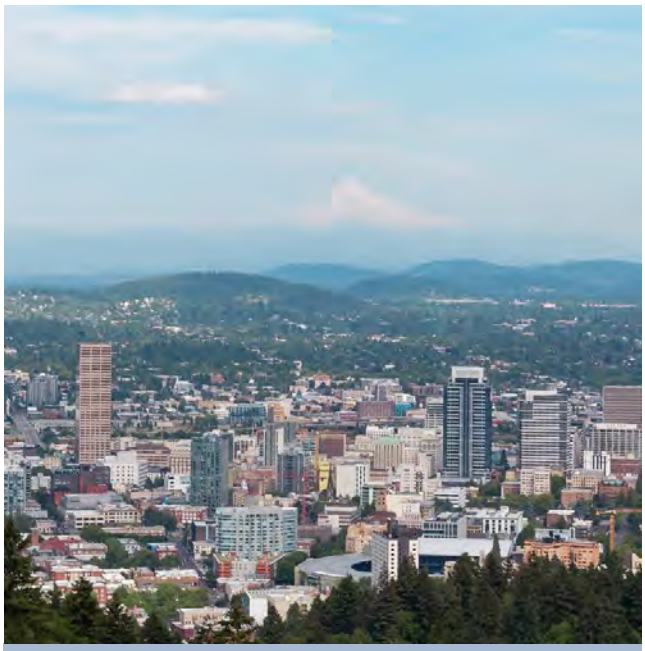

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