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Assessing the Risk of Leachables from Single-Use Systems

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This article is the second in a two-part series on extractables and leachables.

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Assessing the risk posed by leachables from single-use assemblies can be challenging. Disposable assemblies are often constructed of a mix of functional components, containers, tubing, valves, and filters, each made from several materials by complex manufacturing processes. Additionally, the gamma sterilization pretreatments of such assemblies potentially can create trace-level leachables that are nearly impossible to completely identify and quantify. Regardless of these challenges, it is necessary to assess the risk posed by leachables for the following reasons:

- It is a regulatory requirement
- The leachable may affect the drug potency
- The leachable may interfere with the assay of the drug
- The leachable may be toxic.

This article will review the design and engineering steps used to reduce the risk from leachables, investigate methods for assessing the risk from leachables, and present data from two case studies involving single-use systems.

BUILDING QUALITY IN

Quality by design (QbD) is defined as the systematic process to build quality into a product from its inception. This principle can be leveraged during the design and engineering of components of single-use systems to minimize the risk of leachables. Component materials should be nonreactive, 21 *CFR*-cleared material and pass USP Class VI specifications.

PureFlex polyethylene film (EMD Millipore) is an example of a material that can be designed into single-use systems to minimize generation of leachables. PureFlex film is a high-purity, medical-grade coextruded film designed to provide strength, flexibility with maximum resistance to flex-crack, excellent gas-barrier performance, and inert contact. The fluid-contact material is ultra-low-density polyethylene (ULDPE), which was chosen because of its inertness.

The Hansen solubility parameter (HSP) is often used to evaluate solubility and shows that ULDPE is an appropriate choice for fluid contact (1). A chemical with an HSP value similar to the solvent will more likely dissolve in the solvent, according to the principle of "like dissolves like." The HSP value is determined by three forces: the dispersion force of a chemical, the polar force of a chemical, and the hydrogen-bonding force of a chemical. Figure 1 shows a graph in which chemicals are plotted based on the percentage that each force contributes to the total HSP value. As shown in the figure, polyethylene is far removed from water and ethanol, indicating that polyethylene and its monomers would not dissolve in either of these two solvents.

PureFlex film is produced using additive compounds that either help process the film or help protect the film. Typical processing additives include slip agents that reduce the film-to-film friction, thereby allowing faster processing speed. A common slip agent is composed of fatty acid amides (e.g. erucamide). Slip agents are added at a concentration from a few parts per million (PPM) to a few thou-

sand PPM (2). Though these agents may be present at high concentrations, aqueous solubilities are very low (e.g., erucamide has an aqueous solubility of 0.00045 mg/L [3]). Antioxidants comprise another common type of additive. These compounds are added to the polymers to protect the film from ultraviolet radiation, and are typically large molecules (hundreds of Daltons in size) with very low aqueous solubility. Commonly used antioxidants include Irganox 1010 (Ciba Specialty Chemicals, 2-[3,5-di-tert-butyl-4-hydroxyphenyl] propionate), with an aqueous solubility of 2.3 x 10^{-16} mg/L and Irgafos 168 (Ciba Specialy Chemicals, tris[2,4-di-tert-butylphenyl] phosphite), with an aqueous solubility of ⩽ 0.09 mg/L.

Another strategy for incorporating QbD is to use materials cleared as food contact substances (FCS) under 21 *CFR*. There are several ways in which a chemical can obtain clearance as an FCS, such as being a prior sanctioned substance, an indirect food additive, generally recognized as safe (GRAS), obtaining a threshold of regulation (TOR) exemption, or obtaining a food contact notification (FCN).

Prior sanction substances are chemicals for which there was explicit approval by FDA or the United States Department of Agriculture before Sept. 6, 1958. Some of these substances are listed in 21 *CFR* 181.

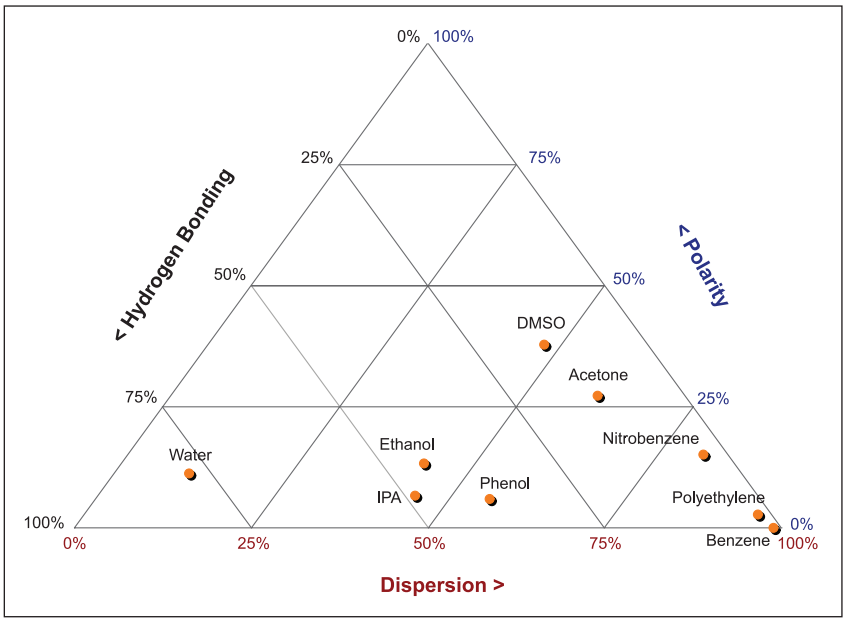
Indirect food additives are substances that come into contact with food as a part of packaging, holding, or processing, but are not intended to be added directly to the food. These substances are listed in 21 *CFR* 175–178 and include adhesives, paper, polymers, and adjuvants.

GRAS substances have been adequately shown to be safe under the conditions of their use. These substances are listed in 21 *CFR* 182–186. TOR exemptions are substances that have been exempted by FDA from a need to submit an FCN because the substances are expected to result in exposures below 1.5 µg/person/day.

Table I: Daily permitted exposures for various classes of chemicals.

Chemical type or class	Threshold concentration (µg/person/day)
Genotoxic compounds	1.5
Neurotoxic organophosphates	18
Cramer Class III	90
Cramer Class II	540
Cramer Class I	1800

Figure 1: Plot of chemicals based on the percentage that each force contributes to the total Hansen solubility parameter value. DMSO is dimethyl sulfoxide, IPA is isopropyl alcohol.



The specific regulations are listed in 21 *CFR* 170.39.

Food contact notification (FCN) is the mechanism by which a substance can be cleared as an indirect food additive. The testing requirements in support of the substance clearance will depend on the expected exposure concentration. If the expected concentration is less than 1.5 µg/person/day, a TOR exemption should be sought. If the expected concentration is between 1.5–150 µg/person/day, only short-term genetic toxicity tests (e.g., Ames test and mouse lymphoma assay) are required. If the expected concentration is between 150–3000 µg/person/day, then more in-depth tests are required. If the expected concentration is greater than 3,000 µg/person/day, a Food Additive Petition should be sought.

Finally, it should be noted that the 1958 Amendment to the *Food Drug and Cosmetic Act* includes the Delaney Clause, which states: "no (substance) could be deemed safe or given FDA approval if found to cause cancer in man or experimental animals (4)." Therefore materials cleared as an FCS under 21 *CFR* will not include any carcinogens.

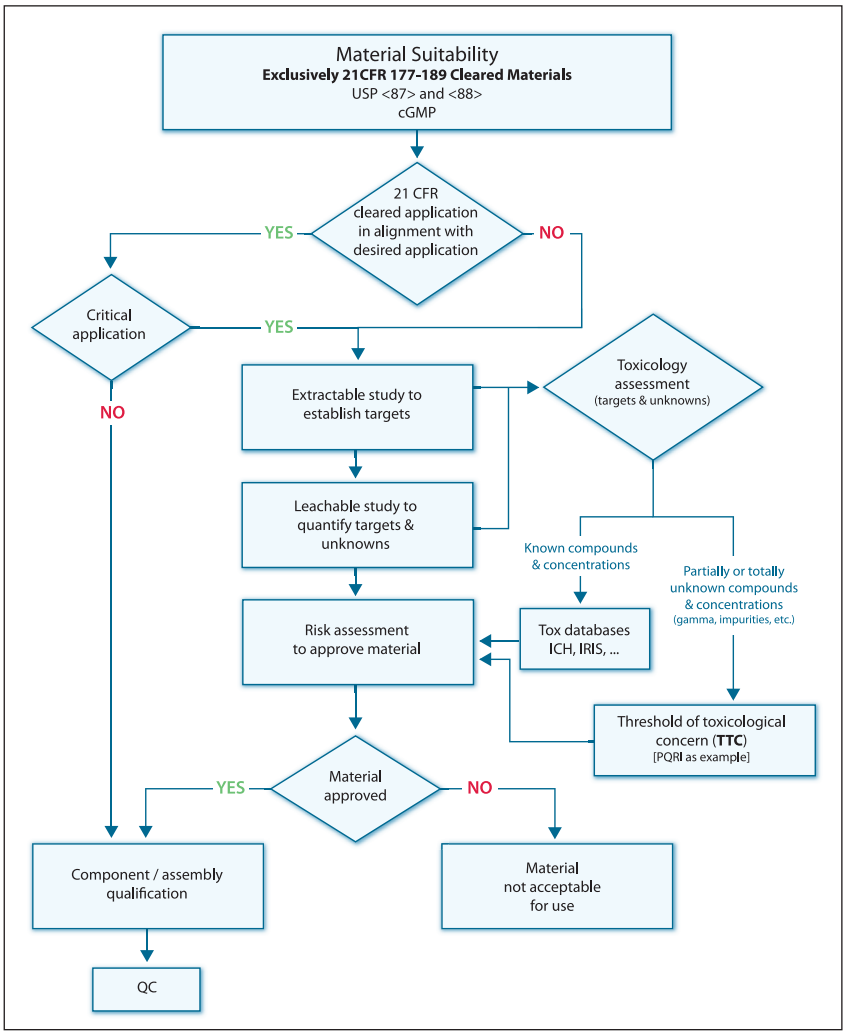
The third strategy to incorporate QbD into single-use components is through use of materials qualified as *US Pharmacopeia* (*USP*) Class VI plastics. The USP sets standards for the quality, purity, strength, and consistency of these products—all critical to the public health (5). One such standard is *USP* <88>

ALL FIGURES ARE COURTESY OF THE AUTHOR

Table II: Leachables generated from five assemblies.

Component	Total organic carbon (mg C)	
	Single assembly	Five assemblies
500-L bag	80.7	403.5
Silicone tubing	2.9	14.5
Opticap Express (SHR)	31.2	156
Total	114.8	574
Final concentration in 200-L batch		2.87 mg C/L

Figure 2: Flowchart for assessing the risk of leachables. ICH is International Conference on Harmonization, IRIS is Integrated Risk Information System, PQRI is Product Quality Research Institute, and QC is quality control.



Biological Reactivity Tests, *In Vivo*. In this standard, extracts of test material are prepared in several solutions, which are then injected into mice and rabbits. The plastic components are classified based on the reaction to a systemic injection test, an intracutaneous test, and an implantation test. Based on the results of the tests, the material is classified as Class I through Class VI, with Class VI being the most stringent.

Another important standard is *USP* <87> Biological Reactivity Tests, *In Vitro*. This standard is designed to determine the biological reactivity of mammalian-cell cultures following contact with plastic materials. The tests performed include the agar diffusion test, the direct contact test, and the elution test. In this standard, the material is found to pass if there is no reaction or only a mild reaction.

No matter how well single-use systems are engineered, leachables will still enter the solution because of the gamma irradiation step. While gamma irradiation is used to reduce the bioburden of these components, the same energy used to destroy bacteria also results in some polymer degradation. An additional complication associated with gamma irradiation is that several compounds will be created depending on the strength of the gamma irradiation, the amount of oxygen present, and the length of time since the gamma treatment.

RISK CHARACTERIZATION

There are several approaches available to evaluate the risk posed by a leachable. The first approach, used for known compounds, is to compare the concentration of the leachable to published limits. A good first source of published limits is Q3C (R3) *Impurities: Guidelines for Residual Solvents* from the International Conference on Harmonization (ICH). The document separates solvents into three groups:

- Class I: Solvents to be avoided—five solvents that should be avoided due to either toxicological concerns or environmental concerns.
- Class II: Solvents to be limited—26 compounds that should be limited because of their inherent toxicity. The allowable concentrations range from 50 PPM to 4840 PPM.
- Class III: Solvents with low toxic potential—28 solvents that should be limited by GMP or other quality-based require-

ments. The ICH limit for Class III solvents is 5000 PPM.

Another source of acceptable limits is published by FDA, Office of Food Additive Safety (OFAS). This publicly available database lists cumulative estimated daily intakes (CEDI) and acceptable daily intakes (ADI) for a large number of substances (6).

A second approach to evaluating risk for a known compound is to calculate an ADI or permitted daily exposure (PDE) concentration (see Equation 1). FDA calculates the ADI by dividing a no observable effect level (NOEL) concentration by a safety factor (usually 100). The safety factor takes into account that the NOEL was determined from an animal study and for variability among humans. Alternatively, ICH calculated the allowable residual concentrations based on PDE. PDE is similar to ADI except for a few variations in the equation (see Equation 2). If NOEL values are not available, then lowest observable effect level (LOEL) values can be used, but with an additional safety factor incorporated.

$$\text{ADI (mg/kg/day)} = \frac{\text{NOEL}}{100} \quad \textbf{[Eq. 1]}$$

$$\text{PDE (mg/day)} = \frac{\text{NOEL} \times \text{BW}}{\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}} \quad \textbf{[Eq. 2]}$$

F1 = Factor to account for extrapolation between species. (Range 2 - 12)
F2 = Factor to account for variability between inviduals (10)
F3 = Factor to account for shorter studies than optimum (Range 1 - 10)
F4 = Factor for cases of servere toxicity. (Range 1 - 10)
F5 = Factor if LOEL was used instead of NOEL (10)

Sources for NOEL values include the Chemical Carcinogenesis Research Information System (CCRIS), Integrated Risk Information System (IRIS) and the International Toxicity Estimates for Risk (ITER).

An approach for evaluating the risk associated with unknown compounds is to use the threshold of toxicological concern (TTC). The TTC is a concept that refers to the establishment of a level of exposure for all chemicals, whether or not there are chemical-specific toxicity data, below which there would be no appreciable risk to human health. The TTC approach is based on the analysis of the toxicological or structural data of a broad range of different chemicals and has been developed as a substitute for substance-specific information.

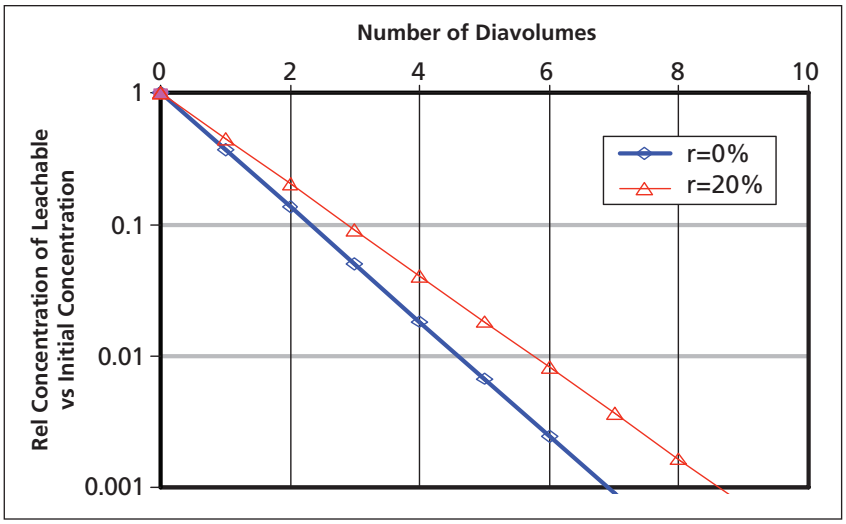
In 1978 Cramer proposed that many chemicals could be categorized into three classes of compounds with three different potentials for toxicological risk based on structural activity relationships (SARs), metabolic mechanisms, chemical reactivity, and other relevant information (7). Cramer Class I substances are those with simple chemical structures and predictable and efficient modes of metabolism that suggest a low order of toxicity. Cramer Class III substances are those that suggest significant toxicity because their chemical structures have similarities to known toxins. Cramer Class II substances are those that cannot be placed in Class I or Class III and are therefore intermediate in expected toxicology. This tier classification was later expanded by the International Life Sciences Institute (ILSI Europe) and its TTC Task Force to include a class for select organophosphates (which have neurotoxicity) (8). Table I summarizes the PDE for the various classes of chemicals using the TTC approach.

In assessing the risk of leachables, no single method needs to be used. Instead a combination of all three methods previously listed can be implemented as shown Figure 2.

Table III: Patient exposure to leachables from five assemblies.

Drug	Type	Dosage (mL)	Frequency (days)	Patient exposure	
				per dose µg/dose	per day µg/person/day
Drug A	Subcutaneous	2	7	14.35	2.050
Drug B	Intravenous infusion	18	56	129.15	2.306
Drug C	Vaccine	0.1	365	0.72	0.002
Drug D	Vaccine	0.5	365	3.59	0.010

Figure 3: Concentration of leachable compounds decreases with each diafiltration volume processed.



ASSESSING THE RISK

The following case studies demonstrate the use of TTC to assess the risk associated with leachables from both upstream and downstream single-use assemblies.

Table IV: Patient exposure to leachables from five assemblies following diafiltration.

Drug	Patient exposure (µg/person/day)			
	(Original) 0 Volumes	2 Volumes	4 Volumes	6 Volumes
Drug A	2.050	0.410	0.082	0.021
Drug B	2.306	0.461	0.092	0.023
Drug C	0.002	0.0004	0.0001	0.00002
Drug D	0.010	0.0020	0.0004	0.00010

Table V: Patient exposure to leachables from final-fill assembly.

Drug	Type	Dosage (mL)	Frequency (days)	Patient exposure	
				per dose µg/dose	per day µg/person/day
Drug A	Subcutaneous	2	7	5.18	0.740
Drug B	Intravenous infusion	18	56	46.64	0.833

Five assemblies, each containing a 500-L PureFlex bag, an Opticap sterile high-retention filter, and silicone tubing were evaluated. Each assembly was used at different stages of the process and may have included buffer solutions for the bioreactor, elutent solutions for the chromatography step, or exchange buffer for the ultrafiltration/diafiltration (UF/DF) step. The final drug product and all leachables were collected in a 200-L tank. No leachables from the tank were included in this case study.

Based on the total organic carbon (TOC) data from several internal studies, the five assemblies would generate the leachables shown in Table II.

Assuming all the TOC is coming from one, unknown compound and that the compound is 40% carbon by weight, the concentration of this compound in the final container would be 7.175 mg/L (9).

The risk of leachables to a patient will depend on how the drug is administered, the dosage, and frequency of dosing. Drug A may be administered subcutaneously once a week at a dose of 2 mL. Drug B may be administered by intravenous infusion once every 18 weeks at a dose of 18 mL. Drug C may be a vaccine administered annually at a dose of 0.1 mL. Drug D may be a vaccine administered annually at a dose of 0.5 mL. The effect on the patient is determined by multiplying the concentration by the dose and dividing by the frequency.

For example, drug A from Table III is delivered at at a volume of 2 mL per dose and a frequency of once per week. The exposure of the leachable to the patient can be calculated as follows:

7.175 mg/L x 2 mL/dose x 1000 µg/mg x 1 L/1000 mL = 14.35 µg/dose **[Eq. 3]**

14.35 µg/dose x 1 dose/7 days = 2.05 µg/person/day **[Eq. 4]**

As seen in Table III, the two vaccine drugs (C and D) have a total daily intake of the leachable that does not pose a risk to the patient (< 0.15 µg/person/day). However, it is uncertain if the total daily intake of the leachable for Drug A and Drug B poses a risk to the patient. Based on the TTC limits shown in Table I, the total daily intake of the leachable would fall between the allowable limit for a genotoxic compound (1.5 µg/person/day) and that of a neurotoxic organophosphate compound (18.0 µg/person/day).

If the potential exposure concentration is unacceptable, one might consider modifying the original assumptions to be more representative of the actual operating conditions. These modifications may include a filter flush step, a shorter residence time in the mixing bag, or accounting for some removal of the leachable during the UF/DF step.

Modification #1: effect of a filter flush step

Most leachables generated from a filter occur during the first few liters. By incorporating a 5-L flush step, the contribution of TOC from the filter would be reduced from 31.2 mg carbon (C)/filter to 2.9 mg C/filter, resulting in a reduction in the final concentration of the unknown leachable from 7.175 mg/L to 5.408 mg/L. The revised patient exposure to the leachable for the four drugs would then be: Drug A at 1.55 µg/person/day; Drug B at 1.74 µg/person/day; Drug C at 0.001 µg/person/day; and Drug D at 0.007 µg/person/day.

Once again, Drugs C and D have a total daily intake of the leachable that does not pose a risk to the patient. However, it is uncertain if the total daily intake of the leachable for drug A and drug B poses a risk to the patient. Based on the TTC limits in Table I, the total daily intake would fall just above the allowable limit for a genotoxic compound (i.e., 1.5 µg/person/day).

Modification #2: effect of residence time

In the initial conditions, it was stated that the assemblies were being used to deliver buffer solutions. As such, it is unlikely that the solutions would be stored for 30 days prior to use. If a one-day residence time is used, the contribution of TOC from the 500-L bag would go from 80.7 mg C/bag down to 35.3 mg C/bag. This reduction in residence time results in the final concentration of the unknown leachable going from 7.175 mg/L

down to 4.340 mg/L. The revised patient exposure to the leachable for the four drugs would then be: Drug A at 1.24 µg/person/day; Drug B at 1.40 µg/person/day; Drug C at 0.001 µg/person/day; and Drug D at 0.006 µg/person/day.

Once again, Drugs C and D have a total daily intake that does not pose a risk to the patient. However, it is uncertain if the total daily intake for Drug A and Drug B pose a risk to the patient. Based on the TTC limits in Table I, the total daily intake concentrations are below the allowable limit for a genotoxic compound (i.e., 1.5 µg/person/day).

Modification #3: effect of UF/DF step

As leachable compounds tend to be small molecules, they will not be retained by the UF/DF membrane. As shown in Figure 3, the concentration of the leachable compounds decreases with every diafiltration volume processed.

Assuming a conservative estimate of compound retention of 20%, the patient exposure will be reduced based on the number diafiltration volumes processed. If two diafiltration volumes are processed, the concentration of the unknown leachable is reduced by 80%, from 7.175 mg/L to 1.435 mg/L. If four diafiltration volumes are processed, the concentration is reduced by 96% to 0.287 mg/L. If six diafiltration volumes are processed, the concentration is reduced by 99% to 0.072 mg/L. Table IV summarizes the patient exposure to the leachable for the four drugs based on various diafiltration volumes processed. By using four diafiltration volumes, patients are no longer exposed to risk from the leachable.

The risk of leachables can also be assessed in downstream processes. In this case study, the total leachables from a fill–finish assembly were assessed. The drug is stored in a 100-L mix bag, which contributes 34.9 mg carbon as leachable. The assembly also has a 2-L bag to control steady state flow, which contributes 2.4 mg carbon as a leachable, a Lynx S2S connector contributing 0.03 mg carbon, intermediate tubing adding 6.7 mg carbon, tubing in the manifold adding 47.1 mg carbon, and an Opticap 4" Durapore filter adding 12.5 mg carbon. The total amount of leachable carbon is 103.6 mg or 1.04 mg C/L of drug product. Using the assumption that the leachable is 40% carbon, we have a leachable concentration of 2.59 mg/L in the drug product.

Table V shows the calculation of patient exposure to leachables for two drugs—one administered subcutaneously and another by IV infusion. The total quantity of extracted compounds is 0.740 µg/person/day for the drug delivered subcutaneously and 0.833 µg/person/day for the IV infusion. Both values are well below the 1.5 µg/person/day limit for a genotoxic compound.

CONCLUSION

The data presented indicate there is little risk from leachables when using single-use systems. The QbD methodology used to identify materials ensures incorporation of components with low aqueous solubilities, materials cleared as food contact substances by FDA, and materials qualified as Class VI by USP.

The low concentrations of leachables that do enter into the product should not affect drug performance or quality-control tests.

As shown in the first case study, if the leachable is introduced upstream, the clarification process will reduce the concentration below the limit of concern. Realistic working conditions allow the level of leachables to fall below the threshold of concern for genotoxic compounds.

The second case study demonstrates that the concentration of leachables from the final formulation and fill–finish assemblies do not pose a risk to patients because concentrations are well below the TTC.

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