# Tuflux® TPE Tubing (Sartorius) vs C-Flex® 374 (Saint-Gobain)

Comparability Guide

SARTORIUS

**Applicable to:**
Tuflux® TPE tubing used for the manufacture of single-use systems as well as standalone coils used in the bioprocessing industry.

The results shown in this comparability protocol and equivalency test report are indicative and do not constitute product specifications.

For official specification please refer to the Tuflux® TPE datasheet, validation guide and extractable guide.

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# 1. Introduction

Sartorius bags and systems are widely used in biopharmaceutical processes in a variety of unit operations for the commercial production of drug products such as vaccines, recombinant proteins and monoclonal antibodies and for the development of future biomolecules in clinical phases.

Buffers and media are increasingly formulated, sterile filtered and stored in single-use FMS (Fluid Management Systems). Product intermediates are also filtered and stored between UF | DF and chromatography purification steps in gamma sterile fluid management systems. FMS are also adopted for the formulation, filtration and aseptic processing of final drug products.

From buffer media preparation, cell culture operations, purification operations up to final formulation, filtration and transfer, thermoplastic tubing such as Tuflux® TPE is a key element for the successful implementation of disposable manufacturing processes.

Tuflux® TPE, Sartorius weldable and sealable tubing, is qualified, manufactured and released according to stringent product validation protocols and quality control testing to offer safe and robust single-use processes to end users for biopharmaceutical applications.

This Guide demonstrates comparability and variances between Thermoplastic Elastomer (TPE) tubing from different suppliers, notably Tuflux® TPE from Raumedic | Sartorius and C-Flex® 374 from Saint-Gobain.

Tuflux® TPE is the Sartorius standard tubing and replaces Saint-Gobain C-Flex® 374 in all new standard FMS.

# 2. General Features

### Physical properties

The following values are determined on standard test specimens punched from a press plate.

| TPE Tubing Type | Color | Hardness Shore A | Operating Temperature Range | Tensile Strength at Break (ISO 527-3) | Elongation at Break (ISO 527) |
|---|---|---|---|---|---|
| Tuflux® TPE (Sartorius) | Translucent | 60 ± 5 (ISO 868) | -55 °C to +135 °C | > 6 MPa | > 750 % |
| C-Flex® 374 (Saint-Gobain) | Translucent | 59 (ASTM D2240) | -45 °C to +135 °C | > 4.0 MPa | > 650 % |

### Material hardness:

### Purpose and test method

A measure of the indentation resistance of elastomeric or soft plastic materials based on the depth of penetration of a conical indentor. Hardness values, represented in Shore A, range from 0 (for full penetration) to 100 (for no penetration).

### Tensile properties:

### Purpose and test method

A tensile test consists in applying an elongation to a tubing specimen and measuring the resulting strength. Mechanical properties can then be defined from the stress-strain curve.

### Tensile Strength at break (TS)

The stress a material can withstand before breaking is calculated by dividing the load at break by the cross section area of the specimen. The tensile strength test is performed with a tensile machine in traction mode.

### Elongation at break:

The elongation is recorded at the moment of specimen rupture and often expressed as a percentage of the original length. Materials with high elongation at break withstand a high deformation before rupture. A high elongation at break often means high flexibility.

| TPE Tubing Type | Weldable and Sealable | Co-Weldable | Dimension Color Coded on tube | Pumpable | Dimension on printed on tube | Coils double bagged in ISO 7 | Sterilization Resistance |
|---|---|---|---|---|---|---|---|
| Tuflux® TPE (Sartorius) | Yes | Yes* | Yes | Yes | Yes | Yes | Gamma-irradiation Autoclave ETO |
| C-Flex® 374 (Saint-Gobain) | Yes | No** | No | No | Not for Sartorius | Yes | Gamma-irradiation Autoclave ETO |

* To C-Flex® & AdvantaFlex®.

** Only to Tuflux® TPE with Tuflux® TPE welding parameters.

### Tubing printing

"a | b + c | d" where a | b is the Internal Dimension and c | d the Outer Dimension of the tubing in inches.

The innocuity of the ink is proven on printed tubing by tests performed according to ISO 10993-5.

### Welding & sealing:

Validated welding parameters for Biowelder® TC and sealing parameters for Biosealer® (TC).

For welding each tube material has its own welding parameter set.

### Co-welding:

Capability of tube being co-welded to other TPE tube materials.

| TPE Tubing Type | STD Welding Parameter | Co-Weldable with Tuflux® TPE Paremeters to ... |
|---|---|---|
| Tuflux® TPE (Sartorius) | Tuflux® TPE | C-Flex® 374 & AdvantaFlex® |
| C-Flex® 374 (Saint-Gobain) | C-Flex® 374 | Tuflux® TPE |
| AdvantaFlex® (AdvantaPure) | AdvantaFlex® | Tuflux® TPE |

# 3. Biocompatibility

**TPE tubing meets the requirements of following biocompatibility tests:**

| TPE Tubing Type | USP <88> Class VI | USP <87> | USP <85> & EP 2.6.14 |
|---|---|---|---|
| Tuflux® TPE (Sartorius) | Compliant | Compliant | Compliant |
| C-Flex® 374 (Saint-Gobain) | Compliant | Compliant | Compliant |

| TPE Tubing Type | USP <661> | ADCF | REACH Compliant |
|---|---|---|---|
| Tuflux® TPE (Sartorius) | Compliant | Yes | Yes |
| C-Flex® 374 (Saint-Gobain) | Compliant | Yes | Yes |

### USP <85>: LAL Endotoxin Test

TPE tubing (non-sterile, t = 0) passed LAL Endotoxin Test according to USP <85> (equivalent to EP 2.6.14).

### Purpose and test method

Biocompatibility tests are performed to demonstrate that the Tubing is biocompatible and meets or exceeds the current USP and ISO requirements.

Tests are carried out on Tuflux® TPE samples before and after gamma irradiation (50 kGy) or autoclave treatment (30 minutes at 123 °C). Tuflux® TPE tubing samples were supplied to an independent testing facility for evaluation under the current USP <88> Class VI, USP <87> and ISO 10993-5 Biocompatibility standards.

### USP <88> Class VI: Biological reactivity tests, in vivo

TPE material meets the requirements of the USP <88> Class VI tests, meaning that biological neutrality has been proven via these experiment tests on non-sterile, gamma irradiated (50 kGy) or autoclaved sterilized (30 minutes at 123 °C) tube samples.

- Intracutaneous test
- Systemic injection test
- Implantation test (5 days)

### Test results of USP <88> Class VI

All material used in the construction of the tubing meet or exceed the requirements of the USP <88> Class VI– 121 °C Plastics tests and are considered as non cytotoxic and non haemolytic.

### USP <87> and ISO 10993-5: biological reactivity tests, in vitro

Within the context of a cytotoxicity test according to USP <87> (equivalent to ISO 10993-5) no substances with cytotoxicity effects were detected. The test has been performed on non-sterile, gamma irradiated (50 kGy) and autoclaved samples (30 min at 123 °C).

### Physico-chemical test

All testing presented in this comparability guide have been performed on gamma irradiated tubing at 50 kGy that represents the maximum doses. If different the tubing conditions will be specified.

### USP <661>
Containers, Physicochemical Tests – Plastic Purpose
Physicochemical tests are designed to determine physical and chemical properties of TPE tubing and their extracts. They are performed on TPE samples before and after irradiation and accelerated ageing conditions.

### Test results
The TPE tubing meets the USP <661> requirements when sterilized at 50 kGy with and without aging conditions corresponding to a shelf life of 3 years.

| Test Description | USP <661> Limits |
|---|---|
| Non-volatile residue | < 15 mg |
| Residue in ignition | < 5 mg |
| Heavy metals | < 1 ppm |
| Buffering capacity | < 10 mL |

### ADCF certified
Tubing Material does not contain any animal derived components.

### REACH
Tubing Material is free from any substances defines as SVHC – Substances of Very High Concern – by the European REACH regulation.

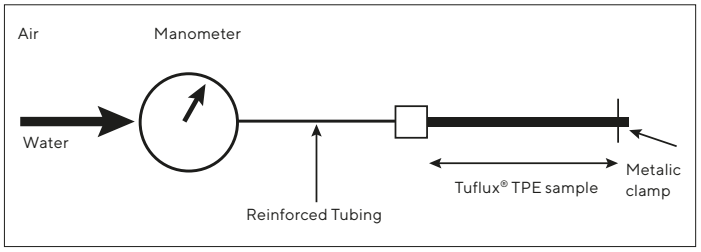
### Absence of chemicals
- Bisphenol A
- Melamine
- Latex
- Heavy Metals acc. to 2011/65/EC
- Phtalates

### Results:
"Conform" or "Yes" for Tuflux® TPE and C-Flex® 374

# 4. Pressure Resistance

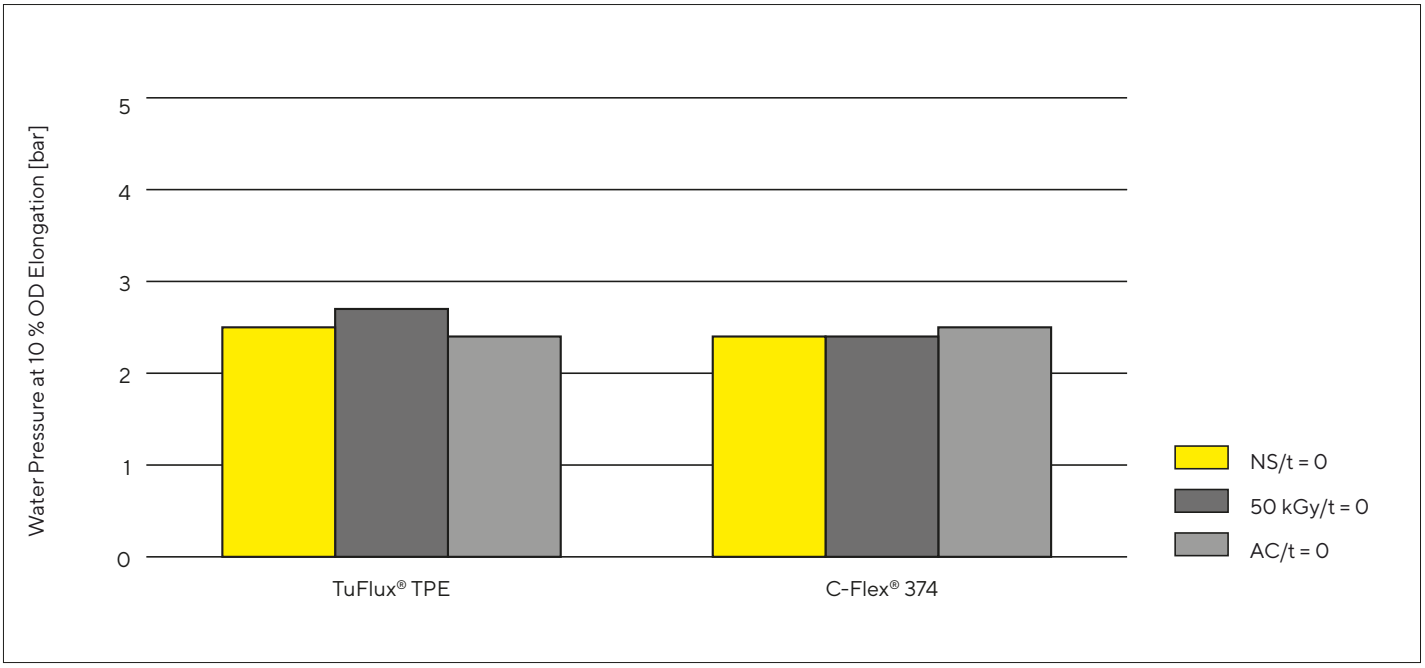
**Purpose**
The goal of the burst pressure test is to assess the pressure resistance of the tubing depending on the tubing dimensions (inside and outside diameter).

**Test method**
The test method is described in the scheme below. Three measurements of the pressure were taken for each tubing reference on gamma sterilized samples at 50 kGy at burst.

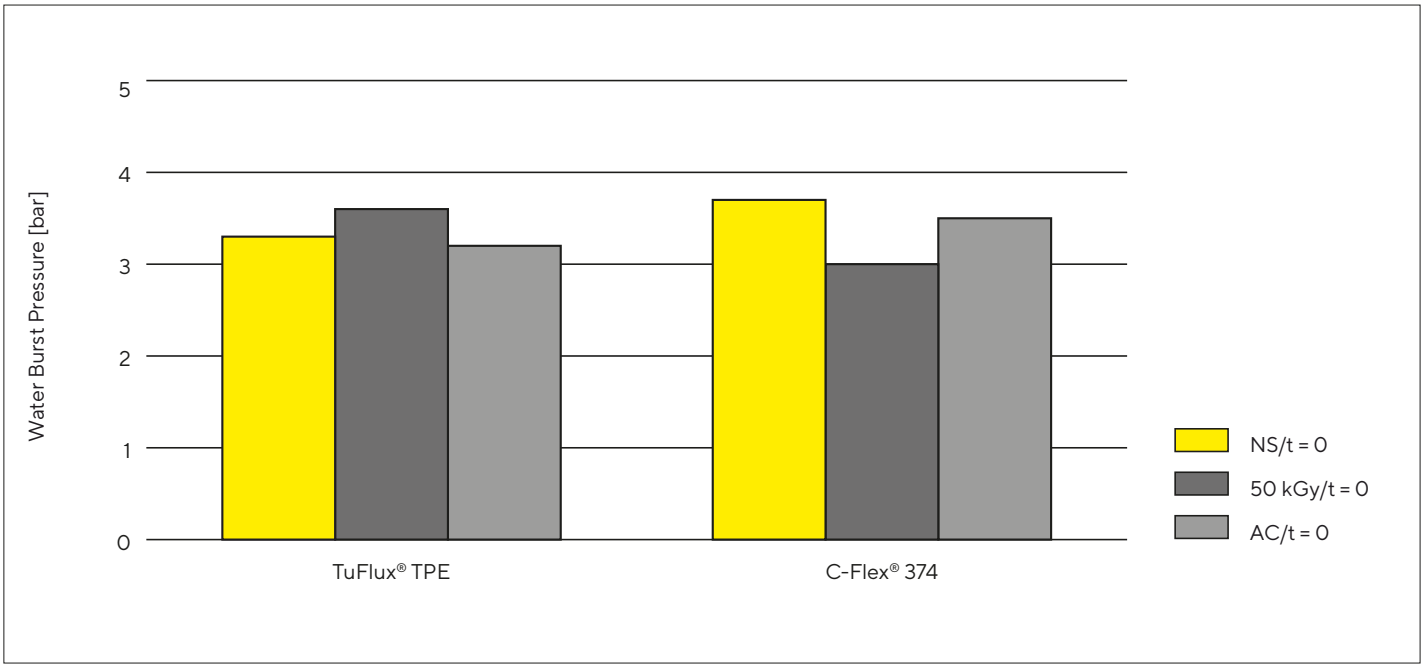


**Pressure resistance results:**
Pressure at which tubings inflate up to 10 % of their initial outer dimension and until tubing bursts.

**Water pressure at 10 % OD elongation for ½''× ¾" TPE tubings at t=0 after different treatments**




**Water burst pressure for ½" × ¾" TPE tubings at t=0 after different treatments**



# 5. Pumping Life Time

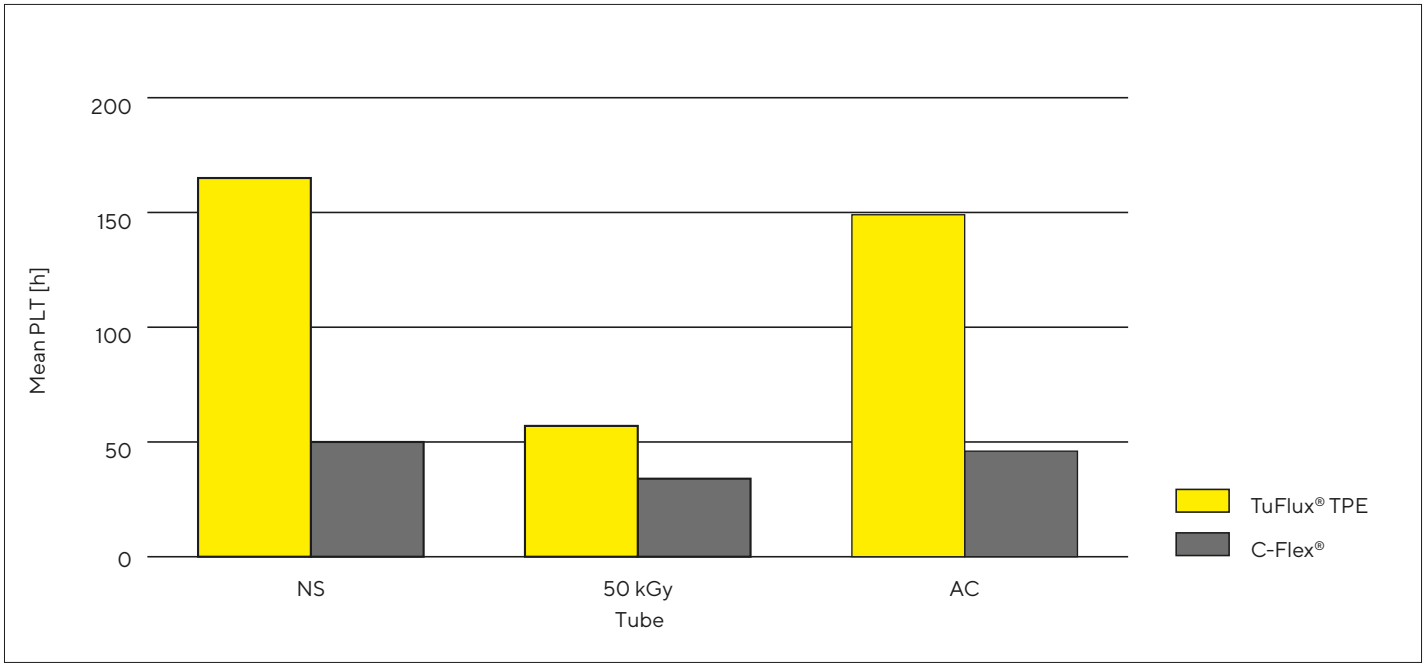
**Purpose**
The goal of the pumping life time test is to assess the mechanical resistance of the tubing under pumping conditions.

**Test method**
The tubing was placed in a Watson Marlow 720 series peristaltic pump and speed was set to maximum (> 300 rpm). Water was pumped through the tubing at ambient temperature between 2 tanks mimicking recirculation conditions.

The test was stopped and time measured when leaking from the tube was observed.

| Tube | Diameter | Sterilization | Ageing | Mean PLT [h] |
|---|---|---|---|---|
| Tuflux® TPE | ½" × ¾" | NS | t 0 | 165 |
| Tuflux® TPE | ½" × ¾" | 50 kGy | t 0 | 57 |
| Tuflux® TPE | ½" × ¾" | AC | t 0 | 149 |
| C-Flex® | ½" × ¾" | NS | t 0 | 50 |
| C-Flex® | ½" × ¾" | 50 kGy | t 0 | 34 |
| C-Flex® | ½" × ¾" | AC | t 0 | 46 |

**Mean pumping lifetime at $t_0$ after different treatments**



# 6. Extractables Comparison

Extractables are compounds that are extracted from materials such as polymers using harsh extraction conditions. Potential extractables are discussed below for C-Flex® 374 and Tuflux® TPE tubing. For this purpose, extractables present in an ethanol extract after 70 days at 40 °C were evaluated. Surface area to extraction volume ratio was set to 6 $cm^2$ to 1 mL. The extracts were generated applying the qualified extractables approach developed by Sartorius. More details can be found in Pahl, I. et al. Development of a Standardized Extractables Approach for Single-Use Components - General Considerations and Practical Aspects. Bioprocess Int. 16, (2018) article.

Sterilization by gamma irradiation is considered as worst-case pre-treatment method generating additional extractables. Therefore, both type of tubing were irradiated at high dose (≥ 50 kGy) before extraction. The ethanol extracts were evaluated using the full set of orthogonal analytical methods. In addition, the headspace GC-MS data for the water extracts is provided since this analysis is not performed for ethanol. Expected extractables level in ethanol are provided as ranges in order to allow an easy assessment. The extractables found are classified to the most prominent substance classes and examples are provided for a representative selected individual extractable.

The comparability guide offers the possibility to obtain an overview of the expected extractables for the compared single-use products. It is recommended to use the Extractables Guide provided by Sartorius or safety assessment of the product and process qualification.

1. Pahl, I. *et al*. Development of a Standardized Extractables Approach for Single-Use Components - General Considerations and Practical Aspects. *Bioprocess Int*. **16**, (2018).

Table 1: Main selected extractables assigned as degradants polyolefin of the C-Flex® and Tuflux® TPE tubing; concentration levels: low < 5 ppm, medium 5-20 ppm, and high > 20 ppm and individual exact concentration in µg/mL

| Compounds | CAS | C-Flex® 374 | Tuflux® TPE | Analytical Methods |
|---|---|---|---|---|
| **Volatiles, for example** | | **low** | **low** | **HS GC-MS (water extract)** |
| *tert*-Butanol | 75-65-0 | 0.41 | 1.1 | |
| Acetone | 67-64-1 | 0.29 | 0.25 | |
| Acetaldehyde | 75-07-0 | 0.14 | 0.22 | |
| | | | | |
| **Saturated acids C1-C6, for example** | **-** | **medium** | **medium** | **IC, GC-MS** |
| Acetic acid | 64-19-7 | 11 | 6.6 | |
| | | | | |
| **Saturated acids C7-C18, for example** | **-** | **low-medium** | **low** | **GC-MS, LC-MS** |
| Octanoic acid | 124-07-2 | 7.7 | 1.4 | |
| Stearic acid | 57-11-4 | 1.2 | 1.4 | |
| | | | | |
| **Releasing agents, for example** | | **low** | **medium** | **GC-MS, LC-MS** |
| Erucamide | 112-84-5 | 0.18 | 4.8 | |
| | | | | |
| **Linear alkanes, for example** | **-** | **low-medium** | **low-medium** | **GC-MS** |
| Dodecane | 112-40-3 | 1.9 | 8.34 | |
| | | | | |
| **Linear ketones, for example** | | **low-medium** | **low-medium** | **GC-MS** |
| 2-Octanone | 111-13-7 | 4.4 | 3.99* | |
| | | | | |
| **Linear aldehydes, for example** | | **low** | **low** | **GC-MS** |
| Heptanal | 111-71-7 | - | 4.11 | |
| | | | | |
| **Hydrocarbons** | | 23,000 | 14,000 | **GC-MS, NVR** |
| **Elemental impurities** | | no elements detected as classified in ICH Q3D | | **ICP-OES/MS** |

* Grouped among alkyl ketones in Extractables guide

Table 2: Main selected extractables assigned as European and United States Pharmacopeia Plastic Additives and Degradants Thereof; concentration levels: low < 1 ppm, medium 5-20 ppm, and high > 20 ppm and individual exact concentration in µg/mL

| Compounds | CAS | C-Flex® 374 | Tuflux® TPE | Analytical Methods |
|---|---|---|---|---|
| **Antioxidants** | | **low-high** | **low-high** | **GC-MS, LC-UV\|MS** |
| Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate) | 6683-19-8 | 19 | 58 | |
| Octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate | 2082-79-3 | 3.0 | - | |
| 7,9-Di-*tert*-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione | 82304-66-3 | 38 | 29 | |
| 2,6-Di-*tert*-butylbenzoquinone | 719-22-2 | 15 | 24 | |
| Tris(2,4-di-*tert*-butylphenyl) phosphate * | 95906-11-9 | 14 | - | |
| Bis(2,4-di-*tert*-butylphenyl) phosphate (**bDtBPP**) * | 69284-93-1 | 42 | - | |
| **Light-stabilizer (HALS)** | | **-** | **low-medium** | **GC-MS, LC-MS** |
| 4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine | 52722-86-8 | - | 12 | |

* Degradants of the secondary antioxidant Tris(2,4-di-*tert*-butylphenyl) phosphite (trade name form example Irgafos® 168).

# 7. Cell-culture Trials

The aim is to assess whether the Tuflux® TPE tube is compatible with cell-growth (not inhibiting it) and then suitable to be used in bag systems or bioreactors for cell culture. The cell-culture trials are performed according to the scheme on Figure.

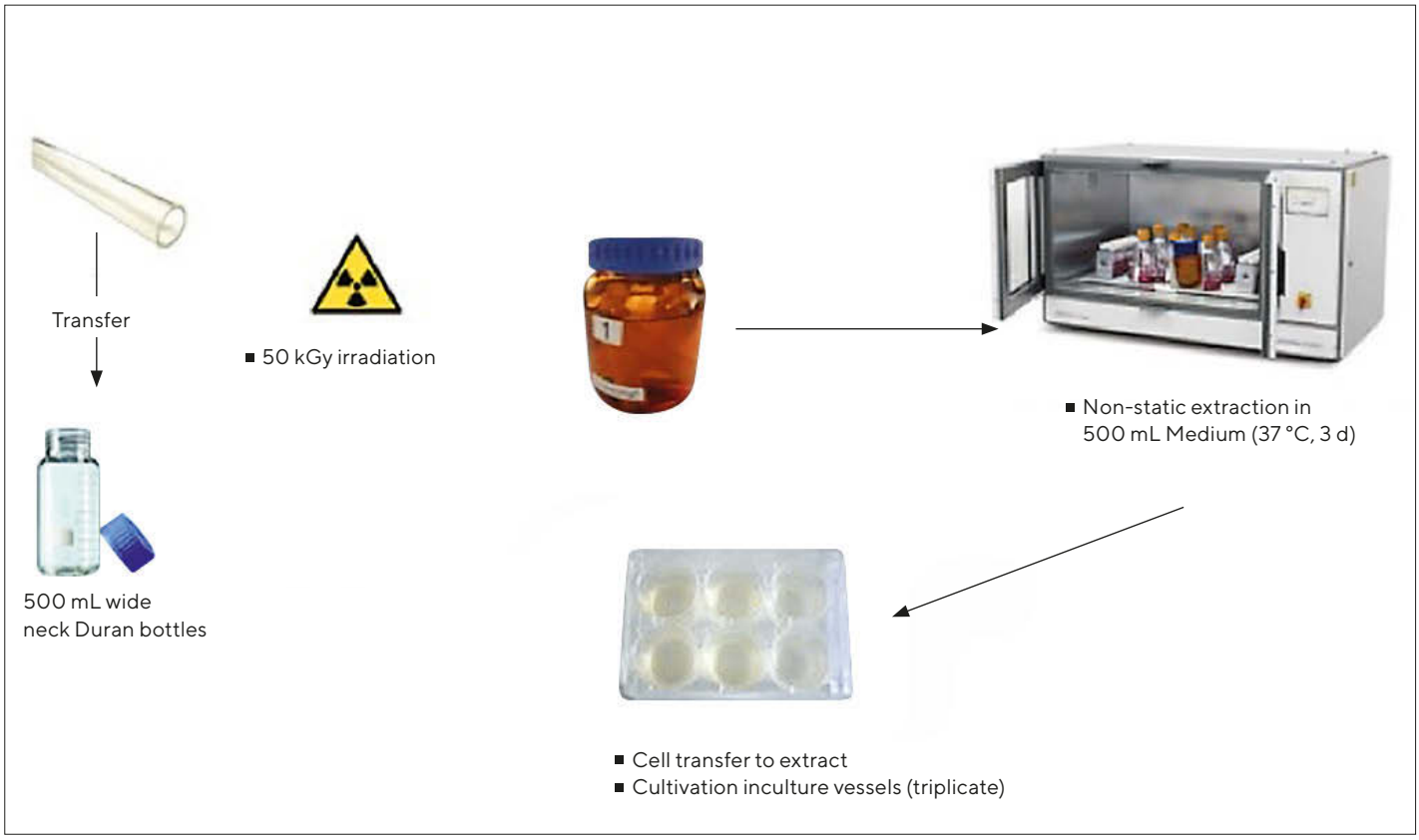
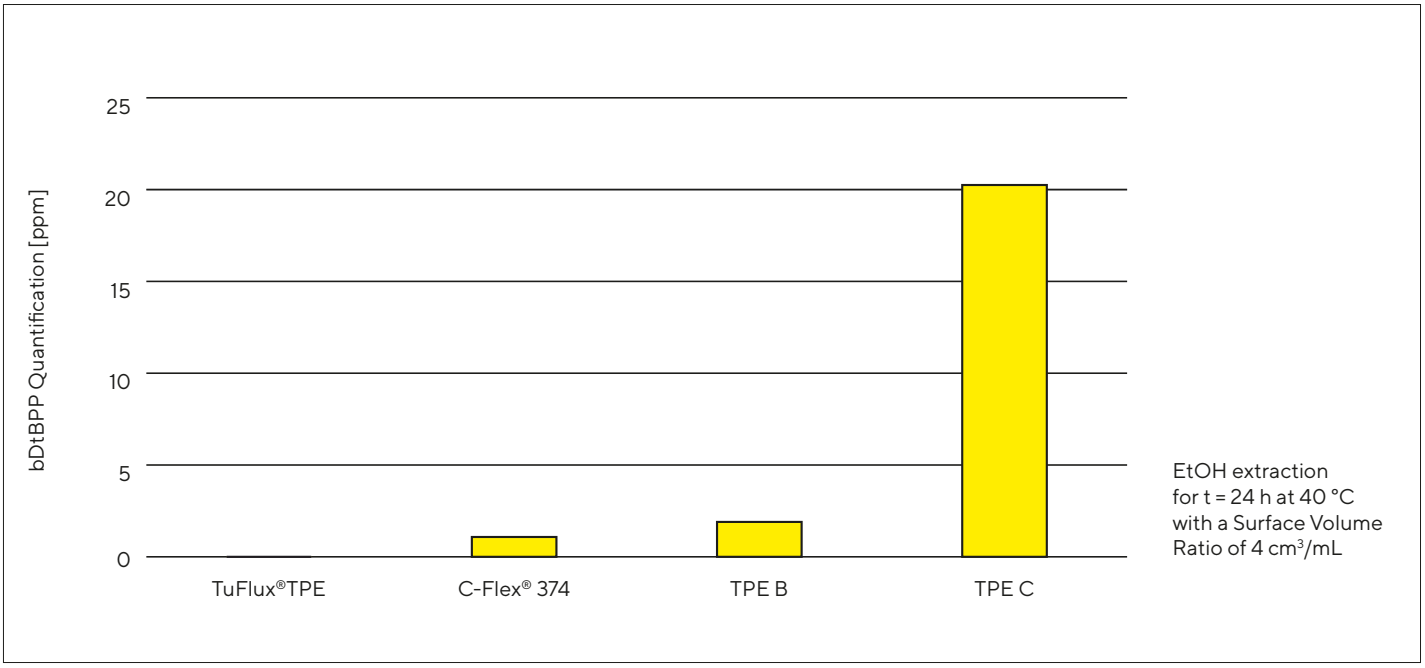


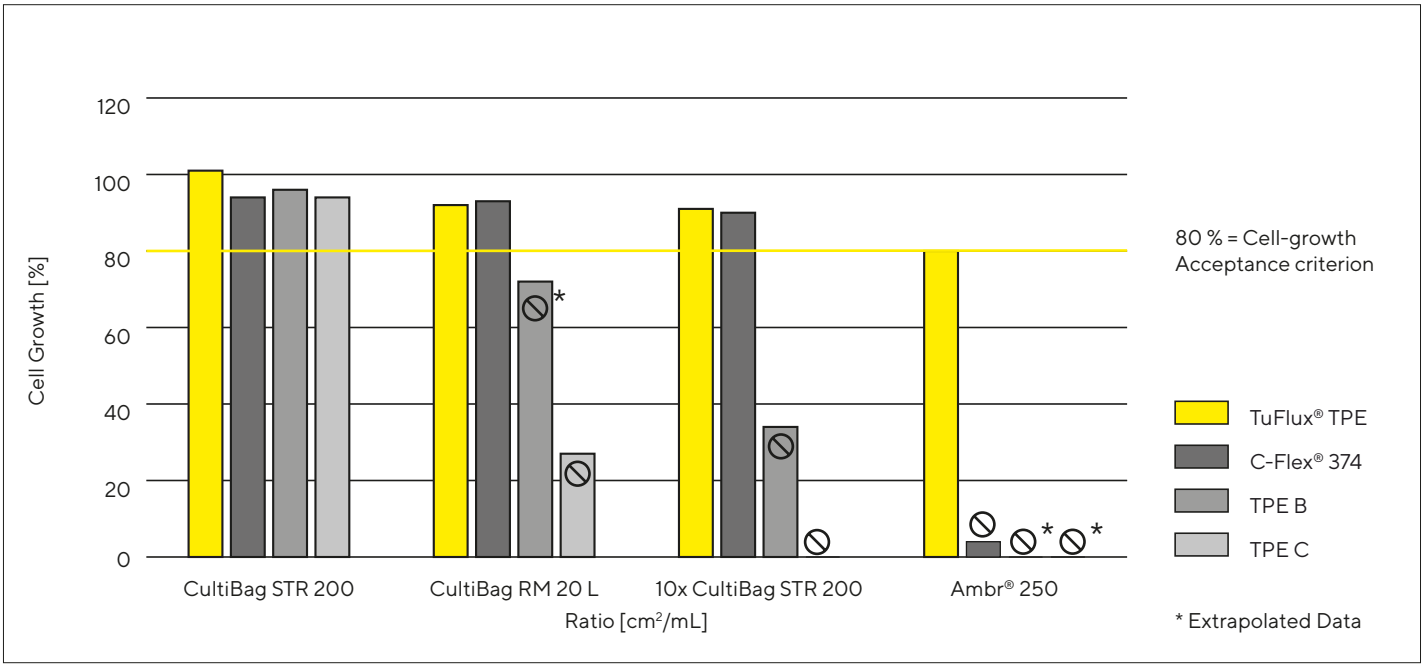

Figure 1: Cell-culture trial scheme

## Focus on additives – bDtBPP quantification




Tuflux® TPE: 1st bDtBPP free TPE tubing in pharmaceutical market

## Cell growth results for worst case sv conditions for different bioreactor scales after 50 kGy irradiation




The higher the bDtBPP content, the less cell viablility when increasing the S/V ratio

**Evaluation and conclusion**

Both tubing are thermoplastic elastomers based on polyolefin. Additional hydrocarbons are added to optimize the physical properties for example to obtain flexibility or to optimize the welding performance. Typical extractables from such materials include mainly compounds linked to stabilizers and the polyolefin material itself. In addition, degradants induced by gamma irradiation such as small organic acids from the polyolefin can be expected. Table 1 summarizes extractables that are typically released by the polyolefin material. The number and quantity of extractables is comparable for both tubing. They typically involve individual hydrocarbons such as dodecane but also relevant amounts of high molecular weight homologues. Organic acids formed during gamma sterilization are for example acetic or octanoic acids. Releasing agent such as Erucamide were also identified and are identical for both tubing.

Several plastic additives – all described in respective USP <661.1> and EP chapter 3.1.13 – and there known degradants were confirmed to be present in the extracts. They are summarized in Table 2. Uniquely for C-Flex® 374 tubing, significant levels of degradants were found of the secondary antioxidant Tris(2,4-di-*tert*-butylphenyl) phosphite (trade name for example Irgafos® 168) including **bDtBPP**. On the other hand, degradants pointing to the polymeric light-stabilizer Poly(4-hydroxy-2,2,6,6-tetramethyl-1-piperidine ethanol-*alt*-1,4-butanedioic acid), trade name Tinuvin® 622, were present in the extracts of Tuflux® TPE. This stabilizer is an approved USP and EP plastic additive. European Medicines Agency states that "... a toxicological data may not be required... (Appendix I and II)" for such approved additives.[2]

In summary, the overall extractable profiles of the C-Flex® 374 and Tuflux® TPE tubing could be considered comparable. The main organic content are hydrocarbons and hydrocarbon-related extractables released by the polyolefin. They differ in their composition because of the use of well-defined synthetic hydrocarbons for Tuflux® TPE compared to naturel paraffin-based hydrocarbons for C-Flex®. The main differences in the additive package are degradants of the secondary antioxidant Irgafos® 168 for C-Flex® 374 compared to molecules from a light-stabilizer present in Tuflux® TPE only.

2. European Medicines Agency (EMEA), Guideline on plastic immediate packaging materials. (2005).

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