Repligen TPE Tubing – Validation Report

Introduction

Purpose of this validation report

The Validation Report presents product information for TPE Tubing from Repligen. As an engineer, scientist, or manufacturer, you may need this information to guide your validation activities, including process development, writing validation protocols, and scaling up systems.

This Validation Report covers all TPE tubing.

Repligen is committed to providing all relevant technical, manufacturing, and quality information, however, only non-confidential information is presented in this document. Confidential details may be made available upon request through a formal confidentiality agreement or as part of a supplier audit.

Where to get help

If you need to know more about the silicone tubing, contact the technical support team at Repligen. The technical support team includes scientists and engineers that can:

a. Answer your technical questions.
b. Assist in the in the selection of silicone tubing.
c. Provide user training programs.

To obtain support, contact your local Repligen sales representative or our customer support team.

Customer Support
customerserviceUS@repligen.com
518-406-5276

Repligen Corporation
1 Fairchild Sq, Ste 11
Clifton Park, NY 12065
www.repligen.com

Quality documentation

Quality policy

Copies of the Repligen quality policy and ISO certificate can be found at repligen.com/resources.

To meet the needs of GMP manufacturing, these products are manufactured in the USA under the following quality standards:

- Repligen maintains an ISO 9001-compliant Quality Management System that is currently certified by TUV Americas and BSI, a copy of the current ISO certifications can be downloaded from repligen.com/resources.
- All materials in the direct fluid contact path meet USP Class VI, and USP <88> requirements for *in vivo* Biological Reactivity.
- All fluid contact components are free from materials of animal origin or compliant with EMA 410/01 Rev 3.

Animal byproduct free

Repligen TPE Tubing is manufactured using materials for production that do not include ingredients of animal origin. Our products do not come in contact with animal product during storage and transportation.

ISO and USP Class VI statements

ISO Statement: Product is manufactured in compliance with Repligen ISO 9001 certified Quality Management System.

USP Class VI Statement: All flow path materials meet USP Class VI biosafety requirements.

Product information

Repligen TPE Tubing is designed to deliver fluids in a variety of bioprocessing applications and offers a safe, clean and durable solution for fluid transfer. Fully compatible with commercial tube welding, sealing equipment, and existing heat sealable tubing like C-Flex® 374, TPE is a translucent weldable and heat sealable flexible thermoplastic elastomer tubing. Sterilization methods include gamma irradiation and autoclaving. Testing includes welding and sealing compatibility, USP Class VI, and extractables per the April 2020 Biophorum (formerly BPOG) document, "Extractables testing Of Polymeric Single-Use Components Used In Biopharmaceutical Manufacturing". TPE Tubing is produced exclusively for Repligen in an ISO-7 cleanroom environment.

Product features

- Sizes from 1/8” to 1” ID
- Sterile weldable and heat sealable
- Animal-derived component free
- Certified to meet:
    1. USP Biological Reactivity Tests, In Vivo USP Class VI.
    2. ISO 10993-11 guidelines for the Systemic Injection Test.
    3. ISO 10993-10 guidelines for the Intracutaneous Reactivity Test.
    4. Classified as nonreactive according to the ISO 10993-6 guidelines for the Implantation Test.
    5. Non hemolytic per ISO 10993-4, ASTM F756.
    6. All analytical testing conducted post-gamma irradiation > 45 kGy.
- Tested for extractables per the April 2020 Biophorum (formerly BPOG) document, "Extractables testing Of Polymeric Single-Use Components Used In Biopharmaceutical Manufacturing.” (Post gamma irradiation > 45 kGy)
- Sterilization methods:
    1. Gamma irradiation up to 45 kGy
    2. Autoclave 121° C for 60 minutes
- Translucent with minimal color change post-irradiation
- Tube welding and tube sealing with C-Flex® 374 settings
- Compatible with C-Flex® 374 and AdvantaFlex® TPE tubing
- Operating pressures equal or greater than other weldable TPE tubing in the industry
- Improved performance in peristaltic pumps over other weldable TPE tubing
- Produced in an ISO-7 cleanroom

Table 1. Physical property data

Test	Standard	TPE
Specific Gravity	ASTM D792	0.89
Durometer, Shore A	ASTM D2240	63
Tensile Strength, PSI	ASTM D412	1230
Elongation, %	ASTM D412	800
Tensile Modulus @ 100%, psi	ASTM D412	297
Tensile Modulus @ 300%, psi	ASTM D412	548
Compression Set (22 hrs @ 70°C), %	ASTM D395B	27
Brittle Point, °C	ASTM D746	-66
Tear Strength lbf/in	ASTM D624B	197
Min Temperature, °F	Internal	-50
Max Temperature, °F	Internal	275

Properties tested post gamma irradiation (28 - 38 kGy).

Table 2. Repligen TPE Tubing ordering information

Part number	Nominal tubing size	Inner diameter		Outer Diameter		Nominal Wall Thickness (Inches)
RT125-250	1/8” x 1/4”	0.125”	3.18 mm	0.25”	6.35 mm	0.0625”
RT250-375	1/4” x 3/8”	0.25”	6.35 mm	0.375”	9.53 mm	0.0625”
RT250-437	1/4” x 7/16”	0.25”	6.35 mm	0.438”	11.11 mm	0.0938”
RT250-500	1/4” x 1/2”	0.25”	6.35 mm	0.50”	12.70 mm	0.125”
RT375-625	3/8” x 5/8”	0.375”	9.53 mm	0.625”	15.88 mm	0.125”
RT500-750	1/2” x 3/4”	0.50”	12.70 mm	0.75”	19.05 mm	0.125”
RT625-875	5/8” x 7/8”	0.625”	15.88 mm	0.875”	22.23 mm	0.125”
RT750-1000	3/4” x 1”	0.75”	19.05 mm	1.00”	25.40 mm	0.125”
RT750-1125	3/4” x 1.125”	0.75”	19.05 mm	1.125”	28.58 mm	0.1875”
RT1000-1375	1” x 1.375”	1.00”	25.40 mm	1.375”	34.93 mm	0.1875”

Typical applications

- Single-use assemblies
- Buffer and media preparation
- Bioreactor processes
- Fluid sampling
- Pharmaceutical production
- Filtration
- Aseptic welding and sealing connections

Weld testing

Tested with various commercially available tube welders and sealers, Repligen TPE Tubing is weldable and sealable with C-Flex® 374 settings (where applicable):

Tube welders

- Terumo SCD® IIB Sterile Tube Welder
  - 1/8” x 1/4” OD tubing size only
  - No thermal settings or adjustments
- Sartorius BioWelder® Total Containment
  - Sizes from 1/8” x 1/4” up to 3/4” x 1”
  - Settings for C-Flex® 374 pre-programmed per tubing size

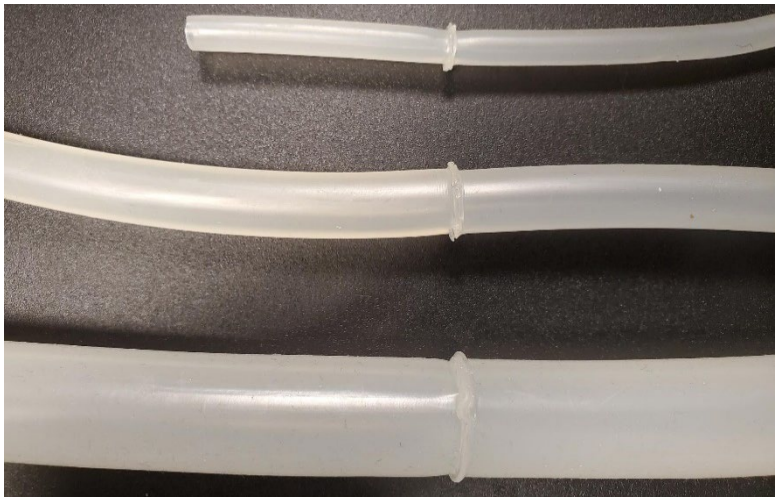

Figure 1. Tube weld example

Tube sealer

Cytiva Hot Lips Tube Sealer™

- Sizes from 1/8” x 1/4” up to 3/4” x 1”
- Settings for C-Flex® 374 pre-programmed per tubing size

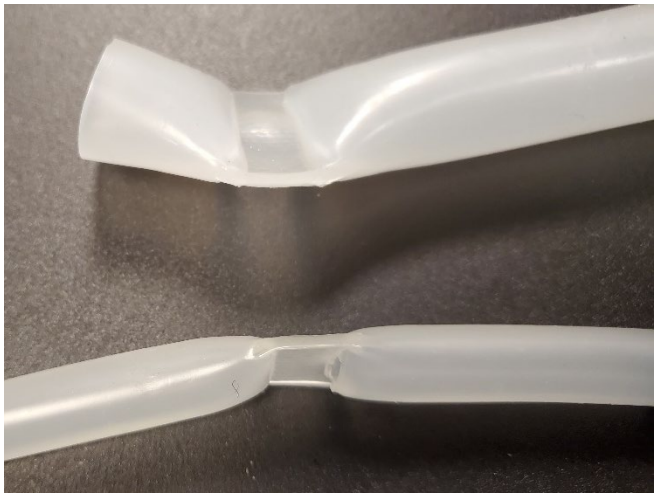

Figure 2. Cytiva Hot Lips Tube Sealer™ example

Weld testing

Weld testing measured pull force, burst pressure and tensile properties of Repligen TPE Tubing welded to itself or to C-Flex® 374 or AdvantaFlex® tubing. Tubing was conditioned as follows: tubing as is; gamma-irradiated to a standard dose (28 - 38kGy); and autoclaved at 121° C for 80 minutes. Gamma irradiation was both the worst-case condition and most common workflow form for welding. In all cases, Repligen TPE outperformed other tubing when welded to itself or to competitive tubing while using C-Flex® 374 settings. High gamma irradiation (> 45 kGy) was performed on selected assemblies to confirm performance.

Burst testing

Welded samples, approximately five inches long, were secured to a regulated pressurized source and clamped off. Pressure was increased approximately 0.2 psi per second until tubing slipped off the barb or burst. The maximum pressure was recorded. For burst testing with Repligen TPE Tubing welded to C-Flex® 374, the failure point was in the C-Flex® 374 and not at the weld or the TPE (Figure 4).

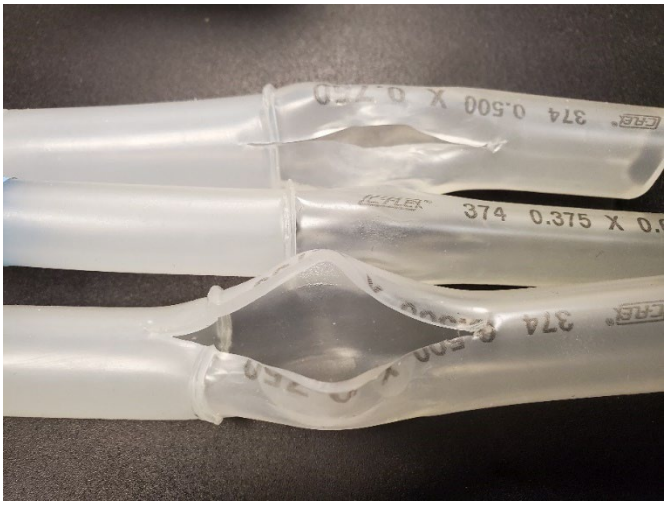

Figure 3. Burst tubing example

Figure 4. Burst away from weld example

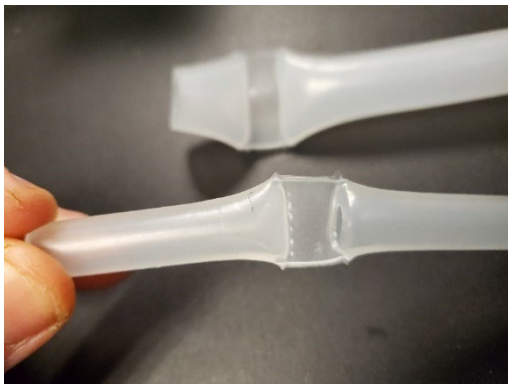

Figure 5. Tube seal burst example

Table 3. Tube sealer burst testing results

Tubing size	Tube sealer burst results (psi)[1]
1/8” x 1/4”	101
1/4” x 7/16”	72
1/4” x 1/2”	68
3/8” x 5/8”	65
1/2” x 3/4”	60
5/8” x 7/8”	48
3/4” x 1”	44
1” X 1.375”	N/A[2]

[1] Samples gamma irradiated to 28 - 37 kGy.
[2] Commercial tube welders not available for 1” x 1.375” size.

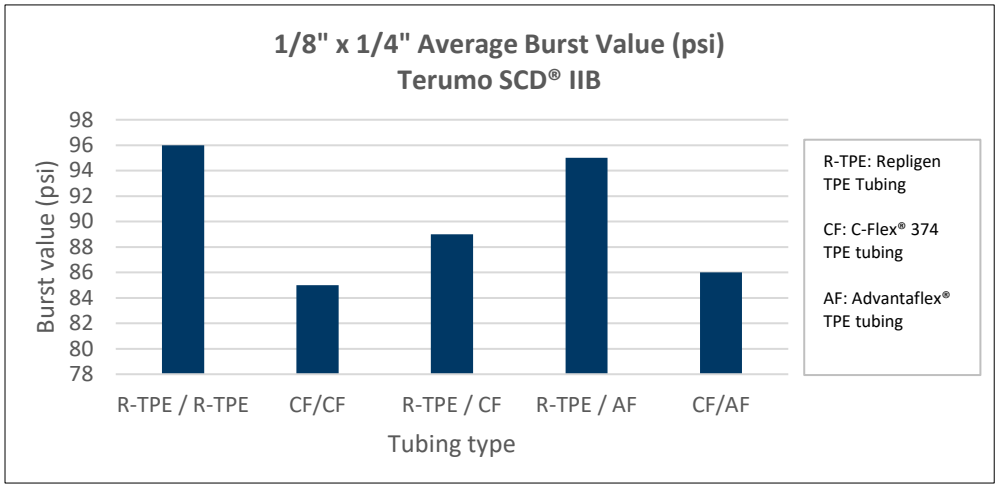

Figure 6. Average burst value Terumo SCD® IIB (⅛” x ¼”)

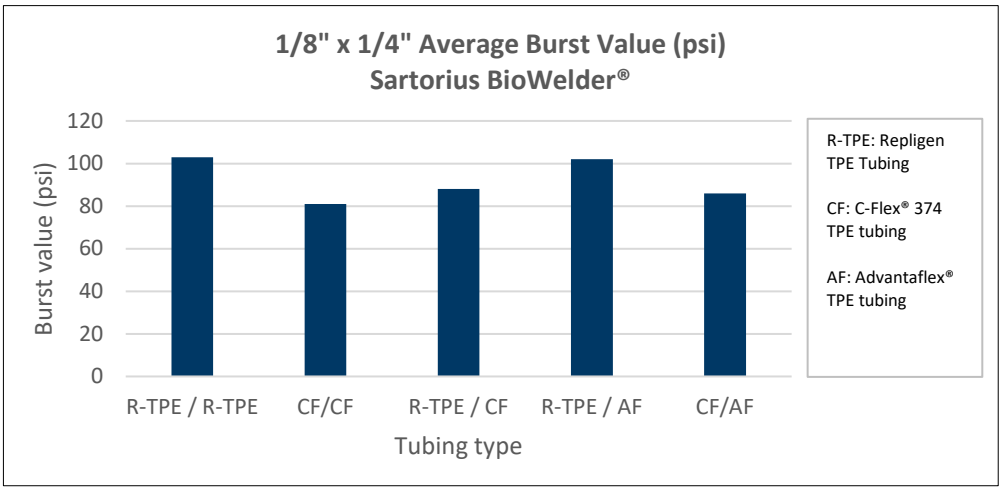

Figure 6. Average burst value Sartorius BioWelder® (⅛” x ¼”)

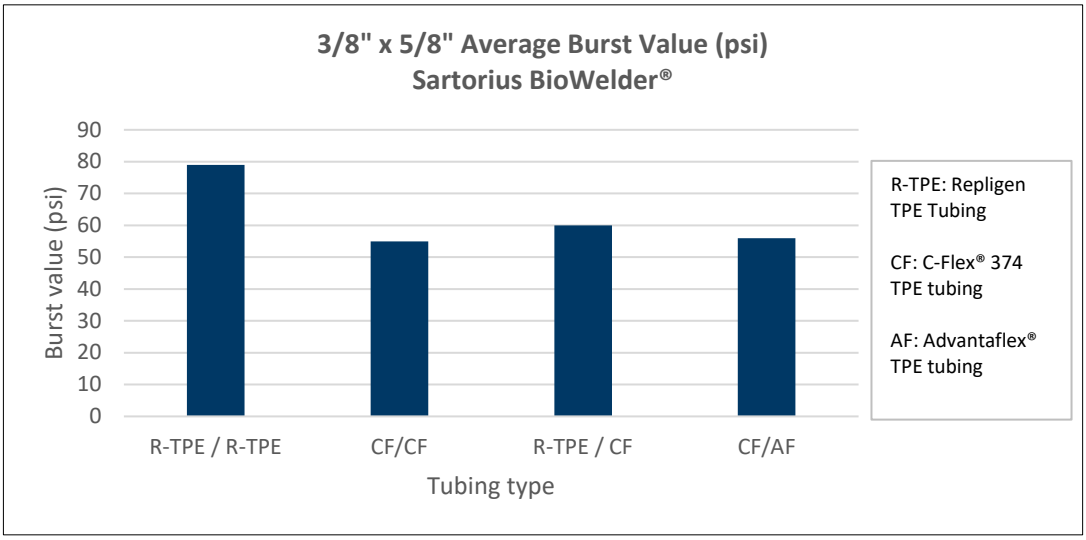

Figure 7. Average burst value Sartorius BioWelder® (3/8” x 5/8”)

Pull testing

Samples from each of four lots were secured into a universal tensile testing machine with mechanical jaws. Test speed of 1 m (39 inches) per minute was used. Maximum force, elongation and failure mode were recorded.

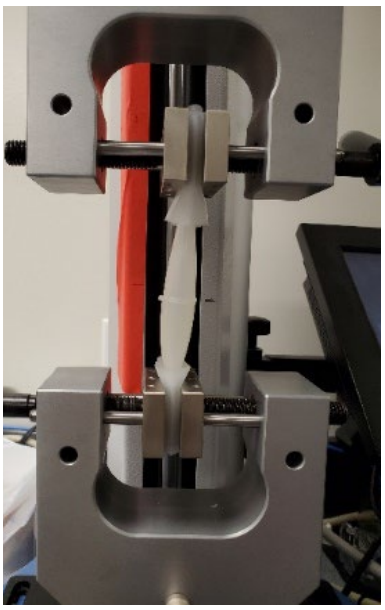

Figure 8. Pull testing example

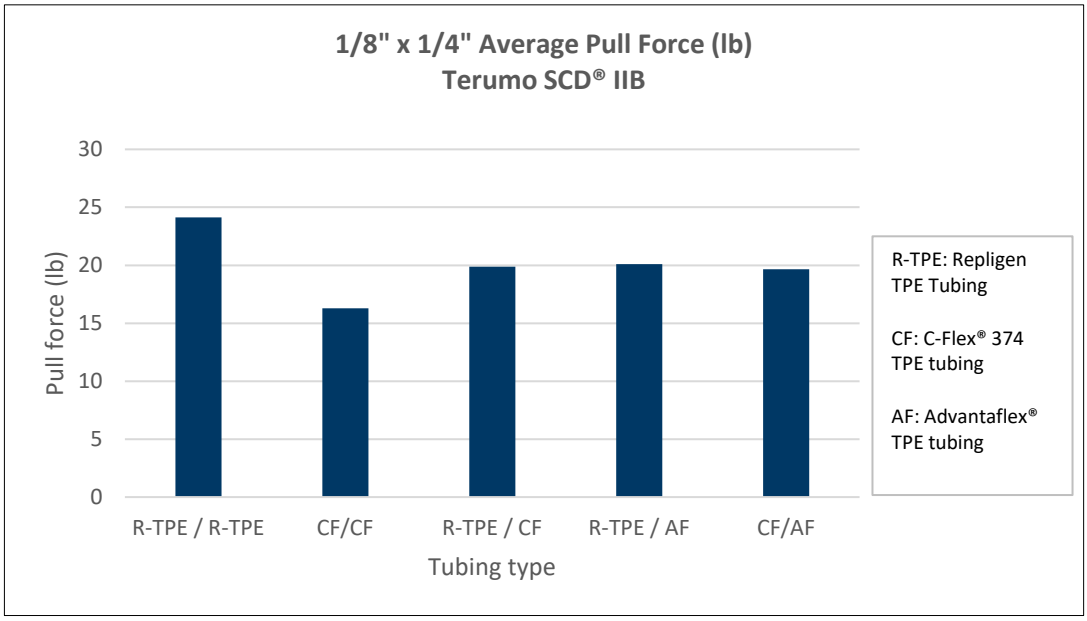

Figure 9. Average pull force Terumo SCD® IIB (⅛" x ¼")

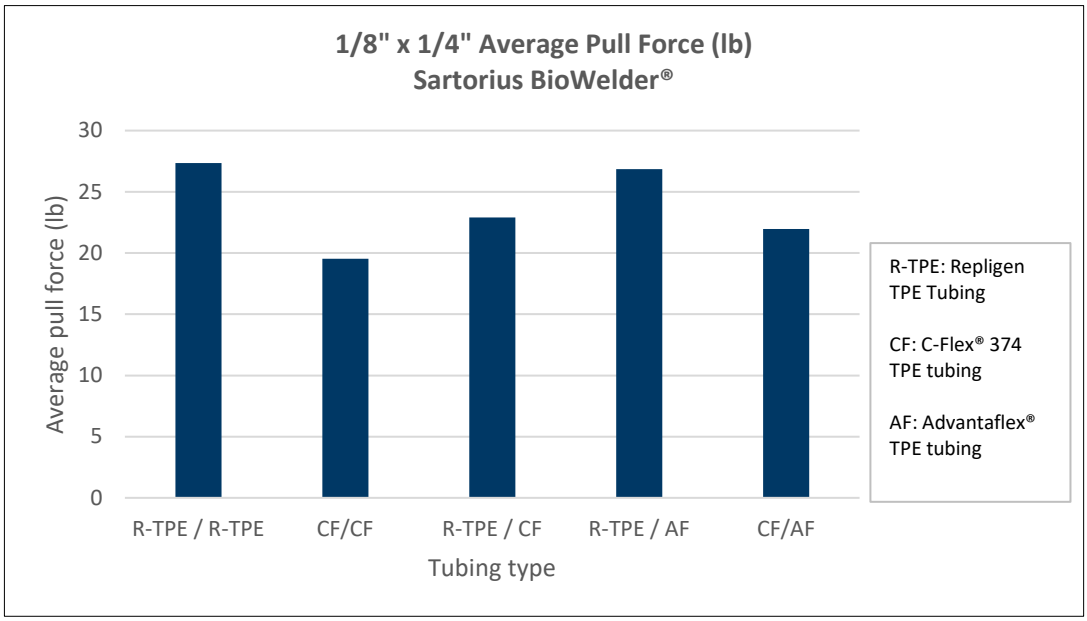

Figure 10. Average pull force Sartorius BioWelder® (⅛" x ¼")

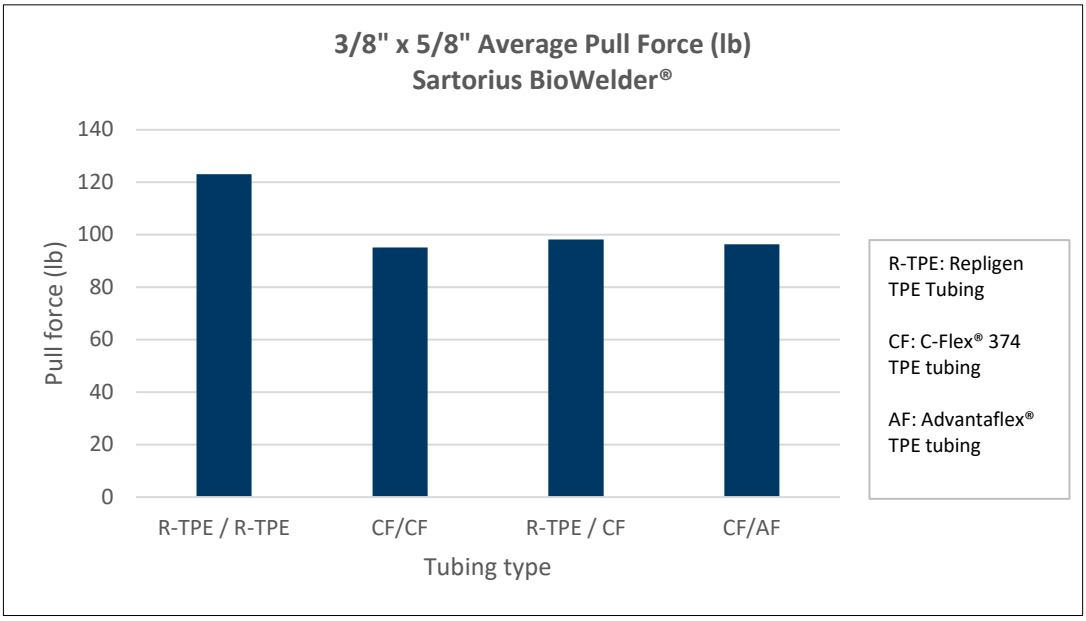

Figure 11. Average pull force Sartorius BioWelder® (3/8” x 5/8”)

Peristaltic pump testing and ratings

Repligen TPE Tubing demonstrated improved performance in peristaltic pumps when compared to other thermoplastic elastomer tubing. Pump performance is similar with non-irradiated and gamma-irradiated samples. Repligen TPE Tubing was placed in Masterflex® I/P and Masterflex® L/S peristaltic pumps set to the maximum of 600 rpm and tested to failure. Wi-Fi-enabled moisture sensors detected moisture outside of the pump and container. Repligen TPE Tubing withstood 96 hours of testing without failure, whereas C-Flex® 374 failed prior to 96 hours.

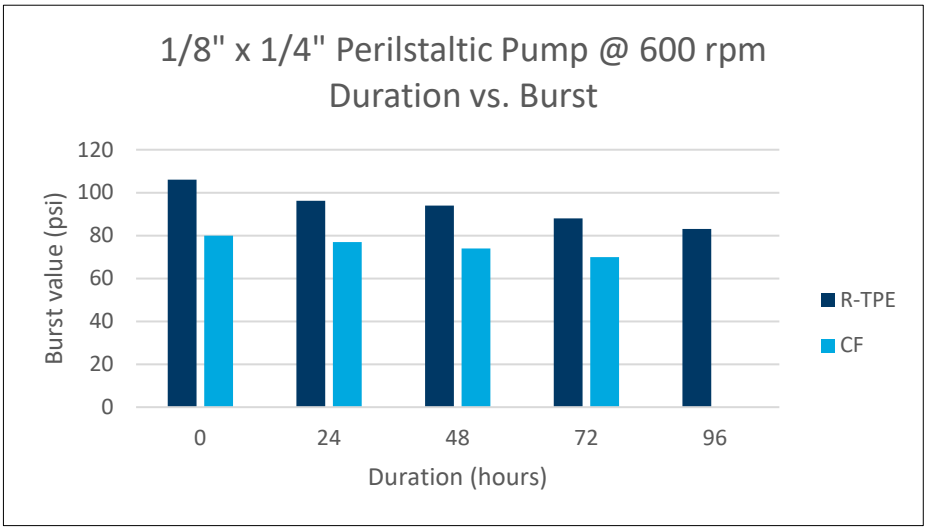

Figure 12. 96-hour duration testing (⅛” x ¼”)

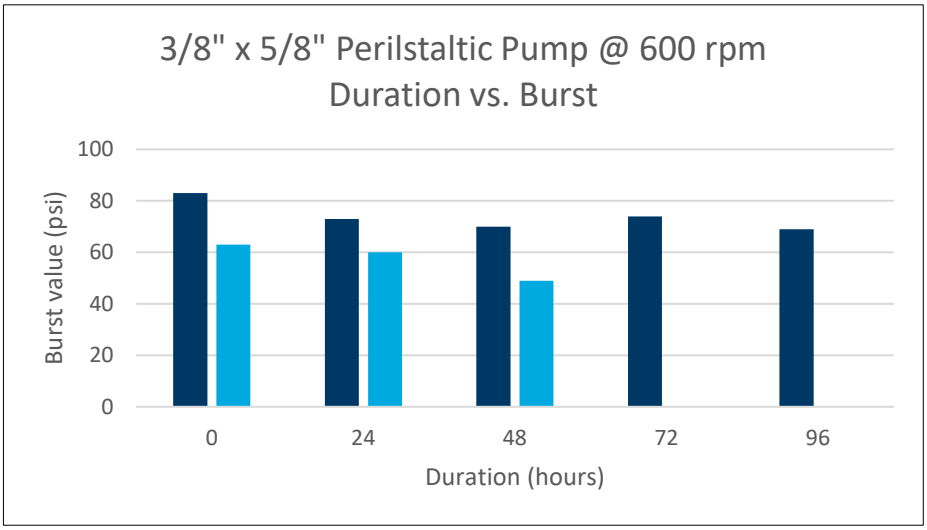

Figure 13. 96-hour duration testing (3/8” x 5/8”)

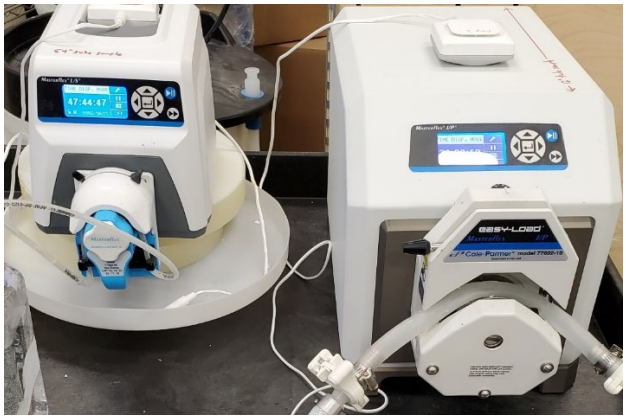

Figure 14. Peristaltic pumps

Table 4. Peristaltic pump ratings

Tubing size	Pump size	Maximum duration	Maximum
1/8” x 1/4”	16	24 hours	650 rpm
1/8” x 7/16”	24		600 rpm
1/4” x 1/2”	26		
3/8” x 5/8”	73		
1/2” x 3/4”	82		500 rpm

Burst testing results and operating pressure

Unwelded samples, approximately 5 inches long, were secured to a regulated pressurized source and clamped off. Pressure was increased approximately 1 psi every 5 seconds until tubing slipped off the barb or burst. The maximum pressure was recorded. Similar results were observed with non-irradiated and gamma-irradiated samples. To determine the operating pressure, a safety factor of ≥ 3X was utilized to ensure proper operation. Pressure ratings were comparable to other thermoplastic elastomer tubing in the industry. In all cases, Repligen TPE Tubing was not rated for high pressure applications.

Table 5. Burst test data

Tubing size	Burst pressure results (psi)	Repligen TPE Tubing operating pressure (psi)
1/8” x 1/4”	106	30
1/4” x 7/16”	89	25
1/4” x 1/2”	113	30
3/8” x 5/8”	83	25
1/2” x 3/4”	65	20
5/8” x 7/8”	54	17
3/4” x 1”	51	17
1” x 1.375”	53	17

Samples irradiated to 29 - 38 kGy.

Table 6. Chemical compatibility

Chemical compatibility guidance	
Strong and weak acids	Acceptable
Strong and weak bases	Acceptable
High purity water	Acceptable
Organic solvents	Not recommended
Alcohols	Not recommended

Repligen TPE Tubing product development

Numerous thermoplastic elastomer formulations were evaluated, compounded, trialed, and tested during product development. Strict criteria were set forth to ensure Repligen TPE Tubing is not only compatible with existing tubing and equipment, but also exhibits optimum performance. Once the final formulation was determined five discrete lots of tubing covering the entire size range were produced and tested.

Raw material and manufacturing environment

TPE Tubing is produced exclusively for Repligen in an ISO-7 environment. The tubing manufacturing process has been qualified via IQ/OQ/PQ qualification.

Repligen conducts extractables testing to the most current industry standards available at the time of sample submission. Repligen TPE Tubing submitted in Q3 2021 was tested for extractables per the April 2020 Biophorum (formerly BPOG) document, "Extractables testing Of Polymeric Single-Use Components Used In Biopharmaceutical Manufacturing.”

Plastic materials have been used in the manufacturing of therapeutics for many decades. Over the last 15 years, focused product development by vendors and biotechnology companies resulted in a plethora of single-use technologies. During this time, the industry witnessed the development and adoption of critical disposable and single-use technologies like mixers, bioreactors, filters, and connectors. As a result, standards and best practices for evaluating component safety have been set for the selection and qualification of plastics. In general, plastics used in biopharmaceutical manufacturing have low defined extractables and have been determined to be non-toxic at equivalent therapeutic doses. Many base standards used for guidance have been set by regulatory

publications, including USP, CFR 21, and EMEA. These basic standards have been elaborated on by industry organizations like the Bio-Process Systems Alliance (BPSA) and Parenteral Drug Association (PDA) as well as product manufacturers through the publication of best practices of testing and assessment of data. In addition, end-user therapeutic manufacturers have become more demanding in their analytical requirements, assessment of data, determination of risk, and minimum threshold for meeting internal standards.

Repligen is sensitive to the demands of the industry and will therefore supply relevant and applicable information about the plastics used in the product contact components of Repligen TPE Tubing.

Extractables: Chemical compounds that migrate from any product-contact material when exposed to an appropriate solvent under exaggerated conditions of time and temperature.

Leachables: A subset of extractables that migrate into a drug formulation from any product contact material because of direct contact under normal process conditions.
There is a consensus that it is the responsibility of the product technology vendor to provide an extractable data package. Recently, there have been efforts to standardize the testing procedures for extractables; wherever possible. It is Repligen's intent to comply with standardization efforts. Additionally, in compliance with CFR 21, Part 211.65, Repligen TPE Tubing is designed such that all product contact materials are not reactive, additive or absorptive so as to alter the safety, identity, strength, quality or purity of the drug product beyond established requirements.

Using the BPSA guidance as a backdrop, Repligen has designed an extractables program to produce a robust data package by conducting extractables testing using four solvents as well as exaggerated time and temperature conditions. Leachables, however, are considered process specific and the responsibility of the end user to define within specific process parameters.

Biocompatibility & Extractables Testing Summary Of Repligen TPE

For biocompatibility & extractables testing, Repligen submitted size 1/2" x 3/4" TPE tubing and irradiated to > 45kGy to facilitate a worst case scenario. Repligen TPE Tubing was tested for extractables per the April 2020 Biophorum (formerly BPOG) document, "Extractables testing Of Polymeric Single-Use Components Used In Biopharmaceutical Manufacturing."

Biocompatibility testing, all passed:

- USP Biological Reactivity Tests, In Vivo (USP Class VI)
- ISO 10993-11 Systemic Injection Test
- ISO 10993-10 Intracutaneous Reactivity Test
- ISO 10993-6 Implantation Test
- Non hemolytic per ISO 10993-4, and ASTM F756

The purpose of the extractables study was to generate a supplier-sponsored extractables profile for Repligen TPE Tubing, specifically for the conditions established in this study. Such an assessment includes the detection, identification and quantitation of compounds or entities that can be extracted from the tubing under the conditions presented. The tubing was extracted by submerging a segment in one of four extraction solvents (Table 7) and incubating at 40 ± 3° C with continuous agitation at 50 rpm for 24 ± 4 hours, 21 ± 1 days and 70 ± 3 days Timepoints. The sample-to-solvent ratio of 5 cm^2 of sample/mL of extraction solvent was achieved. The sample extracts were analyzed for volatile organic extractables by headspace GC-MS, semi-volatile organic extractables by direct injection GC-MS, non-volatile organic extractables by UPLC-PDA-QToF (both APCI and ESI), and inorganic extractables (except 50% EtOH extracts) by ICP-MS. The analytical techniques employed provide a comprehensive strategy for establishing an extractables profile of Repligen TPE Tubing.

The organic extractables discovered with estimated concentrations ≥ 0.1 µg/mL are summarized (Table 9, Table 10).

Table 7. Extraction solvents

Extraction solvent	Description
WFI	Water for injection, reagent grade or higher
0.5 N NaOH	0.5 N sodium hydroxide solution, reagent grade or higher
0.1 M H_3PO_4	0.1 M phosphoric acid in water, reagent grade or higher
50% EtOH	50:50 ethanol: water (v/v), HPLC grade or higher

Timepoint

- 1 day (24 hours)
- 21 Days
- 70 Days

Table 8. Count of extractables at or above reporting threshold per solution

Extraction solvent	Volatile organic extractables	Semi-volatile organic extractables	Non-volatile organic extractables	Inorganic extractables
WFI	3	3	1 (ESI)	5
0.5 N NaOH	4	6	10 (APCI); 2 (ESI)	4
0.1 M H_3PO_4	3	3	1 (APCI); 3 (ESI)	8
50% EtOH	2	8	27 (APCI); 39 (ESI)	0

Table 9. Volatile organic extractables summary

Solvent	RT, minutes	Identification	Cas #	µg/cm²		
				24 Hr.	21 Day	70 Day
WFI	1.73	Acetone	67-64-1	< LOD	< LOD	0.207
	1.81	Isopropyl alcohol	67-63-0	0.078	< LOD	< LOD
	2.06	2-Methyl-2-propanol	75-65-0	< LOD	0.176	0.169
0.5N NaOH	1.73	Acetone	67-64-1	< LOD	0.087	0.091
	1.81	Isopropyl alcohol	67-63-0	< LOD	0.153	0.567
	2.06	2-Methyl-2-propanol	75-65-0	< LOD	0.149	0.162
	4.54	1,1-Diethoxy-ethane	105-57-7	< LOD	0.135	< LOD
0.1 M H_3PO_4	1.73	Acetone	67-64-1	< LOD	< LOD	0.078
	1.81	Isopropyl alcohol	67-63-0	0.096	3.242	0.165
	2.06	2-Methyl-2-propanol	75-65-0	< LOD	0.248	0.447
50% EtOH	4.54	1,1-Diethoxy-ethane	105-57-7	< LOD	< LOD	0.325
	13.55	1,3-Bis(1,1-dimethylethyl)-benzene	1014-60-4	0.061	0.06	0.032

Table 8. Semi-volatile organic extractables summary

Solvent	RT, minutes	Identification	Cas #	µg/cm²		
				24 Hr.	21 Day	70 Day
WFI	4.49	2-Methyl-cyclopentanone	1120-72-5	< LOD	< LOD	0.032
	7.39	Unknown containing phenyl	N/A	< LOD	< LOD	0.022
	9.91	N-Cyclohexyl formamide	766-93-8	< LOD	< LOD	0.025
0.5N NaOH	2.48	1,1-Diethoxy ethane	105-57-7	< LOD	0.031	< LOD
	4.49	2-Methyl-cyclopentanone	1120-72-5	< LOD	< LOD	0.047
	7.39	Unknown containing phenyl	N/A	< LOD	< LOD	0.024
	11.83	4-(1,1-Dimethylpropyl)-phenol	80-46-6	< LOD	0.028	0.023
	13.28	2,4-bis(1,1-Dimethylethyl)-phenol	96-76-4	0.075	0.081	0.092
	16.21	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1620-98-0	0.031	0.099	0.084
0.1 M H_3PO_4	2.23	tert-Butyl hydroperoxide	75-91-2	< LOD	0.03	0.027
	3.2	2,2-Dimethyl propanoic acid	75-98-9	< LOD	0.083	0.096
	4.49	2-Methyl-cyclopentanone	1120-72-5	< LOD	< LOD	0.026
50% EtOH	2.58	Acetic acid	64-19-7	< LOD	0.031	0.059
	9.94	1,3-Bis(1,1-dimethylethyl)-benzene	1014-60-4	0.21	0.209	0.207
	10.05	Unknown acid	N/A	< LOD	< LOD	0.061
	13.28	2,4-bis(1,1-Dimethylethyl)-phenol	96-76-4	0.476	1.144	1.015
	16.21	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1620-98-0	< LOD	< LOD	0.023
	17.79	7,9-di-tert-Butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	82304-66-3	0.051	0.105	0.115
	18.41	Unknown	N/A	< LOD	< LOD	0.025
	19.51	N-Methyl-1-octadecanamine	2439-55-6	< LOD	0.063	0.057