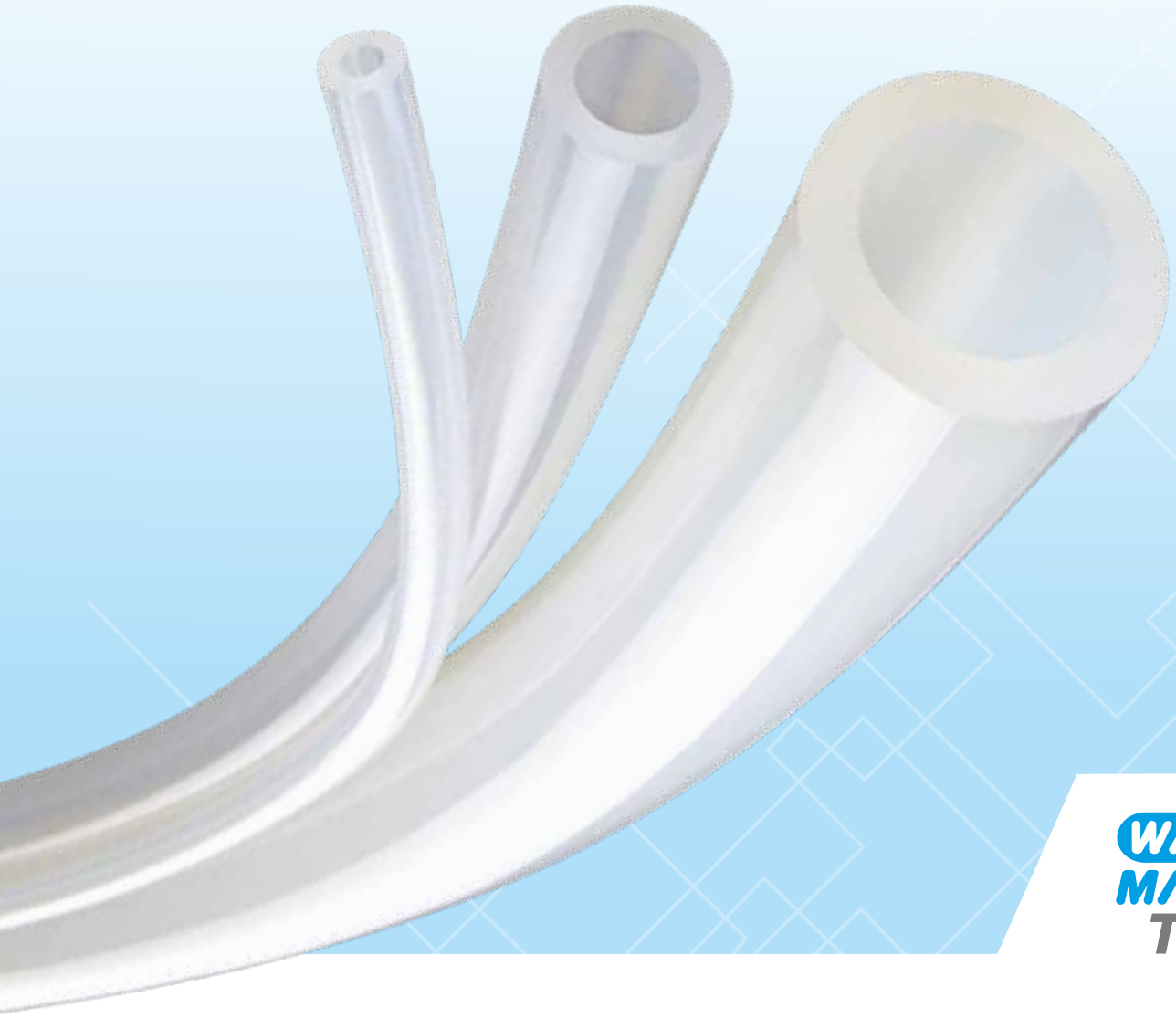
WATSON MARLOW Fluid Technology Solutions



WATSON MARLOW Tubing

A study of the effect of post-curing on the reduction of cyclosiloxanes contaminates in biopharmaceutical products

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“15% of the drug recalls reported by the FDA in the last six months have been due to contamination from processing equipment”

Executive summary

Drug manufacturers are increasingly concerned about the sources of potential contamination from processing equipment. To give a sense of the scale of this problem, 15% of the drug recalls reported by the FDA in the last six months have been due to contamination from processing equipment.

A primary area of concern is the direct contact of plastic processing materials that comprise single-use components with the drug product. It is widely known that this can result in the presence of compounds which may affect the efficacy, safety or quality of the final drug. For manufacturers working on multi-million dollar drug trials and treatments, the implications of this from a product development and brand integrity perspective, are significant.

Focus must therefore be given to the process used to manufacture equipment and ancillaries. Specifically, the manner in which platinum-cured silicone tubing is manufactured must be given attention: the process used to manufacture this tubing has the potential to affect the types of leachables which may migrate into the final product.

Herein, Watson-Marlow Fluid Technology Solutions (WMFTS), fluid path solutions provider of peristaltic pumps and single-use component, outlines the results of a study which makes comparisons between the semi volatiles extractables profile of post-cured and non post-cured samples of its Pumpsil platinum-cured silicone tubing.

At WMFTS, as part of the manufacturing of the Pumpsil silicone tubing, there is an additional post-curing step to the tubing production process. This additional step ensures more cross linking that not only makes the tubing mechanically stronger, but reduces the semi volatile compounds. This extra processing step is not carried out by most other manufacturers of platinum-cured silicone tubing, which means there is a very real risk of cyclosiloxanes migrating into fluids that come into contact with the tubing. This is of course a safety concern and one that drug manufacturers must be aware of.

Focus and outcome of this study

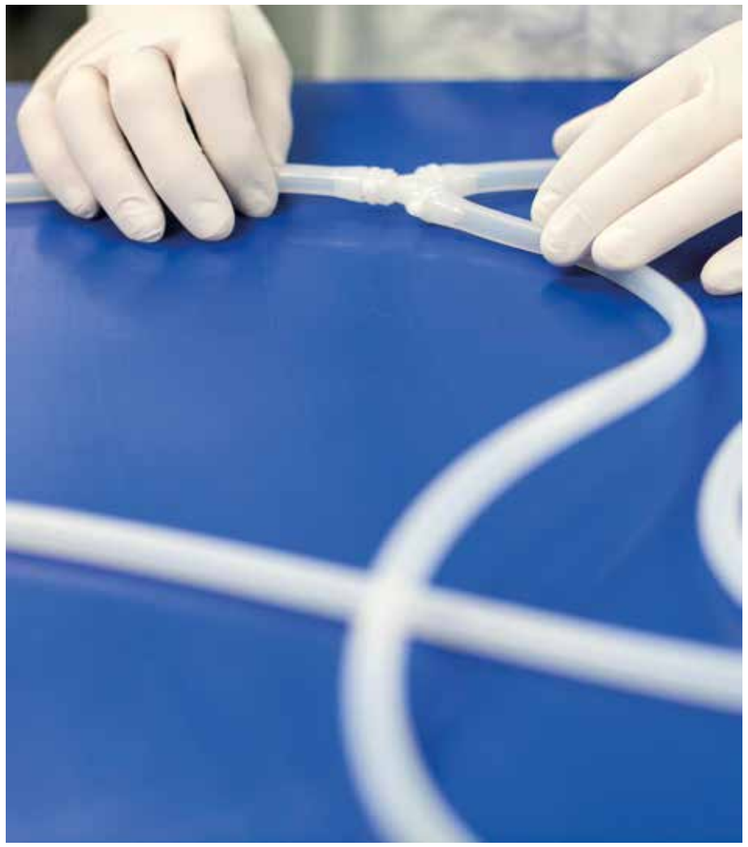
The study reveals noteworthy results that affirm the need for drug manufacturers to adopt post-cured tubing as standard. This paper details that samples of WMFTS Pumpsil tubing (post-cured and non post-cured) were extracted with a 50% ethanol: 50% water solution for 30 minutes at 25C and seven days at 40C.
For the purposes of this paper, the extractables studied were the cyclosiloxanes (mixtures of cyclosiloxanes have been of particular interest due to concerns about their potential toxicity).
The extracts were analysed by Gas Chromatography – Mass Spectrometry (GCMS).
The study demonstrated the presence of cyclosiloxanes was reduced by a minimum of 25% in the post-cured extracts, as compared with non post-cured extracts over a seven day extraction period and by 50% over a 30 minute time point.

Introduction

Tubing has been a part of biopharmaceutical manufacturing processes for many years. Even in the early days of the biopharmaceutical industry, tubing was used for fluid transfer, peristaltic pumping and filling operations.

The advent of single-use technologies has increased the usage of tubing from being a minor player within the manufacturing process, to become a major part of single-use biopharmaceutical manufacturing assemblies, used to link the different technologies such as filters, connectors and biocontainers together. Silicone tubing comprises of polydimethylsiloxanes (PDMS) polymers. Silicone tubing can be divided into two cross-linking types, those manufactured by addition (platinum) and free radical (peroxide) curing systems.

The peroxide-cured silicone tubing has fallen out of favour in recent years due to concerns about the presence of 2-4 dichlorobenzoyl acid as a by-product which has the potential to leach into contacting fluids. Addition cure has no by-products, and as a result the biopharmaceutical industry has transitioned to mostly using platinum-cured tubing.



Manufactured in a class 7 cleanroom

Platinum-cured silicone manufacturing process

Extrusion process

The manner in which silicone tubing is manufactured can affect the types of extractables that may migrate into a contacting fluid. From an academic point of view, it is interesting to know what kind of compounds are present as extractables.

However for a drug producer it is a regulatory requirement to understand the materials in contact with the drug product and its propensity to leach unknown and unwanted compounds. Additionally, there is a requirement to understand and risk assess whether impurities can be of a potential safety concern.

The platinum catalysed system used in Pumpsil Platinum-cured silicon tubing is supplied as four components, prior to extrusion the components are blended to achieve optimum curing and a targeted material hardness. Blending is achieved by milling, the process involves thorough mixing to ensure tubing will cure homogeneously without flaws. During extrusion, the tubing is cured to give a finished product. Most tubing producers would end the tubing manufacture at this stage.

Nonetheless, during processing, not all the polymerisation components are consumed. When there is incomplete polymerisation, low molecular weight polymers (siloxanes) may be present in the processing mixture. If these oligomers remain on the surface of the silicone tubing, then there is a possibility that they could migrate into a contact fluids as an extractable.

One method to remove these residual low molecular weight siloxanes is to post-cure the tubing. This is in addition to the typical curing process all silicone tubing undergoes.

Post-curing to drive off oligomers

Post-curing is achieved after the tubing manufacture. The tubing is subjected to temperatures of 200C/390F for a cycle time of 4 hours.

Post curing of the tubing improves the finished product in the following ways:

- Helps to drive off the majority of volatile compounds (siloxanes), that if left may be potential leachables contaminating the drug product. Therefore, their removal reduces a potential risk factor to the product.
- Tubing performance can be improved by enabling further cross linking. The cross linking can be enhanced by extra curing time at increased temperature. High degree of cross-linking in the tube can lead to a more stable product and longer tube life.

The time to cure silicone depends on a number of factors:

- The concentration of chemical cross linking agent
- The curing temperature

This paper sets out to determine the differences in the levels of siloxane related impurities between post-cured and non post-cured tubing, thereby highlighting how post-curing can provide a degree of risk reduction to a drug manufacturing process. Careful selection of appropriate quality tubing can aid the biopharmaceutical end user to minimise risk to their drug product.

Outline of study

To explore the differences in the extractables levels between the two tubing types, an extractables study was undertaken. The test work comprised extraction of the non post-cured and post-cured tubing using a 50% ethanol solution. The 50% ethanol/50% water solution is one of the extraction fluids recommended in Biophorum Operation Group (BPOG)'s article on the standardised extractables protocol for single-use components, used in biopharmaceutical manufacturing and in the draft guidance of USP 661.3, Plastic Manufacturing components used in Pharmaceutical manufacturing. The 50% ethanol solution was selected due to its high extraction capabilities.

The extracted solutions were analysed using direct injection GCMS. Direct injection GCMS is an analytical technique used to measure the presence of semi volatiles compounds. All test work reported was carried out by Chemic Laboratories Inc. USA.

Experimental method

The test articles evaluated were post-cured and non post-cured silicone tubing samples. The tubes were incubated without any further treatment. The tubing materials were tested using two different temperature and time conditions. The filled tubing was clamped at both ends and placed into individual metal canisters in order to minimise cross contamination and solvent evaporation, over the long extraction time period. The extraction conditions are described in Table 1. The weights of the tubing samples were recorded at various points during the experiment. The volumes of the filling solvent before and after extraction were recorded. Method controls for each time point interval were prepared in glass containers fitted with PTFE caps for assays.

The method controls were incubated, alongside the test materials in an incubator shaker

Table 1: Extraction samples sizes and conditions

Test material	Solvent	Tube surface area (cm2)	Extraction volume (mL)	Temperature (C) / (F)	Extraction period
Tube (post-cured)	50% Ethanol	40	20.1	40 / 104	30mins
Tube (non post-cured)	50% Ethanol	40	20.1	40 / 104	30mins
Tube (post-cured)	50% Ethanol	40	20.1	40 / 104	7 days
Tube (non post-cured)	50% Ethanol	40	20.1	40 / 104	7 days

Analysis

At the end of the extraction time, the extraction fluids were isolated and analysed using direct injection GCMS. The direct injection GCMS instrument was set up as detailed in Table 2.

Table 2: Direct injection GCMS analysis conditions

DI – GC/MS Analysis conditions	
Gas chromatograph	Agilent model 6890
Analytical column	Agilent HP-5MS, 30m x 0.25mm, 0.25um film thickness
Injection port temperature	275C/ 527F
Type of inlet	Splitless (1 minute)
Carrier gas	Helium
Column flow rate	1.3 mL/minute
Injector head pressure	10.11 psi
Oven temperature	
Initial temp	40C / 104F
Initial time	2mins
Rate	8.00C/min
Final	320C / 608F
Final time	5mins
MS quad	150C / 302F
MS source	230C / 446F
Injection volume	1.0µL
Transfer line	280C
Mode	Scan
Scan range	30 - 600amu
Run time	42 mins

All sample extracts and controls were assayed for semi volatile compounds. Known analytes were confirmed using authentic reference standards and the remaining analytes were tentatively identified using a National Institute of Standards and Technology (NIST) library search. For the confirmed analytes, the concentration was also determined using response factors from the reference standards.

Concentrations of all other analytes were determined using the response factor for the internal standard for each sample injection. The internal standards used were Phenathrane-d10 and p-terphenyl-d14.

Figures 1 and 2 show the GCMS chromatograms for the extracts from non post-cured and post-cured tubing after seven days.

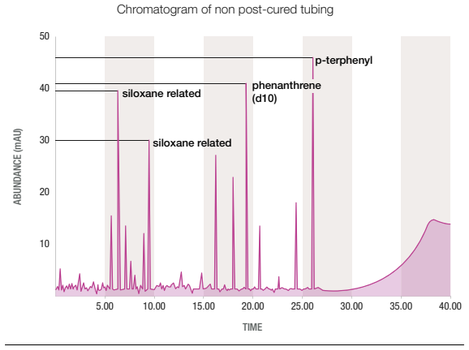


Figure 1: Chromatogram of 50% ethanol extract from non post-cured tubing after seven days

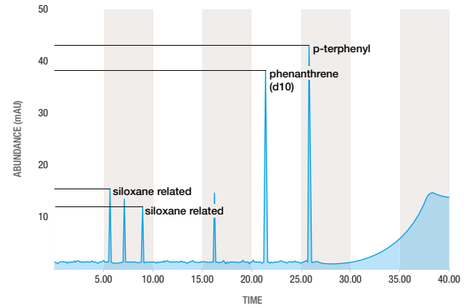


Figure 2: Chromatogram of the 50% ethanol extract from post-cured tubing after seven days

The predominant peaks found in the chromatograms are associated with cyclic oligomers with the general structural formula, [(CH3)2SiO]n. Cyclosiloxanes are small oligomers of polydimethoxysiloxane (PDMS). Direct matches with a mass spectral library allows for the tentative identification of oligomers from n=3 through to n=6.

The most commonly found siloxanes are hexamethylcyclotrisiloxane (D3) octamethylcyclotetrasiloxane (D4), and decamethylcyclopentasiloxane (D5). Further evaluation of retention time and the spectra matches for molecular ions indicative of silicone compounds allows for tentative identification of high molecular weight conformers. These high molecular weight oligomers have been broadly generalised as siloxane related compounds.

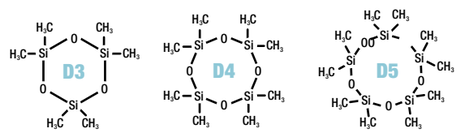


Figure 3: Chemical structure of cyclosiloxanes D3, D4 and D5

Discussion

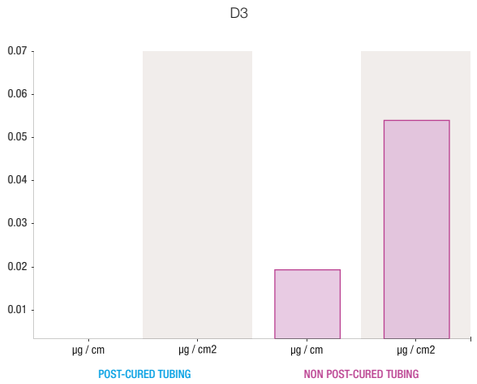
30 minutes extraction

After 30 minutes, it was found that non post-cured tubing had a higher amount of cyclosiloxane and siloxane related compounds than the post-cured tubing. This is shown in Table 3 and represented pictorially in graphs 1 to 4.

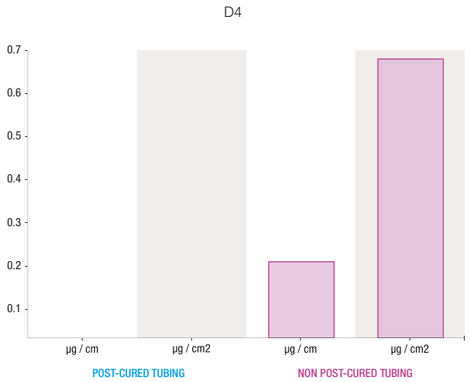
Sample Discussion	Analyte	Concentration (30 minutes) µg/cm	µg/cm2
Non post-cured tubing	D3	0.0185	0.0557
	D4	0.229	0.692
	D5	0.0941	0.284
	Siloxane related	8.69	26.2
Post-cured tubing	D3	ND	ND
	D4	ND	ND
	D5	ND	ND
	Siloxane related	0.174	0.525

Table 3: GCMS summary after 30 minutes extraction at 25C

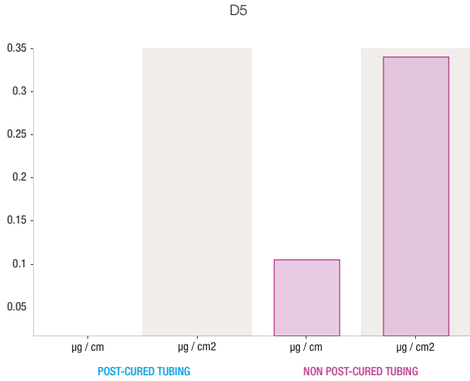
In the post-cured tubing extracts, there are no D3, D4 or D5 cyclosiloxanes identified after a 30 minute extraction period at 25C. The level of siloxane related compounds in post-cured extracts are 50 times lower than the level found in the non post-cured tubing extracts tested under identical conditions.



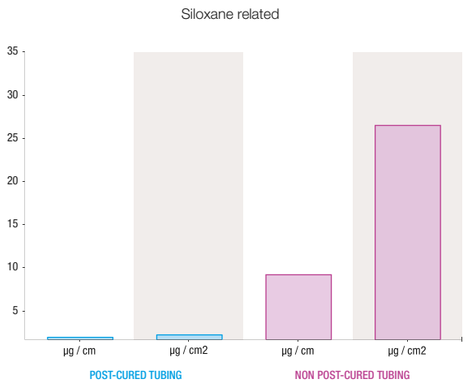
Graph 1: Level of D3 compounds from post-cured and non post-cured tubing after 30 mins extraction



Graph 2: Level of D4 compounds from post-cured and non post-cured tubing after 30 mins extraction



Graph 3: Level of D5 compounds from post-cured and non post-cured tubing after 30 mins extraction



Graph 4: Level of siloxane related compounds from post-cured and non post-cured tubing after 30 mins extraction

“The level of D5 compounds found in the extracts of post-cured tubing is 5 times lower than the level found in the cured tubing extracts”

Seven day extraction

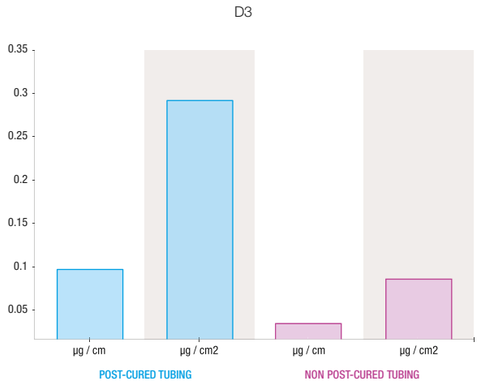
After seven days extraction, the samples from the non post-cured tubing also showed greater amounts of cyclosiloxanes, in comparison to the post-cured tubing as detailed in Table 4 and represented pictorially in Graphs 5 to 8.

The level of D4 compounds found in the extracts of post-cured tubing is 3.5 times lower than the level found in the non post-cured tubing extracts. Similarly, the concentration of D5 compounds in post-cured tubing extracts are 5 times lower than in the non post-cured tubing extracts. The difference in the levels of siloxane related compounds is 5 times higher in the non post-cured tubing extracts as compared to the post-cured tubing.

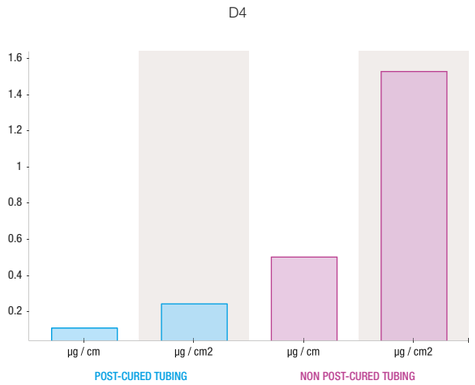
The only anomaly is the level of D3 is higher in the post-cured extracts compared to the non post-cured extracts. This could be due to the breakdown of higher molecular weight oligomers over the prolonged extraction duration. The reason for the difference is not fully understood and may require a further study to determine the variability of this observation.

Sample Discussion	Analyte	Concentration (7 days) µg/cm	µg/cm2
Non post-cured tubing	D3	0.0293	0.0882
	D4	0.510	1.54
	D5	0.151	0.455
	Siloxane related	4.19	11.6
Post-cured tubing	D3	0.10	0.294
	D4	0.140	0.423
	D5	0.03	0.09
	Siloxane related	0.844	2.54

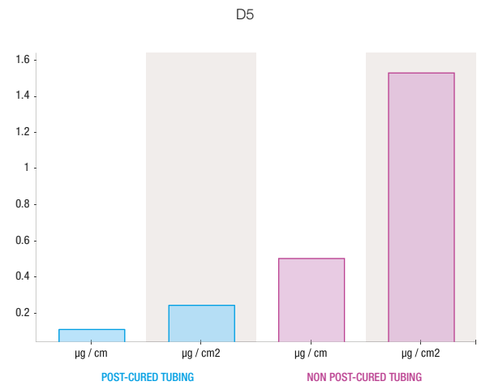
Table 4: GCMS summary after 7 days extraction at 40C



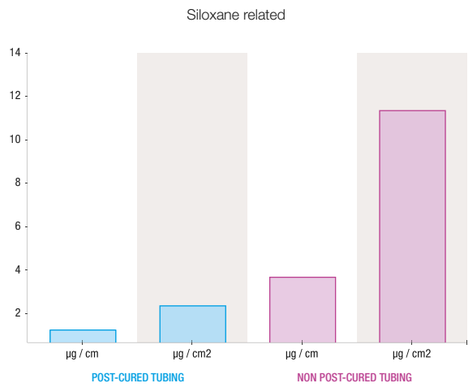
Graph 5: Level of D3 compounds from post-cured and non post-cured tubing after 7 days extraction



Graph 6: Level of siloxane D4 compounds from post-cured and non post-cured tubing after 7 days extraction



Graph 7: Level of siloxane D5 compounds from post-cured and non post-cured tubing after 7 days extraction



Graph 8: Level of siloxane related compounds from post-cured and non post-cured tubing after 7 days extraction

“...the total amount of extractables is greater in the non post-cured tubing extracts in comparison to the post-cured tubing extracts”

Although, the data clearly demonstrates the positive impact that the post-curing process has on the level of extractables from platinum-cured tubing, there are a couple of unexpected results.

For example, after seven days, the amount of the higher congeners siloxanes reduces, a potential reason for this could be that longer extraction time and high temperature leads to the breakdown of higher molecular weight cyclosiloxanes to smaller units. However, what is clearly demonstrated by the study is that overall the total amount of extractables is greater in the non post-cured tubing extracts in comparison to the post-cured tubing extracts regardless of the extraction time or extraction temperature.

Toxicity of cyclosiloxanes

The possible migration of compounds from tubing into drug products can have serious implications for biopharmaceutical manufacturers.

An evaluation of toxicological effects of cyclosiloxanes could be helpful to provide guidance on how extractables data in these compounds can be used for risk assessment purposes.

The toxicity of cyclosiloxanes has been studied extensively. Primarily this has been as a result of their use in cosmetics but more recently because of the controversy around the use of industrial silicone materials used in breast implants. In particular, not only have individual compounds such as Hexamethyltricyclosiloxane (D3) and Octamethyltetracyclosiloxane (D4) been assessed, so has mixtures of the cyclosiloxanes. Research available from the Danish Environmental Protection Agency (DEPA) cites toxicity studies demonstrating the potential carcinogenic effects of cyclosiloxanes, a study reported in 2008, using a mixture of three cyclosiloxanes in a MEM Elution test (ISO 10993-5, 1999- Biological Evaluation of Medical devices Part 5: Tests for In-Vitro Cytotoxicity) and USP 87 showed a severe reactivity response. This response was observed after a 48hr period and was evidence of the cytotoxic mixture of the cyclosiloxanes. The cyclosiloxanes mixture tested was an equal part mixture of D4, D5 and D6.

Specific limits such as permitted daily exposure are available for certain cyclosiloxanes. For example, D3 has a permitted daily exposure (PDE) of 169 mg/day, whilst D4 was found to have a PDE of 0.70 mg/day. Knowledge of these type of values can be used to perform risk assessment calculations as to whether the amount of these cyclosiloxanes detected as part of an extractables study are high enough to exceed the PDE, considering the drug dosing regime and drug treatment duration.

Many tubing types will leach a certain level of cyclosiloxanes depending on the way the tubing has been manufactured. Not all tubing manufacturers are able to provide sufficient detail on the level of cyclosiloxanes necessary for an end user to make a risk assessment based on the PDE.

Conclusion

Drug manufacturers must support the arguments for post-cured silicone tubing; and this white paper presents data to support this position. It offers proof that the post-curing of tubing can significantly reduce the amount of cyclosiloxanes that are present after manufacturing. At a commercial and reputational level, this will help the biopharm industry have greater assurance of the efficacy, safety or quality of their final product.

In summary, the possible contamination from platinum-cured silicone tubing* can be reduced based on the additional processing of the finished product. Post-curing can lead to increased cross-linking of the tubing and a reduction in the levels of potentially toxic cyclosiloxanes.

***Watson-Marlow Fluid Technology Solutions performs an additional post-cure processing step during the manufacture of its Pumpsil tubing. This reduces the potential contamination risk to a customer's product. This is not performed by all tube manufacturers for all their silicone tubing ranges.**

Platinum-cured silicone tubing manufacturing

“Drug manufacturers must embrace the arguments for post-cured silicone tubing”

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