RISK ASSESSMENT OF CYCLIC SILOXANES (D3 TO D19) AS EXTRACTABLES FROM POLYMERIC COMPONENTS IN BIOPHARMACEUTICAL MANUFACTURING

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INTRODUCTION / ABSTRACT

Silicone tubing and polypropylene (PP) connectors are commonly used as polymeric components in the manufacturing of biopharmaceuticals. Because they come into direct or indirect contact with the drug product, it is important to assess the risk of the extractable and leachable (E&L) impurities to patient safety. This presentation will use cyclic siloxanes from extraction studies to illustrate the risk assessment approach on structurally related compounds. Low molecular weight (LMW) cyclic siloxanes (D3 and D4) were detected in extracts from silicone tubing, while higher molecular weight (HMW) cyclic siloxanes (D6 to D8) were detected in extracts from PP connectors. In animal studies, D3 and D4 showed greater systemic toxicity relative to D6-D8, consistent with their different absorption, distribution, metabolism and excretion (ADME) profiles. Based on a literature review on cyclic siloxanes, D3 to D19, 2 categories were developed using OECD (2007, 2009), ECHA (2008) and ECETOC (2012) criteria. The first category included D3 and D4 and the second category included D6 to D19 (ELSIE database). There was no evidence of DNA reactive (mutagenic) potential for any of the compounds. Following the principles and methods of the ICH Q3C approach on impurities, Permitted Daily Exposure (PDE) limits were derived for D4 and D6, as indicator compounds for their respective categories. Using a hypothetical single-use assembly with tubing and connector, a risk assessment on cyclic siloxanes was conducted using conservative assumptions of batch size, product yield and doses per batch. The very conservative estimates of cyclic siloxanes , calculated per dose of a parenteral drug product, were well below the derived PDE limits.

MATERIALS AND METHODS

Commercial platinum-cured silicone tubing and polycarbonate (PP) connect couplings were evaluated in 2 separate studies for their extractable profiles using model solvents under experimental conditions. The tubing and the couplings were components of a single-use assembly and the schematic of a typical assembly is shown in Fig. 1.

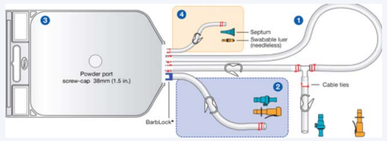


Fig. 1 Example of a Single-use Assembly with bag, tubing, connectors (Source: Google Images on Biocontainers)

The components were certified to meet USP Class VI requirements. The tubing (length: 5 inches, internal diameter: 3/8 inch) was filled with 10 mL deionized water or aqueous organic solvent and stored at room temperature for 3 days. The organic solvent was a mixture of 20% acetonitrile, 20% ethanol and 60% deionized water. Similarly, coupling body and insert were individually immersed in 25 mL water and 15 mL aqueous solvent, respectively and stored at room temperature for 3 days.

The composition of the aqueous organic solvent was to simulate the solvation effect, pH, process conditions and ionic strength of the process fluids that come into direct or indirect contact with the silicone tubing or connect couplings. The extraction conditions were selected to represent the high end of the process applications and product storage conditions, including temperature, contact surface, contact duration, and sterilization methods of the test materials. The experimental conditions are appropriate for biotechnology drug products without impairing the function and integrity of the material contact surfaces. The extracts are analyzed for organic compounds using GC-MS, HPLC-UV, HPLC-MS and ELSD. The identified compounds were quantified.

For the tubing, the mass per length (µg/cm) were converted to mass per surface area (µg/cm^2) based on the internal diameter. Because the coupling body and insert were immersed under experimental conditions, the mass reported for the extract was halved to estimate the mass from internal surface of these components alone.

Literature searches were conducted on the identified organic compounds, supplemented with *in silico* analyses as necessary using Toxtree (IdeaConsult, v 2.6). The sources searched included, but were not limited to, Extractables and Leachables Safety Information Exchange (ELSIE, 2015) database, ExPub, ToxNet, ECHA, Ovid and SciFinder®. The toxicology assessments used decision analysis for genotoxicity (ICH M7, 2014) and non-cancer risk (Cramer et al 1978; Kroes et al, 2004; Benigni & Bossa 2008; Patlewicz et al 2008 and FDA CFSAN 2011).

After the risk of genotoxicity was excluded, Permitted Daily Exposure (PDE) values were derived following the principles and methods in ICH Q3C (2011), with an additional modifying factor for bioavailability to account for parenteral exposure.

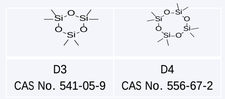
RESULTS AND DISCUSSION

Under the experimental conditions described, cyclic siloxanes were among the predominant compounds extracted from the tubing and the connector. While the extractable profile of the tubing was characterized by the LMW cyclic siloxanes, the profile of the connector, and specifically, the insert, was characterized by the HMW cyclic siloxanes (Table 1).

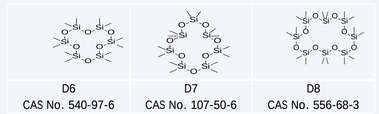
Table 1. Cyclic Siloxanes Extracted from Silicone Tubing, Connector Coupling and Insert

Mass	D3	D4	D6	D7	D8
Tubing, µg/cm^2	0.78	1.53	ND	ND	ND
Coupling Body, µg/unit	ND	ND	ND	ND	ND
Coupling Insert, µg/unit	ND	ND	0.95	3.67	0.89

The structures of the identified cyclic siloxanes are shown in Fig. 1.



D3 CAS No. 541-05-9 | D4 CAS No. 556-67-2



D6 CAS No. 540-97-6 | D7 CAS No. 107-50-6 | D8 CAS No. 556-68-3

Fig. 1. Cyclic Siloxane Structures

For exposure estimates, it was assumed that the hypothetical assembly was connected to 15 cm of tubing via a single connector (coupling and insert). Further, it was assumed that the extracted amounts migrated into the assembly filled with a smallest batch with only 212 product doses. With these assumptions, a product dose would theoretically have no more than 2 µg of D3 and D4 (combined) and no more than 26 µg of D6, D7 and D8 (combined) (See Table 2).

A literature review was conducted on cyclic siloxanes, from D3 to D19. Based on a literature review, 2 categories of cyclic siloxanes were justified using the criteria established by OECD (2007 and 2009). Specifically, OECD defines a chemical category as "a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity." Additional information on grouping chemicals into categories and the use of read-across to fill data gaps can be found in ECETOC (2012) and ECHA (2008).

The first category was justified for D3 and D4, the LMW species and the second category was justified for D6 to D19, the HMW species. Because the literature review showed no evidence of DNA reactive (mutagenic) potential for any of the cyclic siloxanes, Permitted Daily Exposure (PDE) limits were derived following the principles and methods of the ICH Q3C(R5) (2011). A typical body weight of 60 kg was used in the derivation. In addition, a modifying factor (F6) was used to account for route extrapolation as appropriate. Further, D4 was used to read-across for the LMW species and D6 was used to read-across for the HMW species.

Derivation of PDE for D4

Most of the studies on D4 were conducted by whole body inhalation exposure. The target organs of systemic toxicity were the liver and the female reproductive tract. The more sensitive toxicity endpoint was the NOEL of 120 ppm based on reversible liver weight increase in the 3 month nose-only inhalation study in rats (Burns-Naas et al., 2002). The concentration of D4 vapor of 120 ppm was converted to 1.4 mg/L (Patty, 2001):

(120) (296.62/24,450) mg/L = 1.4 mg/L

Based on the default values for respiratory volume (290 L/day) and body weight (0.425 kg) for a rat (ICH Q3C), the NOEL of 1.4 mg/L was calculated to 955 mg/kg/day:

(1.4 mg/L x 290 L/day) / 0.425 kg = 955 mg/kg/day

This was further adjusted based on the exposure conditions used in the study:

955 mg/kg/day x 6 hr/24 hr x 5 days/7 days = 170 mg/kg/day

Retention of [14C] D4 from single dose and repeat dose inhalation studies at day 14 was reported to be 5 - 6% for concentrations between 7 - 700 ppm (Plotzke et al., 2000). A rough estimate of the absorbed (systemic) dose was therefore about 10 mg/kg/day (170 mg/kg/day x 6% = 10 mg/kg/day), which was used for derivation of the PDE using a total modifying factor of 250. No adjustment was made for bioavailability because the systemic dose was used for the derivation. Therefore, the PDE for D4 was estimated to be 2,400 µg/day (Table 2).

Derivation of PDE for D6

The literature review showed D6 to have low order of toxicity in rodents by the oral route. In a repeat dose and reproductive/developmental toxicity screening study in rats, there were no treatment-related adverse effects on systemic toxicity, reproductive performance, and developmental endpoints. The NOAEL was 1,000 mg/kg/day, the highest dose tested in the study (ECHA 2014; Johnson et al., 2011). Therefore, the PDE for D6 was estimated to be 12,000 µg/day using a total modifying factor of 5,000 (Table 2).

Risk Assessment

LMW and HMW cyclic siloxanes were extracted under experimental conditions from a hypothetical assembly constructed with platinum-cured silicone tubing and PP connector. A thorough review of the literature showed no evidence of mutagenic potential. Using D4 and D6 as the indicators for the LMW and HMW species, respectively, the theoretical amounts that could migrate from the assembly into a drug product were shown to be 2 to 3 orders of magnitude below the PDEs (Table 2).

Table 2. Summary Assessment of Cyclic Siloxanes Extracted from a Hypothetical Single-use Assembly

Category/ Compound	Toxicity Endpoint	F1	F2	F3	F4	F5	F6	PDE (µg/day)	Maximum Estimate (µg/dose)
LMW Cyclic Siloxanes: D3 and D4 (Indicator compound: D4)	Rat inhalation NOEL: 122 ppm (absorbed dose: 10 mg/kg/day)	5	10	5	1	1	1	2,400	2
HMW Cyclic Siloxanes: D6-D19 (Indicator compound: D6)	Rat (oral, combined screening repeat dose and reproductive and developmental) NOAEL: 1000 mg/kg/day	5	10	10	1	1	10	12,000	26

CONCLUSION

Based on the extractable profiles and a literature review, the amounts of LMW and HMW cyclic siloxanes extracted from a single-use assembly with silicone tubing and polypropylene connector would not be of toxicological concern.

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