Study Plan for 6:2 FTSB Developed for American Chemistry Council

OECD 105 Study Modified for Consistency with ECETOC Technical Report 122, Section 3 using Simulated Epithelial Lung Fluid

Step 1: Non-GLP Preliminary Solubility Test

- Analytical Method Development
    - Liquid Chromatograph with Tandem Mass Spectrometer (LC/MS/MS) instrumental method will be developed to quantitate the test substance (6:2 FTSB) as a prelude to the definitive water solubility study
    - Effects of SELF/Gamble's Solution on sample chromatography will be investigated and sample composition for instrumental analysis will be optimized
    - Calibration standards will be prepared and assessed for linearity
    - Preliminary Solubility samples will be analyzed to determine what concentration range is most appropriate for definitive calibration curve
- Non-GLP Preliminary Solubility
    - Physical dissolution test will be conducted by placing a small volume of test substance in a clean vessel and increasing volumes of Simulated Epithelial Lung Fluid (SELF)/Gamble's Solution will be used to obtain an estimated solubility value
    - Using the solubility determined in the physical dissolution test, triplicate vessels will be prepared in appropriate containers, with each vessel containing SELF/Gamble's Solution fortified at a nominal concentration that is approximately five times greater than the estimated solubility in reagent water
    - The fortified vessels will be vortexed for 5 minutes, sonicated for 30 minutes, and transferred to a shaking water bath maintained at 37 ± 1°C. Duplicate samples will be collected from each vessel, centrifuged and/or filtered, and diluted as necessary. Samples will be collected at approximately 3, 24 and 48 hours and analyzed for 6:2 FTSB by the appropriate processing and instrumental method
- Summary Report
    - The method development findings will be summarized and communicated in a timely manner to the Sponsor's Representative at a mutually agreed upon frequency.

Step 2: GLP Determination of Biosolubility in Gamble's Solution or other Simulated Epithelial Lung Fluid (SELF)

Test design for this study will be consistent with ECETOC Technical Report 122, Section 3 and/or reports referenced in the literature, such as: Kumar, A. et al., "A Biocompatible Synthetic Lung Fluid Based on Human Respiratory Tract Lining Fluid Composition," Pharm Res (2017) 34:2454–2465, and adapted to Water Solubility: Shake Flask (OECD 105)

- GLP Preliminary Solubility
    - Approximately 0.1g of material will be weighed out and increasing volumes of SELF/Gamble's Solution (previously equilibrated at approximately 37°C) will be added until the material is fully dissolved or until 1L has been added.

- GLP Definitive Solubility
    - Using the solubility determined in the preliminary solubility test, five vessels will be prepared in appropriate containers, with each vessel containing SELF/Gamble's Solution fortified at a nominal concentration that is approximately five times greater than the estimated solubility in reagent water
    - Vessels will be vortexed, sonicated, and placed in a shaking water-bath at 45°C
    - After equilibrating for 24 hours, a single vessel will be transferred into a static water bath maintained at 37°C and allowed to equilibrate.
    - After 24 hours, the sample will be removed from the static water bath, centrifuged/filtered, and processed as appropriate for analysis.
    - This process will be repeated allowing for equilibration times of 24 hours, 48 hours, and 72 hours at 45°C followed by 24 hours at 37°C.
    - If the results from samples taken at 48 and 72 hours of equilibration at 45°C are within 15% of each other, the study will be terminated
    - The definitive study findings will be summarized and communicated in a timely manner to the Sponsor's Representative at a mutually agreed upon frequency.