Executive Summary
- Biodegradation Screening Studies of 8-2 Telomer B Alcohol -

The two studies described in the attached reports (EMSER 13-03/4842 and EMSER 15-03/4842) represent the screening phase for the biodegradability assessment of 8-2 Telomer B Alcohol (CAS# 678-39-7). The unique analytical challenges presented by this chemistry (low water solubility, volatility, and strong adsorption to surface) require a thorough understanding of the inter-relationships of physical-chemical properties and other parameters that may affect the ability to achieve mass balance and to both qualitatively and quantitatively identify potential transformation products in complex matrices. The primary objective of the screening studies was to identify these critical parameters prior to launching the longer-term biodegradability studies, which will have an objective of definitively identifying the ultimate transformation products formed by biotransformation of 8-2 Telomer B Alcohol.

For the screening phase, two cold (non-radiolabeled) studies were conducted in sequence and designed as follows:

1. **Ready Biodegradation (Modified OECD 301D Closed Bottle Test)** to:
    - Achieve the desired test conditions.
        - With unacclimated inoculum from a municipal POTW
        - With test chemical as the only intentional carbon source
    - Test the hypothesis that ready biodegradation, ultimate biodegradation, and primary biodegradation would be unlikely under these test conditions.
    - Confirm the expectation that mass balance will be difficult to achieve in cold studies due to potential abiotic removal mechanisms (e.g., volatility and adsorption).

2. **Accelerated Biodegradation Test** to:
    - Achieve the test conditions with the objective of optimizing conditions for potential biotransformations.
        - With pre-acclimated inoculum from an industrial wastewater treatment plant.
        - With yeast extract added as additional carbon source to enable potential for co-metabolism.
    - Determine if any potential transformation products are formed in the screening phase.
        - To allow development of sampling and analytical methods for identification of potential transformation products. To prepare authentic standards based on the structural information of the identified transformation products to facilitate definitive identification and quantification. These are necessary steps prior to conducting longer-term, more definitive biodegradability studies.

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- Re-confirm the expectation that mass balance will be difficult to achieve in cold studies due to potential abiotic removal mechanisms (e.g., volatility and adsorption).

Ready Biodegradation (Modified OECD 301 D Closed Bottle Test) Results:

1. Parent Compound Monitoring: In both the test system and abiotic control vessels, the concentrations of 8-2 Telomer B Alcohol steadily decreased. At 28 days, the concentrations of 8-2 Telomer B Alcohol were not distinguishable between tests and abiotic control vessels. This indicates that abiotic removal mechanisms - volatilization and/or adsorption are likely to be active and must be accounted for to achieve mass balance.

2. Fluoride Analysis: There were no significant changes in fluoride concentration (17.9 ± 2.6 µg/L at day 0; 17.1 ± 0.8 µg/L at day 7; 17.4 ± 1.4 µg/L at day 14: and 17.5 ± 1.0 µg/L at day 28) over the 28 day test period. Considering that on a molar basis, one mole of 8-2 Telomer B Alcohol converted to one mole of PFOA would yield two moles of fluoride, the absence of an increased fluoride concentration during the test indicated that PFOA is not likely a major biotransformation product of 8-2 Telomer B Alcohol under the Ready Biodegradability test conditions. However, confirmation is needed and quantification of PFOA presence or absence will be accomplished with a definitive study.

Accelerated Biodegradation Test Results:

1. Parent Compound Monitoring:
   a. In the abiotic controls, the concentrations of 8-2 Telomer B Alcohol steadily decreased at rates similar to those observed in the Ready Biodegradability (Modified OECD 301 D Closed Bottle) Test. This indicates that abiotic removal mechanisms - volatilization and/or adsorption must be accounted for to achieve mass balance.
   b. By comparison to the abiotic controls, the test system showed rapid primary biotransformation with loss of parent chemical - 8-2 Telomer B Alcohol at >70% at Day 14 and nearly 100 % at day 28. The loss was clearly as a result of biotransformation and not attributable from the abiotic loss alone.

2. Fluoride Analysis: Results indicate that 8-2 Telomer B Alcohol was rapidly biotransformed to fluorinated acids and other unidentified transformation products as indicated by:
   a. Significant changes in fluoride concentration (10 µg/L at day 0; 64 µg/L at day 14: and 70 µg/L at day 28) over the 28-day test period, a net increase of 60 µg/L due to 8-2 Telomer B alcohol defluorination.

3. Identification of Transformation Products: Four fluorinated acid transformation products were monitored for and were identified in the test system by LC/MS/MS analysis and comparison with known authentic standards:

i. $F(CF_2)_7CF=CHCOOH$ (CAS# 161094-76-4) - most abundant - ≥10 of total mass present
ii. $F(CF_2)_8CH_2COOH$ (CAS# 27854-31-5) - approx. 2% of total mass present
iii. $F(CF_2)_7COOH$ (PFOA, CAS# 335-67-1) - <2% of total mass present
iv. $F(CF_2)_5COOH$ (PFHA, CAS# 307-24-4) - approx. 0.4% of total mass present
   - ➢ It is hypothesized that PFOA may not be a stable transformation product and that defluorination of PFOA may have lead to formation of PFHA during the test.
v. Other potential transformation products that formed during the test were not determined.

Study Conclusions Summary

1. These study results suggest that substantial transformation of 8-2 Telomer B Alcohol to PFOA and/or other transformation products appears to be less likely under "Ready-Biodegradability" conditions. However, the Accelerated Biodegradation studies clearly indicated that at least primary biodegradation of 8-2 Telomer B Alcohol is highly probable under favorable test conditions.

2. The potential transformation products of 8-2 Telomer B Alcohol may include PFOA as an identifiable transformation product. However, this study indicated that PFOA may not be a major biotransformation product and there are indications that PFOA may potentially undergo further defluorination to PFHA. This hypothesis will be explored in the forthcoming long-term biodegradability studies.

3. Mass balance was not a primary objective in these studies and was not achieved. Not all of the potential relevant transformation products have been identified in these studies. Only four specific potential transformation products [$F(CF_2)_7CF=CHCOOH$, CAS# 161094-76-4; $F(CF_2)_8CH_2COOH$, CAS# 27854-31-5; $F(CF_2)_7COOH$, CAS# 335-67-1; and $F(CF_2)_5COOH$, CAS# 307-24-4] were monitored for and were identified in the accelerated study.

4. As anticipated, to achieve mass balance, the test design for long-term biodegradability studies will need to account for abiotic removal mechanisms – volatility and adsorption. Therefore, the use of a ^{14}C labeled study material is necessary to achieve mass balance.

5. The results of these screening studies should be viewed as indicative, not conclusive. It is believed that long-term biodegradability studies (aerobic and anaerobic) using a ^{14}C labeled study material and with a duration of 12-18 months will be needed to identify the stable, ultimate transformation products.