Supporting Information

Targeted Per- and Polyfluoroalkyl Substances (PFAS) Assessments for High Throughput Screening: Analytical and Testing Considerations to Inform a PFAS Stock Quality Evaluation Framework

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Text S1. PFAS concentration determination by LC-MS and GC-MS.

To determine if the provided PFAS stock solutions in DMSO and EtOH were within 20% of the theoretical concentration, 23 unique analytes (10% of total number evaluated) were assessed by LC-MS or GC-MS. DMSO and EtOH standards ranging from 5 to 30 mM were diluted in acetonitrile with matching labelled internal standards. Using a secondary standard for accurate concentration determination, a linear regression fit was applied to a calibration curve. Deviation from expected concentration was then computed for each PFAS stock to provide further knowledge on *in vitro* dose-effect calculations. Additional details for this concentration check are provided in Text S1. 1:1,600,000 by initially diluting the stock 1:200 in matching diluent then mixing all analytes of the same diluent in acetonitrile 1:500. The analyte mix was then diluted 1:4 in a solution containing 10% formic acid, 16 pg/µL labelled internal standards (MPFAC-24ES, M3HFPO-DA), and acetonitrile. After centrifugation at 12,500*g* for 15 minutes, the crashed supernatant was diluted in mobile phase A at a 1:4 ratio. Each sample mix was assessed with a minimum of three technical replicates by LC-MS using MRM transitions for each analyte (Table S2).

Commercially available standards from Wellington Laboratory (catalog number: PFAC-24PAR and HPFO-DA) were used to prepare a calibration curve for quantitation of those chemicals analyzed by LC-MS. Nine concentrations were prepared by diluting the concentrated standard stocks in acetonitrile within the range of 1 to 500 pg/µL (ppb). A crashed matrix match was then performed by diluting the calibration curve points 1:4 in the same crashing solution containing labelled internal standards. After centrifugation at 12,500*g* for 15 minutes, the supernatant was further diluted in mobile phase A 1:4, making the final calibration curve range at LC-MS 0.063 to 31.250 pg/µL (ppb). Specific MS parameters for labelled internal standards are listed in Table S3. The calibration curve was run in duplicate where the response of each analyte (peak area ratio of analyte to associated labelled standard) was fit as a concentration-weighted (1/x) linear curve using MassLynx v4.2 with TargetLynx XS (Waters Corporation, Milford, MA, USA). Deviation from the expected concentration for each assessed PFAS stock solution was determined by subtracting the mean actual concentration determined by LC-MS from expected and dividing this difference by the expected concentration and shown as a percentage.

Similar approaches were applied to GC-able compounds for concentration verification using two GC-MS methods. In the first method, individual 1 mg/mL stocks of 3-(Perfluoro-2-butyl)propane-1,2-diol, Bis(1H,1H-perfluoropropyl)amine, Perfluoropentanamide, and 1H,1H-Heptafluorobutanol were prepared by weighing approximately 10 mg of neat material into 10 mL volumetric flasks. The flasks were filled to volume with acetonitrile and mixed well by shaking and inversion. A working stock was then prepared by transferring an aliquot of each stock, based on the stock concentration, to a 10 mL volumetric flask and subsequently diluting with acetonitrile to a concentration of 10 µg/mL. From this working stock, calibration solutions in a range of 25 to 1000 pg/µL were prepared and 100 µL aliquots were transferred to autosampler vials. The DMSO and EtOH sample stocks were removed from freezer storage and were thawed to room temperature. They were then diluted 1:200 by transferring 5µL of stock to a 2 mL polypropylene centrifuge tube and adding 995 µL of acetonitrile. The dilutions were mixed well by vortex mixing. The stocks were then further diluted by transferring 100µL to 10 mL volumetric flasks and taking to volume with acetonitrile which resulted in solutions with a nominal concentration of 200-441 pg/µL depending on initial concentration and molecular weight. A 100µL aliquot of each stock was transferred to an autosampler vial. 10 µL of a 500 ng/mL internal standard solution containing MFBET, MFHET, and MFOET was transferred to the autosampler vial containing each calibration solution and stock dilution. Samples were then analyzed by GC-MS in EI mode using the conditions listed in Table S4 and S5. Calibration curves were generated using Agilent MassHunter Quantitative Analysis v10.0. A standard, unweighted linear regression model was used for each compound resulting in an R^2 of at least 0.99 for each curve. Calibration checks (500 pg/µL) were run every 10 samples and accuracy ranged from 91-118%.

The second GC-MS method assessed 4:2 Fluorotelomer alcohol (4:2 FTOH), 6:2 Fluorotelomer alcohol (6:2 FTOH), and 8:2 Fluorotelomer alcohol (8:2 FTOH). The neat material was procured from Wellington Laboratories (catalog numbers FBET, FHET, and FOET, respectively; 50 ppm in methanol) and prepared to a concentration of 1 mg/L in MTBE. Using this concentrated stock, a calibration curve was prepared that ranged from 7.81 to 500 ppb (ng/mL). Each 4:2, 6:2, and 8:2 FTOH DMSO and EtOH stock was diluted in deionized water containing 50 ppb MFBET, MFHET, and MFOET (Wellington Laboratories) at 264.1, 364.1, and 464.4 ppb, respectively. Each diluted sample and calibration curve was run by positive chemical ionization with instrument parameters outlined in Table S4 and S5. Deviation from expected concentration for all PFAS assessed by GC-MS was calculated with the method described for the LC-MS samples.

Text S2. Hydrophilic interaction liquid chromatography (HILIC) method conditions for polar PFAS.

A HILIC method was completed using a Waters Corporation (Milford, MA) ACQUITY I Class ultra-high-performance LC modified with the Waters PFAS Solution Installation Kit (P/N 176004548) to optimize the system for better retention of polar PFAS analytes than obtained using the original reverse-phase chromatography method. The chromatographic separation was carried out using a Waters ACQUITY BEH Amide column (2.1 mm x 100 mm, 1.7µm) using a binary mobile phase gradient with mobile phases A (95:5, 2.5 mM ammonium acetate: acetonitrile, pH 9.3 by ammonia solution, 25%) and B (95:5, acetonitrile: 2.5 mM ammonium acetate, pH 9.3 by ammonia solution, 25%). The gradient program was modified from a Waters application note for organic acids (WA60096, June 2009), being 7.5 min total. The gradient and associated flow rate varied over the method program: 99% B (0.3 mL/min, 1.5 min), 99% B (0.6 mL/min, 0.5 min), 99-60% B (0.6 mL/min, 1.5 min), 60-30% B (0.6 mL/min, 0.5 min), 30-99% B (0.6mL/min, 2.0 min), 99% B (0.3 mL/min, 1.5 min). 10 µL of each sample was injected. The mass spectrometry conditions were not modified from original settings, as listed in *Materials and Methods*.

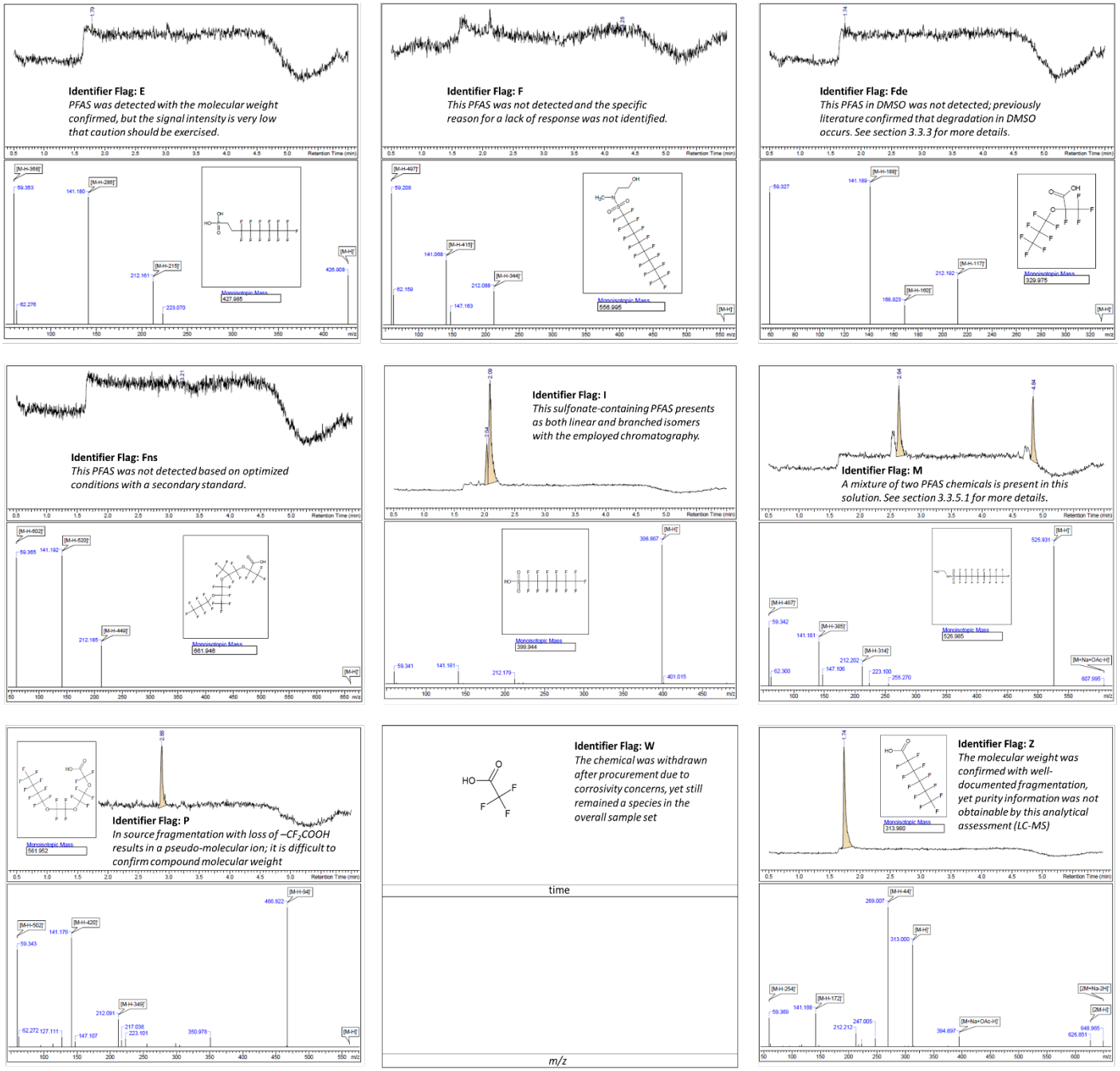


Figure S2. Visualized LC-MS Chromatograms and Mass Spectra for PFAS Flag Identifier. Available descriptions provide additional input to analyst decision on why informational markings were utilized.

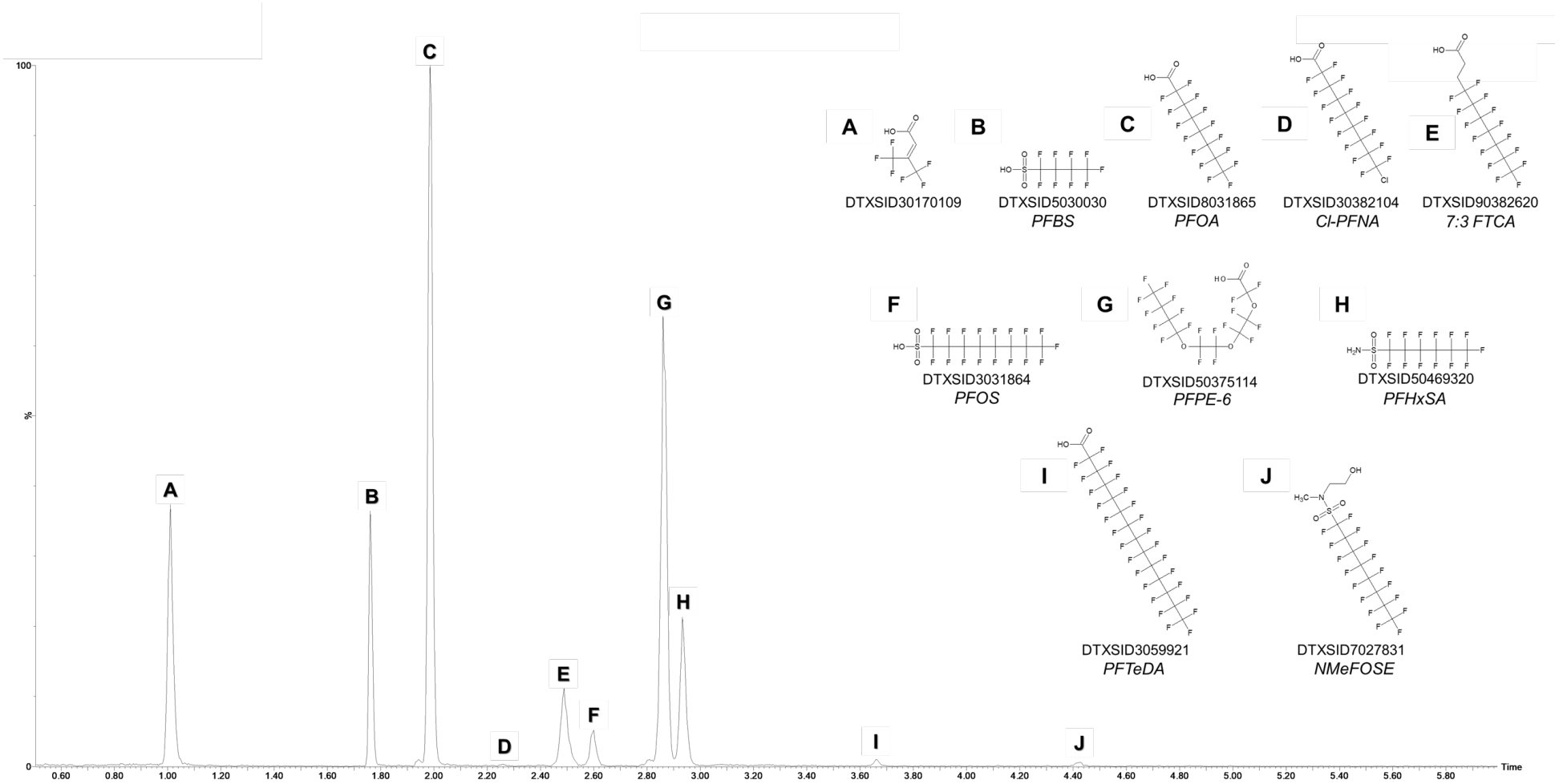


Figure S1. Representative Chromatogram of LC-MS Assessed PFAS. Ten unique PFAS are shown at 10 pg/µL. These varying responses based on the relative height of each peak indicates the sensitivity of each analyte.

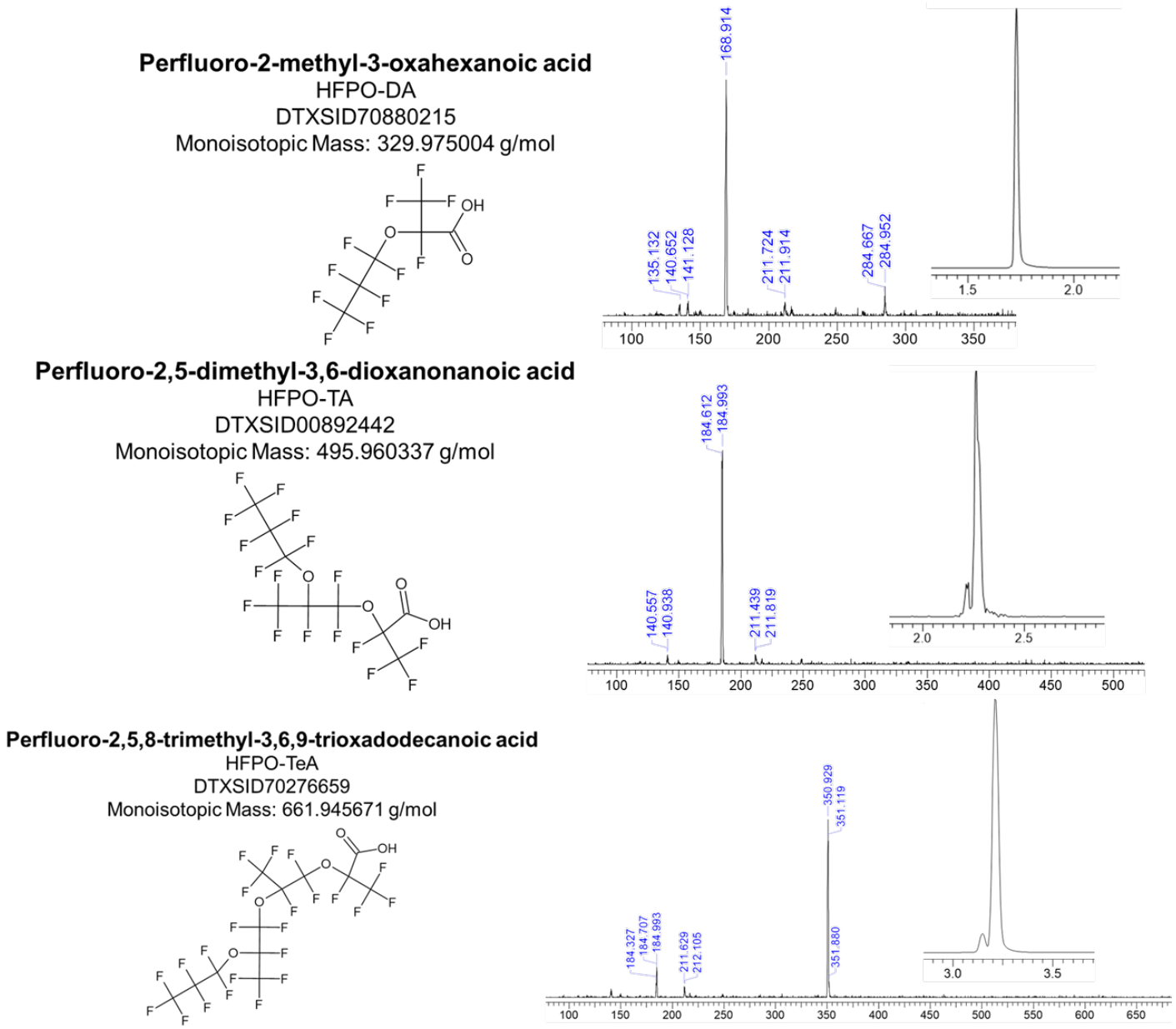


Figure S3. Hexafluoropropylene oxide (HFPO) acids assessed in DMSO, EtOH, and/or water. Chemical structures shown as well as the corresponding mass spectrum obtained in EtOH, which provided passing quality scores. A) Perfluoro-2-methyl-3-oxahexanoic acid (HFPO-DA, DTXSID70880215); B) Perfluoro-2,5-dimethyl-3,6-dioxanonanoic acid (HFPO-TA, DTXSID00892442); C) Perfluoro-(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid (HFPO-TeA, DTXSID70276659). Although the mass spectrum shown do not confirm the parent MW, fragmentation supports the chemical structure, where MRM transitions provided adequate sensitivity for *in vitro* experimentation.

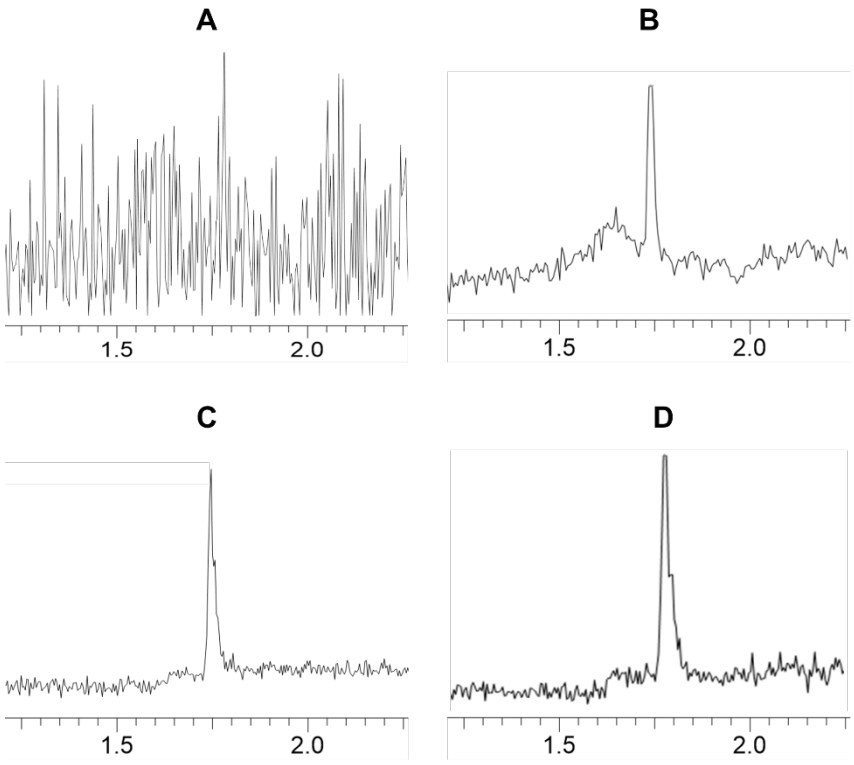


Figure S4. MS2 ESI- TIC analysis of 100 pg/µL Perfluoro-2-methyl-3-oxahexanoic acid (HFPO-DA, DTXSID70880215) in DMSO [A], EtOH [B], water [C], and methanol (certified standard) [D]. HFPO-DA elutes at 1.73 min. No standardization of the response attenuation was applied for the visualization of these detected HFPO-DA solvent samples.

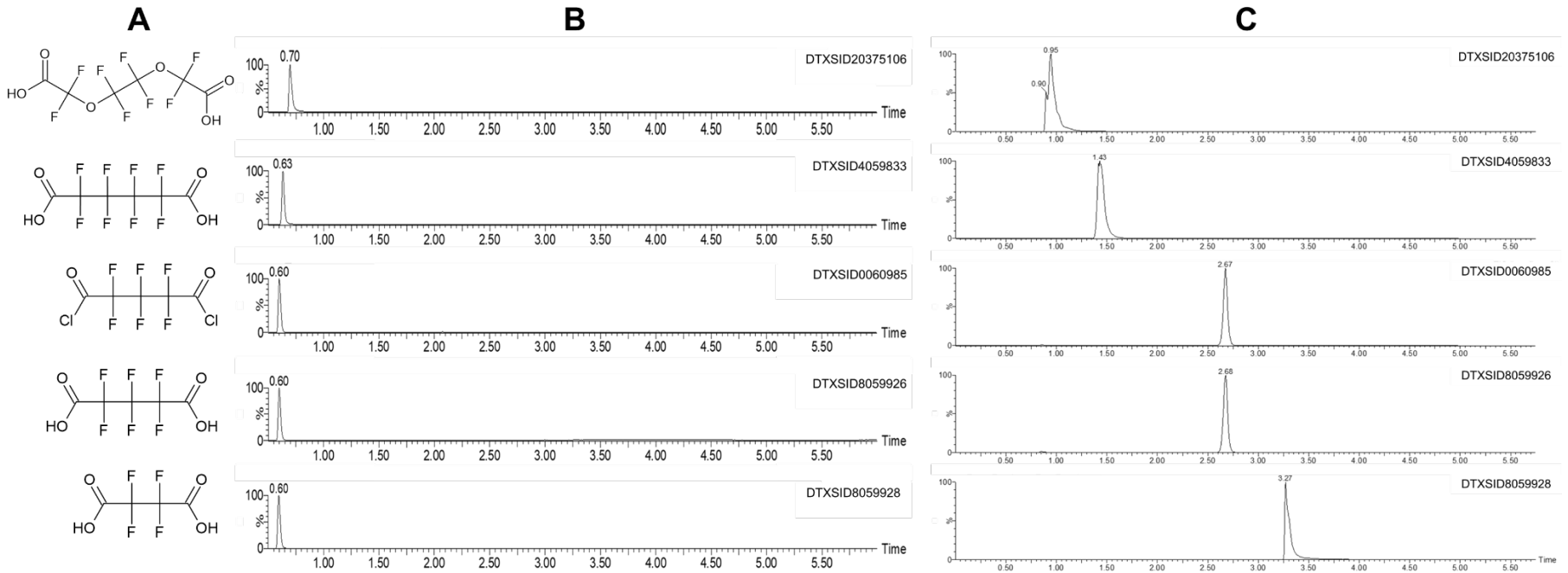


Figure S5. Polar analytes with standard RP chemistry vs HILIC solubilized in DMSO. PFAS diluted to final concentration of 100 pg/uL.

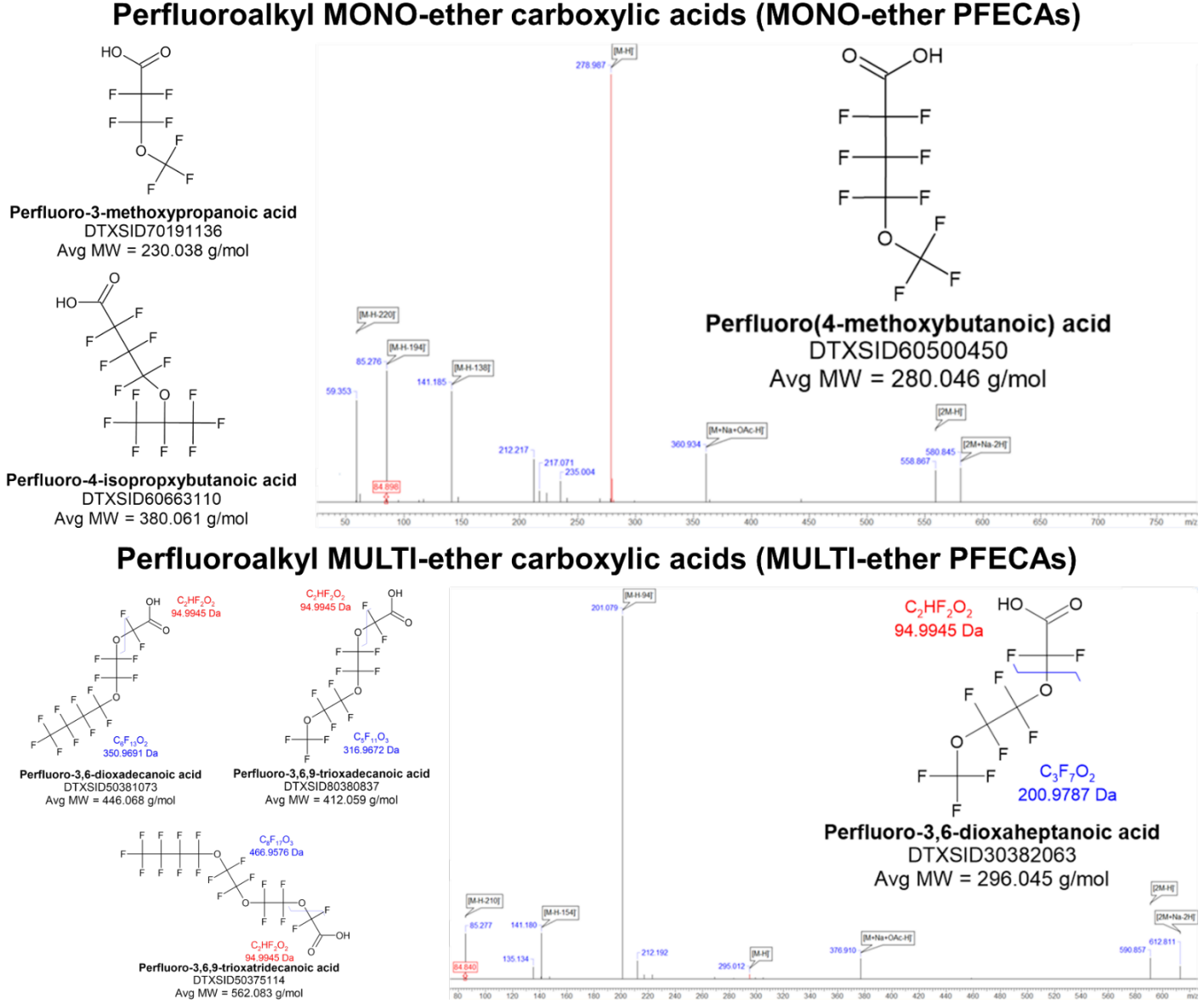
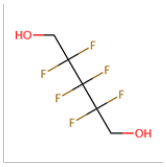


Figure S6. Perfluoroalkyl Ether Acids (PFECAs) Assessed in this PFAS Library. Mass spectrum for two select PFECAs highlighted, showing that in-source fragmentation (loss of 95 Da) results for multi-ether PFECAs for more sensitive quantitation.

DTXSID	CASRN	Preferred_Name	Monoisotopic mass DA
DTXSID3059927	376-90-9	Hexafluoroamylene glycol	212.0272



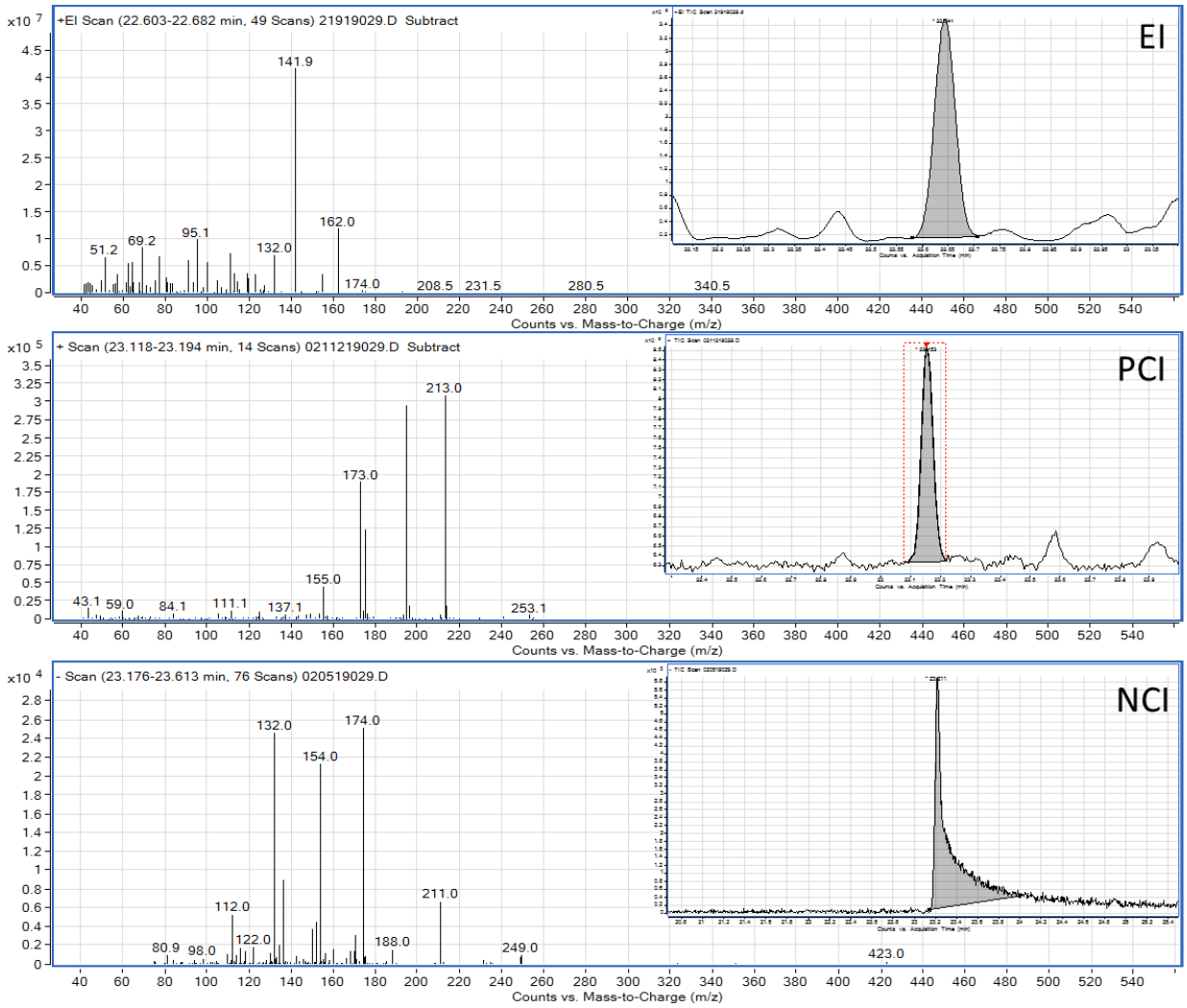
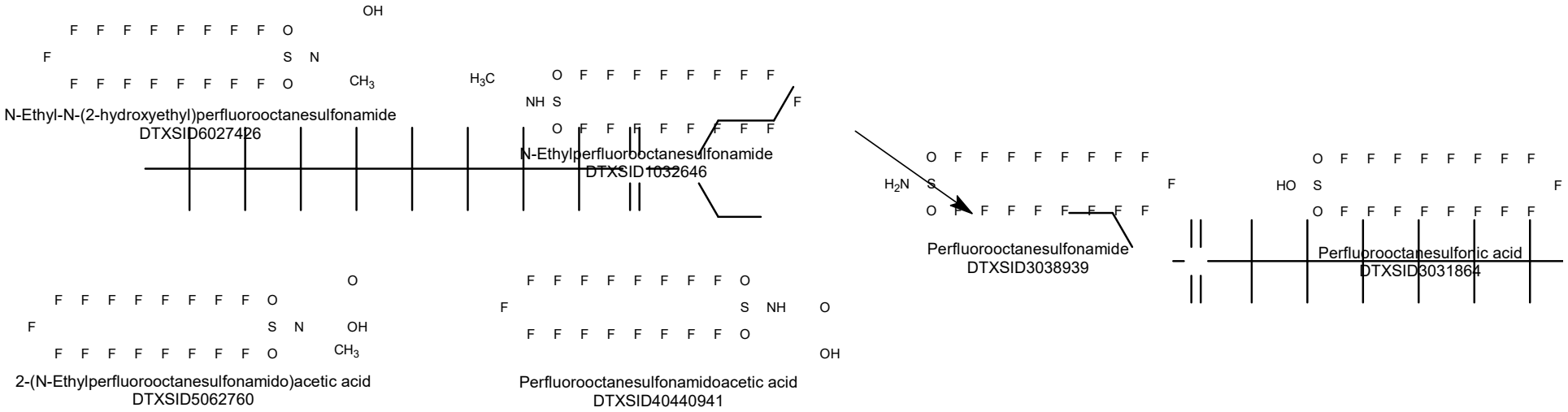
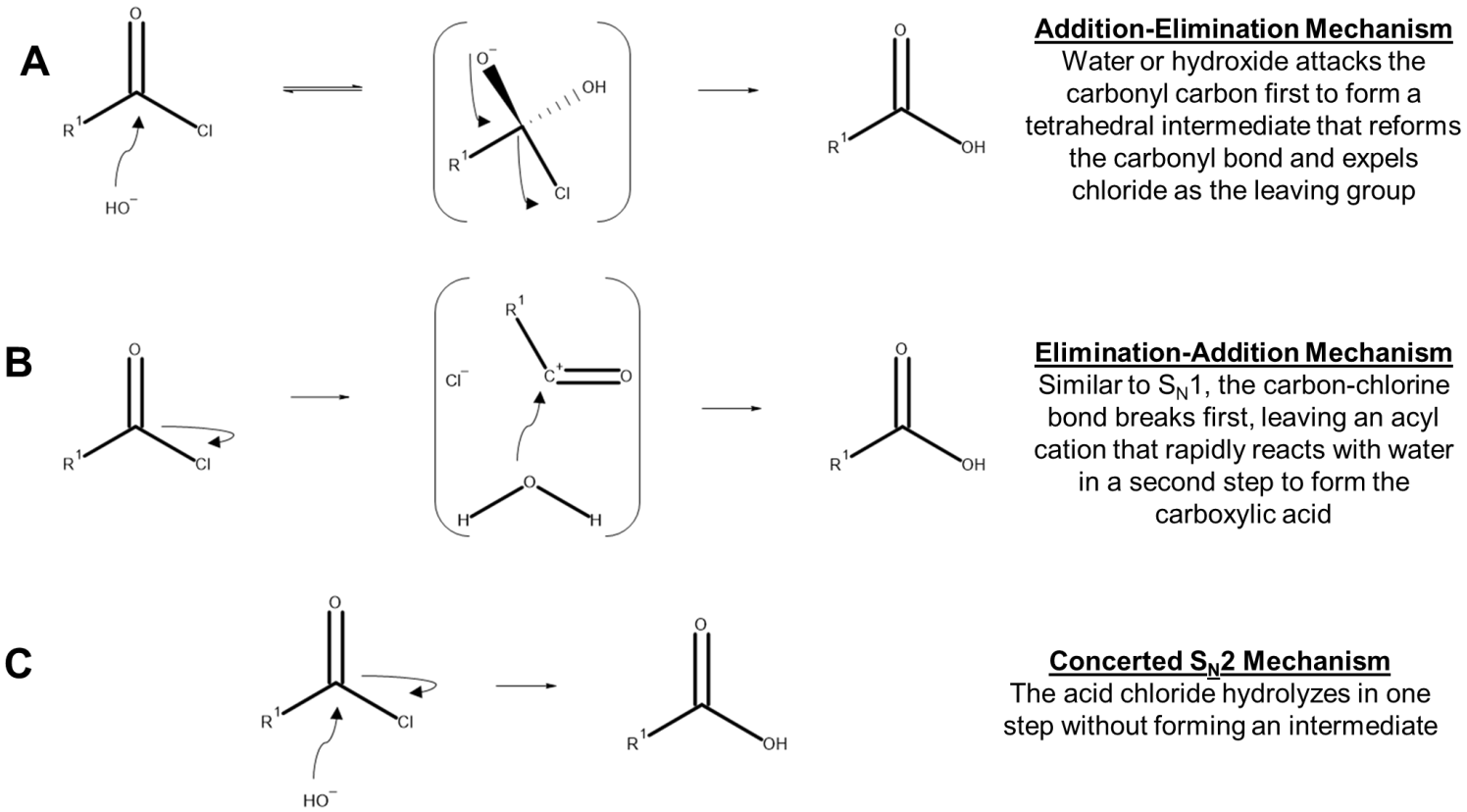


Figure S7. Representative chromatogram of a PFAS chemical assessed by GC-MS. Hexafluoroamylene glycol (DTXSID3059927) was analyzed by EI, PCI, and NCI to determine sample quality score in DMSO and EtOH. Both PCI and NCI allowed for confirmation of the parent molecular weight with respect to the ionization applied, while EI did not well-present the molecular ion.



Scheme S1. Proposed Biotransformation of PFAS Sulfonamides. Based on proposed metabolic trees from Fu *et al.,* 2015, Mejia Avendano and Liu, 2015, and Zhao *et al.,* 2018.



Scheme S2. Potential Nucleophilic Substitution Mechanisms for Acyl Chlorides. The only likely mechanism that is shared between acyl chlorides, acyl fluorides, sufonyl chlorides, and sulfonyl fluorides is the addition-elimination mechanism. These proposed pathways are based Douglas, Cambell, and Wigfield, 1993; Hall 1955; Bunton and Fendler 1966; and Gramstad and Haszeldine 1957.