DAY 2

Leave 1 needle open
flow to 1
Flush ~ 5s

Transmethylation of fatty acids (from Ursula Strandberg) July 2010 – SY updated 27-Nov-2012

1. Begin with evaporated sample from extraction procedure
2. Add 1 ml toluene (glass pipette only)
3. Add 2 ml 1% sulfuric acid in methanol
4. Flush with nitrogen and recap, vortex 10 sec (check to make sure fully mixed)
5. Incubate overnight (about 16 h) in water bath (50 C) set to black mark on dial
6. Remove tubes from the water bath and allow to cool (5-10 minutes).
7. Add 2 ml 2% $KHCO_3$ (Hood)
8. Add 5 ml hexane: diethyl ether 1:1 (Flammables)
9. Cap tubes and shake gently, then release cap to let out CO2.
10. Tighten caps and vortex tubes for 10 sec, then centrifuge 2 min at 1500 rpm
11. Transfer the upper phase into new tube (labeled *sample#*-C), being careful not to remove *any* of the lower layer. Check the bottom of the tube for droplets of the lower layer before evaporating. If *any* droplets are present, pipette the upper phase into a new tube. The lower layer contains sulfuric acid, and will harm the GC column.
12. Add 5 ml hexane: diethyl ether to sample tube B
13. Cap, shake, release cap and CO2, re-cap tube. Then vortex and centrifuge (same time/speed).
14. Transfer the upper phase into tube C.
15. Evaporate off FAMEs tube solvents under nitrogen. Be sure to check for lower layer droplets before evaporation.
16. Add 1.5 ml hexane, vortex to dissolve FAME (OK to store in freezer)
17. Transfer hexane (containing FAMEs) to pre-labeled GC vial – Store this in freezer (-20°C, not -80)
18. Optional: to concentrate sample, evaporate off hexane and add 0.5 ml hexane

*polypropylene pipette tips are ok to use for transferring of chloroform, methanol, 1% sulfuric acid, KHCO3 solutions, hexane, diethyl ether, and BHT. Refer to page 18 of https://fscimage.fishersci.com/images/D16542~.pdf

Toluene must be transferred using a glass pipette.