DECEMBER 1 2012

SUGGESTED LABORATORY PROCEDURES TO DRAW AND ARCHIVE BLOOD SAMPLE FROM INDIVIDUAL WHO SUSPECTS EXPOSURE TO AVIATION ENGINE OIL FUMES. INTENDED FOR POSSIBLE FUTURE ANALYSIS OF TRICRESYLPHOSPHATE METABOLITE(S) IN BLOOD

As of this writing, it has been suggested that it may be optimal to have a blood sample drawn and processed not sooner than 12 hours post-exposure to aviation engine oil fumes, but not longer than 72 hours post-exposure.

Background: Until mid-2012, a research team led by Prof. Clem Furlong at the University of Washington (UW) accepted and archived blood samples from airline crewmembers and passengers, globally, who suspected exposure to aviation engine oil fumes and wanted their blood tested for metabolite(s) of some of the tricresylphosphate constituents of the oil fumes. The blood test development work continues, but the UW lab can no longer archive blood samples due to limited resources and the need to focus exclusively on analytic protocol development. The GCAQE recognizes that some exposed crewmembers may wish to privately arrange for their blood to be drawn and archived after a suspected exposure to aviation engine oil fumes, anticipating that the sample could be analyzed in the future for tricresyl phosphate metabolite(s), once the necessary tests are finalized. As a courtesy, then, what follows are some suggested laboratory procedures to isolate mononuclear cells (MNCs), red blood cells (RBCs), and plasma from whole blood, and to store them appropriately until suitable tests.

Disclaimer: The GCAQE assumes no responsibility for the archived blood samples that are prepared according to these instructions. Further, the GCAQE recommends that each individual ensure that chain-of-custody procedures are documented and appropriate in order to maintain confidence in the validity of any future analyses.

1. Collect approximately 35-40 ml total of whole blood. Either heparin or EDTA tubes would be suitable. Alternatively, the lab may use cell-preparation tubes (CPT). Make note of original blood volume.

2. If using CPT, qualified lab staff will separate the whole blood into MNC, RBC, and plasma fractions, and may then skip to step 11 (below) for storage suggestions. If using heparin or EDTA tubes, use a disposable transfer pipet to transfer blood to 15 ml or 50 ml centrifuge tubes (depending on volume received) and spin down at 1000 RPM at RT for 10 minutes.

3. Remove plasma and set aside on ice to aliquot later. Alternatively, freeze the sample within the temperature range -20 to -80^{o}F.

4. Bring the RBC/MNC cell pellet to twice the original whole-blood volume in phosphate-buffered saline (PBS); e.g., if original blood volume was 10 ml, add PBS to a total final volume of 20 ml.

5. Depending on the volume of the sample, in either a 15 ml or 50 ml centrifuge tube, add Ficoll/Hypaque to the original volume of blood (e.g. if the original volume of blood was 10 ml, add 10 ml F/H). Layer diluted blood on top of the F/H making sure that there are distinct layers of blood and F/H. (If you began with 10 ml of blood, there will be 20 ml diluted blood cells on top of 10 ml F/H = 30 ml. This can be done in a single 50 ml centrifuge tube.)

6. Centrifuge at 400 x g (1500 RPM in the Beckman J-6B) for 30 minutes at room temperature with the brake OFF.

7. Using a transfer pipet, remove the monocyte layer into a 15 ml centrifuge tube. Discard the clear volume (by aspiration into a flask) above the red cell fraction at the bottom of the tube. You will now have one tube of MNCs and one tube of RBCs.

8. Wash the monocytes and the RBCs twice more with PBS. Spin at 400 x g for 15 minutes after each wash.

9. After the last MNC wash, remove the PBS and re-suspend the monocyte pellet in water, at $1/10^{th}$ the original volume of whole blood (e.g., for 10 ml whole blood, re-suspend the pellet in 1 ml water). Can be frozen at this point (-20 to -80^{o}F).

10. After the last RBC wash, remove the clear PBS on top of the RBC layer and re-suspend the RBCs by vortexing. (Alternatively, can simply freeze the RBC pellet at this point (-20 to -80^{o}F)).

11. Aliquot (i.e., separate) the RBCs, MNCs, and previously saved plasma into 2.0 ml centrifuge tubes. Label the tops with the number assigned the sample. Freeze at -20 to -80^{o}F

12. If equipment is not available for these steps, simply centrifuge the sample and save the blood cells and plasma separately as frozen samples (-20 to -80^{o}F).

13. Be sure that the tubes are accurately labeled with a code number that an individual responsible is able to link to the donor. This will need to conform to the local protection of human subjects regulations.

PLEASE COMPLETE THIS QUESTIONNAIRE TO ACCOMPANY YOUR CONSENT FORM TO PARTICIPATE IN THE RESEARCH INTENDED TO DEVELOP A MEANS TO IDENTIFY EVIDENCE OF EXPOSURE TO ENGINE OIL ADDITIVES IN BLOOD

1) Your name: _____ Email: _____

   Today's date: _____ Airline (optional): _____

   PH CONTACT_

2) Is your blood sample connected to a specific and recent smoke/fume event? (circle one) Yes --- No

3) Have you been in one or more fume events in the past, to your knowledge? (circle one) Yes --- No

4) If you are reporting a specific and recent fume event, please answer the following questions:

   Aircraft type: _____ Aircraft number: _____

   Flight number: _____ from: _____ to: _____

   Did you report the incident to the airline? (circle one) Yes --- No

   Did the incident impact the flight schedule/route? (circle any that apply)
   delay --- diversion --- emergency landing --- don't know --- other: _____

   Are you aware of any other documentation about conditions on this flight? (circle any that apply)
   pilot log book – maintenance records – media report – passenger complaints – other: _____

   During what phase of flight did you notice the event? (circle as many as apply)
   gate --- taxi --- takeoff --- ascent --- cruise --- descent – landing --- taxi

   Did you notice an odor? (circle one) Yes --- No
   If yes, describe: _____

   Did you notice a smoke/fume/haze? (circle one) Yes --- No
   If yes, describe: _____

   Did you have symptoms in-flight? (circle one) Yes --- No
   If yes, describe: _____

5) What is the name of the hospital/lab that drew your blood? _____

6) How many hours between the time of your exposure and your blood draw? _____

7) Did you seek medical attention after the flight? (circle one) Yes --- No
   If yes, describe: _____

8) Did you have symptoms the day after the flight? (circle one) Yes --- No
   If yes, describe: _____

9) Did you have symptoms beyond the day after the flight? (circle one) Yes --- No
   If yes, describe _____

Thank you for providing this information. Please fill out both pages and return with your consent form.

10) Diet and medications can influence how your body processes certain chemicals.

(a) Please list any medications/dietary supplements that you take (voluntary):

_____

_____

(b) Please list the foods/drinks you consumed during the 24 hours before the exposure (as best you can):

_____

_____

_____

_____

11) Any other comments:

_____

_____

_____

12) To satisfy US National Institutes of Health survey questionnaire requirements, please answer the following questions:

12a) Ethnicity:
__ Hispanic
__ Not Hispanic
__ Don't know

12b) Race:
__ American Indian/Alaska Native
__ Asian
__ Native Hawaiian/Other Pacific Islander
__ Black or African American
__ White
__ More than one race
__ Other
__ Don't know

13c) Gender:
__ Female
__ Male

Please fill out both pages and return with your signed consent form to: