THE UNIVERSITY OF ADELAIDE AUSTRALIA

SAFE OPERATING PROCEDURE:

TREATING MAMMALIAN CELLS

LOCATION DETAILS

School/Branch: School of Molecular & Biomedical Science

SAFE OPERATING PROCEDURE DETAILS

Treating mammalian cells in culture with aryl hydrocarbon receptor ligands Benzopyrene, Beta-Napthoflavone, & 2,3,7,8-Tetrachlorodibenzodioxin. **REFERENCES:** Risk Assessment: Benzopyrene Risk Assessment: Beta-Napthoflavone Risk Assessment: 2,3,7,8 0 Tetrachlorodibenzodioxin (see Risk Register: www.adelaide.edu.au/mbs/safety/register)	Date Prepared: 05/05/2010

PREPARED BY: Name, position, & Signature (insert names of supervisor, HSO, subject matter expert)

Anne Chapman-Smith – Biochemistry Research Fellow Kate Dixon – Health & Safety Officer	Signature:

RISK ASSESSMENT

Has a risk assessment been completed and all other environmental considerations been made? **YES**	See risk assessment dated: 05/05/2010	Risk Rating: Low ~~Medium~~ ~~High~~ ~~Very High~~

RISKS IDENTIFIED

- Illness/injury due to exposure to chemicals
- Musculoskeletal injury from repetitive work (pipetting)
- Cross contamination/illness or affects from exposure to substances when vessels are in the incubator
- Environmental damage from the waste being exposed to the natural environment

SAFETY PRECAUTIONS

The following control measures MUST be adhered to:

- Persons using the chemicals must have Chemical Management Training (University)
- The appropriate waste disposal procedures must be followed
- Ensure all PPE is worn
- Ensure correct manual handling techniques are used

PERSONAL PROTECTIVE EQUIPMENT REQUIRED

The following PPE must be worn at all times:

- Gloves
- Full length lab coat
- Safety goggles

NB: No part of the skin should be exposed at any time

SAFE OPERATING PROCEDURE

PREPARATION OF CELLS FOR TRANSFECTION

1. One day before the transfection experiment, trypsinize, adjust the cell concentration, and plate the cells in the chosen cell-culture vessel.

Dilute FuGENE® 6 Reagent with serum-free medium (without antibiotics or fungicides)

2. Serum-free medium must be pipetted first. The order and manner of addition is critical.
3. Pipette the FuGENE® 6 Reagent directly into the medium without allowing contact with the walls of the plastic tube.
4. Vortex for one second or flick the tube to mix.
5. Incubate for 5 minutes at room temperature.

Add DNA to diluted FuGENE® 6 Reagent

6. **Mix and incubate the complex:** Tap the tube or vortex for one second to mix the contents.
7. Incubate the transfection reagent:DNA complex for a minimum of 15 minutes at room temperature.
8. **Add complex to the cells:** Remove culture vessel from the incubator. Removal of growth medium is not necessary.
9. Add the transfection reagent:DNA complex to the cells in a drop-wise manner.
10. Swirl the wells or flasks to ensure distribution over the entire plate surface.
11. **Return the cells to the incubator for up to 24 hours.**

Treatment with Ligand (Benzopyrene (B[a]P), Beta-Naphthoflavone (BNF), TCDD)

12. Remove stock solution of ligand from storage. Stock concentration TCDD 10μM; B[a]P 100 μM
13. Dilute stock of ligand into required volume of prewarmed media in tissue culture hood. Final concentration is 1-10 nM TCDD; 100 nM – 1 μM B[a]P; BNF, nM range

i. measure media into screw cap container.
ii. open tube containing ligand solution and pipette up required volume.
iii. close tube containing ligand solution.
iv. dispense ligand solution into media in screw cap container, close container.
v. mix gently by inversion.

14. Pipette tips used for TCDD should be collected separately in a falcon tube and sealed. For B[a]P and BNF, dispose of tips into cardboard waste container, which can then go into normal TC waste for autoclaving.
15. Remove cell-culture vessels from incubator and place in tissue culture hood
16. Aspirate media from cells
17. Gently pipette required volume of diluted ligand in media onto cells in cell-culture vessel.
18. Pipette tips used for TCDD should be collected separately in a falcon tube and sealed. Remaining media containing TCDD is placed in a sealed container for disposal. For B[a]P and BNF, dispose of tips as above (14) and media in normal TC waste for autoclaving.
19. Return vessel to incubator. Vessel should be labelled with ligand warning for safety of other users. (Quantities are minimal at this stage; <<0.1% w/w)
20. Clean work area thoroughly and dispose of this waste and gloves in the same manner as for other material which has been used for handling ligand.
21. Return stock solution of ligand to safe storage.
22. After incubation for up to 24h, transfer cell-culture vessel containing cells growing in the presence of ligand from incubator to the tissue culture hood.
23. Using a pipette, aspirate media containing ligand from cells. For media containing TCDD, dispense the used media into a sealed container (yellow screw cap TC vial or falcon tube) for disposal. Pipette tips used for TCDD should be collected separately in a falcon tube and sealed. Media containing B[a]P or BNF can be aspirated into normal TC waste for disposal. For B[a]P and BNF, dispose of tips into cardboard

waste container, which can then go into normal TC waste for autoclaving.

24. Harvest cells according to next procedure, ie, reporter assays, RNA or protein assays. Cells should contain minimal quantities of the added ligand (<<< 0.1% w/w).
25. Containers with media containing TCDD are placed in fume hood with lid open until liquid has evaporated, then the sealed containers are placed in TCDD waste container for disposal.
26. All plasticware that has been in contact with TCDD is collected into sealed plastic bags which are placed in TCDD waste container for disposal.
27. TCDD WASTE DISPOSAL – ALL MATERIAL FROM THE EXPERIMENT THAT CONTAINS OR HAS BEEN IN CONTACT WITH TCDD MUST BE PLACED IN A SEPARATE SEALED CONTAINER WITH THE TOTAL QUANTITY OF TCDD USED IN THE EXPERIMENT CLEARLY WRITTEN ON THE OUTSIDE TO INDICATE THE MAXIMUM LEVEL OF TCDD THAT REQUIRES DISPOSAL.

OTHER INFORMATION

TCDD WASTE DISPOSAL – ALL MATERIAL FROM AN EXPERIMENT THAT CONTAINS OR HAS BEEN IN CONTACT WITH TCDD MUST BE PLACED IN A SEPARATE SEALED CONTAINER WITH THE TOTAL QUANTITY OF TCDD USED IN THE EXPERIMENT CLEARLY WRITTEN ON THE OUTSIDE TO INDICATE THE MAXIMUM LEVEL OF TCDD THAT REQUIRES DISPOSAL.

ADMINISTRATION

Note: This Safe Operating Procedure must be reviewed :

a) after any accident, incident or near miss;

b) when training new staff;

c) if adopted by new work group;

d) if equipment, substances or processes change; or

e) within 5 years of date of issue.