

Basic training in cell culture for the study of cell proliferation under the effect of Butyl Benzyl Phthalate

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Background: Butyl Benzyl Phthalate (BBP) is an ester with the formula $C^{15}H^{20}O^{04}$; It is a plasticizer, and can be found in items such as conveyor belts, traffic cones, and artificial leather. BBP has been shown to cause cancer in breast tissue. The effect of BBP on a transformed and invasive cell line was tested. The cell line being tested is called C5 or bsMCF (1), a derivative from the non-cancerous epithelial breast cell line MCF10F developed in the BCRL in 1990.

Objective: To provide basic training on how to measure the effect of BBP on the cell proliferation and morphology of the C5 cell line using the cell proliferation assay, Alamar Blue.

Work Performed: BBP stock solution of 70mM was diluted to 3 test concentrations: 0.7nM, 7nM, and 70nM. C5 cells were maintained in high calcium culture medium, and harvested for testing. Testing of C5 cells was performed in 96-well plates, with 5 rows of 8 wells each corresponding to the following conditions: blanks (no cells, no BBP, only media), control (cells + regular medium, no BBP), and the 3 BBP test concentrations (i.e. cells + 0.7, 7, and 70nM, each in a separate column). Each well in all conditions contained a total of 100 μ L of liquid. C5 cells were grown in the 96-well plates for 5 consecutive days; on each day all wells were incubated with 10 μ L of Alamar blue (1/10 volume ratio) for 4 hours. After the 4 hour incubation with Alamar blue the 96-well plates were placed in an Epoch spectrophotometer/plate-reader, which measures the change from blue to pink of the Alamar and this is recorded in the software Gen5. The degree of change from blue to pink in the Alamar blue reflects the amount of cellular respiration the cells in that well were performing at the time, i.e. more pink = more cellular respiration.

Results: During the four weeks in the BCRL the student learned basic techniques on how to measure the effect of BBP on the cell proliferation and morphology of the C5 cell line using the cell proliferation assay, Alamar Blue.

Conclusions: We have learned how to manipulate human breast epithelial cells in culture and how to measure basic parameters like cell proliferation and cell morphology.

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References:

- (1) <http://www.fccc.edu/research/pid/fernandez/research.html> under the title of ' Reversion of the neoplastic transformation'