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CYTOTOXICITY – MEM ELUTION – MG023

Test Article: Comb-18
Test article size used:29.4 sq.cm (1.7 grams), release paper removed .

Procedure: A monolayer of L-929 mouse fibroblast cells was grown to confluency and exposed to an extract of the test article prepared by placing the test article in 20 ml of 55 Minimum Essential Medium and extracting at 37º C for 24 hours. Duplicate MEM aliquots were used as negative controls. The positive control was extracted at 37º C for 24 hours and tested using an end-point titration procedure. After exposure to the extracts, the cells were examined microscopically for cytotoxic effect (CTE). Presence (+) or absence (-) of a confluent monolayer, vacuolization, cellular swelling and crenation and the percentage of cellular lysis were recorded.

CTE Score	Microscopic Appearance of Cells
Nontoxic (N)	A uniform, confluent monolayer, with primarily elongated cells, and discrete intracytoplasmic granules present at the 24 hour observation. At the 48 and 72 hour observation periods, there should be an increasing number of rounded cells as cell population increases and crowding begins. Slight or no vacuolization, crenation or swelling should be present.
Intermediate (I)	Cells may show marked vacuolization, crenation or swelling. Cytolysis (0-50%) of cells that5 results in floating cells and debris in the medium may be present. The remaining cells are still attached to the flask surface.
Toxic (T)	Greater than 50% of all cells have been lysed. Extensive vacuolization, swelling, or crenation are usually present in the cells remaining on the flask surface.

Results:

	Confluent Monolayer	Vacuolization	Swelling	Crenation	%lysis	CTE Score
24 Hours						
Test Medium	(+)	(-)	(-)	(-)	0	N
Neg. Controls	(+)	(-)	(-)	(-)	0	N
48 Hours						
Test Medium	(+)	(-)	(-)	(-)	0	N
Neg. Controls	(+)	(-)	(-)	(-)	0	N
72 Hours						
Test Medium	(+)	(-)	(-)	(-)	0	N
Neg. Controls	(+)	(-)	(-)	(-)	0	N

Positive Control, SCG-7, was toxic at a dilution of 1:16 at 24 hours

Conclusion: NONTOXIC

ORIGINAL ON FILE

Comments: Not Applicable.

Date Prepared: 7-19-93 Date Terminated: 7-23-93

Completed Tech Approved