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REPORT REFERENCE: 136

TITLE: Genetic Evaluation of Dow Corning Q7-2159A Medical Gel  
in the In Vitro Mammalian Cell transformation Assay.  
Dow Corning Tox. File No. 5773-2

Date: February 9, 1989

AUTHORS: R.T. Henrich and J.M. McMahon

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SOURCE: Internal Dow Corning study

MATERIAL: Silicone mammary gel

GLP STATUS: Yes

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ABSTRACT: An ethanolic extract of Q7-2159A was evaluated for an ability to induce morphological transformation of BALB/C-3T3 cells in culture with and without metabolic activation. No activity was observed.

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ADVERSE EFFECTS: None

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DOW CORNING CORPORATION  
Toxicology Department

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PROPRIETA

GENETIC EVALUATION OF DOW CORNING®  
Q7-2167/58\* IN THE IN VITRO MAMMALIAN  
CELL TRANSFORMATION ASSAY

File No.: 5773-2

Reference No.: TX-88-0700-55

Series No.: I-0005-2505

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Date: February 9, 1989

Reference No.: HH038980

GLP/QAU: P. A. Bils'  
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This summary of data and conclusions is based upon the sample received.  
Additional studies may be required as specific uses and formulations are  
developed or if process changes occur.

ABSTRACT

An extract of the test material was evaluated for its ability to induce  
morphological transformation of BALB/C-3T3 cells in culture both with and  
without metabolic activation. The material was found to be inactive in the  
induction of morphologically transformed cells.

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\*Responsive Medical Gel

Distribution

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OBJECTIVE

The objective of this assay is to evaluate the test article for its ability to induce morphological transformation of cultured BALB/C-3T3 cells. Transformation of BALB/C-3T3 cells is recognized by the appearance of dense, piled-up foci of altered cells superimposed on a monolayer of normal cells.

RATIONALE

BALB/C-3T3 mouse cells will multiply in culture until a monolayer is achieved and will then cease further division. These cells, if injected into immunosuppressed, syngeneic host animals will produce neoplastic tumors. However, cells treated in vitro with chemical carcinogens will give rise to foci of cellular growth superimposed on the cell monolayer. If these foci are picked from the culture, grown to larger numbers and injected into animals, a malignant tumor will in most cases be obtained. Thus, the appearance of foci of altered cells is correlated with malignant transformation.

MATERIALS

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A. Indicator Cells

Clone 1931 of BALB/C-3T3 mouse cells was obtained from American Type Culture Collection (ATCC). Further subclones, selected for low spontaneous frequencies of foci formation, are used for assays. Stocks are maintained in liquid nitrogen and laboratory cultures and are checked periodically to ensure the absence of mycoplasma contamination. Cultures are grown and Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% calf serum (CS). They were maintained at 37 ± 2°C in a humidified atmosphere containing approximately 5% CO<sub>2</sub>.

B. Control Articles

1. Solvent/Negative Controls

A solvent control was performed for each portion of the assays by exposing the cells to the solvent only. In the activation portion of the assay, the solvent control cultures were exposed to the S-9 activation mix. The solvent used in this assay was ethanol.

2. Positive Controls

3-Methylcholanthrene (3-MCA) was used as a positive control in the nonactivation assay. This chemical was used at a final concentration of 5 µg/ml.

Benzo(a)pyrene (BP) was used as a positive control in the activation assay. This chemical was used at a final concentration of 12.5 µg/ml.

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EXPERIMENTAL DESIGNA. Preliminary Cytotoxicity Testing

Since the material to be tested was a gel, which was not soluble in any solvent compatible with the biological test system, an extraction procedure was used. Ten grams of the test material was extracted with 10 ml of absolute ethanol on a New Brunswick<sup>™</sup> rotary shaker for 24 hours/37°C/150 rpm. Twelve dose levels were chosen to determine the dose range to be employed in the transformation assay. The growth medium used was DMEM with 10% cs.

Cells were seeded at 200-250 cells/60-mm dish and were cultured for 24 hours in 5 ml of growth medium. The cells were then exposed, in both the presence and absence of S-9 activation, to each dose. The cells being exposed in the absence of metabolic activation were given a three-day exposure period and those in the presence of metabolic activation a four-hour exposure period. After either a three-day or four-hour exposure period, the cells were washed and incubated in fresh growth medium for an additional 7-10 days. The surviving colonies were stained and counted. A relative survival for each dose was obtained by comparing the number of colonies surviving treatment to the colony counts in the solvent control dishes. The highest dose chosen for the subsequent transformation assay would normally have caused no more than a 80-90% reduction in the colony-forming ability of the 3T3 cells (Rundell, J. O., et al, 1983). Since none of the doses caused a 80-90% reduction in the colony-forming ability of the 3T3 cells, the maximum dose used in both transformation assays was 10,000 µg/ml. Three lower doses were also selected for the transformation assays.

B. Transformation Assay1. Nonactivation Assay

The procedure used was adopted from that reported by Kakunaga (1973). Exponentially growing BALB/C-3T3 cells are seeded at 200-250 cells/60-mm dish in three dishes per treatment for the cytotoxicity studies of each treatment and at  $1 \times 10^4$  cells/60-mm dish in a 15 replicate dishes per condition for the transformation assay. After a 24-hour incubation, the dishes were treated for each of the following conditions: four preselected doses of the test material; the positive control; and the solvent control. All testing was carried out in 5 ml of growth medium. The dishes were incubated for three days in the presence of the test material at 37°C. After the exposure period, the medium was removed and the dishes were washed with Hank's Balanced Salt Solution (HBSS). Fresh growth media was added and the dishes were reincubated for approximately 8-10 days for the cytotoxicity study and four weeks for the transformation assay. During this time, the media was changed twice a week.

At the end of the respective incubation periods, all colonies were fixed with methanol and stained with Giemsa. The stained dishes were examined by eye with a microscope to determine the number of surviving colonies for the cytotoxicity assay and the number of foci of transformed cells for the transformation assay.

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2. Activation Assay

The activation assay was performed independently with its own set of solvent and positive controls. The procedure was identical to the nonactivation assay except for the addition of the S-9 fraction of rat liver homogenate, Aroclor® 1254 induced, obtained commercially from Hazleton Laboratories (formerly Litton Bionetics, Incorporated), Kensington, Maryland, and necessary cofactors during the four-hour treatment period.

RESULTS AND DISCUSSION

The results are presented in Tables I-IV. Preliminary cytotoxicity data is presented in Tables I and II. As can be seen, the extract produced no toxicity with or without metabolic activation. Therefore, 10,000 µg/ml was chosen as the starting concentration.

Tables III and IV represent the results of the transformation assays with and without metabolic activation, respectively. No significant increase in the transformation frequency was observed either with or without activation. The test material should be considered inactive in inducing morphological transformation of BA1B/C-3T3 cells.

DATA PRESENTATION

A. Relative Survival

Relative Survival (%) - (Average number of colonies per treated culture/average number of colonies per solvent control dish) x 100%.

B. Plating Efficiency (PE)

Plating Efficiency (%) - (Average number of colonies per dish/number of cells seeded) x 100%.

C. Cell at Risk (CAR)

CAR - Number of dishes x  $1 \times 10^4$  x PE.

D. Transforming Frequency (TF)

TF - Number of foci/CAR.

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STATISTICAL EVALUATION

Statistical tables from Kastenbaum and Bowman (1970) were utilized to determine the statistical significance at each dose level versus the negative control at the 95% or 99% confidence level. The 95% confidence level is the minimum acceptable level for considering the test material to be positive for transforming activity.

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4. Schechtman, L. H. and Kouri, R. E., 1977. Control of Benzo(a)-pyrene-Induced Mammalian Cell Cytotoxicity, Mutagenesis and Transformation by Exogenous Enzyme Fractions. *In: Progress in Genetic Toxicology*, D. Scott, B. A. Bridges, and F. H. Sobels, eds. Elsevier/North-Holland Biomedical Press, NY, pp. 307-316.

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This report constitutes pages 1-8 and Tables I-IV.

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QUALITY ASSURANCE STATEMENT

This report represents data generated by the Toxicology Department, Dow Corning Corporation, Midland, Michigan. This study was conducted according to EPA Toxic Substances Control; Good Laboratory Practices Regulations; 40 CFR, Part 797 Vol. 48, No. 230. The results reported accurately reflect the data generated. All raw data is located at Dow Corning Corporation.

Study Initiated: October 24, 1988  
Study Completed: January 31, 1989  
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Report Issued: February 9, 1989

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TABLE I

Preliminary Cytotoxicity With Metabolic Activation

Plate Number	Concentration $\mu\text{g/ml}$	Cell Count			Average Count	Relative Survival (%)
		Dish Number 1	Dish Number 2	Dish Number 3		
Control	---	112	118	113	114.3	100.0
1	10,000	84	83	92	86.3	75.5
2	5,000	92	98	97	95.7	83.7
3	2,500	108	89	92	96.3	84.5
4	1,250	81	73	83	79.0	69.1
5	625	92	109	108	103.0	90.1
6	312.5	87	81	93	87.0	76.1
7	156.3	93	90	85	89.3	78.2
8	78.1	92	83	96	90.3	79.0
9	39.1	101	93	102	98.7	86.3
10	19.5	102	103	84	96.3	84.3
11	9.8	98	87	93	92.7	81.1
12	4.9	110	111	99	106.7	93.3

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TABLE II

Preliminary Cytotoxicity Without Metabolic Activation

Plate Number	Concentration $\mu\text{g/ml}$	Cell Count			Average Count	Relative Survival (%)
		Dish Number 1	Dish Number 2	Dish Number 3		
Control	---	128	132	127	129.0	100.0
1	10,000	103	115	109	109.3	84.5
2	5,000	108	117	119	114.7	88.9
3	2,500	104	109	113	108.7	84.2
4	1,250	103	100	99	100.7	75.0
5	625	102	97	112	103.7	80.4
6	312.5	95	90	101	95.3	73.9
7	156.3	96	92	117	101.7	78.8
8	78.1	118	103	94	105.0	81.4
9	39.1	82	95	98	91.7	71.7
10	19.5	103	112	107	107.3	83.2
11	9.8	83	88	90	87.0	67.4
12	4.9	97	92	108	99.0	76.7

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TABLE III

Transformation Assay With Metabolic Activation

Treatment	Final Concentration (ug/ml)	Plating Efficiency (%)	Relative Survival (%)	CAR (x 10 <sup>3</sup> )	Foci (x	
<u>Solvent Control</u>						
EtOH <sup>a</sup>	---	54.0	100.0	81.0	6	
<u>Positive Control</u>						
B <sup>b</sup>	12.5	45.4	84.0	68.1	28	
<u>Test Material</u>						
Q7-2167/68	10,000	44.9	83.1	67.4	4	0
	5,000	45.7	84.5	68.6	3	0
	2,500	48.9	90.5	73.4	7	0
	1,250	47.3	87.6	71.0	5	0

<sup>a</sup>EtOH - Absolute Ethanol<sup>b</sup>B<sup>b</sup> - Benzo(a)pyrene

\*Significant Increase, P&lt;0.01

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TABLE IV

Transformation Assay Without Metabolic Activation

Treatment	Final Concentration (µg/ml)	Plating Efficiency (%)	Relative Survival (%)	CAR (x 10 <sup>3</sup> )	Foci
<u>Solvent Control</u>					
ETOH <sup>a</sup>	---	50.7	100.0	76.1	5
<u>Positive Control</u>					
3-MCA <sup>b</sup>	5.0	40.9	80.7	61.4	26
<u>Test Material</u>					
Q7-2157/68	10,000	44.0	86.9	66.0	2
	5,000	47.0	92.8	70.5	3
	2,500	44.5	87.9	66.8	4
	1,250	49.0	96.7	73.5	2

<sup>a</sup>ETOH - Absolute Ethanol

<sup>b</sup>3-MCA - 3-Methylcholanthrene

\*Significant Increase, P<0.01

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