9-9-88

Guideline Series 84 : MUTAGENICITY

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Date: September 6, 1988

DATA EVALUATION REPORT

CHEMICAL: N-Ethyl Perfluorooctanesulfonamide Tox. Chem. No.: 454E

STUDY TYPE: In Vitro Transformation of BALB/3T3 Cells - GX-071

MRID NUMBER: 406126-12

SYNONYMS/CAS No.: GX-071

SPONSOR: Griffin Corporation

TESTING FACILITY: Toxikon Corporation, Norwood, MA

TITLE OF REPORT: In Vitro Transformation of BALB/3T3 Cells - GX-071

AUTHOR(S): Laxman S. Desai, D. Sc.

STUDY NUMBER(S): 86G-0007

REPORT ISSUED: July 25, 1986; amended March 21, 1988

Classification: Unacceptable.

CONCLUSION(S) - Executive Summary: This mutagenicity assay is inadequate in that there are too few flasks scored per test material dose level. Although the Methods' section of the report states that 15 flasks were used per control and test groups, and the Assay Acceptance Criteria state that more than eight flasks per test condition were available for analysis, the tables listing the results show only 2-3 dishes (flasks) per test-dose group. This assay cannot be used to adequately assess the carcinogenic potential of GX-071 via its ability to transform BALB/3T3 cells in vitro.

I. Materials and Methods: GX-071 (Purity-99+%; Lot/Batch No. not provided; white needle crystals) was said to be insoluble in the growth medium, and therefore, DMSO was used as the solvent. BALB/3T3 Clone A_{31} mouse cells were used in the transformation assays. Stocks were said to be checked periodically to ensure the absence of mycoplasma contamination. Routine cultures were grown and passaged weekly in Eagle's Minimum Essential Medium (EMEM) supplemented with 5% calf serum. The assay was performed under activated and non-activated conditions.

Negative controls: 1- Assay procedures performed on untreated cells; and 2- A solvent control (DMSO; final concentration of 0.3% in the growth medium). Fifteen flasks of the negative controls were prepared for each assay.

Positive control: A known carcinogen, 3-methylcholanthrene (MCA), which can be metabolized to the active carcinogen by this mouse cell line, was used in the assay. Fifteen flasks were treated with 5 ug MCA/ml medium.

In the activated assay, S9 reaction mixture was added to the growth medium, together with the test material and incubated for 2 hours, with the exposure period terminated by washing cells twice with saline. The cells were treated subsequently as were those in the non-activated assay (see below). The activated system utilized the S9 fraction derived from the liver of Fischer 344 male rats induced with Aroclor 1254. The S9 fraction contained 1.4 mg NADP, 2.7 mg isocitrate, and 15 ul per growth medium of 2.5% serum. It was stated that commercially available S9 fraction was used whenever found feasible. Note: In a 1983 review of cell transformation by chemical agents *, no validated metabolic activation system was said to be available.

Transformation assay: Twenty-four hours prior to treatment, a series of flasks were seeded with 10^4 cells/flask and incubated at 37° C. Fifteen flasks were treated for each of the following conditions: (a) five preselected doses of test article; (2) positive control; (3) negative control, and the flasks were incubated for 3 days. Following washing, the cells were fed with test-article-free medium and incubated for 4 weeks, with re-feeding twice a week. Termination of the assay was made by fixing the cell monolayers with methanol and staining with Giemsa. The number of transformed foci were scored on the basis of the criteria described in the protocol (copy attached). The flasks were read "blind".

II. Results:

The doses used in the transformation assay were 500 ug to 31.25 ug/ml, based on the results of a range-finding study. The results were presented in two tables (copies attached). Note: Table III, which the study report identifies as the results of the activated assay, is mis-labeled.

It is stated that the test article did not induce a significant increase in the frequency of transformed foci, relative to controls, in either the presence or absence of microsomal activation. Under the conditions of the assay, the author concluded that GX-071 was not mutagenic in the transformation assay. Comment: Metabolic activation had no effect on the 3-MCA response.

III. Conclusion:

TB cannot concur with the author's conclusion. In the Method's section of the report, 15 flasks were to be used for each of the doses of test material and each control. Additionally, the Assay Acceptance Criteria indicate that more than eight flasks per test condition were available for analysis (should have 15-20 flasks). However, the results, as presented in Tables II and III, give results for only 2-3 flasks per test group. This is an inadequate number of samples for analysis.

* Heidelberger, C., Freeman, A.E., Pienta, R.J., Sivak, A., Bertram, J.S., Casto, B.C., Dunkel, V.C., Francis, M.W., Kakunaga, T., Little, J.B., and Schechtman, L.M. Cell Transformation by Chemical Agents - A Review and Analysis of the Literature. Mutation Research 114, 283-385 (1983).

Sulfluramid

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Date: September 6, 1988

DATA EVALUATION REPORT

CHEMICAL: N-Ethyl Perfluorooctanesulfonamide Tox. Chem. No.: 454E

STUDY TYPE: Sister Chromatid Exchange - Chinese Hamster Ovary (CHO) Cells

MRID NUMBER: 406126-14

SYNONYMS/CAS No.: GX-071

SPONSOR: Griffin Corporation

TESTING FACILITY: Toxikon Corporation, Norwood, MA

TITLE OF REPORT: Sister Chromatid Exchange (SCE) In Chinese Hamster Ovary Cells - GX071

AUTHOR(S): Laxman S. Desai, D. Sc.

STUDY NUMBER(S): 86G-0002

REPORT ISSUED: July 25, 1986; amended March 21, 1988

Classification: Unacceptable.

CONCLUSION(S) - Executive Summary: Under the conditions of this assay, GX-071 was found to be negative for induction of sister chromatid exchange in Chinese hamster ovary cells, both with and without metabolic activation, at concentrations up to 1000 ug/ml. However, this top concentration did not result in at least a 50% reduction in the second mitosis, thus indicating that a higher dose should have been utilized. The study should be repeated using higher dose levels.

SISTER CHROMATID EXCHANGE

I. Materials and Methods: GX-071 (Lot # not provided) was described as white needle crystals with a purity of 99+%. The Chinese hamster ovary cells (CHO) were obtained from the American Type Culture Collection (Repository No. CCL61), which is a permanent cell line with an average cycle time of 10 to 12 hours, a model chromosome number of 20, and a plating efficiency of approximately 90%. The cells were grown in "complete" growth medium (Minimal Essential Medium (MEM) supplemented with 10% fetal calf serum. There were two negative controls: assay procedures were performed on untreated cells and dimethyl sulfoxide (DMSO) served as the solvent control. The assays were conducted with and without metabolic activation. The activated systems utilized the S9 fraction derived from the liver of male Fischer 344 rats induced with Aroclor 1254. The S9 reaction mixture contained S9 15 ul/ml, NADP 1.4 mg/ml, and isocitric acid 2.7 mg/ml growth medium with 2.5% serum. The positive control agents used in the assays were Ethylmethane sulfonate (EMS) for the non-activation series and Dimethylnitrosamine (DMN) in the metabolic activation series.

Forty second division cells (M2) were scored for the frequency of SCEs per cell (20 cells each from duplicate cultures per concentration). The proportions of cells in the first, second, and third divisions (i.e., M1, M2, and M3 cells) were also determined by scoring 50 cells. Scoring of slides was performed "blind". A quality assurance statement was provided.

II. Results:

A. Solubility, Stability, and Dose Determination - The test article was insoluble in water. A solvent control was utilized since the test article was insoluble in the growth medium. DMSO was the solvent used and the concentrations tested were 10, 50, 100, 500, and 1000 ug/ml.

B. Sister Chromatid Exchange Assay Without Metabolic Activation - There was some toxicity demonstrated at the top dose level of test material (slight delay in cell cycle time). Results are shown in Table II, attached. No significant increase in SCE was observed at any of the dose levels. GX-071 was considered negative under non-activation conditions for inducing SCE.

C. Sister Chromatid Exchange Assay With Metabolic Activation - Very slight cell cycle delay was reported at the top dose level. There was no significant increase in SCE reported at the concentrations tested (Table III, attached). The test article was considered to be negative for inducing SCE under the conditions of metabolic activation.

III. Conclusion: TB agrees with the author that GX-071, under the conditions of the assay (with and without metabolic activation), was not found to induce sister chromatid exchanges in Chinese hamster ovary cells. However, the dose levels utilized were not sufficiently high to produce a 50% reduction in the second mitosis (significant cell cycle delay). Therefore, this assay is inadequate for the assessment of the mutagenic potential of GX-071 to induce SCEs in cultured CHO cells, with and without metabolic activation.

Note: Table III is mislabeled as presenting the results of the non-activated system, while the text indicates that this table is the activated system and Table II is the non-activated assay results. Additionally, in Table I, the cell number is labeled as number of cells X 10^{-4}; this is misleading as this should perhaps be 10^{4}.

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