84-2 In-Vitro Chinese Hamster Cell (V79) Mutation Assay
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DATA EVALUATION REPORT

Study Type: Gene Mutation in Cultured Chinese Hamster Cells (In-Vitro)

TOX. CHEM. NO.: 2980

Accession No.: 403888-07

MRID NO.:

Test Material: CGA-154281 Technical (Batch No. FL 870211; 94% Purity)

Synonyms:

Study Number (s): 871160

Sponsor: CIBA-GEIGY Corporation Agricultural Division, Greensboro, NC

Testing Facility: CIBA-GEIGY Limited Experimental Pathology Laboratory Basle, Switzerland

Title of Report: Point Mutation Test with Chinese Hamster Cells V79

Author(s): P. Dollenmeier, M. Porlezza and P. Schrag

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Conclusions:

CGA-154281 Technical was nonmutagenic in the in-vitro Chinese hamster cell (V79) mutation assay with or without metabolic activation at the concentrations tested.

Concentrations tested: 0.5, 1, 2, 4, 6, 8 and 10 ug/ml without activation; 6, 12, 24, 48, 72, 96 and 120 ug/ml with activation (Two separate assays).

Classification of Data: Unacceptable

Deficiencies are identified in the detailed review

Title of Study: Point Mutation Test with Chinese Hamster Cells V79
(Test Material: CGA-154281 Technical)
Test No. 871160

I. Materials and Methods:

1. Test Material

The test compound, CGA-154281 Technical (Batch No. FL 870211; 94% Purity), dissolved in DMSO was used in this study. Ethyl methanesulphonate (300 ul/ml) and N-nitroso-dimethylamine (1 ul/ml) were used as the positive controls.

2. Cell Line

The V79 Chinese hamster cell line (clone 65/3), originally obtained from Dr. D. Wild of Friedburg, FRG, was used to assay the mutagenicity of CGA-154281 at the hypoxanthine guanine phosphoribosayl transferase (HGPART) gene locus in this study. The cells were cultivated in 25 ml of growth medium in 75 cm^2 tissue culture flasks and passaged weekly in low numbers (1000 cells/flask). After the 8th passage, the cultures were checked with the Hoechst-dye staining method for mycoplasma contamination and were also cleansed of HGPRT - mutants by culturing in growth medium supplemented with 3 uM aminopterin.

3. Medium

Ham's F10 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml)and streptomycin (100 ug/ml).

4. Activation System

Aroclor 1254-induced rat liver homogenate (S9) was used as the exogenous activation system in this study. The cofactors used were essentially NADP, glucose-6-phospahte, Ca^{++} and Mg^{++} as described by Machanoff et al (Chem-Biol. Intereactions 34: 1-10, 1981). The activation mixture was added to the medium at a concentration of 10% and the final concentration of S9 fraction was 2% in the treatment medium.

5. Cyototoxicity Test

Cultured cells were exposed in multi-well dishes for 5 hours with activation and a range of 7 concentrations of CGA-154281 technical from 2.4 to 156.3 ug/ml, and for 21 hours without activation and a range of 7 concentrations from 0.3 to 19.5 ug/ml. After the treatment, the treated cells were washed with buffer solution, followed by reincubation in fresh growth medium for further 6 days. After the incubation, the cultures were fixed and stained

with Giemsa and the surviving colonies counted with the aid of an electronic colony counter (Fisher Count Model 800). The concentration to be selected as the highest dose for the mutagenicity test is that causing about 90% reduction of viable cells in this test in comparison with the negative control.

6. Mutagenicity Test

The assay was initiated by exposing about 2.5 X 10^6 cells in 75 cm^2 culture flask containing 25 ml growth medium to the test compound at predetermined doses (0.5, 1, 2, 4, 6, 8 and 10 ug/ml without activation; 6, 12, 24, 48, 72, 96 and 120 ug/ml with activation) for 5 hours in the experiment with activation and for 21 hours in the experiment without activation. After the exposure period, the cultured cells were washed with buffer solution, suspended by trypsinization, pelleted, resuspended in fresh growth medium, counted with a hemocytometer, diluted with fresh growth medium and replated into flasks at 10^6 cells. These cultures were incubated for 5 days to express the mutant phenotype. At the end of the expression period, the cultured cells were reseeded at 1.8 X 10^6 cells in the 9.6 cm^2 dish containing 6-thioguanine (8 ug/ml) in the medium for 7-8 days of incubation at 37°C (mutant selection). At the same time, additional aliquots of treated cells and untreated cells were diluted to a density of 100 cells to each dish containing growth medium without 6-TG for the determination of cell viability at the end of the treatment.

7. Criteria for positive Response

A test material is considered mutagenic in this test system if the analysis of the data demonstrates either:

A. a dose-dependent increase in the mutant frequency and an increase in the highest mutant frequency with respect to the negative control by a factor of at least 2.5; or

B. an increase in any mutant frequency by a factor of 3.0 or more with respect to the negative control at any concentration tested together with an absolute difference of at least 20 clones per 10^6 cells plated between the negative control and substance treated dishes.

II. Reported Results:

1. Toxicity Test (Table 1 attached)
In the preliminary toxicity tests, a 90% reduction in the viability of the cells treated with CGA-154281 Technical was obtained between the concentrations of 78.1 ug/ml and 156.3 ug/ml in the experiment with microsomal activation. In the experiment without microsome activation, a 90% reduction in the viability of the cells treated with CGA-154281 Technical was obtained between the concentrations of 4.9 ug/ml and 9.8 ug/ml. Therefore, the mutagenicity test without microsomal activation was consequently performed with concentrations of CGA-154281 Technical ranging from 0.5 ug/ml to 10 ug/ml and the mutagenicity test with microsomal activation was performed with concentrations ranging from 6.0 ug/ml to 120 ug/ml.

2. Mutagenicity Test

A. Original Mutagenicity Test (Tables 2, 3, 4, 5, 6 and 7 attached)

The original mutagencity test was carried out with seven selected concentrations of CGA-154281 Technical (i.e., 0.5, 1, 2, 4, 6, 8 and 10 ug/ml without activation; 6, 12, 24, 48, 72, 96 and 120 ug/ml with activation) in the presence and absence of metabolic activation. Comparison of the mutant frequency in the treated cultures and the negative controls without metabolic activation gave a factor of 2.5 at the highest concentration (10 ug/ml) and factors of 1.1, 1.5, 1.3, 1.0 and 1.0 at the six further concentrations down to the lowest. Comparison of the mutant frequency values of the treated cultures with that of the ngative controls with metabolic activation gave a factor of 1.0 of all concentrations tested with one exception at 72 ug/ml, where a factor of 2.3 was calculated.

B. Confirmatory Mutagenicity Test (Tables 8, 9, 10, 11, 12 and 13 attached)

In the confirmatory mutagenicity test, the same concentrations of CGA-154281 Technical used in the original mutagenicity test were used. Comparison of the mutant frequency in the control and the treated cultures in the experiment without metabolic activation gave a factor of 2.4 at the highest concentration (10 ug/ml) and factors of 1.0, 1.0, 1.8, 1.0, 1.0 and 1.0 at the further six concentrations down to the lowest. In the experiment with microsomal activation, the highest concentration (120 ug/ml) yielded a mutant factor of 2.6 and for the further six lower concentrations factors of 1.0, 1.0, 1.7, 1.9, 1.1 and 1.0 were calculated.

Based on the results obtained from both of these studies, a mutant factor greater than 3.0 together with a difference in the treated and untreated dishes of at least 20 clones per 10^6 cells plated was not detected at any concentration of the test material, and there was also no indication of any dose-related mutant frequency found in either experiment. Therefore, CGA-154281 Technical induced no mutagenic effect in this forward mutation system.

III. Evaluation and Recommendation:

1. The positive controls (EMS and DMN) induced significant increase in the mutant frequency with respect to the corresponding negative controls by a mutant factor of at least 24.6 (i.e., factors in both trials: EMS, 301.6-494.1; DMN, 24.6-41.9) indicating the assay was sensitive to known mutagens in the presence and absence of metabolic activation.

2. The background mutant frequencies (average of the negative and solvent controls) under either the activation or the nonactivation system were found within the normal range for mammalian cell mutation assay (i.e., mutant frequency: below 4×10^{-6} in 1st trial; below 4.2×10^{-6} in 2nd trial).

3. However, the evaluation of mutagenicity of CGA-154281 Technical in the cultured V79 Chinese hamster cell forward mutation assay cannot be accomplished due to the following reporting deficiencies:

   A. Although the highest concentration of test material should give about 10% of control survival (or 90% reduction of control) for this study as recommended by the testing laboratory, the highest concentration of CGA-154281 Technical (120 ug/ml) used in these experiments with microsomal activation was not supported by the data presented (i.e., Cytotoxicity data in Table 1, none; Trial 1 in Table 5, 34.99% of control; Trial 2 in Table 11, 40.45% of control), Therefore, it is questionable whether an appropriate highest concentration (i.e., greater than 120 ug/ml or lower than 156.3 ug/ml) was chosen for the experiments with microsomal activation in this study. In addition, since significant increases in mutant frequency at the highest concentrations (i.e., 10 ug/ml without activation; 120 ug/ml with activation) were observed in both the experiments with and without microsomal activation (Tables 4, 10 and 13) in this study, the test compound must be further tested at dose level extending into the toxic range for the experiments with microsomal activation.

   B. As a rule, the S-9 concentration which gives the greatest cytotoxicity should be chosen for the cultured mammalian cell gene mutation assay (Reference: Hsie et al., Mut. Res. 86: 193-224, 1981; Li et al., Mut. Res. 119: 387-392, 1983). It is unclear whether a specific study was performed to establish the optimal S-9 concentration for this study.

4. Therefore, the submitted report is incomplete and unacceptable in the present form.

4-(Dichloroacetyl)-3,4-Dihydro-3-Methyl-
2H-1,4-Benzoxazine

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