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Accession No. 403888-01
and 403888-03

Review of Registrant's Mutagenicity Overview of CGA-154281 Technical (Author-S.J. Spoede) with the Assessments of Mutagenicity Studies Conducted with CGA-154281 Technical by Ciba-Geigy's Consultant (Author - David Brusick, Hazleton Laboratories America, Inc., Kensington, MD)

I. Ames Salmonella/Microsome Plate Assay

Five submitted studies with CGA-154281 Technical (Study Nos. 840232, 86076, 860840, 871077 and 871080) have been reviewed by the Toxicology Branch as indicated in Table 1.

Spoede's Conclusion: " Three Ames assays (Study Nos. 860840, 86076 and 871080) ... were conducted. The results of the Ames assay range from a weak positive in three strains (TA98, TA1537 and TA1538) to a clear negative in all five strains. "

Brusick's Assessment: " Five studies, some with independent repeat tests, were reviewed. The only study demonstrated unequivocal positive results was the U.S. study using batch FL 860318. The results show clear frameshift mutagenesis both with and without S9 mix (i.e., metabolic enzymes do not activate or deactivate). The responses occurred at 250 ug per plate of the 154281 Technical (Test No. 86076). In this study, the compound prepared in DMSO was completely toxic to the test organisms at 2000 ug/plate. Strains TA98, TA1538 and TA1537 responded. All other Ames tests produced highly variable and predominantly negative responses. Several other batches were used in these tests and none of the studies showed toxicity comparable to the U.S. study (Test No. 871080). The other studies (Test Nos. 860840, 871077 and 840232) produced none or only slight toxicity at 5000 ug/plate and one trial tested up to 8000 ug/plate. It appears to me that both the toxic and mutagenic responses could be a function of the solvent used. All studies with low toxicity and variable responses were performed using acetone as the solvent. This might lead one to suspect differential solubility for CGA-154281 Technical in acetone and DMSO. Solubility information derived by Ciba-Geigy indicates however that both DMSO and acetone are suitable solvent for CGA-154281. One other explanation for the different results may be that CGA-154281 Technical is soluble in both acetone and DMSO but mutagenic impurities that might be present in some of the batches are highly soluble in DMSO and only sparingly soluble in acetone. Such impurities could be responsible for the effects shown in the one positive study and possibly in the marginal studies using higher doses in acetone. At this point, it is not possible to resolve the inconsistent results beyond the solvent differences. Other technical variables might be involved but none could be established from the data as presented. "

Reviewer's Comments: The provided technical explanations for the inconsistent results of the five studies (Accession No. 403888-03) are considered to be reasonable and useful. Toxicology Branch agrees with the priniciple that the discrepency of test results from five different batches of CGA-154281 Technical containing various degree of mutagenic impurities is not a simple problem to resolve assuming that all of these studies have been properly performed to meet the minimum acceptability level (References: Ames et al., Mutation Res., 31: 347-364, 1975; EPA Health Effects Test Guidelines 56/6/83-001). Since two of these studies (Test Nos. 860840 and 86076) showing significantly mutagenic responses were considered acceptable and the remaining three of these studies (Test Nos. 840232, 871080 and 871077) showing no mutagenic effect of the test material were found to be invalid at this point, conclusion of negative results in these studies for the test material (Accession No. 403888-01) was not supported by the data presented. Therefore, Toxicology Branch disagrees that this compound was nonmutagenic in the Ames test with or wihtout metabolic activation.

Recommendation:

1. A comprehensive study with CGA-154281 Technical using the Ames Salmonella/Microsome Plate assay conducted by an independent laboratory (Preference: EPA Genetic Toxicology Research Laboratory, RTP, NC; Microbiological Associates Laboratory, Bethesda, MD; Hazleton Laboratories America, Inc., Kinsington, MD) should be considered. The experimental design and report for this study should include the following specific information:

(a) Test Material: Batch No. HM 4287/144 (99.3% Purity, Test No. 840232); Batch No. FL 870116-USA (99% Purity, Test No. 871077); Batch No. FL 870211-USA (94% Purity, Test No. 871080);

(b) Solvent Used: Acetone and DMSO

(c) Dose Levels Used: 1000, 2000, 3000, 4000, 5000 and 8000 ug/plate;

(d) Bacterial Strains Used: TA98, TA1537 and TA1538:

(e) Positive and Negative Controls;

(f) Details of Protocol Used for Metabolic Activation;

(g) Individual Plate Counts, Mean Number of Revertant Colonies Per Plate, Standard Deviation;

(h) Statistic Evaluation;

(i) Criteria for Determining Positive Results.

2. It is possible that impurities present in the various batches could be responsible for the variable positive/negative results. However, we have identified some deficiencies in these studies which could also have contributed to these results. The Registrant should attempt to identify or clarify the contribution of impurities to the variable results. If impurities are demonstrated to be responsible, they should be removed from the manufactured products.

II. Gene Mutation in Cultured V79 Cells

One submitted study with CGA-154281 Technical (Study No. 871160) has been reviewed by the Toxicology Branch as indicated in Table 1.

Spoede's Conclusion: " ... In the original and confirmatory experiments, comparison of the numbers of mutant colonies in the controls and the cultures treated with CGA-154281 Technical revealed no significant deviations of the mutant frequencies as determined by the screening with 6-TG. Under the given experimental conditions, CGA-154281 and its metabolites induced no mutagenic effects. "

Brusick's Assessment: " I evaluated these studies as negative; however, I noticed a rather reproducible increase at the highest concentrations tested. These increases are not sufficient of define positive responses but might warrant a closer examination of the dose range near the MTD. "

Reviewer's Comments: This study is judged to be unacceptable in the submitted form. Deficiencies are identified in the detailed review.

Recommendation:

No final conclusion of test results can be drawn from this study at the present time.

III. UDS Assays

Six submitted studies with CGA-154281 Technical (Study Nos. 850665, 850666, 860177, 860178, 871076 and 871079) have been reviewed by the Toxicology Branch as indicated in Table 1.

Spoede's Conclusion: " With DNA repair tests on rat hepatocytes, one test was borderline positive (Test No. 860177) and another was negative (Test No. 870179). An unactivated test in human fibroblasts was negative. "

Brusick's Assessment: " While some of the studies reported by Ciba-Geigy concluded that CGA-154281 Technical was active (based on a two-fold increase in the net nuclear grains), my assessment is that none of the studies show positive responses based on more generally used evaluation criteria. Our laboratory as well as many other performing this assay use the following criteria for establishing a positive effect: the average net nuclear grain counts are increased by 6 (or greater) over the spontaneous background. Obviously one would also like to see a dose response. In the studies reported for CGA-154281 Technical, the only values meeting the above criteria were the positive controls. All of the remaining data looked negative. I would recommend that the data from these studies be reevaluated and the studies amended to at least reflect this alternate evaluation criteria as well as that used originally. The in vitro studies conducted in human fibroblasts were considered unacceptable by the EPA because no metabolic activation was used. This test method should be scientifically valid to identify direct acting genotoxicants, and it was suggested from the one positive Ames tests that genotoxic activity of CGA-154281 would be expressed in the absence of S9. Consequently, these tests tend to support the negative evaluation for the rat hepatocytes. "

Reviewer's Comments: The only study with CGA-154281 Technical showing positive response in the rat primary hepatocyte unscheduled DNA synthesis assay (Test No. 860177) was based on the evaluation criteria recommended by the testing laboratory (i.e., The number of net grain per nucleus in relation to the concurrent control value was doubled). Toxicology Branch believes that the conclusion of borderline positive response made by Dr. Spoede for this study (Accession No. 403888-01) was supported by the data presented. However, we agree with Dr. Brusick's evaluation that none of these three studies with CGA-154281 Technical (Study No. 860177, 871076 and 871079 - Accepted by Toxicology Branch) show positive response based on the evaluation criteria recommended by Williams (Cancer Res., 37: 1845-1851, 1977) and generally used by the U.S. Laboratories. All of the in vitro studies conducted in human fibroblasts were considered unacceptable by the Toxicology Branch at this point. Reporting deficiencies for these studies are identified in the detailed review.

Recommendation:

Based on the results of these studies conducted in the rat primary hepatocyte unscheduled DNA synthesis assay, there is insufficient evidence to indicate the test compound was active in these UDS assays. However no final conclusion of the test results can be drawn from those in vitro human fibroblast unscheduled DNA synthesis assays with CGA-154281 technical at the present time.

IV. Micronucleus Assay

One submitted study with CGA-154281 Technical (Study No. 860109) has been reviewed by the Toxicology Branch as indicated in Table 1.

Spoede's Conclusion: "... In both experiments, there were no statistically significant increases in the number of micronucleated polychromatic erythrocytes in comparison with the negative control animals. Under the conditions of this experiment, no evidence of mutagenic effects was obtained in Chinese hamsters treated with CGA-154281 Technical."

Brusick's Assessment: " The results are negative. The EPA was concerned that an appropriate MTD had not been determined prior to the study. This concern seems unwarranted in that (a) the report states that 5000 mg/kg was the highest dose tested not resulting in animal death and (b) 5000 mg/kg should, in my opinion, be sufficiently high to establish nongenotoxicity."

Reviewer's Comments: The registrant's responses to the two deficiencies cited in the previous Toxicology Branch review are considered to be reasonable.

Recommendation:

The test compound is not considered to be clastogenic in the Chinese hamster micronucleus test at the dose levels tested (1250 through 5000 mg/kg). The study is upgraded to acceptable.

Table 1 - Mutagenicity Tests with CGA-154281

Study No.	Study Type	Batch No.	Solvent	Dose Range	Results	Classification of Data
840232	Ames test	HM 4287/ 1+4; 99.3%	Acetone	20-5120 ug/plate	Negative	Unacceptable
86076	Ames test	FL 860318	DMSO	5-1000 ug/plate	Positive	Acceptable
860840	Ames test	93.9%	Acetone	20-5000 ug/plate 500-8000 ug/plate	Positive	Acceptable
871077	Ames test	FL 870116 99%	Acetone	20-5000 ug/plate	Negative	Unacceptable
871080	Ames test	FL 870211 94%	Acetone	20-5000 ug/plate	Negative	Unacceptable
871160	V79 Cell Mutation	FL 870211 94%	DMSO	0.5-10 ug/ml 6-120 ug/ml	Negative	Unacceptable
850665	UDS test in rat hepato-cytes	KGL 3339-6 99.7%	DMSO	1-125 ug/ml	Inactive	Unacceptable
850666	UDS test in human fibro-blasts	KGL 3339-6 99.7%	DMSO	0.25-31.25 ug/ml	Inactive	Unacceptable
860177	UDS test in rat hepato-cytes	93.9%	DMSO	0.12-15 ug/ml 0.125-20 ug/ml	Active	Acceptable
860178	UDS test in human fibro-blasts	93.9%	DMSO	0.5-62.5 ug/ml	Inactive	Unacceptable
871076	UDS test in rat hepato-cytes	FL 870116 98.3%	DMSO	0.008-20 ug/ml 0.0004-10 ug/ml	Inactive	Acceptable
871079	UDS test in rat hepato-cytes	FL 870211 94%	DMSO	0.1-20 ug/ml	Inactive	Acceptable
860109	Micronucleus test in CH cells	93.9%	DMSO	1250-5000 mg/kg	Negative	Acceptable

Title of Study: Chinese Hamster Micronucleus Test with CGA-154281 Technical
CIBA-GEIGY Limited Study No. 860109

Review of Registrant's Response to the Previous Review Comments Concerning the Chinese Hamster Micronucleus Test with CGA-154281 Technical (Toxicology Branch Memorandum of August 27, 1987, J. Chen)

Registrant's Response:

1. The maximum dose used in the preliminary toxicity and definitive mutagenicity study was 5000 mg/kg. The criteria used to select doses for definitive study are a dose which does not cause death in the preliminary study or a limited dose of 5000 mg/kg. No deaths or toxic effects occurred at 5000 mg/kg in the preliminary study. An evaluation of micronuclei in the bone marrow is not routinely performed in the toxicity test. The highest dose 5000 mg/kg was chosen as an appropriate maximum based on a meeting held June 20, 1986 with the EPA. Acceptable upper end-points for in vivo mutagenicity assays were agreed to be an MTD, cytotoxicity, or 5000 mg/kg. (The final minutes of the meeting were sent to Dr. Ted Farber for distribution within the EPA).

2. This study was performed in agreement with OECD guideline 474. clinical observations are not required by this guideline nor by TSCA guidelines (Fedreal Register, Vol. 50, No. 188, Page 39447). Body weights following treatment are not required to be recorded by the 2 guidelines cited above. The initial body weights were reported as ranges on page 8 of 22 in the report. Individual body weights are provided in Attachment 1. Since animals were sacrificed after a maximum of only 48 hours past-treatment and no animal died, no biologically important differences in body weight were likely to occur. Body weights were not taken before the sacrifice times of 16, 24, or 48 hours. CIBA-GEIGY requests that the classification of the micronucleus test be changed to acceptable.

Reviewer's Comments:

The Registrant's response to the two deficiencies cited in the previous Toxicology Barnch review with additional information concerning the individual body weights of test animals at the beginning of treatment for the micronucleus tests are considered to be reasonable.

Recommendation:

The test compound, CGA-154281 Technical (93.9% Purity) is not considered to be clastogenic in the Chinese hamster micronucleus test at the dose levels tested (1250 through 5000 mg/kg). The study is upgraded to acceptable.

Title of Study: Autoradiographic DNA Repair Test on Human Fibroblasts with CGA-154281 Technical
CIBA-GEIGY Limited Study No. 860178

Review of Registrant's Response to the Previous Review Comments Concerning the Autoradiographic DNA Repair Test on Human Fibroblasts with CGA-154281 Technical (Toxicology Branch Memorandum of August 27, 1987, J. Chen)

Registrant's Response:

This test was especially designed only for the detection of direct acting genotoxic agents. To detect promutagens and procarcinogens, respectively, the DNA repair test with rat hepatocytes has been performed. CIBA-GEIGY requests that the classification of the DNA repair test in human fibroblasts to be changed to acceptable for non-activated system.

Reviewer's Comments:

Although the test compound appeared to be inactive in this study under the non-activated system according to the criteria for determining positive response established by the testing laboratory, the submitted data, which were not presented in terms of net grain value (i.e., calculated by subtracting the average grain count over the cytoplasm from the total over the nuclei) as recommended by Brusick (Reference: Assessment of Mutagenicity Studies Conducted with CGA-154281 Technical, Accession No. 403888-03), should be clarified. Furthermore, it should be borne in mind that either negative or positive results obtained from the study without metabolic activation may be of no biological significance to the intact mammalian organisms.

Recommendation:

Until the reporting deficiency cited above is clarified, this study under the non-activated system cannot be upgraded to acceptable.