UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

004600

7/30/85

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mutation Study Which Was Not Included in Memorandum on EPA Experimental Use Permit File Symbol 352-EUP-RER and Pesticide Petition No. 4G3138

FROM: Thomas Edwards, Pharmacologist W Thomas Edwards 7-30-85
Hazard Evaluation Division (TS-769)

TO: Robert Taylor, PM 25
Registration Division (TS-767)

THRU: Clint Skinner, Section Chief Clint Skinner 8-30-85
Review Section III

and

Theodore Farber, Chief
Toxicology Branch, HED (TS-769)

Chemical: DPX-M6316

Caswell No.: 573S

EPA Identification Nos.: 352-EUP-RER, 4G3138

Accession No.: 073010

Attached is the review of the CHO/HGPRT Assay for Gene Mutation, study no. HLO-240-84. This study has been classified unacceptable for reasons stated in the review. The objections should be addressed or the study replaced before permanent registration.

EPA: 68-01-6561
TASK: 86
July 19, 1985

DATA EVALUATION RECORD

DPX/M6316

Mutagenicity - CHO/HGPRT Forward Gene Mutation

STUDY IDENTIFICATION: Summers, J. C. CHO/HGPRT assay for gene mutation with INM-6316-20. (Unpublished study No. 240-84 prepared and submitted by E. I. du Pont de Nemours and Co., Inc., Newark, DE; no date.) Accession No. 072849-15.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-19-85

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1. CHEMICAL: 2-Thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] amino] sulfonyl]-, methyl ester; (INM-6316-20).

2. TEST MATERIAL: INM-6316-20 estimated purity 95.6%.

3. STUDY/ACTION TYPE: Mutagenicity - CHO/HGPRT Forward Gene Mutation.

4. STUDY IDENTIFICATION: Summers, J. C. CHO/HGPRT assay for gene mutation with INM-6316-20. (Unpublished study No. 240-84 prepared and submitted by E. I. du Pont de Nemours and Co., Inc., Newark, DE; no date.) Accession No. 072849-15.

5. REVIEWED BY:

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Signature: Nancy L McCarroll
Date: 7-18-85

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Date: July 18, 1985

6. APPROVED BY:

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W. Thomas Edwards
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Signature: _____
Date: _____

7. CONCLUSIONS:

A. Under the conditions of two independent assays, the mutagenic potential of INM 6316-20 to induce an increase in the mutation frequency of CHO cells cannot be fully assessed.

B. The study as presented is unacceptable. The observed results from the S9 activated phase of one experiment indicated that all doses (1-7 mM) induced increases in mutation frequencies. A reevaluation of statistical analyses confirmed the lack of significant effect or trend; however, the biological significance has not been resolved. We conclude, therefore, that the results are equivocal (test material is a presumptive positive).

8. RECOMMENDATIONS:

Additional studies are required to clarify the equivocal responses observed in the activated phase of one of the experiments. Individual colony counts for the dosed and control groups are required to validate the study information. Information relative to the solubility characteristics of the test material in the test medium are needed for us to assess the rationale used to select the highest dose.

Items 9 and 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for details.

(1) The test material INM-6316-20 had an estimated purity of 95.6%. The solvent of choice, dimethylsulfoxide (DMSO), yielded a solution containing 700 mM of the test material.

(2) Target cells for this assay, Chinese Hamster Ovary (CHO) cells, CHO-K1-BH4 were obtained from Dr. Abraham Hsie, Oak Ridge National Laboratory.

(3) The S9 microsomal fractions used for metabolic activation was derived from the livers of 8 to 9 week old male CD rats injected ip with 500 mg/kg of Aroclor 1254.

(4) Mutagenicity Assay: Seeded cultures (5 x 10^5 cells) were exposed to the appropriate levels of the test material, solvent, or positive controls both in the presence and absence of S9 activation.

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[1] Only items appropriate to this DER have been included.

Cytotoxicity was assessed by plating 200 cells/60 mm dishes (6 dishes) for each treatment. Following 7 days incubation cells were stained and counted.

Expression of 6-thioguanine (6-TG) resistant mutants was accomplished by plating 1 x 10^6 cells/100 mm dishes (1 dish) and subculturing the cells into media containing 6-TG. Following 7 day incubation, colonies were stained and counted. The mutation frequency was calculated by dividing the total number of mutant clones by the number of cells plated, adjusting for the cloning efficiency of the cells at the time of mutant selection.

(5) Evaluation Criteria: The assay was considered to be positive if a) the mutation frequency of one or more of the sample concentrations tested was significantly greater than that of the solvent control, where significance was judged at the 0.01 level, b) the correlation between the mutation frequency and the concentration of the test sample was significantly greater than 0, where significance was judged at the 0.01 level.

(7) Data were analyzed for significance in accordance with the method of Snee and Irr.[2]

B. Protocol:

See Appendix A.

12. REPORTED RESULTS:

Mutagenicity Assay

Based on the results of a concurrent cytotoxicity assay, dose selection for the mutagenicity assay was governed by the solubility characteristics of the test material in DMSO. However, no data were present to support the authors statement that the test material was "tested up to and slightly beyond the limit of solubility in the treatment medium." Two independent mutagenicity assays were conducted with the test material at doses of 1, 3, 5, 6 and 7 mM both in the presence and absence of metabolic activation (30% S9 fraction in the S9 mix).

In the nonactivated assay, the results from two independent trials indicated that the test material did not induce a significant increase in mutations at the HGPRT locus in Chinese Hamster Ovary cells. In the presence of metabolic activation; however, the results from the first study showed increases in mutation frequencies at all test doses (Table 1). These findings were not confirmed in the second independent evaluation conducted with the test material (Table 1). When

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[2] R. D. Snee and J. D. Irr, Mutation Research 85(1981): 77-93.

TABLE 1

TABLE 1. Summarized Results of the CHO Forward Mutation Assay of INM-6316-20 with S9 Activation

Compound	Concentration (mM)	TRIAL ONE Mutants Total	TRIAL ONE Mutants Frequency[a]	Fold[b] Increase	TRIAL TWO Mutants Total	TRIAL TWO Mutants Frequency[a]	Fold[b] Increase
DMSO	0	7	10.1		2	4.0	
		4	7.2	-	3	5.8	-
IMN-6316-20	1.0	26	39.3	4.2	0	0	<1
		24	37.2		2	3.5	
	3.0	15	23.9	3.3	2	3.3	<1
		24	35.5		0	0	
	5.0	16	23.8	2.2	1	1.9	<1
		11	16.5		1	2.2	
	6.0	47	65.6	4.8	3	5.8	1.2
		12	18.0		3	6.1	
	7.0	16	27.7	5.8	2	4.0	<1
		45	76.5		0	0	
DMBA	0.015	178	319.6	31.9	62	112.1	24.8
		140	254.1		72	136.4	

[a]Mutants per 10^6 surviving cells.

[b]Fold Increase = $\frac{\text{Mutation Frequency of Test Group}}{\text{Mutation Frequency of Control}}$

DMBA = 9,10-dimethyl-1,2-benz(a)anthracene.

these data were combined for statistical evaluation; no significant increases or dose-related effect were demonstrated.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded that, "Under the conditions of the assay, INM-6316-20 was not mutagenic in CHO cells either in the presence or the absence of an S9 activation system."

B. A quality assurance statement was not present with this report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the biological significance of the increased mutation frequencies at all S9 activated doses in trial one cannot be fully evaluated. When we reevaluated the individual study results and combined data, we confirmed the statistical analysis of the study author. The small sample size and the intragroup variations in the mutation frequency, particularly noteworthy at 6 and 7mM, appear to be responsible for the lack of a significant effect or trend (Table 1). While presentation of fold-increase in mutation frequencies is not a conventional method for evaluation of data in this type of study, it has been included to illustrate the marked increases in test group mutation frequencies compared to the control in trial one. The results of trial two did not confirm the findings in trial one; however, no explanation was given for the apparent increases and/or variations in trial one.

Selection of the highest dose was based on solubility characteristics of the test material since toxicity was not clearly demonstrated at any level. For cytotoxicity to be considered a valid criterion for dose selection in this system a maximum of 50 percent survival at the highest dose is required. While solubility governed dose selection, no data were presented to suggest that the test material was assayed at doses that slightly exceeded the limits of solubility in the test medium. No criteria for validation of the test system were outlined in the report.

The assayed doses of the test material (1-7 mM) spanned a rather narrow dose range; a wider selection of test concentration covering at least a two-log dose range should have been used.

Information on the method of synthesis, physical appearance or molecular weight was not furnished. The performance of a preliminary cytotoxicity assay, as outlined in the Experimental Design section of this report, was not supported by report data.

The studies, however, satisfied certain established criteria for acceptable assays which include: 1) the positive controls (ethyl methanesulfonate, 0.5mM -S9 and 9, 10-dimethyl-1,2-benz(a)anthracene, 0.015 mM +S9) caused significant increases in the mutation frequency which demonstrated the sensitivity of the test system to detect mutagenic responses, and 2) mutation frequency of the solvent controls was within acceptable ranges.

Item 15 - see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods (Protocol) CBI pp. 1-5.

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APPENDIX A

Materials and Methods (Protocol)

Harmony Reviews

Page _____ is not included in this copy.

Pages 11 through 15 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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___ Sales or other commercial/financial information

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