C04670

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

9-23-85

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: FMC 54800 TECHNICAL

TO: Mr. George LaRocca, PM 15
Registration Division (TS-767)

FROM: Byron T. Backus
Toxicologist
Toxicology Branch
07/19/85

THROUGH: Clint Skinner, Ph.D.
Head, Section III
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)
For CS 09-19-85
9/23/85

Registration number: 279-3055

Registrant: FMC Corporation

Tox. Chem. 463F

Action Requested:

Review of five mutagenicity studies.

Background:

Three mutagenicity studies have been reviewed. The following two are currently still in review:

Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells. Study conducted by Microbiological Associates; study no. A83-1105. Report dated 01/05/84.

CHO/HGPRT Mutation Assay in the Presence and Absence of Exogenous Metabolic Activation. Study conducted by Microbiological Associates; study no. A83-1144. Report dated 7/11/84.

1 3114

DISCUSSION

Comments and Conclusions:

The following is a listing of the studies which have been reviewed (refer to the attached data evaluation reviews), along with a short summary of the comments made regarding their acceptability or unacceptability:

1. Morphological Transformation of BALB/3T3 Mouse Embryo Cells in the Absence of Exogenous Metabolic Activation. Study conducted at Microbiological Associates; study no. T2007.301. Dated October 11, 1983.

The study is unacceptable. Due to the lack of a definitive cytotoxic effect and an insufficient number of replicates used for the test material and solvent control, the results of the nonactivated transformation assay conducted with FMC 54800 cannot be fully assessed.

2. L5178Y $TK^{+}/^{-}$ Mouse Lymphoma Mutagenesis Assay with FMC 54800 Technical Lot No. E-2392-105. Study conducted at Microbiological Associates; study no. T2007.701. Dated October 26, 1983.

This study is acceptable. Doses of Talstar ranging from 0.042 to 0.24 ul/ml without S9 activation and 0.024 to 0.1 ul/ml with S9 activation, induced dose-dependent increases in forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells. Talstar is therefore considered to be mutagenic in the mouse lymphoma forward mutation assay.

3. Sex Linked Recessive Lethal Assay with FMC 54800 in Drosophila melanogaster. Study conducted at Litton Bionetics; study no. LBI 22205. Dated February, 1984.

This study has been classified as unacceptable. While the test material did not induce sex-linked recessive lethals in the post-meiotic germ cells of D. melanogaster at dosage levels of 50 and 100 ug/ml, the effect on pre-meiotic stem cell development was not determined.

Copies of the attached data evaluation reviews should be provided to the registrant.

004670

EPA: 68-01-6561
TASK: 120
September 17, 1985

# DATA EVALUATION RECORD

## TALSTAR

## Mutagenicity-*In Vitro* Transformation of BALB/3T3 Cells Without S9 Activation Assay

**STUDY IDENTIFICATION**: Putman, D. L. and McCarvill, J. T. Morphological transformation of BALB/3T3 mouse embryo cells in the absence of exogenous metabolic activation. (Unpublished Study No. T2007.301 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 11, 1983). Accession No. 254405.

**APPROVED BY**:

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

Signature: I. Cecil Felkner

Date: 9-16-85

1. CHEMICAL: FMC 54800 Technical (A83-980), Talstar.

2. TEST MATERIAL: FMC 54800 technical (A83-980), lot E-2392-105, a light brown solid, purity not reported.

3. STUDY/ACTION TYPE: Mutagenicity - In Vitro Transformation of BALB/3T3 Cells Without S9 Activation Assay.

4. STUDY IDENTIFICATION: Putman, D. L. and McCarvill, J. T. Morphological transformation of BALB/3T3 mouse embryo cells in the absence of exogenous metabolic activation. (Unpublished Study No. T2007.301 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 11, 1983). Accession No. 254405.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 9-16-85

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 9-16-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-16-85

Byron Backus, M.S.
EPA Reviewer

Signature: Byron Backus
Date: 9-19-85

Clint Skinner, Ph.D.
EPA Section Head

Signature:
Date: 9-19-85

7. CONCLUSIONS:

A. Due to the lack of a definitive cytotoxic effect and the insufficient number of replicates used for the test material and solvent control, the results of the nonactivated transformation assay conducted with FMC 54800 cannot be fully assessed.

B. The study was unacceptable.

8. RECOMMENDATIONS:

A. The following recommendations are given to upgrade a repeat study:

(1) Demonstrate a cytotoxic effect (10-20% survival) at the highest assayed dose, limits of solubility, or increase the maximum dose tested to 500 µg/ml.

(2) Increase the number of replicates for all treatment groups to 20 dishes. Increasing the number of replicates per dose will ensure that a sufficient sample size is available for statistical evaluation of the results.

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, FMC 54800 technical (A83-980), lot E-2392-105 was described as a light brown solid. Purity and stability were not specified. The test material was stored at room temperature and dissolved in acetone on the day of use.

2. BALB/3T3 Clone A 31-1 mouse embryo cells were obtained from Dr. T. Kakunaga, National Cancer Institute, Bethesda, MD.

3. Cytotoxicity/Transformation Assay:

Exponentially growing cells were seeded at 250 cells/60 mm dish in triplicate for the cytotoxicity test and at $1x10^4$ cells/60 mm dish (12-15 replicates/dose) for the transformation assay. Prepared cultures were exposed to four doses of the test material, solvent or positive control in the absence of S9 activation for 24 hours. At the conclusion of the exposure period, the cells were washed and refed with

---

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

growth medium. After 7-10 days, the surviving cells exposed to the appropriate doses of the test material in the concurrent cytotoxicity test were fixed, stained and counted. Throughout the 4-6 week incubation period of the transformation assay, the cells were refed twice weekly with growth medium. The assay was terminated by fixing and staining the monolayers; the number of foci per dish were counted and scored as Type II or Type III morphological transformants according to the method of Reznikoff et al.[2]

4. Evaluation Criteria:

The assay was considered positive if the transformation frequencies in the test material treated groups were statistically increased ($p \leq 0.05$) relative to the solvent control group.

5. The statistical method used was a Modified Poisson Distribution.

8. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity/Transformation Assay:

The doses selected for the nonactivated cytotoxicity/transformation assay (3, 10, 30, and 100 µg/ml of the test material) were selected based on a preliminary dose-range finding clonal cytotoxicity study; however, neither the dose levels assayed nor the results were provided by the authors. Relative cell survival, following a 24-hour exposure to the four doses of the test material, ranged from 67 percent at 100 µg/ml to 100 percent at 3 µg/ml (Table 1). Results from the transformation assay showed that the four nonactivated doses of the test material did not cause a significant increase in morphological transformation of BALB/3T3 cells. From these results the authors concluded that the test material lacked cell transformation properties. Representative data are presented in Table 1.

---

2 C. A. Reznikoff, J. S. Bertram, D. S. Brankow and C. Heidelberger, "Quantitative and Qualitative Studies of Chemical Transformation of Cloned C3H Mouse Embryo Cells Sensitive to Post-confluence Inhibition of Cell Division," Cancer Research 33 (1973): 3239-3249.

TABLE 1. Representative Results of the Nonactivated Transformation Assay with FMC 54800 Technical

| Substance | Dose µg/ml | Relative[a] Survival (%) | Total Foci/Total Dishes Type II | Type III | Transformation[b] Frequency x $10^4$ |
|---|---|---|---|---|---|
| **Negative Control** | | | | | |
| Acetone 0.17 | 2 µl/ml | | 100.0 | 1/14 | 1/14 |
| **Positive Control** | | | | | |
| N-methyl-N'-nitro N-nitrosoguanidine | 0.5 | 9.5 | 9/15 | 12/15 | 20.00* |
| **Test Material** | | | | | |
| FMC 54800 | 100.0[c] | 66.7 | 0/13 | 1/13 | 0.27 |

[a] $\frac{\text{Number of colonies/number of cells seed in test groups}}{\text{Number of colonies/number of cells seed in solvent control}} \times 100$

[b] Number of Type III foci per surviving cells.

[c] Highest dose tested; doses below this level (3-30 µg/ml) gave transformation values comparable to acetone control.

* Significantly different than control value at $p \leq 0.01$, Modified Poisson Distribution.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors stated, "Under the conditions of the assay described in this report, the data suggest that T2007 [FMC 54800] does not induce morphological transformation in BALB/3T3 cells and is negative in the BALB/3T3 cell transformation assay."

B. A quality assurance statement was present, signed, and dated November 14, 1983.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. It is our assessment that the assay did not conform to recommended BALB/3T3 transformation assay procedures for the following reasons:

(1) Definitive cytotoxicity (10-20% cell survival) was not demonstrated at the highest test dose. In accordance with the recommended guidelines for dose selection, if neither cytotoxicity (10-20% cell survival) nor solubility are achieved, 500 µg/ml can be used as the highest test dose.[3]

(2) The number of replicate plates (13) at two test doses and the solvent control plates (14) was lower than the number of recommended plates/dose (15-20). It is critical that a sufficient number of replicate plates be used since the incidence of spontaneously occurring transformed cells is historically low. While 15 to 20 replicates per dose is considered a statistically adequate sample size, at least 20 plates per treatment is preferred.[3]

The ability of the test system to detect chemically induced morphological transformation was demonstrated by the statistically significant increase in transformation frequency reported for cells exposed to the positive control, N-methyl-N'-nitro-N-nitrosoguanidine (0.5 µg/ml-S9).

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol), CBI, pp. 3-6.

---

3 Charles Heidelberger, Aaron E. Freedman, Roman J. Pienta, Andrew Sivak, John S. Bertram, Bruce C. Castro, Virginia C. Dunkel, Mary W. Francis, Takeo Kakunaga, John B. Little and Leonard M. Schechtman, "Cell Transformation by Chemical Agents - A Review and Analysis of the Literature, A Report of the U.S. Environmental Protection Agency Gene-Tox Program," Mutation Research 114 (1983): 283-385.

# APPENDIX A

(Materials, Method, and Protocol)

Page _____ is not included in this copy.

Pages 10 through 13 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

004670

004670

EPA: 88-01-6561
TASK: 120
September 17, 1985

# DATA EVALUATION RECORD

## TALSTAR

## Mutagenicity - Mouse Lymphoma Mutagenesis Assay

STUDY IDENTIFICATION: Kirby, P. E., and Royers-Back, A. M. L5178Y TK$^{+/-}$ mouse lymphoma mutagenesis assay with FMC 54800 technical lot No. E-2392-105. (Unpublished study No. T2007.701 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 26, 1983.) Accession No. 254405.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 9-16-85

004670

1. CHEMICAL: Talstar technical.

2. TEST MATERIAL: FMC 54800 technical, Lot No. E-2392-105, a light brown solid of unspecified purity.

3. STUDY/ACTION TYPE: Mutagenicity - Mouse Lymphoma Mutagenesis Assay.

4. STUDY IDENTIFICATION: Kirby, P. E., and Royers-Back, A. M. L5178Y $TK^{+/-}$ mouse lymphoma mutagenesis assay with FMC 54800 technical lot No. E-2392-105. (Unpublished study No. T2007.701 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 26, 1983.) Accession No. 254405.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 9-16-85

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 9-16-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-16-85

Byron Backus, M.S.
EPA Reviewer

Signature: Byron Backus
Date: 9-19-85

Clint Skinner, Ph.D.
EPA Section Head

Signature: Clint Skinner
Date: 9-19-85

7. CONCLUSIONS:

A. Under the conditions of the assay, doses of Talstar (FMC 54800 technical) ranging from 0.042 to 0.24 µl/ml without S9 activation and 0.024 to 0.1 µl/ml with S9 activation, induced dose-dependent increases in forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells. Talstar is, therefore, considered to be mutagenic in the mouse lymphoma forward mutation assay.

B. The study is acceptable.

Items 8 through 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for details.

(1) The test material, FMC 54800 technical, lot No. E-2392-105, was described as a light brown solid; purity was unspecified. The test material was stored at room temperature under desiccation and protected from light. Test solutions were prepared on the day of use by liquefying the test material at 50°C and diluting in acetone.

(2) The L5178Y $TK^{+/-}$ mouse lymphoma cells, Clone 3.7.2c, were obtained from Dr. D. Clive, Burroughs Wellcome Company, Research Triangle Park, NC. The cells were maintained, grown, and cleansed as described by Clive et al[2].

(3) The S9 fraction used for metabolic activation was prepared from the livers of male Sprague-Dawley rats induced with a 2:1 mixture of Aroclor 1242 and 1254.

(4) Cytotoxicity: Cytotoxicity was determined from the reduction in cell population after dosing with the test material. The assay was performed with and without S9 metabolic activation. Cells were washed after the 4-hour dose exposure, resuspended

---

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

[2] Donald Clive and Joanne Spector, "Laboratory Procedures for Assessing Specific Locus Mutations at the TK Locus in Cultured L5178Y Mouse Lymphoma Cells," Mutation Research 31(1975) 17-29.

in F10P medium, and incubated for a maximum of 48 hours. Cells were counted at 24 and 48 hours and from these results 16 dose levels were selected for evaluation in the mutagenicity assay.

(5) Mutagenicity Assay: Actively growing, precleansed cells, adjusted to a density of $1x10^6$ cells/ml, were exposed to 16 doses of the test material, the solvent, or the positive controls with or without S9 activation. Four hours post-exposure, cells were washed, resuspended, and incubated for a 2-day expression period. Cell population adjustments were made daily throughout the expression period. Cells exposed to the appropriate doses of the test material both in the presence and absence of S9 activation were selected for cloning. For determination of the mutant population, cells exposed to each test dose and control cells were plated in three (replicate) selective medium plates containing trifluorothymidine (TFT) to yield $1x10^6$ cells/plate. The total population was determined by plating at a dilution estimated to contain 200 cells from each test dose and controls in three (replicate) nonselective viability plates. After 10-12 days incubation, colonies were counted; cloning efficiency, total survival, and mutation frequency were determined.

(6) Evaluation Criteria: The assay was considered positive for mutagenicity if a dose-related response was obtained, and the mutation frequencies of one or more of the three highest doses were two-fold higher than the background level.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity Assay: The preliminary cytotoxicity assay was performed in the presence and absence of S9 activation with 0.001, 0.01, 0.1, 1.0 and 10.0 µl/ml of the test material. Complete cytotoxicity was observed in the absence of S9 activation at 10 and 1 µl/ml of the test material; in the presence of S9 activation, 0% survival was recorded at 10.0, 1.0, and 0.1 µl/ml.

Mutagenicity Assay: Based on the results from the cytotoxicity assay, 16 doses of the test material were assayed in the mutagenicity assay. The ten nonactivated doses of the test material, chosen for cloning (0.24 to 0.018 µl/ml), spanned a 5 to 78% survival range. Survival percentages of cells exposed to the ten selected S9 activated doses (0.0075 to 0.1 µl/ml) ranged from 93 to 30%.

In the absence of metabolic activation, 0.24, 0.18, 0.13, 0.1, 0.075, and 0.024 µl/ml of the test material elicited a dose-related increase in mutation frequency that ranged from 4.2 at the high dose to 2.0 at the low dose compared to the mean mutation frequency of the

solvent control (Table 1). As shown in Table 2, a two-fold increase in mutation frequency was also recorded for the highest S9-activated dose (0.1 µl/ml). While two-fold increases were not seen at the remaining activated doses, dose-related increases, ranging from 1.4 to 1.6, were calculated at four test concentrations (0.032, 0.042, 0.056, and 0.075 µl/ml). The authors concluded that dose-related increases in the mutation frequencies of mouse lymphoma cells occurred both with and without S9 activation.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "under the conditions of this test, test article FMC 54800 technical produced a positive response in the presence and absence of exogenous metabolic activation."

B. A quality assurance statement from the laboratory performing the study was present, signed, and dated October 19, 1983. A quality assurance statement from the sponsor's Toxicology Department was present, signed, and dated October 31, 1983.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was conducted properly, and that the authors interpreted the data correctly. As shown in Table 1, the highest mutation frequencies were calculated in the nonactivated assay at test material doses having less than 10% suspension growth (0.24, 0.18 and 0.13 µl/ml). However, there were sufficient test doses with greater than 10% suspension growth meeting the criteria for a positive effect (see Evaluation Criteria, pp. 31 and 32 of Appendix A). Similarly, representative data presented in Table 2 show that S9 activated mouse lymphoma cells gave a positive mutagenic response at test material doses ranging from 0.024 to 0.1 µl/ml, as specified by the authors' criteria.

Data presented in Tables 1 and 2 show that the response to the positive controls, Ethyl Methanesulfonate at 0.5 µl (-S9) and 7,12-Dimethylbenz(a)anthracene at 7.5 µg/ml (+S9), adequately measured the sensitivity of the test system to detect mutagenic activity. The spontaneous mutation frequency of the solvent control was within acceptable ranges (0.2-1.0 mutants per $10^4$ survivors)[2].

Item 15 - see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 3-9.
Protocol, CBI pp. 22a-37.

TABLE 1. Representative Results from the Mouse Lymphoma Forward Mutation Assay with FMC 54800 Technical without S9 Activation

| Substance | Dose | S9 Activation | Average Mutant Counts | Average Viability Counts | % Suspension[a] Growth |
|---|---|---|---|---|---|
| Solvent Control | | | | | |
| Acetone | - | - | 61 | 203 | 100 |
| Positive Control | | | | | |
| Ethyl Methanesulfonate | 0.5 µl/ml | - | 339 | 36 | 38 |
| Test Material | | | | | |
| FMC 54800 | 0.032 µl/ml[d] | - | 51 | 146 | 37 |
| | 0.042 | - | 75 | 140 | 22 |
| | 0.056 | - | 65 | 123 | 19 |
| | 0.075 | - | 74 | 127 | 23 |
| | 0.10 | - | 74 | 106 | 13 |
| | 0.13 | - | 90 | 85 | 8 |
| | 0.18 | - | 82 | 90 | 6 |
| | 0.24 | - | 85 | 69 | 5 |

[a] % Suspension Growth = $\frac{\text{Total Suspension Growth of Test Culture}}{\text{Total Suspension Growth of Solvent Control Culture}}$ X 100.

[b] Mutation Frequency = $\frac{\text{Average No. of Mutant Counts}}{\text{Average No. of Viability Counts}}$ X 2.

[c] Fold Increase = $\frac{\text{Mutation Frequency of Test Dose}}{\text{Mutation Frequency of Solvent Control}}$; Calculated by our reviewers.

[d] Highest test dose showing no effect.

TABLE 2. Representative Results from the Mouse Lymphoma Forward Mutation Assay with FMC 54800 Technical with S9 Activation

| Substance | Dose | S9 Activation | Average Mutant Counts | Average Viability Counts | % Suspension[a] Growth |
|---|---|---|---|---|---|
| **Solvent Control** | | | | | |
| Acetone | | + | 60 | 167 | 100 |
| **Positive Control** | | | | | |
| 7, 12-Dimethyl benz(a) anthracene | 7.5 µg/ml | + | 302 | 58 | 16 |
| **Test Material** | | | | | |
| FMC 54800 | 0.018 µl/ml[d] | + | 59 | 162 | 90 |
| | 0.024 | + | 79 | 185 | 84 |
| | 0.032 | + | 78 | 157 | 76 |
| | 0.042 | + | 73 | 154 | 64 |
| | 0.056 | + | 78 | 137 | 39 |
| | 0.075 | + | 72 | 135 | 44 |
| | 0.1 | + | 76 | 106 | 30 |

[a] % Suspension Growth = $\frac{\text{Total Suspension Growth of Test Culture}}{\text{Total Suspension Growth of Solvent Control Culture}}$ X 100.

[b] Mutation Frequency = $\frac{\text{Average No. of Mutant Counts}}{\text{Average No. of Viability Counts}}$ X 2.

[c] Fold Increase = $\frac{\text{Mutation Frequency of Test Dose}}{\text{Mutation Frequency of Solvent Control}}$; Calculated by our reviewers.

[d] Highest test dose showing no effect.

# APPENDIX A

## Materials and Methods
## Protocol

Page _____ is not included in this copy.

Pages 22 through 44 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA: 68-01-6587 004670
TASK: 120
September 17, 1985

# DATA EVALUATION RECORD

## TALSTAR

## Mutagenicity - Sex Linked Recessive Lethal Assay In Drosophila melanogaster

STUDY IDENTIFICATION: Benson, S. E. and Myhr, B. C. Sex linked recessive lethal assay with FMC 54800 in Drosophila melanogaster. (Unpublished study No. LBI 22205 prepared by Litton Bionetics, Inc., Kensington, MD, for FMC Corporation, Princeton, NJ; dated February, 1984.) Accession No. 254405.

APPROVED BY:

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

Signature: I. Cecil Felkner
Date: 9-16-85

004670

1. CHEMICAL: Talstar.

2. TEST MATERIAL: FMC 54800 technical, a light brown, waxy solid; purity 88.35%.

3. STUDY/ACTION TYPE: Mutagenicity - Sex linked recessive lethal assay in Drosophila melanogaster.

4. STUDY IDENTIFICATION: Benson, S. E. and Myhr, B. C. Sex linked Recessive Lethal Assay with FMC 54800 in Drosophila melanogaster. (Unpublished study No. LBI 22205 prepared by Litton Bionetics, Inc., Kensington, MD, for FMC Corporation, Princeton, NJ; dated February, 1984.) Accession No. 254405.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 9-16-85

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-16-85

6. APPROVED BY:

William L. McLellan, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: _____
Date: _____

Byron Backus, M.S.
EPA Reviewer

Signature: Byron Backus
Date: 9-19-85

Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: 9/19/85

7. CONCLUSIONS:

A. Under the conditions of this assay, 50 and 100 µg/ml FMC 54800 technical did not induce sex-linked recessive lethals in the post-meiotic germ cells of D. melanogaster. The test material is, therefore, considered to be nonmutagenic to the post-meiotic germ cells of D. melanogaster. We concluded, however, that the mutagenic potential of the test material was not fully investigated in this system since the effect on pre-meiotic stem cell development was not determined.

B. The study is inconclusive.

8. RECOMMENDATIONS:

It is recommended that the test material be reevaluated in a sex-linked recessive lethal assay which includes a third round of mating. Before the mutagenic potential of FMC 54800 in this test system can be assessed, the effect of the test material on early spermatids and spermatocytes at the time of exposure is required.

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, FMC 54800 technical, was described as a light brown, waxy solid with a purity of 88.35%. The insolubility of the test material in an aqueous medium prompted the preparation of unsalted butter suspensions of the test material. The suspensions were achieved by dispersing concentrations of the test material up to 500 µg/ml in liquified butter.

(2) D. melanogaster, wild-type male and Basc female cultures were maintained at Litton Bionetics, Inc., Kensington, MD.

(3) Preliminary Toxicity Assay:

a. Culture Preparation. D. melanogaster wild-type males were collected within 16 hours of emergence and aged 2-3 days.

---

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

b. Dosing: The appropriate concentrations of the test material, prepared in liquified butter or the carrier, were pipetted in 1.5 ml volumes into feeding vials. The vials were coated with the suspensions and chilled. Fifty $P_1$ males/vial were exposed to the experimental compound for 24 hours; feeding behavior and toxic effects were monitored.

(4) Definitive Toxicity/Fertility Assay:

Six selected concentrations of the test material, established from the findings of the preliminary toxicity assay, and the vehicle were prepared and were fed to aged $P_1$ males. The dosed males were mated through the sequence of spermatogenesis described for the Sex Linked Recessive Lethal Assay. The percent fertility was determined for all fly populations.

(5) Sex Linked Recessive Lethal Assay:

a. Culture Preparation and Dosing:

The methods described above were used for culture preparation and dosing. Two dose levels of the test material, selected from the findings of the toxicity/fertility test, the vehicle or the positive control were fed to $P_1$ males. At least 200 $P_1$ male flies were used for each dose level of the test compound and negative control to insure that an ample number of chromosomes were available for statistical interpretation.

b. Mating:

1) Brood I. Twenty-four hours after the dosing interval, individual $P_1$ males were mated with three virgin Basc females for a 3-4 day period. This constituted Brood I and represented the sampling of germ cells that were primarily mature sperm at the time of treatment.

2) Brood II. The individual $P_1$ males were transferred to fresh food vials and remated with three virgin females for a second 3-4 day mating interval. This constituted Brood II and represented the sampling of germ cells that were primarily immature sperm at the time of treatment.

c. $F_2$ Generation:

The desired number of $F_1$ females from each culture of each brood were pair-mated to their brothers. The progeny ($F_2$) of the selected $F_1$ females were scored.

d. Scoring the $F_2$:

The progeny ($F_2$) of the selected $F_1$ females from each brood were examined for the presence of lethal or non-lethal cultures and the frequency of X-linked recessive lethals were calculated.

(6) Evaluation Criteria:

The assay was considered negative if a) the increase in the lethal frequency of the treated group over the control was less than 0.2% and the sample size was large enough to allow detection of a statistically significant increase of 0.2%; or b) none of the broods analyzed shows a positive result, the increases in the lethal frequency of the treated group of at least two broods was large enough to allow the detection of a statistically significant increase of 0.4%.

(7) The Kastenbaum-Bowman test was used to determine the significance of the results.[2]

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Preliminary Toxicity Assay:

Fifty $P_1$ wild-type males per group were allowed to feed for 24 hours on 1.0, 10.0, and 100.0 µg/ml suspensions of the test material, which were prepared in liquified butter. The $LD_{50}$ was not achieved at any test dose; therefore, a second preliminary toxicity test was performed with 62.5, 125, 250, and 500 µg/ml of the test material. No flies survived exposure to 500 µg/ml of the test material; the approximate $LD_{50}$ for the test material was established at 125 µg/ml.

B. Definitive Toxicity/Fertility Assay:

Based on the findings from the second preliminary toxicity assay, six doses of the test material (10, 25, 50, 100, 125, and 200 µg/ml) were selected for the definitive toxicity/fertility assay. Following a 24-hour feeding interval, representative males exposed to 25, 50, 100, and 125 µg/ml of the test material were sequentially mated to yield Broods I and II, $F_1$ progeny. A dose-related reduction in percent fertility ranging from 72 to 56% for Brood I and 60 to 57% for Brood II accompanied exposure of parental males to increasing doses of the test material in the concentration range of 25 to 125 µg/ml.

[2] M. A. Kastenbaum and K. O. Bowman, "Tables for determining the statistical significance of mutation frequencies," Mutation Research 9(1970): 527-549.

C. Sex-Linked Recessive Lethal Assay:

Based on the findings of the definitive toxicity/fertility assay, 50 and 100 µg/ml of the test material were examined in the mutation assay. Randomly selected $P_1$ wild-type males were exposed to the two selected doses of the test material, the test material vehicle or the positive control for a 24-hour period. Treated males were mated sequentially to virgin females, which yielded Broods I and II $F_1$ progeny. A final round of brother-sister mating resulted in the $F_2$ generation.

Single mutations were scored for all broods within each treatment group. Multiple lethals, which arose from single males, were observed in Brood II from the vehicle control group, in Broods I and II from the 50 µg/ml test material group, and Brood I from the 100 µg/ml test material group. In accordance with the study design, clusters of mutations, which were derived from individual males, were considered to have arisen from a spontaneous mutation and were, therefore, counted as a single event. From the results, the authors concluded that the test material did not induce a mutagenic effect. Representative data are shown in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "FMC 54800 administered via feeding does not induce sex linked recessive lethals in the post-meiotic germ cells of Drosophila melanogaster."

B. A Quality Assurance statement from the study authors' laboratory was present, signed and dated February 13, 1984. A Quality Assurance statement from the sponsor was also present, signed and dated February 24, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

While it is our assessment that the authors' interpretation of the data was correct, a third round of mating was not performed; therefore, the effect of the test material on early spermatids and spermatocytes could not be assessed. Analysis of the offsprings from treated spermatogonial stem cells is essential since Vogel has shown that at this stage many procarcinogens exhibit peak mutagenic activity.[3] The authors, however, only reported the findings relative to post-meiotic cells.

The results of the positive control (Ethylmethane sulfonate, 0.7 µl/ml) fell within the laboratory's expected range for this mutagen (20 to 30% sex-linked recessive lethals); thus, the sensitivity of the test system to detect chemically induced mutagenic events was demonstrated.

---

[3] E. Vogel, "Some aspects of the detection of potential mutagenic agents in Drosophila," Mutation Research 29(1975): 241-250.

TABLE I Representative Results of the Sex Linked Recessive Lethal Assay in D. melanogaster with FMC 54800 Technical

| Treatment | Dose | Brood[a] I | Percent[b] Lethals | Brood[a] II | Percent[b] Lethals | Combined[c] | Combined[d] Percent Lethals |
|---|---|---|---|---|---|---|---|
| Vehicle Control | | | | | | | |
| Unsalted Butter | | 3/3972 | 0.08 | 2/3971[e] | 0.05 | 5/7943 | 0.06 |
| Positive Control | | | | | | | |
| Ethylmethane sulfonate | 0.7 µl/ml | 46/169 | 27.2 | 43/129 | 33.3 | 89/298 | 29.87 |
| Test Material | | | | | | | |
| FMC 54800 | 50 µg/ml | 5/3924[e] | 0.13 | 4/3293[e] | 0.12 | 9/7217 | 0.12 |
| | 100 µg/ml | 3/3590[e] | 0.08 | 4/3404 | 0.12 | 7/6994 | 0.10 |

[a] Number of Lethals/Number of Tests (Number of Nonlethals + Lethals).

[b] $\frac{\text{Number of Lethals}}{\text{Number of Tests}} \times 100$

[c] Total Number of Lethals/Total Number of Tests.

[d] $\frac{\text{Total Number of Lethals}}{\text{Total Number of Tests}} \times 100$

[e] Cluster of Lethals, counted as one lethal.

Item 15 - See footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol) CBI pp. 8-12.

# APPENDIX A

Materials and Methods (Protocol) CBI pp. 8-12



53

Page _____ is not included in this copy.

Pages 54 through 58 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

004670

EPA: 68-01-6561
TASK: 120
September 17, 1985

# DATA EVALUATION RECORD

## TALSTAR

## Mutagenicity - Sex Linked Recessive Lethal Assay In *Drosophila melanogaster*

**STUDY IDENTIFICATION:** Benson, S. E. and Myhr, B. C. Sex linked recessive lethal assay with FMC 54800 in *Drosophila melanogaster*. (Unpublished study No. LBI 22205 prepared by Litton Bionetics, Inc., Kensington, MD, for FMC Corporation, Princeton, NJ; dated February, 1984.) Accession No. 254405.

**APPROVED BY:**

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

Signature: I. Cecil Felkner

Date: 9-16-85



59

1. CHEMICAL: Talstar.

2. TEST MATERIAL: FMC 54800 technical, a light brown, waxy solid; purity 88.35%.

3. STUDY/ACTION TYPE: Mutagenicity - Sex linked recessive lethal assay in Drosophila melanogaster.

4. STUDY IDENTIFICATION: Benson, S. E. and Myhr, B. C. Sex linked Recessive Lethal Assay with FMC 54800 in Drosophila melanogaster. (Unpublished study No. LBI 22205 prepared by Litton Bionetics, Inc., Kensington, MD, for FMC Corporation, Princeton, NJ: dated February, 1984.) Accession No. 254405.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 9-16-85

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-16-85

6. APPROVED BY:

William L. McLellan, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: _____
Date: _____

Byron Backus, M.S.
EPA Reviewer

Signature: Byron Backus
Date: 9-19-85

Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: 9.19.85



60
(60)

7. CONCLUSIONS:

A. Under the conditions of this assay, 50 and 100 µg/ml FMC 54800 technical did not induce sex-linked recessive lethals in the post-meiotic germ cells of D. melanogaster. The test material is, therefore, considered to be nonmutagenic to the post-meiotic germ cells of D. melanogaster. We concluded, however, that the mutagenic potential of the test material was not fully investigated in this system since the effect on pre-meiotic stem cell development was not determined.

B. The study is inconclusive.

8. RECOMMENDATIONS:

It is recommended that the test material be reevaluated in a sex-linked recessive lethal assay which includes a third round of mating. Before the mutagenic potential of FMC 54800 in this test system can be assessed, the effect of the test material on early spermatids and spermatocytes at the time of exposure is required.

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, FMC 54800 technical, was described as a light brown, waxy solid with a purity of 88.35%. The insolubility of the test material in an aqueous medium prompted the preparation of unsalted butter suspensions of the test material. The suspensions were achieved by dispersing concentrations of the test material up to 500 µg/ml in liquified butter.

(2) D. melanogaster, wild-type male and Basc female cultures were maintained at Litton Bionetics, Inc., Kensington, MD.

(3) Preliminary Toxicity Assay:

a. Culture Preparation. D. melanogaster wild-type males were collected within 16 hours of emergence and aged 2-3 days.

---

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

b. Dosing: The appropriate concentrations of the test material, prepared in liquified butter or the carrier, were pipetted in 1.5 ml volumes into feeding vials. The vials were coated with the suspensions and chilled. Fifty $P_1$ males/vial were exposed to the experimental compound for 24 hours; feeding behavior and toxic effects were monitored.

(4) Definitive Toxicity/Fertility Assay:

Six selected concentrations of the test material, established from the findings of the preliminary toxicity assay, and the vehicle were prepared and were fed to aged $P_1$ males. The dosed males were mated through the sequence of spermatogenesis described for the Sex Linked Recessive Lethal Assay. The percent fertility was determined for all fly populations.

(5) Sex Linked Recessive Lethal Assay:

a. Culture Preparation and Dosing:

The methods described above were used for culture preparation and dosing. Two dose levels of the test material, selected from the findings of the toxicity/fertility test, the vehicle or the positive control were fed to $P_1$ males. At least 200 $P_1$ male flies were used for each dose level of the test compound and negative control to insure that an ample number of chromosomes were available for statistical interpretation.

b. Mating:

1) Brood I. Twenty-four hours after the dosing interval, individual $P_1$ males were mated with three virgin Basc females for a 3-4 day period. This constituted Brood I and represented the sampling of germ cells that were primarily mature sperm at the time of treatment.

2) Brood II. The individual $P_1$ males were transferred to fresh food vials and remated with three virgin females for a second 3-4 day mating interval. This constituted Brood II and represented the sampling of germ cells that were primarily immature sperm at the time of treatment.

c. $F_2$ Generation:

The desired number of $F_1$ females from each culture of each brood were pair-mated to their brothers. The progeny ($F_2$) of the selected $F_1$ females were scored.

d. Scoring the $F_2$:

The progeny ($F_2$) of the selected $F_1$ females from each brood were examined for the presence of lethal or non-lethal cultures and the frequency of X-linked recessive lethals were calculated.

(6) Evaluation Criteria:

The assay was considered negative if a) the increase in the lethal frequency of the treated group over the control was less than 0.2% and the sample size was large enough to allow detection of a statistically significant increase of 0.2%; or b) none of the broods analyzed shows a positive result, the increases in the lethal frequency of the treated group of at least two broods was large enough to allow the detection of a statistically significant increase of 0.4%.

(7) The Kastenbaum-Bowman test was used to determine the significance of the results.[2]

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Preliminary Toxicity Assay:

Fifty $P_1$ wild-type males per group were allowed to feed for 24 hours on 1.0, 10.0, and 100.0 µg/ml suspensions of the test material, which were prepared in liquified butter. The $LD_{50}$ was not achieved at any test dose; therefore, a second preliminary toxicity test was performed with 62.5, 125, 250, and 500 µg/ml of the test material. No flies survived exposure to 500 µg/ml of the test material; the approximate $LD_{50}$ for the test material was established at 125 µg/ml.

B. Definitive Toxicity/Fertility Assay:

Based on the findings from the second preliminary toxicity assay, six doses of the test material (10, 25, 50, 100, 125, and 200 µg/ml) were selected for the definitive toxicity/fertility assay. Following a 24-hour feeding interval, representative males exposed to 25, 50, 100, and 125 µg/ml of the test material were sequentially mated to yield Broods I and II, $F_1$ progeny. A dose-related reduction in percent fertility ranging from 72 to 56% for Brood I and 60 to 57% for Brood II accompanied exposure of parental males to increasing doses of the test material in the concentration range of 25 to 125 µg/ml.

---

[2] M. A. Kastenbaum and K. O. Bowman, "Tables for determining the statistical significance of mutation frequencies," Mutation Research 9(1970): 527-549.

C. Sex-Linked Recessive Lethal Assay:

Based on the findings of the definitive toxicity/fertility assay, 50 and 100 µg/ml of the test material were examined in the mutation assay. Randomly selected $P_1$ wild-type males were exposed to the two selected doses of the test material, the test material vehicle or the positive control for a 24-hour period. Treated males were mated sequentially to virgin females, which yielded Broods I and II $F_1$ progeny. A final round of brother-sister mating resulted in the $F_2$ generation.

Single mutations were scored for all broods within each treatment group. Multiple lethals, which arose from single males, were observed in Brood II from the vehicle control group, in Broods I and II from the 50 µg/ml test material group, and Brood I from the 100 µg/ml test material group. In accordance with the study design, clusters of mutations, which were derived from individual males, were considered to have arisen from a spontaneous mutation and were, therefore, counted as a single event. From the results, the authors concluded that the test material did not induce a mutagenic effect. Representative data are shown in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "FMC 54800 administered via feeding does not induce sex linked recessive lethals in the post-meiotic germ cells of Drosophila melanogaster."

B. A Quality Assurance statement from the study authors' laboratory was present, signed and dated February 13, 1984. A Quality Assurance statement from the sponsor was also present, signed and dated February 24, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

While it is our assessment that the authors' interpretation of the data was correct, a third round of mating was not performed; therefore, the effect of the test material on early spermatids and spermatocytes could not be assessed. Analysis of the offsprings from treated spermatogonial stem cells is essential since Vogel has shown that at this stage many procarcinogens exhibit peak mutagenic activity.[3] The authors, however, only reported the findings relative to post-meiotic cells.

The results of the positive control (Ethylmethane sulfonate, 0.7 µl/ml) fell within the laboratory's expected range for this mutagen (20 to 30% sex-linked recessive lethals); thus, the sensitivity of the test system to detect chemically induced mutagenic events was demonstrated.

---

[3] E. Vogel, "Some aspects of the detection of potential mutagenic agents in Drosophila," Mutation Research 29(1975): 241-250.

TABLE I Representative Results of the Sex Linked Recessive Lethal Assay in D. melanogaster with FMC 54800 Technical

| Treatment | Dose | Brood[a] I | Percent[b] Lethals | Brood[a] II | Percent[b] Lethals | Combined[c] | Combined[d] Percent Lethals |
|---|---|---|---|---|---|---|---|
| **Vehicle Control** | | | | | | | |
| Unsalted Butter | | 3/3972 | 0.08 | 2/3971[e] | 0.05 | 5/7943 | 0.06 |
| **Positive Control** | | | | | | | |
| Ethylmethane sulfonate | 0.7 µl/ml | 46/169 | 27.2 | 43/129 | 33.3 | 89/298 | 29.87 |
| **Test Material** | | | | | | | |
| FMC 54800 | 50 µg/ml | 5/3924[e] | 0.13 | 4/3293[e] | 0.12 | 9/7217 | 0.12 |
| | 100 µg/ml | 3/3590[e] | 0.08 | 4/3404 | 0.12 | 7/6994 | 0.10 |

[a] Number of Lethals/Number of Tests (Number of Nonlethals + Lethals).

[b] $\frac{\text{Number of lethals}}{\text{Number of Tests}} \times 100$

[c] Total Number of Lethals/Total Number of Tests.

[d] $\frac{\text{Total Number of Lethals}}{\text{Total Number of Tests}} \times 100$

[e] Cluster of Lethals, counted as one lethal.

Item 15 - See footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol) CBI pp. 8-12.

# APPENDIX A

Materials and Methods (Protocol) CBI pp. 8-12

Page _____ is not included in this copy.

Pages 68 through 72 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

004670

004670

EPA: 68-01-6561
TASK: 120
September 17, 1985

DATA EVALUATION RECORD

TALSTAR

Mutagenicity - Mouse Lymphoma Mutagenesis Assay

STUDY IDENTIFICATION: Kirby, P. E., and Royers-Back, A. M. L5178Y TK+/- mouse lymphoma mutagenesis assay with FMC 54800 technical lot No. E-2392-105. (Unpublished study No. T2007.701 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 26, 1983.) Accession No. 254405.

APPROVED BY:

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

Signature: I Cecil Felkner
Date: 9-16-85

Copy



73

1. CHEMICAL: Talstar technical.

2. TEST MATERIAL: FMC 54800 technical, Lot No. E-2392-105, a light brown solid of unspecified purity.

3. STUDY/ACTION TYPE: Mutagenicity - Mouse Lymphoma Mutagenesis Assay.

4. STUDY IDENTIFICATION: Kirby, P. E., and Royers-Back, A. M. L5178Y $TK^{+/-}$ mouse lymphoma mutagenesis assay with FMC 54800 technical lot No. E-2392-105. (Unpublished study No. T2007.701 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 26, 1983.) Accession No. 254405.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: [signature]
Date: 9-16-85

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: [signature]
Date: 9-16-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: [signature]
Date: 9-16-85

Byron Backus, M.S.
EPA Reviewer

Signature: [signature]
Date: 09-19-85

Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: _____

74

7. CONCLUSIONS:

A. Under the conditions of the assay, doses of Talstar (FMC 54800 technical) ranging from 0.042 to 0.24 µl/ml without S9 activation and 0.024 to 0.1 µl/ml with S9 activation, induced dose-dependent increases in forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells. Talstar is, therefore, considered to be mutagenic in the mouse lymphoma forward mutation assay.

B. The study is acceptable.

Items 8 through 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for details.

(1) The test material, FMC 54800 technical, lot No. E-2392-105, was described as a light brown solid; purity was unspecified. The test material was stored at room temperature under desiccation and protected from light. Test solutions were prepared on the day of use by liquefying the test material at 50°C and diluting in acetone.

(2) The L5178Y $TK^{+/-}$ mouse lymphoma cells, Clone 3.7.2c, were obtained from Dr. D. Clive, Burroughs Wellcome Company, Research Triangle Park, NC. The cells were maintained, grown, and cleansed as described by Clive et al[2].

(3) The S9 fraction used for metabolic activation was prepared from the livers of male Sprague-Dawley rats induced with a 2:1 mixture of Aroclor 1242 and 1254.

(4) Cytotoxicity: Cytotoxicity was determined from the reduction in cell population after dosing with the test material. The assay was performed with and without S9 metabolic activation. Cells were washed after the 4-hour dose exposure, resuspended

---

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

[2] Donald Clive and Joanne Spector, "Laboratory Procedures for Assessing Specific Locus Mutations at the TK Locus in Cultured L5178Y Mouse Lymphoma Cells," Mutation Research 31(1975) 17-29.

in $F_{10}P$ medium, and incubated for a maximum of 48 hours. Cells were counted at 24 and 48 hours and from these results 16 dose levels were selected for evaluation in the mutagenicity assay.

(5) Mutagenicity Assay: Actively growing, precleansed cells, adjusted to a density of $1x10^6$ cells/ml, were exposed to 16 doses of the test material, the solvent, or the positive controls with or without S9 activation. Four hours post-exposure, cells were washed, resuspended, and incubated for a 2-day expression period. Cell population adjustments were made daily throughout the expression period. Cells exposed to the appropriate doses of the test material both in the presence and absence of S9 activation were selected for cloning. For determination of the mutant population, cells exposed to each test dose and control cells were plated in three (replicate) selective medium plates containing trifluorothymidine (TFT) to yield $1x10^6$ cells/plate. The total population was determined by plating at a dilution estimated to contain 200 cells from each test dose and controls in three (replicate) nonselective viability plates. After 10-12 days incubation, colonies were counted; cloning efficiency, total survival, and mutation frequency were determined.

(6) Evaluation Criteria: The assay was considered positive for mutagenicity if a dose-related response was obtained, and the mutation frequencies of one or more of the three highest doses were two-fold higher than the background level.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity Assay: The preliminary cytotoxicity assay was performed in the presence and absence of S9 activation with 0.001, 0.01, 0.1, 1.0 and 10.0 µl/ml of the test material. Complete cytotoxicity was observed in the absence of S9 activation at 10 and 1 µl/ml of the test material; in the presence of S9 activation, 0% survival was recorded at 10.0, 1.0, and 0.1 µl/ml.

Mutagenicity Assay: Based on the results from the cytotoxicity assay, 16 doses of the test material were assayed in the mutagenicity assay. The ten nonactivated doses of the test material, chosen for cloning (0.24 to 0.018 µl/ml), spanned a 5 to 78% survival range. Survival percentages of cells exposed to the ten selected S9 activated doses (0.0075 to 0.1 µl/ml) ranged from 93 to 30%.

In the absence of metabolic activation, 0.24, 0.18, 0.13, 0.1, 0.075, and 0.024 µl/ml of the test material elicited a dose-related increase in mutation frequency that ranged from 4.2 at the high dose to 2.0 at the low dose compared to the mean mutation frequency of the

solvent control (Table 1). As shown in Table 2, a two-fold increase in mutation frequency was also recorded for the highest S9-activated dose (0.1 µl/ml). While two-fold increases were not seen at the remaining activated doses, dose-related increases, ranging from 1.4 to 1.6, were calculated at four test concentrations (0.032, 0.042, 0.056, and 0.075 µl/ml). The authors concluded that dose-related increases in the mutation frequencies of mouse lymphoma cells occurred both with and without S9 activation.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "under the conditions of this test, test article FMC 54800 technical produced a positive response in the presence and absence of exogenous metabolic activation."

B. A quality assurance statement from the laboratory performing the study was present, signed, and dated October 19, 1983. A quality assurance statement from the sponsor's Toxicology Department was present, signed, and dated October 31, 1983.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the study was conducted properly, and that the authors interpreted the data correctly. As shown in Table 1, the highest mutation frequencies were calculated in the nonactivated assay at test material doses having less than 10% suspension growth (0.24, 0.18 and 0.13 µl/ml). However, there were sufficient test doses with greater than 10% suspension growth meeting the criteria for a positive effect (see Evaluation Criteria, pp. 31 and 32 of Appendix A). Similarly, representative data presented in Table 2 show that S9 activated mouse lymphoma cells gave a positive mutagenic response at test material doses ranging from 0.024 to 0.1 µl/ml, as specified by the authors' criteria.

Data presented in Tables 1 and 2 show that the response to the positive controls, Ethyl Methanesulfonate at 0.5 µl (-S9) and 7,12-Dimethylbenz(a)anthracene at 7.5 µg/ml (+S9), adequately measured the sensitivity of the test system to detect mutagenic activity. The spontaneous mutation frequency of the solvent control was within acceptable ranges (0.2-1.0 mutants per $10^4$ survivors)[2].

Item 15 - see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 3-9.
Protocol, CBI pp. 22a-37.

77



77

TABLE 1. Representative Results from the Mouse Lymphoma Forward Mutation Assay with FMC 54800 Technical without S9 Activation

| Substance | Dose | S9 Activation | Average Mutant Counts | Average Viability Counts | % Suspension[a] Growth |
|---|---|---|---|---|---|
| **Solvent Control** | | | | | |
| Acetone | | - | 61 | 203 | 100 |
| **Positive Control** | | | | | |
| Ethyl Methanesulfonate | 0.5 µl/ml | - | 339 | 36 | 38 |
| **Test Material** | | | | | |
| FMC 54800 | 0.032 µl/ml[d] | - | 51 | 146 | 37 |
| | 0.042 | - | 75 | 140 | 22 |
| | 0.056 | - | 65 | 123 | 19 |
| | 0.075 | - | 74 | 127 | 23 |
| | 0.10 | - | 74 | 106 | 13 |
| | 0.13 | - | 90 | 85 | 8 |
| | 0.18 | - | 82 | 90 | 6 |
| | 0.24 | - | 85 | 69 | 5 |

[a] % Suspension Growth = $\frac{\text{Total Suspension Growth of Test Culture}}{\text{Total Suspension Growth of Solvent Control Culture}}$ X 100.

[b] Mutation Frequency = $\frac{\text{Average No. of Mutant Counts}}{\text{Average No. of Viability Counts}}$ X 2.

[c] Fold Increase = $\frac{\text{Mutation Frequency of Test Dose}}{\text{Mutation Frequency of Solvent Control}}$; Calculated by our reviewers.

[d] Highest test dose showing no effect.

TABLE 2. Representative Results from the Mouse Lymphoma Forward Mutation Assay with FMC 54800 Technical with S9 Activation

| Substance | Dose | S9 Activation | Average Mutant Counts | Average Viability Counts | % Suspension[a] Fr Growth |
|---|---|---|---|---|---|
| Solvent Control | | | | | |
| Acetone | | + | 60 | 167 | 100 |
| Positive Control | | | | | |
| 7, 12-Dimethyl benz(a) anthracene | 7.5 µg/ml | + | 302 | 58 | 16 |
| Test Material | | | | | |
| FMC 54800 | 0.018 µl/ml[d] | + | 59 | 162 | 90 |
| | 0.024 | + | 79 | 185 | 84 |
| | 0.032 | + | 78 | 157 | 76 |
| | 0.042 | + | 73 | 154 | 64 |
| | 0.056 | + | 78 | 137 | 39 |
| | 0.075 | + | 72 | 135 | 44 |
| | 0.1 | + | 76 | 106 | 30 |

[a] % Suspension Growth = $\frac{\text{Total Suspension Growth of Test Culture}}{\text{Total Suspension Growth of Solvent Control Culture}}$ X 100.

[b] Mutation Frequency = $\frac{\text{Average No. of Mutant Counts}}{\text{Average No. of Viability Counts}}$ X 2.

[c] Fold Increase = $\frac{\text{Mutation Frequency of Test Dose}}{\text{Mutation Frequency of Solvent Control}}$; Calculated by our reviewers.

[d] Highest test dose showing no effect.

# APPENDIX A

## Materials and Methods
## Protocol

Page _____ is not included in this copy.

Pages 81 through 103 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

004670

EPA: 68-01-6561
TASK: 120
September 17, 1985

# DATA EVALUATION RECORD

## TALSTAR

## Mutagenicity-In Vitro Transformation of BALB/3T3 Cells Without S9 Activation Assay

STUDY IDENTIFICATION: Putman, D. L. and McCarvill, J. T. Morphological transformation of BALB/3T3 mouse embryo cells in the absence of exogenous metabolic activation. (Unpublished Study No. T2007.301 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 11, 1983). Accession No. 254405.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 9-16-85

Copy



104

004670

1. CHEMICAL: FMC 54800 Technical (A83-980), Talstar.

2. TEST MATERIAL: FMC 54800 technical (A83-980), lot E-2392-105, a light brown solid, purity not reported.

3. STUDY/ACTION TYPE: Mutagenicity - In Vitro Transformation of BALB/3T3 Cells Without S9 Activation Assay.

4. STUDY IDENTIFICATION: Putman, D. L. and McCarvill, J. T. Morphological transformation of BALB/3T3 mouse embryo cells in the absence of exogenous metabolic activation. (Unpublished Study No. T2007.301 prepared by Microbiological Associates, Bethesda, MD, for FMC Corporation, Princeton, NJ; dated October 11, 1983). Accession No. 254405.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 9-16-85

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 9-16-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-16-85

Byron Backus, M.S.
EPA Reviewer

Signature: Byron Backus
Date: 09/19/85

Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: _____

105



105

7. CONCLUSIONS:

A. Due to the lack of a definitive cytotoxic effect and the insufficient number of replicates used for the test material and solvent control, the results of the nonactivated transformation assay conducted with FMC 54800 cannot be fully assessed.

B. The study was unacceptable.

8. RECOMMENDATIONS:

A. The following recommendations are given to upgrade a repeat study:

(1) Demonstrate a cytotoxic effect (10-20% survival) at the highest assayed dose, limits of solubility, or increase the maximum dose tested to 500 µg/ml.

(2) Increase the number of replicates for all treatment groups to 20 dishes. Increasing the number of replicates per dose will ensure that a sufficient sample size is available for statistical evaluation of the results.

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, FMC 54800 technical (A83-980), lot E-2392-105 was described as a light brown solid. Purity and stability were not specified. The test material was stored at room temperature and dissolved in acetone on the day of use.

2. BALB/3T3 Clone A 31-1 mouse embryo cells were obtained from Dr. T. Kakunaga, National Cancer Institute, Bethesda, MD.

3. Cytotoxicity/Transformation Assay:

Exponentially growing cells were seeded at 250 cells/60 mm dish in triplicate for the cytotoxicity test and at $1x10^4$ cells/60 mm dish (12-15 replicates/dose) for the transformation assay. Prepared cultures were exposed to four doses of the test material, solvent or positive control in the absence of S9 activation for 24 hours. At the conclusion of the exposure period, the cells were washed and refed with

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

growth medium. After 7-10 days, the surviving cells exposed to the appropriate doses of the test material in the concurrent cytotoxicity test were fixed, stained and counted. Throughout the 4-6 week incubation period of the transformation assay, the cells were refed twice weekly with growth medium. The assay was terminated by fixing and staining the monolayers; the number of foci per dish were counted and scored as Type II or Type III morphological transformants according to the method of Reznikoff et al.[2]

4. Evaluation Criteria:

   The assay was considered positive if the transformation frequencies in the test material treated groups were statistically increased ($p \leq 0.05$) relative to the solvent control group.

5. The statistical method used was a Modified Poisson Distribution.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity/Transformation Assay:

The doses selected for the nonactivated cytotoxicity/transformation assay (3, 10, 30, and 100 µg/ml of the test material) were selected based on a preliminary dose-range finding clonal cytotoxicity study; however, neither the dose levels assayed nor the results were provided by the authors. Relative cell survival, following a 24-hour exposure to the four doses of the test material, ranged from 67 percent at 100 µg/ml to 100 percent at 3 µg/ml (Table 1). Results from the transformation assay showed that the four nonactivated doses of the test material did not cause a significant increase in morphological transformation of BALB/3T3 cells. From these results the authors concluded that the test material lacked cell transformation properties. Representative data are presented in Table 1.

---

2 C. A. Reznikoff, J. S. Bertram, D. S. Brankow and C. Heidelberger, "Quantitative and Qualitative Studies of Chemical Transformation of Cloned C3H Mouse Embryo Cells Sensitive to Post-confluence Inhibition of Cell Division," Cancer Research 33 (1973): 3239-3249.

TABLE 1. Representative Results of the Nonactivated Transformation Assay with FMC 54800 Technical

| Substance | Dose µg/ml | Relative[a] Survival (%) | Total Foci/Total Dishes Type II | Total Foci/Total Dishes Type III | Transformation[b] Frequency x $10^4$ |
|---|---|---|---|---|---|
| Negative Control | | | | | |
| Acetone 0.17 | 2 µl/ml | | 100.0 | 1/14 | 1/14 |
| Positive Control | | | | | |
| N-methyl-N'-nitro N-nitrosoguanidine | 0.5 | 9.5 | 9/15 | 12/15 | 20.00* |
| Test Material | | | | | |
| FMC 54800 | 100.0[c] | 66.7 | 0/13 | 1/13 | 0.27 |

[a] $\frac{\text{Number of colonies/number of cells seed in test groups}}{\text{Number of colonies/number of cells seed in solvent control}} \times 100$

[b] Number of Type III foci per surviving cells.

[c] Highest dose tested; doses below this level (3-30 µg/ml) gave transformation values comparable to acetone control.

* Significantly different than control value at $p \leq 0.01$, Modified Poisson Distribution.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors stated, "Under the conditions of the assay described in this report, the data suggest that T2007 [FMC 54800] does not induce morphological transformation in BALB/3T3 cells and is negative in the BALB/3T3 cell transformation assay."

B. A quality assurance statement was present, signed, and dated November 14, 1983.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. It is our assessment that the assay did not conform to recommended BALB/3T3 transformation assay procedures for the following reasons:

(1) Definitive cytotoxicity (10-20% cell survival) was not demonstrated at the highest test dose. In accordance with the recommended guidelines for dose selection, if neither cytotoxicity (10-20% cell survival) nor solubility are achieved, 500 µg/ml can be used as the highest test dose.[3]

(2) The number of replicate plates (13) at two test doses and the solvent control plates (14) was lower than the number of recommended plates/dose (15-20). It is critical that a sufficient number of replicate plates be used since the incidence of spontaneously occurring transformed cells is historically low. While 15 to 20 replicates per dose is considered a statistically adequate sample size, at least 20 plates per treatment is preferred.[3]

The ability of the test system to detect chemically induced morphological transformation was demonstrated by the statistically significant increase in transformation frequency reported for cells exposed to the positive control, N-methyl-N'-nitro-N-nitrosoguanidine (0.5 µg/ml-S9).

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol), CBI, pp. 3-6.

---

3 Charles Heidelberger, Aaron E. Freedman, Roman J. Pienta, Andrew Sivak, John S. Bertram, Bruce C. Castro, Virginia C. Dunkel, Mary W. Francis, Takeo Kakunaga, John B. Little and Leonard M. Schechtman, "Cell Transformation by Chemical Agents - A Review and Analysis of the Literature, A Report of the U.S. Environmental Protection Agency Gene-Tox Program," Mutation Research 114 (1983): 283-385.

# APPENDIX A

(Materials, Method, and Protocol)


110

Page _____ is not included in this copy.

Pages 111 through 114 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.