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

004522

MAY 31 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Mutagenicity Studies Submitted in Response to Registration Standard on EPTC

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767)

THRU: Robert P. Zendzian, Ph.D.
Acting Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Chad B. Sandusky, Ph.D.
Pharmacologist
Toxicology Branch
Hazard Evaluation Division (TS-769)

Compound: EPTC Tox. Chem. #: 435

Registrant#: 476-2140
476-2165 Registrant: Stauffer Chemical Company

Accession #: 255674

Action Requested:

Stauffer Chemical Company has submitted for review 7 mutagenicty studies on EPTC. Two of these seven reports were from the published literature and did not contain sufficient detail to be useful in the evaluation of the mutagenic potential of EPTC (see references). Therefore, they were not reviewed.

Conclusion:

Detailed reviews on 5 of these reports are contained in the attached DER's. Also attached is an overall summary of the mutagenic potential of EPTC based on the data in these 5 reports. Overall, EPTC should be considered mutagenic. However, not all categories normally tested for mutagenic activity, i.e., other mutagenic mechanisms, had adequate data submitted, since none of the in vitro assays in this category included a metabolic activation system.

The results from the mouse lymphoma assay (T-11907) showed that a postive response was obtained only if S9-mix was included in the assay. Hence, the rec assay with B. subtilis and the strand-break and nick-translation assays in human fibroblasts should be performed using S9-activation. In addition, at least one in vivo mammalian assay should be conducted, e.g., mouse micronucleus using two dosings and multiple sampling in both sexes (according to: Heddle, J. and Salamone, M.F. in eds. H.F. Stich and R.H.C. San. Short-Term Tests for Chemical Carcinogenicity, Springer-Verlag, NY/Heidedberg/Berlin pp. 246-252.). Filling these data gaps will provide useful information regarding the mutagenic potential of Eptam/EPTC technical.

Background:

These 7 mutugenicity reports were submitted in response to the Registration Standard on EPTC.

Discussion:

A detailed discussion of these data are attached (mutagenicity Overview of EPTC) as well as DER's on 5 of the 7 studies.

DER's:

The following items are attached:

A. Mutagenicity Overview on the Herbicide Eptam/EPTC.

B. DER's

1) Shirasu, Y., Moriya, M., and Miyazaua, T. Mutagenicity testing on EPTC in microbial systems. (Unpublished Study No. T-6617 by the Institute of Environmental Toxicology, Tokyo, Japan for Staffer Chemical Company; dated July 24, 1978.) Accession No. 255674.

2) Jagannath, D.R. and Brusick, D.J. Mutagenicity evaluation of Eptam Tech 3905-35. (Unpublished Study No. T6314 prepared by Litton Bionetics, Inc., Kensington, MD for Stauffer Chemical Corporation, Western Research Centers, Richmond, CA; dated October 27, 1977.) Accession No. 255674.

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3) Majeska, J.B., Hertzel, K., and Matheson, D.W. Mutagenicity evaluation in mouse lymphoma multiple endpoint test forward mutation assay with Eptam technical. (Unpublished report No. T-11907 prepared by Agricultural Chemical Division of Stauffer Chemical Co. for Stauffer Chemical Co., Farmington, CT; dated September 18, 1984.) Accession No. 255674.

4. Majeska, J.B., Hertzel, K., and Matheson, D.W. Mutagenicity evaluation in mouse lymphoma multiple endpoint test cytogenetic assay. (Unpublished report No. T-11908 prepared by Stauffer Chemical-Toxicology Section for Stauffer Chemical Co., Farmington, CT; dated September 7, 1984.) Accession No. 255674.

5. Snyder, R.D. and Matheson, D.W. Effects of Eptam on Human Fibroblast DNA. (Unpublished Study No. T-11909 by the In Vitro Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmington, CT for Stauffer Chemical Co.; dated September 19, 1984.) Accession No. 255674.

References:

The following two mutagenicity reports from the published literature were submitted but not reviewed (due to insufficient detail - screening reports only):

1) Anderson, K.J., et al., Evaulation of Herbicides for possible mutagenic activity, J. Agr. Food Chem. 20: 649, 1972.

2) Plant Mutation Assays, in: Genetic Toxicology, an Agricultural Perspective, ed. R.A. Flack and A. Hollander, Plenum Press, New York, 1972, pp. 327-352.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 89
April 1, 1985

OVERVIEW

EPTAM/EPTC

Mutagenicity

STUDY IDENTIFICATION: Mutagenicity Overview on the [redacted] Herbicide EPTAM/EPTC.

APPROVED BY:

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

Signature: I Cecil Felkner

Date: 4-1-85

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1. CHEMICAL: EPTC/EPTAM.

2. TEST MATERIAL: EPTAM (97.2% to 98.6%).

3. STUDY/ACTION TYPE: Overview - Registration Action.

4. STUDY IDENTIFICATION: Mutagenicity Overview on the Herbicide EPTC/EPTAM.

5. REVIEWED BY:

I. Cecil Felkner, Ph.D.
Principal Author
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Date: 4-1-85

Satish Bhalla, Ph.D.
Independent Reviewer
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6. APPROVED BY:

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

Signature: William L. McLellan
Date: 4-1-85

Chad Sandusky, Ph.D.
EPA Reviewer

Signature: Chad B. Sandusky
Date: 5/23/85
with changes as per C. Felkner 5/23/85

Robert Zendzian, Ph.D.
Acting Section Head

Signature:
Date: 5/28/85

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MUTAGENICITY OVERVIEW ON THE Herbicide EPTAM/EPTC

Introduction: Under FIFRA Guideline Subdivision F: Pesticide Assessment Guidelines: Hazard Evaluation - Human and Domestic Animals, dated 11-30-82, an overview (Section 80-1) is required for the various subdivisions of toxicology. "This subdivision details the toxicity data recommended to support the registration of pesticide products," and should meet the requirements of good laboratory practice (40 CFR Part 160), if applicable.

For each test substance, bioassays must be performed to assess its "potential to affect the qualitative or quantitative integrity of human genetic material." A battery of tests to assess mutagenicity is therefore required with the objectives of:

1. Detecting, with great sensitivity, the capacity of a test material to alter cellular genetic material.

2. Determining the relevance of genetic alterations to mammals.

3. Incorporating positive genetic findings into the risk assessments for heritable effects, carcinogenicity, and possibly other health endpoints.

There are three categories of genetic effects that must be addressed by the test battery.

1. Gene mutations.

2. Structural chromosomal aberrations.

3. Other mutagenic mechanisms (e.g., direct DNA damage, microtubule/spindle fiber inhibition) as deemed appropriate for the test material.

Mutagenicity data as required by 40 CFR Section 158.135 are to be submitted to support the registration of each manufacturing-use product and of certain end-use products. The assays are to be performed with the technical grade of each active ingredient in the product. The product must be tested in nonactivated and metabolically activated in vitro assays, and should also be assayed using in vivo mammalian systems with all appropriate positive and negative controls.

Summary of Study Evaluations: Five mutagenicity studies were conducted on the [redacted] product, EPTAM/EPTC. Two studies were conducted using the Salmonella typhimurium/microsome assay of Ames, et al.[1] and two other studies using Escherichia coli WP2 and Saccharomyces cerevisiae D4, respectively, with the same method[1]

[1] Ames, B.N., et al. Mutation Res. 31:347-364, 1975.

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(studies no. T-6617 and T6314). One study, T-11907, was conducted using the mouse lymphoma L5178Y forward mutation assay of Clive, et al.[2] The S. typhimurium, E. coli, S. cerevisiae and mouse lymphoma $TK^{+/-}$ forward mutation assays, respectively, meet the requirement of category No. 1, gene mutations. There was one mouse lymphoma assay submitted for review which meets the criteria of category no. 2, structural chromosomal aberrations (T-11908), and two studies (T-11909 consisting of strand-break and nick-translation two assays) and a B. subtilis rec assay (T-6617) in category 3, other genotoxic effects. A summary of the results for all assays is presented in Table 1.

The test materials assayed included either liquid or an unspecified form with purities ranging from 97.2 to 98.6% (or unspecified). The identities according to lot and study number are as follows: Unspecified (T-6617); Lot No. 3905-35 (T-6314); Lot No. 4921-4-10 (T-11907); Lot No. 4921-4-10 (T-11908); and Lot No. 4921-4-10 (T-11909).

All bacterial assays using Salmonella or E. coli in category 1 (T-6617 and T6314) were negative for gene mutations; the gene mutation assay with S. cerevisiae D4 was also negative. However, the assays in study no. T6314 were considered unacceptable. The forward mutation assay in mouse lymphoma cells (T-11907) was positive for mutagenicity at 50 or 60 µg/ml when EPTAM was activated by S9, but was negative at doses between 12.5 and 150 µg/ml in the nonactivated system. The results show that EPTAM technical is mutagenic in mammalian cell culture in the presence of metabolic activation, but that gene mutations were not induced in bacteria with or without S9 activation, or in mammalian cell culture in the absence of metabolic activation.

One study meeting the category 2 requirement (T-11908) showed an equivocal positive response for chromosomal aberrations in mouse lymphoma cells at 25 to 100 µg/ml in the presence of S9 activation. In the nonactivated assay, the test material did not induce aberrations at doses from 5 to 60 µg/ml.

One bacterial rec assay[3] (T-6617) and a study using human fibroblasts[4] (T-11908) to detect DNA strand breaks and DNA nick translation met the criteria for category 3. At doses of 1 to 100% v/v EPTAM, B. subtilis was negative for rec-repair. At doses of 50 to 100 µg/ml EPTAM did not induce strand breaks in human fibroblast DNA or nick translation at 100 µg/ml in the absence of metabolic activation. These results indicate that repairable DNA damage was not induced in bacterial or mammalian (human) cell cultures.

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[2] Clive, D., et al. Mutation Res. 31:17-29, 1975.

[3] Kada, T., et al. Mutation Res. 16: 165, 1972.

[4] Snyder and Matheson. Environ. Mutagenesis, submitted.

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From the results of all studies, the data indicate that EPTAM technical can induce gene mutations and possibly chromosomal aberrations after metabolic activation with a liver microsomal fraction. The compound should therefore be considered a potential mutagen for mammals.

Since the human fibroblast studies and studies with the B. subtilis rec assay did not include S9 activation, a complete evaluation of the genotoxic potential of EPTAM technical cannot be made. This information gap is important because data from the other studies submitted for our reviewers, indicate that metabolic activation is required to produce an active mutagen from a promutagen.

Recommendations: The test material should be considered mutagenic; however, there are data gaps in category 3 (other mutagenic mechanisms) because none of the in vitro assays qualifying in this category included a metabolic activation system. The results from the mouse lymphoma assay (T-11907) showed that a positive response was obtained only if S9-mix was included in the assay. Hence, the rec assay with B. subtilis and the strand-break and nick-translation assays in human fibroblasts should be performed using S9-activation. In addition, at least one in vivo mammalian assay should be conducted, e.g., mouse micronucleus[5] using two dosings and multiple sampling in both sexes. Filling the data gaps would provide useful information regarding the mutagenic potential of EPTAM/EPTC technical.

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[5] Heddle, J. and Salamone, M.F. in eds. H.F. Stich and R.H.C. San. Short-Term Tests for Chemical Carcinogenicity, Springer-Verlag, NY/Heidedberg/Berlin pp. 246-252.

TABLE 1. Summary of EPTAM/EPTC Mutagenicity Assays

Study No. (Mutagenicity Category)	Chemical Form/ Purity/Lot No.	Lowest Mutagenic Dose or Test Range[a]/Test Organism(s)
T-6617 (1)	Unspecified[b]/97.2%/ Unspecified	Negative (10 to 5000) S. typhimurium, Ames and E. coli WP2 uvrA
(3)[c,d]		Negative (1 to 100%/v/v, -S9) B. subtilis H17/M45, rec assay
T-6314 (1)[d]	Liquid/unspecified/ 3905-35	Negative (1-5000) S. typhimurium, Ames
(1)[d]		Inconclusive (1-5000) S. cerevisiae D4
T-11907 (1)	Liquid/98.6%/4921-4-10	Positive; 50 and 60, +S9 mouse lymphoma L5178Y $TK^{+/-}$ cells
		Negative (12.5 to 150; -S9) mouse lymphoma L5178Y $TK^{+/-}$ cells.
T-11908 (2)	Liquid/98.6%/4921-4-10	Equivocal positive (25 to 100; -S9) mouse lymphoma L5178Y, chromosome aberration assay

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TABLE 1. Summary of EPTAM/EPTC Mutagenicity Assays (Continued)

Study No. (Mutagenicity Category)	Chemical Form/ Purity/Lot No.	Lowest Mutagenic Dose or Test Range[a]/Test Organism(s)
T-11909 (3)[c,d]	Liquid/98.6%/4921-4-10	Negative (50 to 100; -S9) Human fibroblast, strand-break
(3)[d]		Negative (100; -S9) Human fibroblast, nick-translation

[a] S. typhimurium, E. coli WP2 uvrA, or S. cerevisiae D4 = µg per plate; B. subtilis = volume % per disc; mouse lymphoma or human fibroblasts = µg/ml.

[b] Assumed to be liquid.

[c] S9-activation system was not assayed.

[d] Study was unacceptable.

~~READING FILE~~

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 89
April 1, 1985

DATA EVALUATION RECORD

EPTAM

Chromosome Aberration in Mouse Lymphoma Cells

STUDY IDENTIFICATION: Majeska, J.B., Hertzel, K., and Matheson, D.W. Mutagenicity evaluation in mouse lymphoma multiple endpoint test cytogenetic assay. (Unpublished report No. T-11908 prepared by Stauffer Chemical-Toxicology Section for Stauffer Chemical Co., Farmington, CT; dated September 7, 1984.) Accession No. 255674.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 4-1-85

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1. CHEMICAL: Eptam technical, EPTC.

2. TEST MATERIAL: The test material, Eptam technical, Lot No. 4921-4-10, was described as a pale yellow liquid with a purity of 98.6%.

3. STUDY/ACTION TYPE: Chromosome Aberration in Mouse Lymphoma Cells

4. STUDY IDENTIFICATION: Majeska, J.B., Hertzel, K., and Matheson, D.W. Mutagenicity evaluation in mouse lymphoma multiple endpoint test cytogenetic assay. (Unpublished report No. T-11908 prepared by Stauffer Chemical-Toxicology Section for Stauffer Chemical Co., Farmington, CT; dated September 7, 1984.) Accession No. 255674.

5. REVIEWED BY:

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Date: 4-1-85

William L. McLellan, Ph.D.
Independent Reviewer
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Date: 5/23/85

Robert Zendzian, Ph.D.
EPA, Acting Section Head

Signature:
Date: 5/25/85

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7. CONCLUSIONS:

Under the conditions of the assay, Eptam technical induced sporadic positive responses at dose levels of 0.025 µl/ml and 0.1 µl/ml in the nonactivated system; however, neither response was dose related and, therefore, Eptam technical was not considered mutagenic/clastogenic in the mouse lymphoma cells L5178Y, TK+/-, over the dose ranges of 0.0125 to 0.15 µl/ml in the nonactivated system or 0.005 to 0.06 µl/ml with S9 activation.

8. RECOMMENDATIONS:

The authors should provide more information on the procedure by which the mitotic index was utilized in the evaluation of chromosome aberrations in the mouse lymphoma cell cytogenetic assay.

9. BACKGROUND:

In an initial rangefinding assay with Eptam technical, Lot No. 4921-4-10, a 63 and 77% reduction in relative growth (RG), indicating cytotoxicity, was seen at doses of $\geq$ 0.023 µl/ml (dose range 0.006 to 3.0 µl/ml) with and without S9 activation, respectively.

Item 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for details.

1. The test material, Eptam technical, Lot No. 4921-4-10, was described as a pale yellow liquid with a purity of 98.6%. The test material was diluted with dimethylsulfoxide (DMSO), the solvent control, to final concentrations ranging from 0.0125 to 0.15 µl/ml in the nonactivation system and 0.005 to 0.06 µl/ml in the S9 activated system. The dose ranges were selected based on a cytotoxicity assay.

2. The mouse lymphoma cell line used in the assay was L5178Y, TK+/-, derived from the Fischer L5178Y cell line provided by Dr. Donald Clive.

3. The mouse lymphoma cells were treated for 4 hours with either the test material, solvent or positive control with or without S9 activation. The cells were incubated for 20 hours

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

in the medium described by Clive, et al.,[2] supplemented with 10^{-4}M BrdU, then harvested for the cytogenetic assay in accordance with the procedure reported by Lebowitz, et al. 1977.[3]

4. Cell Scoring - Samples (50 cells) from the five highest doses with readable metaphase cells were scored for chromosome aberrations. The mitotic index (MI) was calculated from 500 cells in groups of 100 where the number of mitoses/500 cells was recorded.

5. Evaluation Criteria - Structural aberrations were analyzed on a per cell basis using the Student's t-test. A test material was considered positive if it showed a dose-related response over 3 consecutive doses and its increase at the highest dose was significantly ($p < 0.01$) different from the solvent control.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity Assay - The dose ranges selected for Eptam in the cytotoxicity assay (see Item 9, Background - Rangefinding: page 3 of this report) were 0.0063 to 0.2 µl/ml in the nonactivated system and 0.0025 to 0.08 µl/ml in the S9 activated system. The test material had a reduction in % RG of 64 to 14% at dose levels of 0.05 to 0.2 µl/ml in the nonactivated system. In the S9 activated system the test material was cytotoxic at dose levels of 0.04 to 0.08 µl/ml with a % RG range of 55 to 15% (Table 1).

Cytogenetic Assay - Cultures dosed with Eptam at 0.0125 to 0.15 µl/ml in the nonactivated system induced a significant increase ($p < 0.05$) in structural aberrations at a dose level of 0.025 µl/ml and a significant increase ($p < 0.01$) in numerical aberrations at a dose level of 0.1 µl/ml. No increase in aberrations were seen in cultures dosed with 0.005 to 0.06 µl/ml with S9 activations.

The nonactivated positive control, EMS (0.5 µl/ml), had a significant ($p < 0.01$) increase in structural aberrations. DMN (0.05 µl/ml), the positive control in the S9 activated system, also had a significant ($p < 0.01$) increase in structural aberrations (Table 2).

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[2]Clive et al. Mutation Res. 31: 17-29 (1975).

[3]Lebowitz et al., 1977. Complete reference was not reported.

TABLE 1. Cytotoxicity Results of Eptam Technical in the Cytogenetic Assay with Mouse Lymphoma Cells

Substance	Avg. Suspension Growth x 10^5/ml	Avg. % Relative Growth
Nonactivated		
Medium Control	7.1	108
Solvent Control	6.6	100
Eptam (µl/ml)		
0.0063	5.2	79
0.0125	5.4	82
0.0250	5.1	77
0.0500	4.2	64
0.1000	3.2	48
0.1500	2.3	35
0.2000	0.9	14
Positive Control[a]		
EMS 0.5 µl/ml	7.1	108
Activation		
Medium Control	10.5	108
Solvent Control	9.7	100
Eptam (µl/ml)		
0.0025	8.9	92
0.0050	8.9	92
0.0100	9.1	94
0.0200	8.2	85
0.0400	5.3	55
0.0600	2.5	26
0.0800	1.5	15
Positive Control[a]		
DMN 0.05 µl/ml	8.7	90

[a] = Data from one culture.
Avg. = Average of duplicate cultures.
EMS = Ethylmethanesulfonate.
DMN = N-nitrosodimethylamine.

TABLE 2. Cytogenetic Assay Results with Eptam Technical

Substance	Total # of Cells	# Cells w/ Aberrations (%)	# Cells w/ 2 or more Aber. (%)	Mitotic Index (%)
Nonactivated				
Medium Control	50	2(4) 0[a]	0	8.0
Solvent Control	50	1(2) 0[a]	1(2)	7.4
Positive Control				
EMS (0.5 µl/ml)	50	19(38)**[b]	8(16)	10.4
S9 Activation				
Medium Control	50	1(2)	0	10.6
Solvent Control	50	1(2)	0	7.2
Positive Control				
DMN (0.05 µl/ml)	50	18(36)**[b]	12(24)	8.8
Eptam (nonactivated)				
0.025 µl/ml	50	6(12)*[b]	2(4)	6.2
0.100 µl/ml	50	5(10)**[a]	1(2)	7.0

[a]Numerical aberrations.
[b]Structural aberrations.
*Significantly different from control value at $p < 0.05$.
**Significantly different from control value at $p < 0.01$.
EMS = Ethylmethanesulfonate.
DMN = N-nitrosodimethylamine.

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13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Eptam technical was not mutagenic/clastogenic to L5178Y mouse lymphoma cells with or without S9 activation. The increases in aberrations noted at dose levels 0.0250 and 0.1 µl/ml were considered to be the result of an effect on the cell as a whole, interfering with the mitotic process. "Neither increase was dose-related and is not considered an indicator of significant clastogenic activity."

B. A quality assurance statement was present and dated August 29, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The authors concluded that Eptam technical did not induce a dose-related statistically significant increase in chromosome aberrations compared to the concurrent solvent control, although there were statistically significant positive responses in sporadic doses, i.e., in the nonactivated system the 0.025 µl/ml dose induced structural aberrations and the 0.1 µl/ml dose induced numerical aberrations. It is our assessment that the authors interpreted their data correctly, because a dose-response increase in aberrations was not induced by the test material. The positive controls induced significant ($p < 0.01$) clastogenic responses; therefore, the sensitivity of the assay was appropriate.

Item 15 - see footnote 1.

16. CBI APPENDIX: Appendix A, CBI; page 1 and pp. 7-13.

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APPENDIX A

(Materials and Methods)

Eptam

Page _____ is not included in this copy.

Pages _19_ through _26_ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

__X__ 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.

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RCB

EPA: 68-01-6561
TASK: 89
April 1, 1985

DATA EVALUATION RECORD

EPTAM

Forward Mutation in Mouse Lymphoma Cells

STUDY IDENTIFICATION: Majeska, J.B., Hertzel, K., and Matheson, D.W. Mutagenicity evaluation in mouse lymphoma multiple endpoint test forward mutation assay with Eptam technical. (Unpublished report No. T-11907 prepared by Agricultural Chemical Division of Stauffer Chemical Co. for Stauffer Chemical Co., Farmington, CT; dated September 18, 1984.) Accession No. 255674.

APPROVED BY:

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

Signature: I Cecil Felkner

Date: 4-1-85

27

1. CHEMICAL: Eptam technical; EPTC.

2. TEST MATERIAL: The test material, Eptam technical, Lot No. 4921-4-10, was described as a pale yellow liquid with a purity of 98.6%.

3. STUDY/ACTION TYPE: Forward Mutation in Mouse Lymphoma Cells.

4. STUDY IDENTIFICATION: Majeska, J.B., Hertzel, K., and Matheson, D.W. Mutagenicity evaluation in mouse lymphoma multiple endpoint test forward mutation assay with Eptam technical. (Unpublished report No. T-11907 prepared by Agricultural Chemical Division of Stauffer Chemical Co. for Stauffer Chemical Co., Farmington, CT; dated September 18, 1984.) Accession No. 255674.

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Date: 5/20/85

Robert Zendzian, Ph.D.
EPA, Acting Section Head

Signature: Robert Zendzian
Date: 5-15-85

28

7. CONCLUSIONS:

Under the conditions of the assay, Eptam technical, Lot No. 4921-4-10, induced a mutagenic effect at dose levels of 0.05 and 0.06 µl/ml in the presence of S9 activation. Eptam was not mutagenic over a dose range of 0.0125 to 0.15 µl/ml in the nonactivated system.

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, Eptam technical, Lot No. 4921-4-10, was described as a pale yellow liquid with a purity of 98.6%. The test material was diluted with dimethylsulfoxide (DMSO), the solvent control, to final concentrations of 0.0125 to 0.15 µl/ml in the nonactivated system and 0.005 to 0.06 µl/ml with S9 activation.

2. The mouse lymphoma cell line used in this assay was L5178Y $TK^{+/-}$ derived from the Fischer L5178Y line provided by Dr. Donald Clive.

   Laboratory cultures were periodically screened for mycoplasma, and methotrexate was used to select against spontaneously occurring $TK^{-/-}$ revertants.

3. Media: The growth medium used was RPMI 1640 (10% horse serum, glutamine, penicillin-streptomycin, sodium pyruvate, and pluronic). Treatment medium was growth medium with (5%) horse serum, cloning medium was growth medium and agar without pluronic, and selective medium was cloning medium with 5-trifluorothymidine (TFT) added.

4. The cytotoxicity of the test material was determined with $TK^{+/-}$ cells with or without S9 activation at 10 dose levels ranging from 0.006 to 3 µl/ml.

5. Evaluation Criteria: To establish a positive response the test material was required to induce a dose-related increase in mutation frequency, that was 2.5 times greater than the solvent control. If the induced mutation response was not dose-related over an increasing dose-range, and occurred only

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Footnote 1: Only items appropriate to this DER have been included.

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at the highest testable level[2] but was reproducible, then the test material was mutagenic.

6. The method of Clive[3] was used to assess the test material's ability to induce forward mutation.

B. Protocol:

See Appendix A.

12. REPORTED RESULTS:

A. Cytotoxicity assay - Treatment with Eptam over a dose range of 0.006 to 3.0 µl/ml for 20 hr. resulted in a reduced relative growth. This showed there was a dose-related cytotoxicity at dose levels greater than or equal to 0.023 µl/ml with and without S9 activation (Table 1).

B. Mutagenicity assay - In the nonactivated system, Eptam was assayed using duplicate plates over a dose range of 0.0125 to 0.1500 µl/ml. The percent relative growth (% RG) range was 76 to 16 percent and the average mutation frequency range was 29-35 x 10^6 cells/ml. The solvent and medium controls had % RG percentages of 100 and 99, respectively; mutation frequencies for these controls were 24 x 10^6 and 25 x 10^6 cells/ml, respectively. The positive control, EMS at 0.05 µl/ml, induced an average mutation frequency of 523 x 10^6 cells/ml (Table 2).

The data for Eptam in the S9 activation system were compiled from two separate assays, each performed with duplicate plates over a dose range of 0.005 to 0.06 µl/ml. The average % RG range was 89 to 4 percent and the average mutation frequencies ranged from 33 x 10^6 to 158 x 10^6 cells/ml. The solvent and medium controls had average % RG of 100/107 and 114 and their mutation frequencies were 31/24 x 10^6 and 29 x 10^6 cells/ml, respectively. The positive control, n-nitrosodimethylamine (DMN) at 0.05 µl/ml, induced average mutation frequencies of 352 x 10^6 and 380 x 10^6 cells/ml in the two assays, respectively. (Table 3).

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[2] The highest testable dose, based on excessive cytotoxicity and/or maximum solubility.

[3] Clive, et al. Mutation Res. 3:17-29, 1975.

TABLE 1. Cytotoxicity Results of the Mouse Lymphoma Assay with Eptam Technical

Substance	S9 Activation	Viable Cell Count (x 10^5 cells/ml)	% Relative Growth Post 20 Hr.
Solvent Control	-	8.4[a]	100
	+	10.8[a]	100
Eptam (µl/ml)			
0.006	-	8.6	103
	+	9.7	90
0.012	-	8.5	102
	+	9.0	84
0.023	-	6.4	77
	+	6.8	63
0.047	-	3.2	39
	+	1.6	15
0.094	-	2.7	32
	+	0.7	6
0.188	-	0.7	8
	+	0.4	4
0.375	-	0.1	1
	+	0.3	3
0.750	-	0.1	1
	+	0.1	1
1.500	-	0.1	1
	+	0.2	2
3.000	-	0.1	1
	+	0.2	2

[a] Average of duplicates calculated by reviewers

TABLE 2. Mouse Lymphoma Results with Eptam Technical Nonactivated System

Substance	Av.[a] Mutant Clones	Av.[a] Viable Clones	Av.[a] % RG[b]	Av. Mutation Freq. x 10^6
Medium Control	77	608	99	25
Solvent Control	80	676	100	24
Positive Control EMS[c] (0.05 µl/ml)	1418	570	43	523
Eptam (µl/ml)				
0.0125	83	570	76	29
0.0250	85	565	62	30
0.0500	99	642	47	31
0.1000	116	720	25	32
0.1500	115	667	16	35

[a] Av. = average of duplicate plates

[b] % RG = percent relative growth

[c] EMS = ethyl methane sulfonate; data from single sample

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TABLE 3. Mouse Lymphoma Results with Eptam Technical S9 Activation (compiled from two assays)

Substance	Av.[a] Mutant Clones	Av. Viable Clones	Av. % RG[b]	Av. Mutant Freq. x 10^6
Medium Control[d]	71	619	107	24
[e]	100	702	100	29
Solvent Control[d]	102	669	100	31
[e]	104	676	100	31
Positive Control				
DMN[c] (0.05µl/ml)[d]	347	197	18	352
[e]	460	242	23	380
Eptam (µl/ml)[d]				
0.0050	89	543	76	33
0.0100	124	633	89	40
0.0200	155	668	84	47
0.0400	158	584	38	55
0.0600	293	565	9	105
Eptam (µl/ml)[e]				
0.020	129	610	81	42
0.030	170	592	58	57
0.040	192	659	44	58
0.050	327	573	13	114
0.060	303	388	4	158

[a] Av. = average of duplicate plates

[b] % RG = percent relative growth

[c] DMN = N-nitrosodimethylamine; data from single sample

[d] results from assay 1

[e] results from assay 2

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Eptam technical was not mutagenic in the nonactivated system over a range of 15 to 75% relative cell survival. However, in the S9 activated system Eptam induced a reproducible increase in mutant frequency at doses greater than or equal to 0.030 µl/ml. The increase reached significant levels greater than 2.5 times the solvent control at a dose of 0.050 µl/ml. Relative survival at greater than or equal to 0.50 µl/ml was reduced to 13% or less of controls. At survival levels 37 to 70 percent (considered by the authors more relevant) the increase in mutants was smaller (only twice the solvent control). This pattern indicates that Eptam technical was weakly mutagenic at the thymidine kinase locus in L5178Y mouse lymphoma cells with S9 activation.

B. A quality assurance statement was present, signed, and dated (7-22-84) which listed dates of inspections (5-22-84 and 7-13-84).

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The authors concluded that Eptam technical induced a slight mutagenic effect in L5178Y ($TK^{+/-}$) mouse lymphoma cells with S9 activation. Eptam technical was not mutagenic in the nonactivated system.

Our assessment is that the authors interpreted their results correctly. The negative control results were within the acceptable published[3] ranges, and the positive controls at the concentrations tested confirmed that the test system had an appropriate level of sensitivity.

Item 15 - see Footnote 1.

16. CBI APPENDIX: Appendix A, CBI pp. 11-16.

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[3] Clive, et al. Mutation Res. 3:17-29, 1975.

APPENDIX A

(Materials and Methods)

Eptam

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_____ Identity of the source of product ingredients.

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EPA: 68-01-6561
TASK: 89
April 1, 1985

DATA EVALUATION RECORD

EPTC

Mutagenicity - Rec Assay in Bacillus subtilis;
Reverse Mutation in Salmonella typhimurium and Escherichia coli

STUDY IDENTIFICATION: Shirasu, Y., Moriya, M., and Miyazaua, T. Mutagenicity testing on EPTC in microbial systems. (Unpublished Study No. T-6617 by the Institute of Environmental Toxicology, Tokyo, Japan for Stauffer Chemical Company; dated July 24, 1978.) Accession No. 255674.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 4-1-85

4/2

1. CHEMICAL: EPTC; Ethyl-di-n-propylthiocarbamate; Eptam.

2. TEST MATERIAL: EPTC with a purity of 97.2%.

3. STUDY/ACTION TYPE: Mutagenicity - rec assay in B. subtilis; reverse mutation in S. typhimurium and E. coli.

4. STUDY IDENTIFICATION: Shirasu, Y., Moriya, M., and Miyazaua, T. Mutagenicity testing on EPTC in microbial systems. (Unpublished Study No. T-6617 by the Institute of Environmental Toxicology, Tokyo, Japan for Stauffer Chemical Company; dated July 24, 1978.) Accession No. 255674.

5. REVIEWED BY:

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Date: 4-1-85

Chad Sandusky, Ph.D.
EPA Reviewer

Signature: Chad B. Sandusky
Date: 5/21/85

Robert Zendzian, Ph.D.
EPA, Acting Section Head

Signature:
Date: 5/28/85

43

7. CONCLUSIONS:

A. EPTC did not induce DNA damage in B. subtilis rec⁻ M45 strain at concentrations from 1 to 100% (v/v), but without S9 activation only.

B. EPTC did not induce a mutagenic response in Salmonella typhimurium or Escherichia coli strains with or without S9 activation at doses from 10 to 5,000 µg/plate.

C. For both assays, the test material was tested to the limit of cytotoxicity or solubility.

8. RECOMMENDATIONS:

B. subtilis rec assay: This assay should have been conducted with a minimum of duplicate plates and also with metabolic (S9) activation.

9. BACKGROUND: Not applicable.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material was described as EPTC with a purity of 97.2%. The test material was dissolved in dimethylsulfoxide (DMSO) the solvent control.

2. Bacillus subtilis strains M45 and H17 were used in the recombination assay. Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and Escherichia coli WP2 hcr (uvrA) with and without metabolic (S9) activation were used in the reverse mutation assay.

3. The method of Shirasu[1] was employed to determine DNA damage in the B. subtilis assay at dose levels of 1 to 100% (v/v). The Ames[2] method was used to determine mutagenicity at dose levels of 10 to 5000 µg/plate.

B. Protocol: See Appendix A.

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[1]Sharisu, Y., et al., Mutation Res. 40:19-30, 1976.

[2]Ames, B.N., et al., Mutation Res. 31:347-364, 1975.

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12. REPORTED RESULTS:

A. B. subtilis rec assay: EPTC at concentrations of 1 to 100 percent (v/v), induced similar zones of inhibition in both the wild type strain (H17) and the rec⁻ strain (M45); likewise the negative control (Kanamycin) also induced similar zones of inhibition in both strains. Mitomycin C, the positive control induced an increased zone of inhibition (11 mm) in the rec⁻ strain as compared to the zone of inhibition (0 mm) of the wild type strain (Table 1).

B. S. typhimurium and E. coli reverse mutation assays: EPTC, did not induce an increase in the number of revertants over the solvent control with or without S9 activation.

The number of revertant colonies for the solvent control was within an acceptable range for all tester strains with and without S9 activation. The marked increase in the number of revertants of the positive controls over the solvent controls confirmed that the assay had an appropriate level of sensitivity.

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

A. The authors concluded that "EPTC was negative in the mutation tests including rec-assay, reverse mutation tests with and without the metabolic activation system assay".

B. Quality assurance measures were not reported.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. It is our assessment that EPTC did not induce DNA damage in B. subtilis rec deficient strain M45 when compared to the wild type strain H17 in the nonactivated assay at concentrations of 1 to 100% EPTC. Cytotoxicity was observed at the two highest concentrations (50 and 100%) with equal zones of inhibition in both strains. The positive control, 0.1 µg/disc of Mitomycin C induced a DNA damaging response confirming that the assay had an appropriate level of sensitivity and the solvent control did not induce any inhibition in either strain. The antibiotic control, 10 µg/disc of Kanamycin, was cytotoxic and since strain M45 was inhibited slightly more than H17 it was also considered as positive for DNA damage induction.

B. Salmonella typhimurium and Escherichia coli-reverse mutation: It is our assessment that the authors interpreted their data correctly, and that EPTC was not mutagenic at the dose levels of 10 to 500 µg/plate with or without S9 activation in Salmonella strains TA1535, TA1537, TA1538, TA98, or TA100; likewise EPTC was not mutagenic in E. coli strain WP2 hcr.

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The cytotoxicity (complete killing or a reduction in revertants) reported for the highest dose tested (5000 µg/plate) showed that dosing was adequate. For all tester strains, the solvent control had a number of revertant colonies within the range of published values.[1] The positive controls for all tester strains induced large numbers of revertants over the solvent control, confirming that the assay had an appropriate level of sensitivity.

15. COMPLETION OF ONE-LINER FORM FOR STUDY: Not applicable.

16. CBI APPENDIX:

Appendix A, Materials and Method, CBI pp. 1-3.

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[1] Sharisu, Y., et al. Mutation Res. 40:19-30, 1976.

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TABLE 1. Solvent and Positive Control Results in *Bacillus subtilis* Assay with EPTC

	Inhibition Zone (mm)	
	M45 (*rec*⁻)	H17 (wild type)
Solvent Control		
DMSO	0	0
Negative Control		
Kanamycin (10 µg/disc)	7	5
Positive Control		
Mitomycin C (0.1 µg/disc)	11	0

TABLE 2. Solvent and Positive Control Results in the Reverse Mutation Assay with EPTC

Test Substance	Dose/ plate	S9 Activation	Revertants/Plate[a] WP2 hcr	TA1535	TA100	TA1537	TA1538	TA98
DMSO (Solvent)	100 µl	-	23	2	126	5	11	19
		+	23	2	109	11	12	13
Positive Controls								
AF2	0.25µg	-	2210					
2-AA	10 µg	+	82					
B-propiolactone	50 µg	-		148				
2-AA	10 µg	+		259				
AF2	.05 µg	-			1542			
2-AA	10 µg	+			>3000			
9-AA	200 µg					>10,000		
2-AA	10 µg					435		
2-NF	50 g	-					>3000	
2-AA	10 µg	+					>3000	
2-AF	0.1 µg	-						447
2-AA	10 µg	+						>3000

[a] = The average of duplicate plates (averaged by reviewer).
B = Beta-propiolactone
AF2 = 2-(2-furyl)-3-(5-nitro-2-)furyl acrylamide.
2-AA = 2-aminoanthracene
9-AA = 9-aminoacridine
2-NF = 2-nitrofluorene

APPENDIX A

(Methods and Materials)

Eptam

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NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 89
April 1, 1985

DATA EVALUATION RECORD

EPTAM TECHNICAL 3905-35

Mutagenicity-Reverse Mutation in Salmonella

STUDY IDENTIFICATION: Jagannath, D.R. and Brusick, D.J. Mutagenicity evaluation of Eptam Tech 3905-35. (Unpublished study No. T6314 prepared by Litton Bionetics, Inc., Kensington, MD for Stauffer Chemical Corporation, Western Research Centers, Richmond, CA; dated October 27, 1977.) Accession No. 255674.

APPROVED BY:

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

Signature: I Cecil Felkner

Date: 4-1-85

53

1. CHEMICAL: Eptam Technical 3905-35, EPTC.

2. TEST MATERIAL: Eptam Technical 3905-35, described as an amber liquid. No purity was given.

3. STUDY/ACTION TYPE: Mutagenicity (reverse mutation in Salmonella).

4. STUDY IDENTIFICATION: Jagannath, D.R. and Brusick, D.J. Mutagenicity evaluation of Eptam Tech 3905-35. (Unpublished study No. T6314 prepared by Litton Bionetics, Inc., Kensington, MD for Stauffer Chemical Corporation, Western Research Centers, Richmond, CA; dated October 27, 1977.) Accession No. 255674.

5. REVIEWED BY:

Brenda Worthy, M.T.
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Date: 4-1-85

Chad Sandusky, Ph.D.
EPA Reviewer

Signature: Chad B. Sandusky
Date: 5/22/85

Robert Zendzian, Ph.D.
EPA, Acting Section Head

Signature:
Date: 5/28/85

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7. CONCLUSIONS:

A. Under the conditions of the assay Eptam Tech 3905-35 was not mutagenic in Salmonella typhimurium with or without S9 activation at dose levels ranging from 0.001 to 5.0 µl/plate.

B. No conclusions can be drawn from the study with Saccharomyces cerevisiae D4 because it could not be confirmed: 1) that the dose range used was adequate; 2) what materials and methods were used to assay mutations in S. cerevisiae strain D4.

8. RECOMMENDATIONS:

A. Salmonella typhimurium assay: The study is unacceptable. This assay should have been conducted with at least duplicate plates.

B. Saccharomyces cerevisiae: The study is unacceptable.

1. Not enough replicates for each sample.

2. The procedures used for mutagenicity were not given.

9. BACKGROUND: Not applicable.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for details.

1. The test material, Eptam Tech 3905-35 was described as an amber liquid. No purity was given for the test material. The test material was diluted in dimethylsulfoxide (DMSO) the solvent control to final dose levels of 0.001, 0.01, 0.1, 1.0, and 5.0 µl/plate.

2. The following microbial strains were used: Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and the yeast strain Saccharomyces cerevisiae strain D4.

3. Mutagenic response was assayed by the method of Ames[1] with and without metabolic (S9) activation.

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[1] Ames B.N., et al. Mutation Res. 31:347-364, 1975.

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4. Evaluation Criteria: If a chemical produced a positive dose response over three concentrations with the lowest increase equal to twice the solvent control in strains TA1535, TA1537, or TA1538, the chemical was considered mutagenic. If a chemical produced a positive dose response over three concentrations with the highest increase equal to twice the solvent control in strains TA98, TA100, or D4, it was considered mutagenic.

B. Protocol:

See Appendix A.

12. REPORTED RESULTS: (data ?)

A. Cytotoxicity Test: The test material was tested over a series of concentrations, which produced evidence of either a quantitative or qualitative chemically-induced physiological effect at the high-dose level. The low dose was below a concentration which did not cause any toxic effect.

B. Mutagenicity Assay: Five dose levels of the test material, 0.001, 0.01, 0.1, 1.0, and 5.0 µl/plate with and without S9 activation were singularly plated with each of the 6 tester strains.

The following results were noted after a 48-hr incubation: The test material, at the highest dose level was toxic to strains TA1535, TA1537 without S9 activation and toxic to strain TA100 with and without S9 activation.

The results of the assay conducted with Eptam Technical with and without S9 activation were all negative.

The number of revertants in the solvent control were similar to the values published by Ames et al.[1] The positive controls, used at recommended dose levels increased numbers of revertants over the solvent control showing that the test system had an appropriate level of sensitivity (Table 1).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that Eptam Tech 3905-35, "did not demonstrate mutagenic activity in the activation and nonactivation assays conducted in this evaluation: " This conclusion includes interpretations from data on S. typhimurium tester strains and S. cerevisiae strain D4.

B. Quality assurance measures were not reported.

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TABLE 1. Results of Controls Used in the Reverse Mutation Assay with Eptam Technical 3905-35

Substance	Dose Per/Plate	S9 Activation	Strains Revertants/Plate[a] TA1535	TA1537	TA1538	TA98	TA100	D4[b]
DMSO	50 µl	-	18	22	19	31	187	32
		+	27	19	34	39	198	23
MNNG	10 µg	-	>1000					
ANTH	100 µg	+	234					
QM	10 µg	-		22				
AMQ	100 µg	+		424				
NF	100 µg	-			>1000			
AAF	100 µg	+			673			
NF	100 µg	-				>1000		
AAF	100 µg	+				>1000		
MNNG	10 µg	-					>1000	
ANTH	100 µg	+					>1000	
MNNG	10 µg	-						573
DMNA	100 µM	+						48

[a] Results from a single plate.
[b] Trp^+. Convertants per single plate.

MNNG = N-methyl-N'-nitro-N-nitrosoguanidine.
ANTH = 2-Anthramine.
QM = Quinacrine mustard.
AMQ = 8-Aminoquinoline.
NF = 2-Nitrofluorene.
AAF = 2-Acetylaminofluorene.
DMNA = was not identified by authors; it is assumed to be Dimethylnitrosamine.

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14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. It is our assessment that Eptam Technical did not induce a mutagenic response in the Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 with or without S9 activation at the dose levels tested under the study conditions. However, to be acceptable the assay should have been conducted with a minimum of duplicate plates to confirm reproducibility of the negative response.

B. The authors reported that Eptam was also negative in the Saccharomyces strain D4; however there were no methods and materials reported nor any toxicity data to ascertain if the dose range used was adequate, thus it can only be assumed that Saccharomyces assay was performed using the Ames[1] method. Since gene conversion is the presumed endpoint, certain modifications would be required to conduct an assay with Saccharomyces cerevisiae strain D4; therefore, further descriptions are needed. We cannot draw any conclusions from this study based on the data reported.

C. Neither the S. typhimurium nor the S. cerevisiae assays were considered to be acceptable because the number of replicate plates assayed was insufficient. Also, description of S. cerevisiae D4 and the procedural and/or media differences required to assay for the trp^+ mutation were not given or even referenced. Therefore, the study should be repeated and properly reported.

15. COMPLETION OF ONE-LINER FORM FOR STUDY: Not applicable.

16. CBI APPENDIX: Appendix A. CBI pp 1-3.

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[1] Ames B.N., et al. Mutation Res. 31:347-364, 1975.

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APPENDIX A

(Methods and Materials)

Eptam

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EPA: 68-01-6561
TASK: 89
April 1, 1985

DATA EVALUATION RECORD

EPTAM

Mutagenicity - Human Fibroblast DNA

STUDY IDENTIFICATION: Snyder, R.D. and Matheson, D.W. Effects of Eptam on Human Fibroblast DNA. (Unpublished Study No. T-11909 by the In Vitro Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmington, CT for Stauffer Chemical Co.; dated September 19, 1984.) Accession No. 255674.

APPROVED BY:

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

Signature: I Cecil Felkner

Date: 4-1-85

63

1. CHEMICAL: Eptam/EPTC; S-Ethyl dipropylthiocarbamate.

2. TEST MATERIAL: Eptam technical, selective herbicide, 98.6% pure; pale yellow liquid of unspecified stability; identified as Eptam (Lot No. 4921-4-10), EHC-0525-39.

3. STUDY/ACTION TYPE: Registration Action. In vitro mutagenicity in human fibroblasts.

4. STUDY IDENTIFICATION: Snyder, R.D., and Matheson, D.W. Effects of Eptam on Human Fibroblast DNA. (Unpublished Study No. T-11909 by the In Vitro Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmington, CT for Stauffer Chemical Co.; dated September 19, 1984.) Accession No. 255674.

5. REVIEWED BY:

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Date: 4-1-85

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

Signature: William L. McLellan
Date: 4-1-85

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Technical Quality Control
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Date: 4-1-85

Chad Sandusky, Ph.D.
EPA Reviewer

Signature: Chad B. Sandusky
Date: 5/25/85

Robert Zendzian, Ph.D.
EPA, Acting Section Head

Signature: _____
Date: _____

64

7. CONCLUSIONS:

A. Under the conditions of the DNA strand-break and nick translation assays, we assess that nonactivated Eptam did not induce damage to DNA or elicit repair in response to DNA lesions in human diploid fibroblasts at concentrations of 0.05 or 0.10 µl/ml (strand-break) or 0.10 µl/ml (nick translation). However, the assay was not conducted with S9 activation, so a complete interpretation of the mutagenic potential was not possible.

8. RECOMMENDATIONS:

The study provides some useful information, but should be repeated using the positive controls, repair inhibitors, and a concurrent rat liver S9 activated system in order to be considered acceptable.

9. BACKGROUND:

The authors stated that doses higher than 0.10 µl/ml Eptam caused fibroblasts to detach from the tissue culture dishes and precluded testing at higher levels. From this statement, a preliminary cytotoxicity assay was implied; however, no preliminary cytotoxicity data were presented.

11.[1] MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material was Eptam, Lot No. 4921-4-10, ECH-0525-39, a pale yellow liquid which was 98.6% pure, provided by Stauffer Chemical Company. The test material was evidently added directly to the media-cell mixture.

2. The assay cells were human-foreskin fibroblasts (HSBP) grown in Eagle's Medium with 10% fetal-bovine serum at 5% CO_2 and at 37° C for 2 days, and the cell DNA was labelled by 24-hour incubation with 0.7 µCi[^{3}H]-thymidine or 0.17 µCi-[^{14}C]-thymidine per ml.

3. The test material was added to the cell-media mixture at 0.05 µl or 0.10 µl/ml for the alkaline sucrose sedimentation (strand-break) studies and at 0.1 µl/ml for the nick-translation assay.[2] For the strand-break studies, the

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

[2] Nose and Okamoto 1983 (no reference given by authors).

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[^{3}H]-labelled cells received the test material, and the [^{14}C]-labelled cells served as controls.

4. For both the alkaline sucrose sedimentation and nick-translation studies, the cell-test material mixtures were incubated for 30 minutes at 37° C.

5. After sedimentation and fractionation onto paper filter strips, the alkaline sucrose gradient fractions were counted by liquid scintillation, DNA molecular weights determined, and DNA strand-break frequencies calculated.

   After suitable time of reaction (30 minutes), the nick-translation bioassay mixture was precipitated onto discs (acid insoluble fractions), processed, and counted by liquid scintillation procedures.

   (See Appendix A for detailed description of methods.)

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. From the alkaline sucrose velocity-sedimentation analyses, the molecular weights of DNA from Eptam-dosed fibroblasts were 2.22×10^{8} and 2.41×10^{8} daltons at 0.05 and 0.10 µl/ml, respectively. The [^{14}C]-labelled control fibroblast DNA molecular weights were 2.36×10^{8} and 2.3×10^{6} daltons, respectively. When the formula $2 \times \Delta MW-1$ was used to calculate excess strand breaks per 10^{8} daltons DNA, the values were 0.00 and 0.04 for 0.05 and 0.10 ml of Eptam. These excess strand-break frequencies were considered to be within the expected normal range. Comparable data for a positive control, e.g., dimethylsulfate, was not presented.

   From the nick-translation damage assay, dosing with 0.1 µl/ml of Eptam for 30 minutes in the presence of E. coli DNA polymerase I and [^{3}H]-labelled triphosphates did not result in a 20 percent increase in incorporation of [^{3}H] compared to the zero time value. Furthermore, if Eptam was removed and repair synthesis measured for an additional 1.5 hours there was no increase [^{3}H] incorporation compared to a control in which repair synthesis was inhibited with ara-C and hydroxyurea (given as AH, see Appendix B). Treatment with 0.2 µl/ml dimethyl sulfate for 30 min gave a 2.59-fold [^{3}H]incorporation relative to the control.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that Eptam "did not induce DNA damage or elicit a repair response in human diploid fibroblasts." The authors also stated that it was possible that addition of a metabolic activation system could have produced a positive response, and that studies with metabolic activation were not conducted.

B. A quality assurance statement was present, signed, dated on 8-21-84, and reported to the study director and to management on 8-27-84 and 8-28-84, respectively.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We assess that the authors' conclusions are correct and based on the proper interpretation of their data; however, there are some gaps in the data that should be addressed. 1. In the strand-break assay, a positive control, e.g., dimethyl sulfate, should have been included to demonstrate sensitivity of the assay to give a positive response. 2. In the nick-translation assay, more than one concentration of the test material should have been used, and a concurrent assay using dimethyl sulfate with ara-C and hydroxyurea should have been conducted to demonstrate how these inhibitors affected [^{3}H] incorporation into acid precipitable DNA; i.e., an increase in "DNA strand breaks which are recognized by the polymerase resulting in a further increase in radioactive incorporation."

B. We agree that metabolic activation of Eptam might have produced a positive response in either or both of the assays, and recommend that this data be produced; we also recommend that the non-activated assays be repeated concurrently, incorporating the additional assay components listed as data gaps. These features should ensure an appropriate sensitivity level for the assays.

C. Although the report stated that in nick translation the normal variation of the assay is ±20%, it could not be determined if the assay was done in replicate because the radioactive incorporation data values were presented as percent relative to control.

16.[1] CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-7; Appendix B, Nick Translation for DNA Damage and Repair, CBI p. 6, Table II.

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

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APPENDIX A

Materials and Methods

Eptam

Page _____ is not included in this copy.

Pages _69__ through _73__ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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

__X__ FIFRA registration data.

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

_____ The document is not responsive to the request.

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FEB 25 1985 Toxicology Branch/HED Review

Caswell file

To: Robert Taylor PM #25

Registration No(s).:

Pesticide Petition No(s).: 476-2140/2165

Caswell No(s).: 435

Chemical(s): EPTC / Eptam

RAC(s) - tolerance(s): N/A

Inert(s) cleared 180.1001: N/A

% of ADI occupied: Existing: N/A Resulting:

Resulting % increase in TMRC: N/A

Attached (?): ADI printout: YES/~~NO~~ TOX "one-liner": YES/~~NO~~; DER: YES/~~NO~~

Existing regulatory actions against registration: N/A

RPAR status: N/A

New Data: 14-week subchronic inhalation study in rats; submitted 6-a-2 by Reg. Div. - suspected because of a possible 6-a-2 effect noted earlier (neuropath ~~myopathy~~)

~~Data considered in setting the ADI~~:

Data gaps: N/A

Recommendation: A screen of data did not show any neuropathy or neuropathy seen in chronic studies in rats with EPTC. This effect is seen only in older rats, i.e., 1 year or older. However, a myocardial degener

~~Comments~~: was noted at the 2 highest dose groups in greater frequency and degree. Therefore, this histopathological effect should be closel examined and this study should be resubmitted to Tox. Br. for an in-depth review. This review will require additional turn-a-round t Data is being retained and a new Registration cover sheet should be s

Reviewer: [signature] 2/20/85 Date: 2/22/85

Section Head: [signature] 2/26/85

FEB 25 1985 74

(435)

Shaughnessy #: 041401

EAB Logout Date: JAN 11 1985

Signature:

TO: R. Taylor
Product Manager #25
Registration Division (TS-767)

FROM: Lionel A. Richardson, Chief
Environmental Chemistry Reveiw Section #3
Exposure Assessment Branch (TS-769C)
Hazard Evaluation Division

Attached please find the EAB Review of...

Reg./File No.: 476-2140 and 2165

Chemical: EPTC (EPTAM)

Type Product: H

Product Name:

Company Name: Stauffer

Submission Purpose: Response to R.S.

ZBB Code: ACTION CODE: 660

Date In: 11/21/84 EAB # 5119 and 5120

Date Completed: 1/10/85 TAIS (level II) Days

44 2

Defferals To:

_____ Ecological Effects Branch

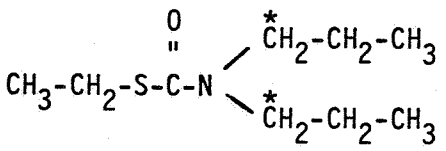
_____ Residue Chemistry Branch

_____ Toxicology Branch

75

SUBJECT: EPTC (Eptam) - S-Ethyldipropylthiocarbamate; Field accumulation - rotational crops

MATERIALS AND METHODS



*Denotes position of ^{14}C

1.0 ^{14}C-Eptam, 10.2uC/mM, prepared by Stauffer Chemical Co's. DeGuine Technical Center, having a purity of 98.7% was used in this study. The soil was Keeton sandy loam (source not mentioned) with a pH of 7.0 and organic matter content of 4.5%.

2.0 Three clay pots, approximately 7" high and 8" diameter at the top, were filled up to 6" with soil; 3" of the soil was removed from each pot and treated with 8.8 mg of ^{14}C-Eptam in 0.8 ml pentane (4ppm or 5.4 lb. ai/acre) and mixed. The treated soil was then returned to the pots (above the untreated portions) and maintained in the greenhouse with weekly watering (level not described) for one year. Three other pots with untreated soil were also maintained as controls.

At the end of one year any weeds found growing in the pots were cut and discarded and the soils (upper and lower portions) in each pot were mixed and fertilized prior to planting.

Soil samples were collected and frozen until later analysis. Samples were also taken after harvest for analysis.

Individual pots were seeded with soybeans (Glycine max var. Bragg), wheat (Tritieum aestivum var Anza) and sugar beets (Beta vulgaris var. Holly), and the plants were grown in the greenhouse. Mature plants were harvested at the appropriate stage, except that soybeans were harvested early due to an infestation of spider mites. Samples were taken and stored frozen until analyzed.

3.0 Plant samples were pulverized under liquid nitrogen and 150 mg samples taken for radioactivity analysis - air-dried soil samples were also analyzed for radioactivity - all samples being combusted in a Model 306 Packard Tri-Carb Sample Oxidizer. Combustion efficiency was determined by internal spiking with known quantities of ^{14}C-hexadecane. Residues of Eptam were calculated from the measured amounts of ^{14}C recovered.

RESULTS

1.0 Wheat plants were harvested at 5-week and 9-week intervals and at maturity. Total ^{14}C in the leaves ranged from 0.1084 ppm at 9 weeks to 0.0708 at maturity; in the chaff, ^{14}C measured 0.1241 at maturity. Total ^{14}C-Eptam in whole grain measured 0.0116 ppm equivalents at maturity. ^{14}C-Eptam equivalents in wheat soil dropped from 0.1811 ppm before planting to 0.1491 ppm at harvest.

2.0 ^{14}C-Eptam residues in mature sugarbeets at harvest ranged from 0.0019 to 0.0030 ppm. Residues in leaves ranged from 0.0054 to 0.0065 ppm. Residues in sugarbeet soil dropped from 0.1813 to 0.1473 ppm, planting to harvest.

3.0 Total ^{14}C-Eptam residues in soybean plants at harvest were found to be 0.0249 ppm in the leaves, 0.0058-0.0097 ppm in the stems, and 0.0065-0.0075 ppm in the buds. As mentioned previously, the soybeans were harvested before maturity because of a mite infestation. Soybean soil assayed 0.2108-0.1857 ppm ^{14}C-Eptam equivalents, planting to harvest respectively.

CONCLUSION

1.0 This study was scientifically valid and the results meet EPA Guidelines for Registering Pesticides Requirements (1983) (Sec. 165-1) by showing that EPTC is unlikely to be accumulated in the rotated crops grown on previously treated soil. However, it does not meet Sec. 164-1 of the Guidelines for Field (Terrestrial) Dissipation. This statement is made to prevent any misunderstanding about the latter.

HB

Hudson Boyd
1-9-85

CONFIDENTIAL

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Shaughnessy No.: 041401

Due date: 12/3/84

Init: [signature] for SMC

EAB Log Out Date 06 DEC 1984

To: R. Taylor
Product Manager 25
Registration Division (TS-767)

From: Dr. Lionel A. Richardson, Chief
Environmental Review Section #3
Exposure Assessment Branch
Hazard Evaluation Division (TS-769C)

Attached, please find the EAB review of:

Reg./File No.: 476-2140 & 476-2165

Chemical: EPTC

Type Product: H

Product Name:

Company Name: Stauffer

Submission Purpose: Response to Registration Standard

ZBB Code:

Action Code: 660

Date In: 10/2/84

EAB No.: 5003, 5004

Date Completed: 11/15/84

TAIS (Level II) Days

Typed: 12/5/84

42 1

Deferrals To:

_____ Ecological Effects Branch

_____ Residue Chemistry Branch

_____ Toxicology Branch

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Eptam

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__X__ FIFRA registration data.

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