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

NOV 5 1991

008762

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Toxicology Data on Two Bifenthrin Technical Impurities (FMC 78161 and FMC 78162)

TO: Heyward/LaRocca, PM 13
Registration Division (H7505C)

FROM: Byron T. Backus, Ph.D., Toxicologist Byron T. Backus 10/24/91
Toxicology Branch 2
HED (H7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section Head, Review Section 2
Toxicology Branch 2
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch 2 Marcia van Gemert 10/30/91
HED (H7509C)

DP Barcode D167590 Case 051804

Submission S400938 Project No. 1-2020

Tox. Chem. 463F (impurities)

Action Requested:

Review acute oral LD50 determinations and mutagenicity assays on two impurities (designated as FMC 78161 and FMC 78162) in Bifenthrin. These studies have been submitted in support of a change in the manufacturing process for Bifenthrin which will result in a reduction in waste generated, but will also result in incorporation of the two impurities in technical Bifenthrin.

Comments and Recommendations:

1. The oral LD50 determinations, and all the mutagenicity assays (Ames Assay, L5178Y TK+/- Mouse Lymphoma Assay, Mouse Micronucleus Assay) on each of these two impurities, have been classified as acceptable. The computer estimations of oral LD50 values and Ames assay mutagenicity potential have been classi-

fied as core supplementary data. A summarization of the studies and review findings is given below:

STUDY	TEST MATERIAL	STUDY FINDINGS	CLASSIFICATION
Oral LD50 (rat)	FMC 78162	LD50(M) > 1000 mg/kg LD50(F) = 1065 mg/kg	Core Minimum
Oral LD50 (computer est.)	FMC 78162	rat LD50 = 30.9 g/kg	Supplementary
Ames Assay	FMC 78162	Negative, with and without S9, at up to 10000 µg/plate	Acceptable
Ames Assay (computer est.)	FMC 78162	Probability value of 0.004 (based on compound structure) indicates FMC 78162 should be negative.	Supplementary
Mouse Micro-nucleus Assay	FMC 78162	Negative at IP doses up to 322 mg/kg with 24, 48, and 72-hr sacrifice.	Acceptable
TK+/- Mouse Lymphoma Assay	FMC 78162	Negative at up to 0.10 µl/ml without S9 and 0.12 µl/ml with S9.	Acceptable
Oral LD50 (rat)	FMC 78161	LD50(M) ≈ 4900 mg/kg LD50(F) = 4900 mg/kg	Core Minimum
Oral LD50 (computer est.)	FMC 78161	rat LD50 = 96.9 g/kg	Supplementary
Ames Assay	FMC 78161	Negative, with and without S9, at up to 10000 µg/plate	Acceptable
Ames Assay (computer est.)	FMC 78161	Probability value of 0.007 (based on compound structure) indicates FMC 78161 should be negative.	Supplementary
Mouse Micro-nucleus Assay	FMC 78161	Negative at IP doses up to 160 mg/kg with 24, 48, and 72-hr sacrifice.	Acceptable
TK+/- Mouse Lymphoma Assay	FMC 78161	Negative at up to 56 µg/ml without S9 and 32 µg/ml with S9.	Acceptable

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2. Based on the negative findings in all mutagenicity assays, as well as the relatively high oral LD50 values for these two impurities, Toxicology Branch II has no objection to the change in manufacturing process as proposed by the registrant.

3. The chemical formulas and structures of these two impurities are given on the next page of this memorandum. The registrant has stated that this information is to be considered confidential.

Page 4 is not included in this copy.

Pages _____ through _____ 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.

008762

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/15/91
Section 2, HFASB (H-7509C)

Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, HFASB (H-7509C)

DATA EVALUATION REPORT I

STUDY TYPE: Acute oral LD_{50} - rats (81-1)

TOX CHEM NO. 463F (impurity)

MRID NO: 419685-13

TEST MATERIAL: FMC 78162 Technical

STUDY NUMBER(S): A90-3172

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Corporation
Toxicology Laboratory
Box 8
Princeton, NJ 08543

TITLE OF REPORT: Acute Oral Toxicity Study in Rats

AUTHOR(S): Freeman, C.

REPORT ISSUED: May 3, 1990

CLASSIFICATION: Core Minimum Data. This study satisfies the data requirements (81-1) for an acute oral LD50 study on FMC 78162 Technical. The test material is in toxicity category III by this exposure route.

CONCLUSIONS:

1. The study adequately demonstrates a rat oral LD_{50} > 1 gm/kg for males (one mortality among 5 animals receiving this dose) and a female LD_{50} of 1065 mg/kg (95% confidence limits of 804-1327 mg/kg). The test material is in toxicity category III by the oral exposure route.

2. The study is classified as core minimum data.

A. MATERIALS:

1. Test material: FMC 78162 technical, reference no. E6680-46-3. The chemical formula and structure of this material are considered confidential by the registrant (FMC 78162 is an inert impurity in Bifenthrin). Both the chemical formula and structure are given in an attachment to the cover memorandum to this DER.

   The test material consisted of beige crystals. According to information in MRID 419685-11 the average purity of the test material was 97.3% (by solution injection) and 97.2% (by solid sample injection) on 3/19/90; the corresponding values 30 days later were 97.1 and 96.9%.

2. Test animals: Identified (p. 7) as young adult Sprague-Dawley rats from Taconic Farms, Germantown, New York. Males weighed between 271-298 g, and females between 205 and 270 g on day of dosage.

B. STUDY DESIGN:

1. Dosing of the test material: From p. 8: "The test material was melted in a water bath at 50°C, then maintained in a water bath at 50°C during dosing. The test material was administered undiluted. The density of the test material was determined to be 1.18 g/ml."

2. Observations: "The animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 hours on the day of dosing and twice daily thereafter for thirteen days; on day 14 they were observed once. Body weights were taken on days 0, 7 and 14 of the study. Animals dying intercurrently were weighed upon discovery of death."

3. Quality assurance: There is a signed and dated (p. 4) Quality Assurance Statement. There is a Certification of Good Laboratory Practice on p. 3 of the report.

C. RESULTS:

Mortality and symptoms: The following mortalities occurred:

Dose Level (Mg/kg)	#Dead/#Dosed	
	Males	Females
1000	1/5	2/5
1500	-	5/5
2000	-	5/5

All mortalities occurred within 3 days of dosage.

Symptoms: these included abdominalgenital staining, chromorhinorrhea, diarrhea, oral discharge, and (some females only) tremors.

"All survivors gained weight by the end of the study."

"All animals appeared normal at necropsy."

D. DISCUSSION:

The study adequately defines an oral LD_{50} > 1000 mg/kg in male rats and 1065 (95% C.L. of 804-1327) mg/kg in females for technical FMC 78162, which would then be in toxicity category III by the oral exposure route. However, it is noted that FMC 78162 is only present as an impurity (upper limit: [redacted] in technical bifenthrin.

008762

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/15/91
Section 2, HFASB (H-7509C)

Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, HFASB (H-7509C)

DATA EVALUATION REPORT II

STUDY TYPE: Computer Estimation of Acute Oral LD_{50} - rats (81-1)

TOX CHEM NO. 463F (impurity)

MRID NO: 419685-12

TEST MATERIAL: FMC 78162 Technical

STUDY NUMBER(S): A90-3166

SPONSOR: FMC Corporation

TESTING FACILITY: Health Designs, Inc.
183 East Main Street
Rochester, NY 14604

TITLE OF REPORT: Computer Estimation of Rat Oral LD50

AUTHOR(S): Blake, B.

REPORT ISSUED: March 9, 1990

CLASSIFICATION: Core Supplementary Data. This report attempts to estimate a rat oral LD50 value for FMC 78162 using structure-activity relationships. It is noteworthy that the TOPKAT program value (30.9 g/kg) - to which "a moderate level of confidence has been assigned" - suggests a considerably less toxic material than that indicated by actual testing (LD50 value of about 1 g/kg) of FMC 78162. The computer-generated LD50 value does not satisfy the 81-1 acute toxicity data requirement for FMC 78162.

CONCLUSIONS:

1. The report is of some interest as it estimates a rat oral LD50 value for FMC 78162 based on compound structure, while a second report (refer to DER I of this review package) involved actual testing. The TOPKAT program used for estimating the oral LD50 value yielded a value of 30.9 g/kg; this value is "interpreted to mean the probable rat oral LD50 is greater than 5 g/kg." The actual value, as determined by toxicity testing, is approximately 1 g/kg.

2. The report is classified as core supplementary data.

DISCUSSION:

The chemical formula and structure of FMC 78162 are considered confidential by the registrant (FMC 78162 is an inert impurity in Bifenthrin). Both the chemical formula and structure are given in an attachment to the cover memorandum to this DER.

The report is of some interest as it estimates a rat oral LD50 value for FMC 78162 based on compound structure, while a second report (refer to DER I of this review package) involved actual testing. The TOPKAT program used for estimating the oral LD50 value yielded a value of 30.9 g/kg; this value is "interpreted to mean the probable rat oral LD50 is greater than 5 g/kg." The actual value, as determined by toxicity testing, is approximately 1 g/kg.

It is noted that the actual equation used in the TOPKAT program, as well as derivations of particular values associated with structural aspects of the molecule, are not given in this report.

MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/11/91
Section 2, Tox Branch 2 (H7509C)
Secondary reviewer: John H. Chen, D.V.M. John H Chen 10/17/91
Section 1, Tox Branch 2 (H7509C)
Tertiary reviewer: K. Clark Swentzel K Clark Swentzel 10/28/91
Section 2, Tox Branch 2 (H7509C)

DATA EVALUATION REPORT III

CHEMICAL: FMC 78162 Tox. Chem. No. 463F

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION or MRID NUMBER: 419685-15

SYNONYMS/CAS No:

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Corporation
GENETIC TOXICOLOGY LABORATORY
Box 8
Princeton, NJ 08543

TITLE OF REPORT: FMC 78162 SALMONELLA/MAMMALIAN-MICROSOME PLATE INCORPORATION MUTAGENICITY ASSAY (AMES TEST)

AUTHOR(S): Batt, K.

STUDY NUMBER(S): A90-3170

REPORT ISSUED: 18 April 1990

CONCLUSION(S):

1. In two separate experiments FMC 78162 was assayed at exposure levels of 100, 333, 1000, 3333, and 10000 µg/plate, both with and without S9 activation, using Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100. The findings indicate that FMC 78162 is negative for mutagenic activity under the conditions of this assay.

2. This study, with its negative findings, is acceptable. This study satisfies the guideline requirements for a Salmonella typhimurium reverse mutation assay under 84-2(b)(1).

A. MATERIALS

1. Test Material: FMC 78161 technical, lot no. E6680-43. Description: a solid. According to information in MRID 419685-11 the average purity of the test material was 97.3% (by solution injection) and 97.2% (by solid sample injection) on 3/10/90; the corresponding values 30 days later were 97.1 and 96.9%.

   Contaminants: none reported
   Solvent used: Dimethylsulphoxide (DMSO)

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration:
   Positive: Non-activation:

Sodium azide	5.0	µg/plate	TA100, TA1535
2-Nitrofluorene	5.0	µg/plate	TA98, TA1538
9-Aminoacridine	75.0	µg/plate	TA1537

   Activation:
   2-Aminoanthracene (2-anthramine) 4.0 µg/plate
   TA98, TA100, TA1535, TA1537, TA1538

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat*	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

   *Male Sprague-Dawley

   Describe S9 mix composition (if purchased, give details):

S9 fraction	1.00 ml
0.4M $MgCl_2$/1.65M KCl	0.20 ml
0.1M NADP	0.40 ml
0.2M NaH_2PO_4, pH 7.4	5.00 ml
1.0M Glucose-6-phosphate	0.05 ml
H_2O	3.35 ml
	10.00 ml

4. Test organisms: S. typhimurium strains
___ TA97 X TA98 X TA100 ___ TA102 ___ TA104 X TA1535 X TA1537 X TA1538 ; list any others: (none).
Properly maintained? Yes (From p. 9: "Tester strain stocks were stored in a REVCO freezer (-80°C) or liquid nitrogen refrigerator (-196°C) and fresh cultures were inoculated directly from these, frozen stocks. Broth cultures were grown overnight at 37 ± 3°C with shaking. At the time of its use in the mutagenicity assay each culture was checked, as described by Ames, for the presence of rfa wall mutation, and strains TA98 and TA100 were checked for the presence of the pkM101 plasmid.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes (From p. 9: "At the time of its use in the mutagenicity assay each culture was checked, as described by Ames, for the presence of rfa wall mutation, and strains TA98 and TA100 were checked for the presence of the pkM101 plasmid."). Strain characterization data are presented in pages 21 and 27. The uvrB is described (p. 9) as a "stable mutation resulting in the loss of an excision repair system."

5. Test compound concentrations used:
Non-activated conditions: 100, 333, 1000, 3333, 10000 µg/plate (both assays)
Activated conditions: 100, 333, 1000, 3333, 10000 µg/plate (both assays)

B. TEST PERFORMANCE

1. Type of Salmonella assay:
- _x_ standard plate test
- ___ pre-incubation (___ minutes)
- ___ "Prival" modification (i.e. azo reduction method)
- ___ spot test
- ___ other (describe in a.)

a. Protocol (brief description, or attach copy to appendix, if inappropriate; e.g. include mediums used, incubation times, assay evaluation):

Refer to appended p. 1 for mediums used, to appended p. 2 for the preliminary toxicity determination and plating procedures for the mutagenicity assay, and to appended p. 3 and 4 for colony counting and analysis of data.

2. Preliminary cytotoxicity assay:

Refer to appended p. 5 for results of the preliminary toxicity evaluation. There were no indications of toxicity, expressed either as a thinning of the background lawn for strain TA100, or as a decrease in number of revertants/plate. At the 3 highest exposure levels (3333, 6667 and 10000 µg/plate) there were oily droplets present. These oily droplets apparently did not interfere with machine counting of colonies, as there is no indication (see p. 13 of the report text) that it was necessary to hand count these particular plates.

3. Mutagenicity assay

Neither of the two separately conducted assays gave any indication of either cytotoxicity or increased number of revertants in any of the tester strains (either with or without metabolic activation) at any of the dose levels assayed. The positive controls elicited the appropriate responses. Refer to appended pages 6 and 7 for average revertant counts. It is noted that oily droplets were reported at 3333 and 10000 µg/plate concentrations of the test material in the first assay, but not in the second (refer to report pages 20 and 26); however, this is not considered significant enough to impact on the acceptability of the study.

4. Laboratory Evaluation of Mutagenicity Assay Data

From p. 13 of the report: "For a test article to be considered positive, it must cause at least a doubling in the mean number of revertants per plate of at least one strain. This increase in the mean number of revertants per plate must be accompanied by a dose response to increasing concentrations of the test article. In those cases where the observed dose response increase in TA1537 revertants per plate is less than three-fold, the response must be reproducible."

5. There is a signed and dated Quality Assurance Statement on p. 4 of the report, along with a Statement of Compliance with EPA Good Laboratory Practice Standards on p. 3.

C. DISCUSSION

Under the conditions of this assay the test material (FMC 78162 technical) gave no indications of mutagenic activity in any of the five S. typhimurium strains (TA1535, TA1537, TA1538, TA98, TA100) used, either in the presence or absence of S9. There was no indication of cytotoxicity in any of the strains at the highest concentration (10,000 µg/plate) at which the test material was assayed. This level is substantially above the 5,000 µg/plate which the Agency normally accepts as a sufficiently adequate dose limit in this assay. The test material was tested in two separate assays, with triplicate plates at each dose level. The positive controls elicited the appropriate responses.

It is concluded that the assay and its negative findings are acceptable. This study satisfies the guideline requirements [84-2(b)(1)] for a gene mutation study.

Page _____ is not included in this copy.

Pages 14 through 20 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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_____ Description of quality control procedures.

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_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/11/91
Section 2, Tox Branch 2 (H7509C)
Secondary reviewer: John H. Chen, D.V.M. John H Chen 10/17/91
Section 1, Tox Branch 2 (H7509C)
Tertiary reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, Tox Branch 2 (H7509C)

DATA EVALUATION REPORT IV

CHEMICAL: FMC 78162 Tox. Chem. No. 463F

STUDY TYPE: Salmonella/mammalian activation gene mutation assay (computer estimation)

ACCESSION or MRID NUMBER: 419685-14

SYNONYMS/CAS No:

SPONSOR: FMC Corporation

TESTING FACILITY: HDI
183 East Main Street
Rochester, New York 14604

TITLE OF REPORT: COMPUTER ESTIMATION OF MUTAGENICITY (AMES)

AUTHOR(S): Blake, B.

STUDY NUMBER(S): A90-3167

REPORT ISSUED: 9 March 1990

CONCLUSION(S):

1. This is a computer estimation (TOPKAT program) of the Salmonella mutagenicity of FMC 78162 based on its structure. According to the report text, analysis of the FMC 78162 structure yields a numerical value of 0.004, with the explanation that "probability values between 0.00 and 0.30 are considered negative, probability values between 0.70 and 1.00 are considered positive, and probability values between 0.30 and 0.70 are considered indeterminate where no judgment as to positivity or negativity can be made." This report is of some interest because the registrant has also submitted an acceptable (with negative findings) Ames study on FMC 78162

2. This computer estimation is classified as core supplementary data. This report does not satisfy the guideline requirements [84-2(b)(1)] for a gene mutation study for FMC 78162.

MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/21/91
Section 2, Tox Branch 2 (H7509C)
Secondary reviewer: John H. Chen, D.V.M. John H Chen 10/21/91
Section 1, Tox Branch 2 (H7509C)
Tertiary reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, Tox. Branch 2 (H7509C)

DATA EVALUATION REPORT V

CHEMICAL: FMC 78162 Tox. Chem. No. 463F

STUDY TYPE: Mouse Micronucleus Assay

ACCESSION or MRID NUMBER: 419685-17

SYNONYMS/CAS No:

SPONSOR: FMC Corporation

TESTING FACILITY: Microbiological Associates
5221 River Road
Bethesda, MD 20816

TITLE OF REPORT: Micronucleus Cytogenetic Assay in Mice

AUTHOR(S): Putman, D. L.

STUDY NUMBER(S): Laboratory Study No. T9490.122019
Sponsor Study No. A90-3335

REPORT ISSUED: 22 July 1991

CONCLUSION(S):

1. Groups of ICR mice were injected IP with 81, 161, or 322 mg/kg of FMC 78162, with sacrifice of 5M and 5F/dose level at 24, 48 and 72 hours after treatment. At the highest dose level (322 mg/kg) of FMC 78162 1/20 males and 3/20 females died within 72 hours of test material administration. A single group of 5M and 5F were injected IP with 30 mg cyclophosphamide and sacrificed 24 hours later. Following sacrifice, slides were prepared from each animal and polychromatic erythrocytes (PCEs) were scored for presence of micronuclei. The number of micronucleated normocytes in a field of 1000 PCEs was determined, as well as the proportion of PCEs to total erythrocytes.

2. Under the conditions of this assay, there were no indications of an increased incidence of micronucleated PCEs at any of the dose levels of FMC 78162. Exposure to CP elicited the appropriate positive response.

3. This study, with its negative findings, is acceptable. This study satisfies the guideline requirements for an in vivo cytogenetics assay (using rodent bone marrow) under 84-2(b)(3)(B).

A. MATERIALS

1. Test Material: FMC 78162, lot no. E6680-46-3, assigned code no. T9490. "Purity was listed by the Sponsor as 97.3%. The test article was described as stable at room temperature."

"The test article was characterized by the Sponsor as a solid material which becomes a clear liquid upon heating." "Upon receipt, the test article was described as a straw colored, clear liquid and was stored at room temperature. The Sponsor was notified upon receipt that the test article was liquid in form rather than solid. The Sponsor verified that the test material may take both forms, depending upon the temperature."

Contaminants: none reported
Solvent used: corn oil

2. Control Materials:
Negative: vehicle control (corn oil)
Positive: cyclophosphamide (CP)

3. Test animals:

ICR Mice, obtained from Harlan Sprague Dawley, Inc., Frederick, MD. "At the initiation of the study, the mice were 6-8 weeks old." Body weights for the toxicity studies and micronucleus assay ranged from 25-37 grams (males) and 20-30 grams (females).

4. Test compound concentrations:

Two analyses were performed (samples received May 9 and 10th from the performing laboratory), with the following results (from p. 33 of the report text):

	analytical values for FMC 78162 (mg/mL)	
Nominal concentrations:	May 9	May 10
FMC 78162, 32.2 mg/mL	28.2	28.2
FMC 78162, 8.1 mg/mL	7.49	6.95

B. TEST PERFORMANCE

1. Preliminary toxicity assay

From p. 10: "Two toxicity studies were required to determine an LD_{50}. For the toxicity studies, animals were randomly assigned

to groups of five males and five females each... All mice in the experimental and control groups were weighed immediately prior to dose administration and the dose volume based on individual body weights. Animals were observed after dose administration and daily thereafter for 7 days for clinical signs of chemical effect. Body weights were recorded prior to dose administration and no less than 1 and 3 days after dose administration." The test material was administered IP.

2. Micronucleus assay

From p. 11: "For the micronucleus assay, the animals were assigned to thirteen experimental groups of five males and five females each based on a computer-generated randomization program. An additional group of 5 males and 5 females were designated as replacement animals in the event of mortality prior to the scheduled sacrifice time and were dosed with the test article high dose level..."

The following is an outline of dose levels (the test article-vehicle mixture, the vehicle alone, or the positive control was administered by IP injection at a constant volume of 10 ml/kg body weight) and sacrifice schedule:

	Sacrifices by Animals/Sex after Dose Administration		
	24 hr	48 hr	72 hr
Vehicle control	5	5	5
Low dose (81 mg/kg)	5	5	5
Mid dose (161 mg/kg)	5	5	5
High dose (322 mg/kg)	5	5	5
CP, 30 mg/kg	5	-	-

3. Slide preparation:

From p. 11: "At the scheduled sacrifice time, five mice per sex were sacrificed by CO_2 asphyxiation. Immediately following sacrifice, the femurs were exposed, cut just above the knee, and the bone marrow was aspirated into a syringe containing fetal bovine serum. The bone marrow cells were transferred to a capped centrifuge tube containing approximately 1 ml FBS. The bone marrow cells were pelleted by centrifugation at approximately 100 x g for five minutes and the supernatant was drawn off, leaving a small amount of serum with the remaining cell pellet. The cells were resuspended by aspiration with a capillary pipet and a small drop of bone marrow suspension was spread onto a clean glass slide. Two to four slides were prepared from each animal. The slides were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted."

4. Scoring, evaluation of test results and criteria for valid test:

Refer to appended p. 1.

5. There are two signed and dated Quality Assurance Statements in this report, one on p. 4 from the performing laboratory and one on p. 5 from the sponsor. In addition, there is a Statement of Compliance (with Good Laboratory Practice Regulations) on p. 3 of the report.

C. DISCUSSION

Under the conditions of this assay (involving IP injection with 81, 161, or 322 mg/kg) the test material gave no indications of mutagenic activity (which would have been evidenced by an increased incidence of micronucleated polychromatic erythrocytes). There was a reduction in ratio of polychromatic erythrocytes to total erythrocytes in some animals administered 322 mg/kg of the test substance and sacrificed at 72 hours, suggesting bone marrow toxicity in these mice. The highest dose level of test material (322 mg/kg) resulted in mortality in 1/20 males and 3/20 females within 72 hours of administration, while administration of 500 mg/kg administered in a preliminary toxicity test resulted in mortality in 4/5 males and 5/5 females within 48 hours. These mortality incidences indicate the test material was administered at a sufficiently high level.

The positive control (cyclophosphamide at 30 mg/kg, with 24-hour sacrifice) elicited an appropriate mutagenic response, indicating the sensitivity of the assay system to a known clastogen. The spontaneous rates of micronuclei in the PCEs of the vehicle (corn oil) controls at the 72-hour sacrifice interval were found to be 0.02% (males) and 0.04% (females). These results were within the normal range for the mouse micronucleus assay as described by Heddle et al. (Mutation Res. 123: 61-118, 1983).

It is concluded that the assay and its negative findings are acceptable. This study satisfies the guideline requirements for an in vivo cytogenetics assay (using rodent bone marrow) under 84-2(b)(3)(B).

Page _____ is not included in this copy.

Pages 26 through 30 are not included in this copy.

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Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/10/91
Section 2, Tox Branch 2 (H7509C)
Secondary reviewer: John H. Chen, D.V.M. John H Chen 10/10/91
Section 1, Tox Branch 2 (H7509C)
Tertiary reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, Tox. Branch 2 (H7509C)

DATA EVALUATION REPORT VI

CHEMICAL: FMC 78162 Tox. Chem. No. 463F

STUDY TYPE: TK+/- Mouse Lymphoma Mutagenesis Assay

ACCESSION or MRID NUMBER: 419685-16

SYNONYMS/CAS No:

SPONSOR: FMC Corporation

TESTING FACILITY: Microbiological Associates
5221 River Road
Bethesda, MD 20816

TITLE OF REPORT: L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay with Confirmation

AUTHOR(S): Bigger, C., and Clarke, J.

STUDY NUMBER(S): Laboratory Study No. T9490.701020
Sponsor Study No. A90-3336

REPORT ISSUED: 26 March 1991

CONCLUSION(S):

1. Under the conditions of this assay (involving L5178Y cell exposure in the first trial to FMC 78162 at 0.0075, 0.01, 0.013, 0.018, 0.024, 0.032, 0.042, 0.056, 0.075, and 0.1 µl/ml both with and without S9, and, in the second trial, 0.06, 0.07, 0.08, 0.09 and 0.10 µl/ml without S9 activation, and 0.06, 0.07, 0.08, 0.09, 0.10 and 0.12 µl/ml with S9) there was no indication at any dose level of a significant increase in revertant frequency relative to the vehicle controls.

2. FMC 78162 was tested at sufficiently high concentrations, as evidenced by considerable cytotoxicity at 0.1 and/or 0.12 µl/ml, both in the absence and presence of S9 activation. The positive controls (ethyl methanesulfonate at 0.25 and 0.5 µl/ml in the absence of S9 activation, 7,12-Dimethylbenz(a)anthracene at 2.5 and 5 µg/ml in the presence of S9 activation) elicited at least

4X increases in mutant frequencies.

3. This study, with its negative findings, is acceptable. This study satisfies the guideline requirements for a mammalian cells in culture forward gene mutation assay (mouse lymphoma L5178Y cells, thymidine kinase gene locus, maximizing assay conditions for small colony expression) under 84-2(b)(2)(A).

A. MATERIALS

1. Test Material: FMC 78162, lot no. E6680-46-3, assigned code no. T9490. From p. 9 of the report: "The test article was characterized by the Sponsor as a clear liquid upon heating, which should be stored at room temperature with no expiration date. Its purity was not provided." [According to information on page 9 of MRID 419685-17 FMC 78162, lot number E6680-46-3, used in the micronucleus assay, had a purity "listed by the Sponsor as 97.3%"].

"Upon receipt, the test article was described as a straw-colored, clear liquid and was stored at room temperature, protected from exposure to light. At the time of dose administration, the test article was dissolved in ethanol... The sponsor has assumed responsibility for the determination of the stability of the test article."

Contaminants: none reported

2. Control Materials:

Negative: solvent (ethanol) control (1% v/v, the same concentration as that for the test article-treated groups).

Positive (without S9): Ethyl methanesulfonate (EMS), diluted in DMSO to exposure concentrations of 50 and 25 µl/ml.
Positive (with S9): 7,12-Dimethylbenz(a)anthracene (7,12-DMBA), diluted in DMSO to concentrations of 500 and 250 µg/ml.

3. S-9 and S-9 mix:

From p. 11: "The S-9 was prepared according to established procedures. Adult male Sprague-Dawley rats, 200-250 gm, were induced by a single intraperitoneal injection of a 2:1 mixture of Aroclor-1242 and Aroclor-1254 at a dosage of 500 mg/kg body weight five days prior to sacrifice. The excised tissue was rinsed three times in cold sterile 0.25 M sucrose and then homogenized in a Polytron Tissuemizer at a concentration of 1:3 (w/v) in 0.25 M sucrose. The supernatant fraction (S-9) was collected following centrifugation at 9000 x g for 10 minutes at 4±2°C, portioned into aliquots for daily use, and stored frozen at <-70°C until used. Each bulk preparation of S-9 was

assayed for sterility and for its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to Salmonella typhimurium TA100."

"Immediately prior to use, the S-9 was mixed with the cofactors and F_0P to contain 250 µl S-9, 6.0 mg NADP, 11.25 mg DL-isocitric acid and 750 µl F_0P/ml S-9 activation mixture and kept on ice until used. The cofactor mixture was adjusted to pH 7.0 and filter sterilized prior to the addition of S-9."

3. Test Cells:

From p. 10: "L5178Y cells, clone 3.7.2C, were obtained directly from Dr. Donald Clive, Burroughs Wellcome Company, Research Triangle Park, NC. Prior to using L5178Y cells for the mutation assay, the cells were cleansed of spontaneous TK -/- cells by adding a restrictive agent to the culture medium which selectively kills TK -/- mutants. Cryopreserved L5178Y cells were rapidly thawed and resuspended in flasks at a concentration of $1x10^5$ cells/ml in 100 ml culture medium. Once normal growth was observed, THMG (thymidine, hypoxanthine, methotrexate and glycine) was added to each flask at a concentration of 9 µg/ml thymidine, 15 µg/ml hypoxanthine, 0.3 µg/ml methotrexate, and 22.5 µg/ml glycine. The flasks were gassed with 5±1% CO_2 in air and incubated at 37±1°C in an environmental incubator shaker at 125 rpm. After approximately 24 hours, the THMG was removed by pelletizing the cells and decanting the supernatant. The cells were rinsed in 20 ml $F_{10}P$ and reinstated in culture at 3×10^4 cells/ml in 100 ml of $F_{10}P$ with THG (3 µg/ml thymidine, 5 µg/ml hypoxanthine and 7.5 µg/ml glycine). After approximately 72 hours, the cells were ready to be used in the mutagenesis assay."

4. Test compound concentrations:

Two analyses were performed (samples received May 9 and 10th from the performing laboratory), with the following results (from p. 33 of the report text):

	analytical values for FMC 78162 (mg/mL)	
Nominal concentrations:	May 9	May 10
FMC 78162, 32.2 mg/mL	28.2	28.2
FMC 78162, 8.1 mg/mL	7.49	6.95

B. TEST PERFORMANCE

1. Preliminary toxicity assay

In the following discussion F_0P = Fischer's Medium with 0.1% Pluronics for Leukemic Cells of Mice; $F_{10}P$ = the same supplemented with 10% horse serum and 4mM L-glutamine.

From p. 11: "The optimal dose levels for the mutagenesis assay were selected following a preliminary toxicity test based on cell population growth relative to the solvent controls. L5178Y cells were exposed to solvent alone and eight concentrations of test article ranging from 100 to 0.01 µl/ml for 4 hours in the absence and presence of an exogenous source of metabolic activation. Each tube was gassed with 5 ± 1% CO_2 in air and placed on a Bellco roller drum apparatus rotating at approximately 25 rpm. The final solvent concentration in the culture medium was 1% by volume. The test solutions were prepared under amber lights and kept in darkness during the entire exposure period. After approximately 4 hours, the test article in solution was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting the supernatant. The cells were washed twice in 10 ml of $F_{10}P$, resuspended in 20 ml $F_{10}P$, gassed with 5 ± 1% CO_2 in air, and replaced on the roller drum apparatus. Cell population density was determined 24 and 48 hours after the initial exposure to the test article by removing a sample from each treatment tube, diluting in 0.1% trypsin, incubating at 37 ± 1°C for 10 minutes, and counting the samples with an electronic cell counter. The cultures were adjusted to 3 x 10^5 cells/ml (if the cell population exceeded 3 x 10^5 cells/ml) at approximately 24 hours after treatment only."

Refer to appended page 1 for results of the initial toxicity test.

2. Mutation assay

In the following TFT = trifluorothymidine, a restrictive agent.

From p. 11: "The mutation assay was performed according to a protocol described by Clive et al. (1975). L5178Y mouse lymphoma cells were cleansed as described previously and resuspended at a cell density of 1x10^6 cells/ml. Six ml aliquots were dispensed into centrifuge tubes to yield 6x10^6 cells/centrifuge tube.

Cells were exposed to sixteen concentrations of the test article ranging from 0.1 to 0.0013 µl/ml for 4 hours at 37±1°C. Two control tubes received solvent only and the positive controls were treated with EMS (0.5 and 0.25 µl/ml) and 7,12-DMBA (5 and 2.5 µg/ml). The treatment medium consisted of 4 ml of F_0P containing various concentrations of test article for the nonactivated and 4 ml of reaction mixture containing various concentrations of test article for the activated cultures. The final solvent concentration in the culture medium was 1% by volume. After the treatment period, the cells were washed twice with $F_{10}P$ by centrifuging the cultures at 1000 rpm for 10 minutes and decanting the supernatant. The cells were resuspended in $F_{10}P$, gassed with 5±1% CO_2 in air and placed on the roller drum apparatus at 37±1°C.

For expression of the mutant phenotype, the cultures were counted and adjusted to $3x10^5$ cells/ml (if the cell population exceeded $3x10^5$ cells/ml) at approximately 24 and 48 hours after treatment.

For expression of the TK -/- cells, cells were placed in cloning medium (C.M.) containing 0.23% granulated agar. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT or V.C. (viable count). Each flask was prewarmed to 37±1°C, filled with 100 ml C.M., and placed in an incubator shaker at 37±1°C until used. The cells were centrifuged at 1000 rpm for 10 minutes, and the supernatant, except for 2 ml, was removed by pipetting. The cells were resuspended in the remaining 2 ml of medium and placed in a TFT flask labeled with the corresponding concentration of the test article. A $2x10^{-4}$ dilution was carried out by adding 1.0 ml of the TFT flask suspension to a test tube containing 4 ml of $F_{10}P$, adding 1.0 ml of this to 9 ml of $F_{10}P$, and adding 1.0 ml of that dilution to the appropriate V.C. flask containing 100 ml of C.M. After the dilution, 1.0 ml of stock solution of TFT was added to the TFT flask (final concentration of 3 µg/ml) and both this flask and the V.C. flask were placed on the shaker at 125 rpm and 37±1°C. After 15 minutes, the flasks were removed one at a time, and 33 ml of the cell suspension was pipetted into each of three appropriately labeled petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 20 minutes. The plates were removed and incubated at 37±1°C in a humidified 5 ± 1% CO_2 atmosphere for 10-12 days..

A confirmatory assay was performed following the initial assay. Eight dose levels ranging from 0.1 to 0.03 µl/ml for the non-activated cultures and from 0.12 to 0.04 µl/ml for the S-9 activated cultures were used to treat duplicate cultures."

Refer to appended pages 2 through 13 for results of the first (pages 2-7) and second (pages 8-13).

3. Scoring, evaluation of test results and criteria for valid test:

Refer to appended p. 14.

4. There are two signed and dated Quality Assurance Statements in this report, one on p. 4 from the performing laboratory and one on p. 5 from the sponsor. In addition, there is a Statement of Compliance (with Good Laboratory Practice Regulations) from the laboratory on p. 3 of the report.

C. DISCUSSION

Under the conditions of this assay (involving cell exposure in the first trial to 0.0075, 0.01, 0.013, 0.018, 0.024, 0.032, 0.042, 0.056, 0.075, and 0.1 µl/ml both with and without S9, and, in the second trial, 0.06, 0.07, 0.08, 0.09 and 0.10 µl/ml without S9 activation, and 0.06, 0.07, 0.08, 0.09, 0.10 and 0.12 µl/ml with S9) there was no indication of any significant increase in mutant frequency per 10^6 surviving cells relative to the vehicle controls at any dose level. FMC 78162 was tested at sufficiently high concentrations, as evidenced by considerable cytotoxicity at 0.1 and/or 0.12 µl/ml, both in the absence and presence of S9 activation. The positive controls (EMS at 0.25 and 0.5 µl/ml in the absence of S9 activation, 7,12-DMBA at 2.5 and 5 µg/ml in the presence of S9 activation) elicited at least 4X increases in mutant frequencies, indicating the assay system was sensitive to known mutagens in the presence and absence of metabolic activation.

It is concluded that the assay and its negative findings are acceptable. This study satisfies the guideline requirements for a mammalian cells in culture forward gene mutation assay (mouse lymphoma L5178Y cells, thymidine kinase gene locus, maximizing assay conditions for small colony expression) under 84-2(b)(2)(A).

Page _____ is not included in this copy.

Pages 37 through 50 are not included in this copy.

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_____ 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.

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_____ 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.

008762

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/15/91
Section 2, HFASB (H-7509C)

Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, HFASB (H-7509C)

DATA EVALUATION REPORT VII

STUDY TYPE: Acute oral LD50 - rats (81-1)

TOX CHEM NO. 463F (impurity)

MRID NO: 419685-06

TEST MATERIAL: FMC 78161 Technical

STUDY NUMBER(S): A90-3173

SPONSOR: FMC Corporation

TESTING FACILITY: FMC Corporation
Toxicology Laboratory
Box 8
Princeton, NJ 08543

TITLE OF REPORT: Acute Oral Toxicity Study in Rats

AUTHOR(S): Freeman, C.

REPORT ISSUED: May 3, 1990

CLASSIFICATION: Core Minimum Data. This study satisfies the data requirements (81-1) for an acute oral LD50 study on FMC 78161 Technical. The test material is in toxicity category III by the oral exposure route.

CONCLUSIONS:

1. The study adequately demonstrates a rat oral LD_{50} in females of 4900 (95% confidence limits of 4027-5774) mg/kg; while a value for the male LD_{50} was not determined, the incidence (3/5) of mortality at 5000 mg/kg was the same as for females, and the symptoms were essentially the same. The findings establish that the test material is in toxicity category III by the oral exposure route.

2. The study is classified as core minimum data.

A. MATERIALS:

1. Test material: FMC 78161 technical, reference no. E6680-43. The chemical formula and structure of this material are considered confidential by the registrant (FMC 78161 is an inert impurity in Bifenthrin). Both the chemical formula and structure are given in an attachment to the cover memorandum to this DER.

   The test material was a white powder. The purity of the test material is not given in this report; however, according to information on page 9 of MRID 419685-10, FMC 78161, lot number E6680-43, used in the micronucleus assay, had a purity "listed by the Sponsor as 99.4%."

2. Test animals: Identified (p. 8) as young adult Sprague-Dawley (Tac:N[SD]fBR) rats from Taconic Farms, Germantown, New York. Males weighed between 277 and 299 g, and females between 206 and 262 g on day of dosage.

B. STUDY DESIGN:

1. Dosing of the test material: From p. 8: "The test material was ground in a mortar and pestle then prepared as a 25% (w/v) mixture in margarine... The test material was introduced directly into the stomach of each animal using a ball-tipped intubation needle."

2. Observations: "The animals were observed for mortality and clinical signs at 0.5, 1, 2, 3, 4 and 6 hours on the day of dosing and twice daily thereafter for thirteen days; on day 14 they were observed once. Body weights were taken on days 0, 7 and 14 of the study. Animals dying intercurrently were weighed upon discovery of death."

3. Quality assurance: On page 4 there is a signed and dated Quality Assurance Statement. On page 3 there is a Certification of Good Laboratory Practice.

C. RESULTS:

Mortality and symptoms: The following mortalities occurred:

Dose Level (Mg/kg)	#Dead/#Dosed	
	Males	Females
5000	3/5	3/5
4500	-	1/5
4000	-	1/5

All mortalities occurred within 3 days of dosage.

Symptoms: these included abdominogenital staining, bloody oral discharge, chromorhinorrhea, and decreased locomotion.

"All survivors gained weight by the end of the study."

"All animals appeared normal at necropsy."

D. DISCUSSION:

The study adequately defines an oral LD_{50} of 4900 (95% confidence limits of 4027-5774) mg/kg in females for technical FMC 78161. While a value for the male LD_{50} was not determined, the incidence (3/5) of mortality at 5000 mg/kg was the same as for females, and the symptoms were essentially the same. The findings establish that the test material is in toxicity category III by the oral exposure route. It is noted that FMC 78161 is only present as an impurity (upper limit: [redacted] in technical bifenthrin.

A. MATERIALS

1. Test Material: FMC 78161 technical, lot no. E6680-43. Description: a solid. From information on p. 6 the purity was "determined; results not reported." According to information on p. 9 of MRID 419685-10, FMC 78161, lot number E6680-43, used in the micronucleus assay, had a purity "listed by the Sponsor as 99.4%."

   Contaminants: none reported
   Solvent used: Dimethylsulphoxide (DMSO)

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration:
   Positive: Non-activation:

Sodium azide	5.0	µg/plate	TA100, TA1535
2-Nitrofluorene	5.0	µg/plate	TA98, TA1538
9-Aminoacridine	75.0	µg/plate	TA1537

   Activation:
   2-Aminoanthracene (2-anthramine) 4.0 µg/plate
   TA98, TA100, TA1535, TA1537, TA1538

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat*	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

   *Male Sprague-Dawley

   If other, describe below
   Describe S9 mix composition (if purchased, give details):

S9 fraction	1.00 ml
0.4M $MgCl_2$/1.65M KCl	0.20 ml
0.1M NADP	0.40 ml
0.2M NaH_2PO_4, pH 7.4	5.00 ml
1.0M Glucose-6-phosphate	0.05 ml
H_2O	3.35 ml
	10.00 ml

4. Test organisms: S. typhimurium strains
   ___ TA97 X TA98 X TA100 ___ TA102 ___ TA104 X TA1535 X TA1537 X TA1538 ; list any others: (none).
   Properly maintained? Yes (From p. 9: "Tester strain stocks were stored in a REVCO freezer (-80°C) or liquid nitrogen refrigerator (-196;C) and fresh cultures were inoculated directly from these frozen stocks. Broth cultures were grown overnight at 37 ± 3°C with shaking. "

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes (From p. 9: "At the time of its use in the mutagenicity assay each culture was checked, as described by Ames, for the presence of rfa wall mutation, and strains TA98 and TA100 were checked for the presence of the pkM101 plasmid."). Strain characterization data are presented in pages 21 and 27. The uvrB is described (p. 9) as a "stable mutation resulting in the loss of an excision repair system."

5. Test compound concentrations used:
Non-activated conditions: 100, 333, 1000, 3333, 10000 µg/plate (both assays).
Activated conditions: 50, 167, 500, 1667, 5000 µg/plate (first assay); 100, 333, 1000, 3333, 10000 µg/plate (second assay).

B. TEST PERFORMANCE

1. Type of Salmonella assay:
- _x_ standard plate test
- ___ pre-incubation (___ minutes)
- ___ "Prival" modification (i.e. azo reduction method)
- ___ spot test
- ___ other (describe in a.)

a. Protocol (brief description, or attach copy to appendix, if inappropriate; e.g. include mediums used, incubation times, assay evaluation):

Refer to appended p. 1 for mediums used, to appended p. 2 for the preliminary toxicity determination and plating procedures for the mutagenicity assay, and to appended p. 3 and 4 for colony counting and analysis of data.

2. Preliminary cytotoxicity assay:

Refer to appended p. 5 for results of the preliminary cytotoxicity evaluation. In the presence of S9, there was slightly reduced lawn (as well as some reduction in number of TA100 revertants) at 3333, 6667, and 10000 µg/plate, along with a concomitant slight precipitate at the two lower dose levels, and moderate precipitation at 10000 µg/plate. While similar precipitates were observed at these levels in the absence of S9, there were no indications of cytotoxicity (either as a reduction in lawn or decrease in number of reverstants).

3. Mutagenicity assay

In neither of the two separately conducted assays was there any indication of either cytotoxicity or increased number of revertants in any of the tester strains (either with or without metabolic activation) at any of the dose levels. The positive controls elicited the appropriate responses. Refer to appended pages 6 and 7 for average revertant counts.

4. Laboratory Evaluation of Mutagenicity Assay Data

From p. 13 of the report: "For a test article to be considered positive, it must cause at least a doubling in the mean number of revertants per plate of at least one strain. This increase in the mean number of revertants per plate must be accompanied by a dose response to increasing concentrations of the test article. In those cases where the observed dose response increase in TA1537 revertants per plate is less than three-fold, the response must be reproducible."

5. There is a signed and dated Quality Assurance Statement on p. 4 of the report, along with a Statement of Compliance with EPA Good Laboratory Practice Standards on p. 3.

C. DISCUSSION

Under the conditions of this assay the test material (FMC 78161 technical) gave no indications of mutagenic activity in any of the five S. typhimurium strains (TA1535, TA1537, TA1538, TA98, TA100) used, either in the presence or absence of S9. There was no indication of cytotoxicity in any of the strains at the highest concentration (5000 µg/plate in the presence of S9 in the first assay; 10,000 µg/plate in the absence of S9 in both assays and in the presence of S9 in the second assay) of test material. The Agency normally accepts 5,000 µg/plate as a sufficiently adequate dose limit in this type of assay. The test material was tested in two separate assays, with triplicate plates at each dose level. The positive controls elicited the appropriate responses.

It is concluded that the assay and its negative findings are acceptable. This study satisfies the guideline requirements [84-2(b)(1)] for a gene mutation study.

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Pages 57 through 63 are not included in this copy.

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MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/16/91
Section 2, Tox Branch 2 (H7509C)
Secondary reviewer: John H. Chen, D.V.M. John H Chen 10/17/91
Section 1, Tox Branch 2 (H7509C)
Tertiary reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, Tox Branch 2 (H7509C)

DATA EVALUATION REPORT X

CHEMICAL: FMC 78161 Tox. Chem. No. 463F (impurity)

STUDY TYPE: Salmonella/mammalian activation gene mutation assay (computer estimation)

ACCESSION or MRID NUMBER: 419685-07

SYNONYMS/CAS No:

SPONSOR: FMC Corporation

TESTING FACILITY: HDI
183 East Main Street
Rochester, New York 14604

TITLE OF REPORT: COMPUTER ESTIMATION OF MUTAGENICITY (AMES)

AUTHOR(S): Blake, B.

STUDY NUMBER(S): A90-3168

REPORT ISSUED: 9 March 1990

CONCLUSION(S):

1. This is a computer estimation (TOPKAT program) of the Salmonella mutagenicity of FMC 78161 based on its structure. According to the report text, analysis of the FMC 78161 structure yields a numerical value of 0.007, with the explanation that "probability values between 0.00 and 0.30 are considered negative, probability values between 0.70 and 1.00 are considered positive, and probability values between 0.30 and 0.70 are considered indeterminate where no judgment as to positivity or negativity can be made." This report is of some interest because the registrant has also submitted an acceptable (with negative findings) Ames study on FMC 78161.

2. This computer estimation is classified as core supplementary data. This report does not satisfy the guideline requirements [84-2(b)(1)] for a gene mutation study for FMC 78161.

MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/21/91
Section 2, Tox Branch 2 (H7509C)
Secondary reviewer: John H. Chen, D.V.M. John H. Chen 10/21/91
Section 1, Tox Branch 2 (H7509C)
Tertiary reviewer: K. Clark Swentzel K. Clark Swentzel 10/28/91
Section 2, Tox. Branch 2 (H7509C)

DATA EVALUATION REPORT XI

CHEMICAL: FMC 78161 Tox. Chem. No. 463F

STUDY TYPE: Mouse Micronucleus Assay

ACCESSION or MRID NUMBER: 419685-10

SYNONYMS/CAS No:

SPONSOR: FMC Corporation

TESTING FACILITY: Microbiological Associates
5221 River Road
Bethesda, MD 20816

TITLE OF REPORT: Micronucleus Cytogenetic Assay in Mice

AUTHOR(S): Putman, D. L.

STUDY NUMBER(S): Laboratory Study No. T9498.122019
Sponsor Study No. A90-3323

REPORT ISSUED: 22 July 1991

CONCLUSION(S):

1. Groups of ICR mice were injected IP with 40, 80, or 160 mg/kg of FMC 78161, with sacrifice of 5M and 5F/dose level at 24, 48 and 72 hours after treatment. At the highest dose level (160 mg/kg) of FMC 78161 2/20 males and 1/20 females died within 72 hours of test material administration. A single group of 5M and 5F were injected IP with 30 mg cyclophosphamide and sacrificed 24 hours later. Following sacrifice, slides were prepared from each animal and polychromatic erythrocytes (PCEs) were scored for presence of micronuclei. The number of micronucleated normocytes in a field of 1000 PCEs was determined, as well as the proportion of PCEs to total erythrocytes.

2. Under the conditions of this assay, there were no indications of an increased incidence of micronucleated PCEs at any of the dose levels of FMC 78161. Exposure to CP elicited the appropriate positive response.

3. This study, with its negative findings, is acceptable. This study satisfies the guideline requirements for an in vivo cytogenetics assay (using rodent bone marrow) under 84-2(b)(3)(B).

A. MATERIALS

1. Test Material: FMC 78161, lot no. E6680-43, assigned code no. T9490. "Purity was listed by the Sponsor as 99.4% and it was described as stable at room temperature."

"Upon receipt, the test article was described as a white granular solid... At the time of use, the test article was suspended in corn oil... A concentration of 16 mg/ml was prepared for use as the dosing solution for the high dose level. Dilutions were carried out in corn oil to yield concentrations of 8 and 4 mg/ml for the dosing solutions for the mid and low dose levels, respectively."

Contaminants: none reported

2. Control Materials:

Negative: vehicle control (corn oil)
Positive: cyclophosphamide (CP), dissolved in sterile distilled water at a concentration of 3 mg/ml.

3. Test animals:

ICR Mice, obtained from Harlan Sprague Dawley, Inc., Frederick, MD. "At the initiation of the study, the mice were 6-8 weeks old." Body weights for the toxicity studies and micronucleus assay ranged from 29-39 grams (males) and 21-31 grams (females).

4. Test compound concentrations:

Analyses were performed on four dosing solutions, with the following results (from p. 33 of the report test):

	analytical values for FMC 78161 (mg/mL)	
Nominal concentrations:	May 9	May 10
FMC 78161, 4.0 mg/mL	3.42	3.38
FMC 78161, 16.0 mg/mL	14.7	17.2

B. TEST PERFORMANCE

1. Preliminary toxicity assay

From p. 10: "Three toxicity studies were required to determine an LD_{50}. For the toxicity studies, animals were randomly assigned to groups of five males and five females each... All

mice in the experimental and control groups were weighed immediately prior to dose administration and the dose volume based on individual body weights. Animals were observed after dose administration and daily thereafter for 7 days for clinical signs of chemical effect. Body weights were recorded prior to dose administration and no less than 1 and 3 days after dose administration." The test material was administered IP. All mice dosed at 346 mg/kg and above died; at 247 mg/kg 4/5M and 4/5F died (refer to appended p. 1).

2. Micronucleus assay

From p. 10: "For the micronucleus assay, the animals were assigned to thirteen experimental groups of five males and five females each based on a computer-generated randomization program. An additional group of 5 males and 5 females were designated as replacement animals in the event of mortality prior to the scheduled sacrifice time and were dosed with the test article high dose level..."

The following is an outline of dose levels (the test article-vehicle mixture, the vehicle alone, or the positive control was administered by IP injection at a constant volume of 10 ml/kg body weight) and sacrifice schedule:

	Sacrifices by Animals/Sex after Dose Administration		
	24 hr	48 hr	72 hr
Vehicle control	5	5	5
Low dose (40 mg/kg)	5	5	5
Mid dose (80 mg/kg)	5	5	5
High dose (160 mg/kg)	5	5	5
CP, 30 mg/kg	5	-	-

3. Slide preparation:

From p. 11: "At the scheduled sacrifice time, five mice per sex were sacrificed by CO_2 asphyxiation. Immediately following sacrifice, the femurs were exposed, cut just above the knee, and the bone marrow was aspirated into a syringe containing fetal bovine serum. The bone marrow cells were transferred to a capped centrifuge tube containing approximately 1 ml FBS. The bone marrow cells were pelleted by centrifugation at approximately 100 x g for five minutes and the supernatant was drawn off, leaving a small amount of serum with the remaining cell pellet. The cells were resuspended by aspiration with a capillary pipet and a small drop of bone marrow suspension was spread onto a clean glass slide. Two to four slides were prepared from each animal. The slides were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted."

4. Scoring, evaluation of test results and criteria for valid test:

   Refer to appended p. 2.

5. Assay results:

   Refer to appended pages 3, 4, 5 and 6.

6. There are two signed and dated Quality Assurance Statements in this report, one on p. 4 from the performing laboratory and one on p. 5 from the sponsor. In addition, there is a Statement of Compliance (with Good Laboratory Practice Regulations) on p. 3 of the report.

C. DISCUSSION

Under the conditions of this assay (involving IP injection with 40, 80, or 160 mg/kg) the test material gave no indications of mutagenic activity (which would have been evidenced by an increased incidence of micronucleated polychromatic erythrocytes). The highest dose level of test material (160 mg/kg) resulted in mortality in 2/20 males and 1/20 females within 72 hours of administration, while administration of 177 mg/kg in a preliminary toxicity test resulted in mortality in 2/5 males and 0/5 females, and administration of 247 mg/kg resulted in mortality in 4/5 males and 4/5 females. These mortality incidences indicate the test material was administered at a sufficiently high level.

The positive control (cyclophosphamide at 30 mg/kg, with 24-hour sacrifice) elicited an appropriate mutagenic response, indicating the sensitivity of the assay system to a known clastogen. The spontaneous rates of micronuclei in the PCEs of the vehicle (corn oil) controls at the 72-hour sacrifice interval were found to be 0.02% (males) and 0.04% (females). These results were within the normal range for the mouse micronucleus assay as described by Heddle et al. (Mutation Res. 123: 61-118, 1983).

It is concluded that the assay and its negative findings are acceptable. This study satisfies the guideline requirements for an in vivo cytogenetics assay (using rodent bone marrow) under 84-2(b)(3)(B).

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MUTAGENICITY

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus 10/10/91
Section 2, Tox Branch 2 (H-7509C)
Secondary reviewer: John H. Chen, D.V.M. John H Chen 10/21/91
Section 1, Tox Branch 2 (H-7509C)
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DATA EVALUATION REPORT XII

CHEMICAL: FMC 78161 Tox. Chem. No. 463F

STUDY TYPE: TK+/- Mouse Lymphoma Mutagenesis Assay

ACCESSION or MRID NUMBER: 419685-09

SYNONYMS/CAS No:

SPONSOR: FMC Corporation

TESTING FACILITY: Microbiological Associates
5221 River Road
Bethesda, MD 20816

TITLE OF REPORT: L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay with Confirmation

AUTHOR(S): Bigger, C., and Clarke, J.

STUDY NUMBER(S): Laboratory Study No. T9498.701020
Sponsor Study No. A90-3334

REPORT ISSUED: 26 March 1991

CONCLUSION(S):

1. Under the conditions of this assay (involving L5178Y cell exposure in the first trial to FMC 78161 at 2.4, 3.2, 4.2, 5.6, 7.5, 10, 13, 18, 24, 32, and 42 µg/ml without activation, and at 2.4, 3.2, 4.2, 5.6, 7.5, 10, 13, 18, 24, and 32 µg/ml with activation; and, in the second trial, exposure to 18, 24, 32, 42 and 56 µg/ml without S9 activation, and 10, 13, 18, 24, and 32 µg/ml with activation) the test material was found to be negative for mutagenic activity. In the first trial there were noticeable increases in mutant frequency at the two highest test article concentrations (32 and 42 µg/ml) evaluated in the absence of S9 activation, and at 32 µg/ml in the presence of S9 activation, although mean mutant frequencies were not doubled (a criterion for a positive response) relative to the respective solvent control values. There were no increases observed at the same doses in the second trial.

2. FMC 78161 was tested at sufficiently high concentrations, as evidenced by considerable (approximately 90%) reductions in total growth at the highest assayed concentrations, both in the absence and presence of S9 activation. The positive controls (ethyl methanesulfonate at 0.25 and 0.5 µl/ml in the absence of S9 activation, 7,12-Dimethylbenz(a)anthracene at 2.5 and 5 µg/ml in the presence of S9 activation) elicited at least 3X (usually greater) increases in mutant frequencies.

3. This study, with its negative findings, is acceptable. This study satisfies the guideline requirements for a mammalian cells in culture forward gene mutation assay (mouse lymphoma L5178Y cells, thymidine kinase gene locus, maximizing assay conditions for small colony expression) under 84-2(b)(2)(A).

A. MATERIALS

1. Test Material: FMC 78161, lot no. E6680-43, assigned code no. T9498. From p. 9 of the report: "The test article was characterized by the Sponsor as a granular solid, which should be stored at room temperature. Its purity and expiration date were not given." [According to information on page 9 of MRID 419685-10 FMC 78161, lot number E6680-43, used in the micronucleus assay, had a purity "listed by the Sponsor as 99.4%"].

   "Upon receipt, the test article was described as a white, granular solid and was stored at room temperature, protected from exposure to light. At the time of dose administration, the test article was dissolved in dimethylsulfoxide... The sponsor has assumed responsibility for the determination of the stability of the test article."

   Contaminants: none reported

2. Control Materials:

   Negative: solvent control (1% v/v DMSO, the same concentration as that for the test article-treated groups).

   Positive (without S9): Ethyl methanesulfonate (EMS), diluted in DMSO to exposure concentrations of 50 and 25 µl/ml.
   Positive (with S9): 7,12-Dimethylbenz(a)anthracene (7,12-DMBA), diluted in DMSO to concentrations of 500 and 250 µg/ml.

3. S-9 and S-9 mix:

   From p. 11: "The S-9 was prepared according to established procedures. Adult male Sprague-Dawley rats, 200-250 gm, were induced by a single intraperitoneal injection of a 2:1 mixture of Aroclor-1242 and Aroclor-1254 at a dosage of 500 mg/kg body

weight five days prior to sacrifice. The excised tissue was rinsed three times in cold sterile 0.25 M sucrose and then homogenized in a Polytron Tissuemizer at a concentration of 1:3 (w/v) in 0.25 M sucrose. The supernatant fraction (S-9) was collected following centrifugation at 9000 x g for 10 minutes at 4±2°C, portioned into aliquots for daily use, and stored frozen at <-70°C until used. Each bulk preparation of S-9 was assayed for sterility and for its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to Salmonella typhimurium TA100."

"Immediately prior to use, the S-9 was mixed with the cofactors and F_0P to contain 250 µl S-9, 6.0 mg NADP, 11.25 mg DL-isocitric acid and 750 µl F_0P/ml S-9 activation mixture and kept on ice until used. The cofactor mixture was adjusted to pH 7.0 and filter sterilized prior to the addition of S-9."

4. Test Cells:

From p. 10: "L5178Y cells, clone 3.7.2C, were obtained directly from Dr. Donald Clive, Burroughs Wellcome Company, Research Triangle Park, NC. Prior to using L5178Y cells for the mutation assay, the cells were cleansed of spontaneous TK -/- cells by adding a restrictive agent to the culture medium which selectively kills TK -/- mutants. Cryopreserved L5178Y cells were rapidly thawed and resuspended in flasks at a concentration of $1x10^5$ cells/ml in 100 ml culture medium. Once normal growth was observed, THMG (thymidine, hypoxanthine, methotrexate and glycine) was added to each flask at a concentration of 9 µg/ml thymidine, 15 µg/ml hypoxanthine, 0.3 µg/ml methotrexate, and 22.5 µg/ml glycine. The flasks were gassed with 5±1% CO_2 in air and incubated at 37±1°C in an environmental incubator shaker at 125 rpm. After approximately 24 hours, the THMG was removed by pelletizing the cells and decanting the supernatant. The cells were rinsed in 20 ml $F_{10}P$ and reinstated in culture at 3×10^4 cells/ml in 100 ml of $F_{10}P$ with THG (3 µg/ml thymidine, 5 µg/ml hypoxanthine and 7.5 µg/ml glycine). After approximately 72 hours, the cells were ready to be used in the mutagenesis assay."

5. Test compound concentrations:

Analyses were performed for four solutions, with the following results (from p. 52 of the report text):

	analytical values for FMC 78161 (µg/mL)	
Nominal concentrations:	Value	% Standard Deviation
FMC 78161, 178 µg/mL	178	2.90
FMC 78161, 12000 µg/mL	12000	1.24
FMC 78161, 563 µg/mL	584	1.63
FMC 78161, 7500 µg/mL	7056	2.42

B. TEST PERFORMANCE

1. Preliminary toxicity assay

In the following discussion F_0P = Fischer's Medium with 0.1% Pluronics for Leukemic Cells of Mice; $F_{10}P$ = the same supplemented with 10% horse serum and 4mM L-glutamine.

From p. 11: "The optimal dose levels for the mutagenesis assay were selected following a preliminary toxicity test based on cell population growth relative to the solvent controls. L5178Y cells were exposed to solvent alone and eight concentrations of test article ranging from 6667 to 0.1 µg/ml for 4 hours in the absence and presence of an exogenous source of metabolic activation. Each tube was gassed with 5 ± 1% CO_2 in air and placed on a Bellco roller drum apparatus rotating at approximately 25 rpm. The final solvent concentration in the culture medium was 1% by volume. The test solutions were prepared under amber lights and kept in darkness during the entire exposure period. After approximately 4 hours, the test article in solution was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting the supernatant. The cells were washed twice in 10 ml of $F_{10}P$, resuspended in 20 ml $F_{10}P$, gassed with 5 ± 1% CO_2 in air, and replaced on the roller drum apparatus. Cell population density was determined 24 and 48 hours after the initial exposure to the test article by removing a sample from each treatment tube, diluting in 0.1% trypsin, incubating at 37 ± 1°C for 10 minutes, and counting the samples with an electronic cell counter. The cultures were adjusted to 3 x 10^5 cells/ml (if the cell population exceeded 3 x 10^5 cells/ml) at approximately 24 hours after treatment only."

Refer to appended page 1 for results of the initial toxicity test.

2. Mutation assay

In the following TFT = trifluorothymidine, a restrictive agent.

From p. 11-12: "The mutation assay was performed according to a protocol described by Clive et al. (1975). L5178Y mouse lymphoma cells were cleansed as described previously and resuspended at a cell density of 1x10^6 cells/ml. Six ml aliquots were dispensed into centrifuge tubes to yield 6x10^6 cells/centrifuge tube.

Cells were exposed to sixteen concentrations of the test article ranging from 120 to 1.8 µg/ml for 4 hours at 37±1°C. Two control tubes received solvent only and the positive controls were treated with EMS (0.5 and 0.25 µl/ml) and 7,12-DMBA (5 and

2.5 μg/ml). The treatment medium consisted of 4 ml of $F_o P$ containing various concentrations of test article for the nonactivated and 4 ml of reaction mixture containing various concentrations of test article for the activated cultures. The final solvent concentration in the culture medium was 1% by volume. After the treatment period, the cells were washed twice with $F_{10}P$ by centrifuging the cultures at 1000 rpm for 10 minutes and decanting the supernatant. The cells were resuspended in $F_{10}P$, gassed with 5±1% CO_2 in air and placed on the roller drum apparatus at 37±1°C.

For expression of the mutant phenotype, the cultures were counted and adjusted to $3x10^5$ cells/ml (if the cell population exceeded $3x10^5$ cells/ml) at approximately 24 and 48 hours after treatment.

For expression of the TK -/- cells, cells were placed in cloning medium (C.M.) containing 0.23% granulated agar. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT or V.C. (viable count). Each flask was prewarmed to 37±1°C, filled with 100 ml C.M., and placed in an incubator shaker at 37±1°C until used. The cells were centrifuged at 1000 rpm for 10 minutes, and the supernatant, except for 2 ml, was removed by pipetting. The cells were resuspended in the remaining 2 ml of medium and placed in a TFT flask labeled with the corresponding concentration of the test article. A $2x10^{-4}$ dilution was carried out by adding 1.0 ml of the TFT flask suspension to a test tube containing 4 ml of $F_{10}P$, adding 1.0 ml of this to 9 ml of $F_{10}P$, and adding 1.0 ml of that dilution to the appropriate V.C. flask containing 100 ml of C.M. After the dilution, 1.0 ml of stock solution of TFT was added to the TFT flask (final concentration of 3 μg/ml) and both this flask and the V.C. flask were placed on the shaker at 125 rpm and 37±1°C. After 15 minutes, the flasks were removed one at a time, and 33 ml of the cell suspension was pipetted into each of three appropriately labeled petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 20 minutes. The plates were removed and incubated at 37±1°C in a humidified 5 ± 1% CO_2 atmosphere for 10-12 days..

A confirmatory assay was performed following the initial assay. Duplicate cultures were exposed to eight concentrations of the test article ranging from 75 to 10 μg/ml for the non-activated cultures and from 42 to 5.6 for the S-9 activated cultures."

Refer to appended pages 2 through 13 for results of the first (pages 2-7) and second (pages 8-13) trial.

3. Scoring, evaluation of test results and criteria for valid test:

Refer to appended p. 14.

4. There are two signed and dated Quality Assurance Statements in this report, one on p. 4 from the performing laboratory and one on p. 5 from the sponsor. In addition, there is a Statement of Compliance (with Good Laboratory Practice Regulations) from the laboratory on p. 3 of the report.

C. DISCUSSION

Under the conditions of this assay (involving cell exposure to FMC 78161 in the first trial at 2.4, 3.2, 4.2, 5.6, 7.5, 10, 13, 18, 24, 32, and 42 μg/ml without activation, and at 2.4, 3.2, 4.2, 5.6, 7.5, 10, 13, 18, 24, and 32 μg/ml with activation; and, in the second trial, exposure to 18, 24, 32, 42 and 56 μg/ml without S9 activation, and 10, 13, 18, 24, and 32 μg/ml with activation) the test material was found to be negative for mutagenic activity. In the first trial there were noticeable increases in mutant frequency (per 10^6 cells) at the two highest test article concentrations (32 and 42 μg/ml) evaluated in the absence of S9 activation, and at 32 μg/ml in the presence of S9 activation, although in no case was there a doubling of mean mutant frequencies (a criterion for a positive response) relative to the respective solvent control values. There was no increase observed at the same concentrations in the second trial.

The test material (FMC 78161) was tested at sufficiently high concentrations, as evidenced by considerable (approximately 90%) reductions in total growth at the highest assayed concentrations, both with and without S9 activation. The positive controls (ethyl methanesulfonate at 0.25 and 0.5 μl/ml in the absence of S9 activation, 7,12-Dimethylbenz(a)anthracene at 2.5 and 5 μg/ml in the presence of S9 activation) elicited 3X (and usually greater) increases in mutant frequencies, indicating the assay system was sensitive to known mutagens in the presence and absence of metabolic activation.

It is concluded that the assay and its negative findings are acceptable. This study satisfies the guideline requirements for a mammalian cells in culture forward gene mutation assay (mouse lymphoma L5178Y cells, thymidine kinase gene locus, maximizing assay conditions for small colony expression) under 84-2(b)(2)(A).

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