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

9/6/84

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDOM

SUBJECT: Request For An Extension Of Experimental Use Permit (279-EUP-93) And Request For Temporary Tolerance (4G2987) For FMC-57020 (Command) Soybeans. Caswell No. 463D

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

FROM: Carolyn Gregorio, Toxicologist
Toxicology Branch/ HED (TS-769) CG 9-6-84

THRU: Robert P. Zendzian PhD, Acting Section Head
Review Section III
Toxicology Branch/ HED (TS-769)
9/6/84 WPB 9-6-84

Petitioner: FMC Corporation

Action Requested: The Petitioner has requested an extension of the EUP-crop destruct (279-EUP-93) for use of the 4E formulation of FMC-57020 until March 1, 1986 and to establish a Temporary Tolerance (4G2987) of 0.05 ppm on soybeans.

Background: Previous reviews (Holder to Taylor, dated January 3, 1983) had recommended approval for the proposed EUP-crop destruct on soybeans.

The identified FMC-57020 (4E formlation) formula inerts were previously cleared (Holder to Taylor, dated January 3, 1983).

FMC-57020 is the Petitioner's code number for 2-(2-chlorophenyl)methyl-4, 4-dimethyl-3-isoxazolidinone. Common and trade names have not been established.

DATA REVIEWED/PREVIOUSLY REVIEWED (Technical- 88.8% a.i.)

Study Type	Results
Acute Oral-rats*	LD50= 2077 mg/kg (male) LD50= 1369 mg/kg (female)
Acute Dermal-rabbit*	LD50= greater than 2000 mg/kg
Acute Inhalation-rat*	LC50= 6.25 mg/L (male)

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	LC50= 4.23 mg/L (female)
Dermal Irritation-rabbit*	Minimally Irritating
Dermal Sensitization-guinea pig*	Not Sensitizer
Eye Irritation-rabbit*	Slightly Irritating
One-year Feeding dog 3-month interim sacrifice	NOEL cannot be determined insufficient animals sacrificed
Chronic Feeding mouse 3-month interim sacrifice	NOEL Not Established liver toxicity
Chronic Feeding rat 3-month interim sacrifice	Report incomplete
Teratology-rabbit	NOEL= 240 mg/kg/day for maternal toxicity, not teratogneic at 700/1000mg/kg HDT
Mutagenicity- Bacterial Gene Mutation	Partially acceptable Not mutagenic for acceptable tests
Mutagenicity- In Vivo Cytogenetic Assay	Acceptable Not mutagenic
Mutagenicity- Unscheduled DNA	Acceptable Not mutagenic
Mutagenicity- Ames*	Acceptable

* Previously reviewed by James Holder (memo to Robert Taylor, dated January 3, 1983).

DATA PREVIOUSLY REVIEWED (4E Formulation)

Study Type	Results
Acute Oral-rat*	LD50= 2343 mg/kg (male) LD50= 1406 mg/kg (female)
Acute Dermal-rabbit*	LD50= greater than 2000 mg/kg
Acute Inhalation-rat*	LC50= 4.47 mg/L (male) LC50= 4.70 mg/L (female)
Dermal Irritation-rabbit*	Moderate to Severe
Dermal Sensitization-guinea pig*	Not Sensitizer
Eye Irritation-rabbit*	Moderate to Severe

* Previously reviewed by James Holder (memo to Robert Taylor, dated January 3, 1983).

DATA GAPS

1.) Clarification of the 90-Day Feeding study in rats (Toxigenics, Study No. 410-0816, FMC Study No. A81-650, dated April 12, 1983). The Petitioner's submitted text was incomplete. The following pages were missing:

Accession No. 072043: 1, 3, 8, 11, 12, 17, 18, 20, 21, 25b,35, 37, 38, 39, 43, 45, 46, 48, 50, 52, 53, 55, 56, 58, 66, 68

Accession No. 072045: C-1, C-3, C-4, C-5, C-7, C-8

Accession No. 072048: G-1, G-3, G-6, G-11, G-13, G-22, G-25, G-26, G-28, G-40, G-41, G-42, G-44, G-45, G-50, G-64, G-65, G-66, G-67, G-70

2.) 90-Day Feeding Study- Rodent. The available 90-day feeding study in mice (90-day/3-month Interim Report for 90-day Subchronic Toxicity Dietary and 24-month Chronic Toxicity and Oncogenicity Dietary Study in Mice Utilizng FMC 57020 Technical. Toxigenics Study No. 410-0817, April 11, 1983) did not establish a NOEL. In the examination of the individual animal data submitted, megalocytosis of the liver was observed at 20(LDT), 2000, and 8000 ppm in the treated males and at 8000 ppm in the treated females. This lesion is an indication of cell degeneration which may be an adaptive change rather than a toxic reaction and should be futher examined in the final outcome of the 2-year feeding study.

Dose-related increased liver weight was observed in both sexes. This became apperant when the liver weights were expressed as percent of control. In the males absolute liver weight was increased in a dose-related fashion at all doses, relative to body weight at 100ppm and and higher doses and relative to brain weight at all doses. In the females, liver weight, absolute and relative to body weight was increased at 100ppm and higher doses and relative to brain weight at 500ppm amd higher doses.

3) 90-day feeding study dog. The available 90-day feeding study in the dog, (Interim Report: One-year chronic oral toxicity study in dogs according to EPA guidelines. HRI. Study No. 6124-101 FMC Study No. A82-759) was not sufficient to establish a NOEL. Only two animals per sex per dose were sacrificed and 4/sex/dose are required for proper evaluation.

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RECOMMENDATION

1.) The data available are sufficient to toxicologically support the requested extension of the EUP-crop destruct.

2.) The data available are NOT sufficient to toxicologically support the establishment of the requested Temporary Tolerance on soybeans (0.05 ppm) since the data did not permit establishment of a NOEL.

3.) The registrant has submitted a data package for tolerances of FMC-57020(Command). This new package contains the final report of the chronic rat, chronic mouse and one-year dog feeding studies for which 90-interim reports were reviewed in this submission. Because the new submission essentially supercedes the interim reports, no further data should be requested from the Registrant at this time in relation to this submission.

DERs ATTACHED

Ninety day final/3 month interim report for ninety day subchronic toxicity dietary and twenty four month chronic toxicity and oncogenicity dietary study in rats utilizing FMC-57020 technical. Submitted to FMC Corporation (FMC study No. A81-650). Submitted by: Toxigenics' Inc. (Toxigenics Study No. 410-0816) April 12, 1983

Interim Report: One-year chronic oral toxicity study in dogs according to EPA guidelines. HRI. Study No. 6124-101 FMC Study No. A82-759

90-day/3-month interim report for 90-day subchronic toxicity dietary and 24-month chronic toxicity and oncogenicity dietary study in mice utilizing FMC-57020 technical. Submitted to FMC Corporation (FMC study No. A81-651). Submitted by: Toxigenics' Inc. (Toxigenics Study No. 410-0817) April 11, 1983

A teratology study in rabbits with FMC 57020, K.M. Werchowski, M.D. Nemec & D.E. Rodwell, Wil Research Laboratories, Inc. Project No. WIL-81157, FMC No. A81/655, Sept 14, 1982

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Citation: Ninety Day Final/3 Month Interim Report For Ninety Day Subchronic Toxicity Dietary And Twenty Four Month Chronic Toxicity And Oncogenicity Dietary Study In Rats Utilizing FMC-57020 Technical. Submitted To: FMC Corporation (FMC Study No. A81-650). Submitted By: Toxigenics', Inc. (Toxigenics' Study No. 410-0816). Dated April 12, 1983.

Report Preparation by Leslie D. Monroe; Report Approval by Gale D. Taylor.

Study Type: 90-Day Feeding- rats

Accession No.: 072043, 072045, 072048

MRID No.: Not Assigned

Registrant: FMC Corporation (Study No. A81-650)

Contracting Lab: Toxigenics', Inc. (Study No. 410-650)

Date: April 12, 1983

Test Material: FMC-57020 (Technical, 88.8% a.i.)

No Data Evaluation Record is being prepared for this study because the Petitioner's submitted text is incomplete. The following pages were missing:

Accession No. 072043: 1,3,8,11,12,17,18,20,21,25b, 35,37,38,39,43,45,46,48,50,52,53,55,56,58,66,68

Accession No. 072045: C-1,C-3,C-4,C-5,C-7,C-8

Accession No. 072048: G-1,G-3,G-6,G-11,G-13,G-22,G-25, G-26,G-28,G-40,G-41,G-42,G-44,G-4,G-50,G-64,G-65,G-66, G-67,G-70

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Citation: Interim Report: One-year Chronic Oral Toxicity Study in Dogs According to EPA Guidelines. HRI Study No. 6124-101. FMC Study No. A82-759.

Study Type: 90-Day Feeding - Dogs (Interim Report of 1-year Feeding)

Accession No.: 072062

MRID No.: Not Reported

Registrant: FMC Corporation

Contracting Lab: Hazleton Labs. (HRI Study No. 6124-101; FMC Study No. A82-759)

Date: September 7, 1983

Test Material: FMC - 57020 (88.8% Purity) (Lot # Reference EL 756-146)

Test Compound Stability: Not Reported

Test Compound Stability In Diet: "The stability of the test material at the four original dose levels was determined on the day of preparation, 8 days at room temperature, and 14 and 28 days frozen. The results were all low (due to analytical methodology); however, they do indicate that FMC is stable in the basal diet for at least 8 days at room temperature and for at least 28 days frozen."

The "Revised Analytical Procedure", as indicated in the Registrant's Table, 38, does indicate that the diets were stable. Only one reading of the 500 ppm dose was outside the theoretical concentration (430 pm on 12-22-82).

Homogeneity of test compound: "The first set of analyses (12-15-82) were low and variable. Therefore, the homogeneity analyses were repeated with the revised analytical procedure (Registrant text table 36) (2-22-83)."

Protocol: Young adult Beagles (4-6 months of age, weighing 6.0 to 9.0 kg at study initiation) were acclimated for 2 weeks. The dogs (6/sex/dose) were fed FMC-57020 in the diet at doses of 0, 100, 500, 2500 or 7500/5000 ppm (7500 ppm reduced to 5000 ppm after 1 week on study) for 1 year. Two dogs/sex were sacrificed after 90 days.

Animals were observed "at least twice daily." Bodyweight and food consumption were monitored once a week. Ophthalmic examinations were conducted prior to initiation of the study and at 3 months.

Hematology, blood chemistry, and urinalysis data were collected at initiation, 1 and 3 months of the study.

A complete necropsy and histopathological examination was conducted at 3 months on 2 dogs/sex/dose.

Results:

Daily Observations: "During the first week of test, the 75000 ppm dose groups defecated infrequently, and feces, when present, were usually mucoid and/or bloody." The dose level was therefore reduced to 5000 ppm after 1 week. "With the reduction in dose level, the incidence of these observations returned to normal."

Body Weight and Food Consumption: Mean body weight (Table 1) and mean food consumption (Table 2) were decreased dramatically in the 7500 ppm males and females after 1 week of feeding, which prompted the contracting lab to drop the high dose from 7500 ppm to 5000 ppm ppm for the duration of the study. Although weight gains in the 5000 ppm group females were slightly slower in the first 5 weeks, these animals essentially "caught up" with control females.

The 5000 ppm males, however, continued to lag behind controls (about 10% less) throughout the 90 days on study.

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Table 1. Mean Body Weight (KG) Over Selected Time

	Males a/					Females a/				
week	0	1	5	10	13	0	1	5	10	13
Dose (ppm)										
0	7.6	7.9	9.3	10.5	10.6	7.0	7.1	8.0	8.5	8.5
100	7.5	7.8	9.2	10.4	10.6	7.1	7.5	8.6	9.6	9.5
500	7.5	7.8	9.0	9.8	10.2	7.0	7.4	8.3	9.2	9.2
2500	7.6	7.8	9.0	10.1	10.3	6.9	7.5	8.3	9.2	9.3
7500/5000 b/	7.7	6.3	7.9	9.4	9.5	7.1	5.9	7.7	8.8	8.8

a/ 6 animals/sex/dose
b/ The dose level was reduced from 7500 to 5000 ppm after 1 week.

Note: This table is comprised from portions of Registrant's submission.

Table 2. Mean Food Consumption (G) Over Selected Time

	Males a/					Females a/				
weeks	0	1	5	10	13	0	1	5	10	13
dose (ppm)										
0	1952	2035	2015	1894	1739	1629	1660	1827	1662	153
100	1957	1905	211[illegible]	1883	1676	1912	1889	2114	1722	16[illegible]
500	1894	1920	1986	1847	1797	1710	1750	1948	1807	15[illegible]
2500	1979	1834	2230	2025	1946	1866	1958	2079	1999	184
7500/5000 b/	2019	32	2045	2014	1806	1848	95	2442	1698	15[illegible]

a/ 6 animals/sex/dose
b/ The dose level was reduced from 75000 to 5000 ppm after 1 week.

Note: This table is comprised from portions of Registrant's submission.

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Ophthalmic Examination: No unusual lesions reported for any animal.

Hematology: Erythrocyte count, hematocrit, hemoglobin, leukocyte count (total), leukocyte differential (neutrophil, lymphocyte, monocyte, eosinophil, basophil, bands, segments), platelet count, and nucleated red blood cell values showed normal variation among all groups.

Blood Chemistry: Albumin, blood urea nitrogen, calcium, chloride, creatinine, gamma glutamyl transpeptidase, glucose, inorganic phosphorous, potassium, serum glutamic oxaloacetic transaminase, sodium, tota' bilirubin, and total protein values showed normal variation among all groups.

Mean cholesterol values for males and females were higher for males and ferales in the 2500 and 5000 ppm treated groups at 1 month (males: 21.3 and 27.1%, respectively; females: 14 and 36.2%, respectively) and at 3 months (males: 18.9 and 26.9%, respectively; females: 20.4 and 27%, respectively). See Table 3 for values.

Table 3. Mean Cholesterol (mg/dl) values

	Males			Females		
Time (month)	0	1	3	0	1	3
0 ppm	136.9	122.1	123.4	116.8	105.6	108.6
100 ppm	159.4	133.1	147.3	117.9	110.2	114.5
500 ppm	144.5	120.6	130.5	137.1	119.6	116.5
2500 ppm	142.4	155.1	152.2	119.3	123.0	136.6
5000 ppm	151.1	168.2	168.8	130.6	164.7	149.4

Note: This table is abstracted from portions of the Registrant's submission.

Mean creatine phosphokinase and serum glutamic pyruvic transaminase were decreased at the 1 and 3 month intervals (Table 4) for the 5000 ppm females only.

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Table 4. Selected Mean Blood Chemistry Values - Females

Dose (ppm)	0	100	500	2500	5000
Creative Phosphokinase (IU/L)					
0	100.6	91.0	90.2	100.1	116.5
1	174.6	165.7	154.2	167.2	137.4
3	120.9	147.5	104.6	144.2	101.4
Serum Glutamic Pyruvic Transaminase (IU/L)					
0	11.1	11.9	12.2	12.5	11.8
1	16.2	16.3	15.5	14.0	13.9
3	19.2	20.2	18.4	18.3	15.2

Note: This table is comprised from portions of the Registrant's submission.

Urine Analysis: The specific gravity and pH were similar among all groups.

The Registrant submitted text indicated that the urine was to be analyzed for bilirubin, blood, glucose, ketones, protein, urobilinogen and microscopic examination of sediments; no values were reported for any of these parameters.

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Organ Weights: Mean absolute and mean relative liver weights were increased for the 2500 and 5000 ppm male and female when compared to concurrent controls (Table 5 and Table 6).

There was a slight increase in the thyroid/parathyroid absolute and relative to body weight ratio in the 2500 and 5000 ppm males. Ovary absolute and relative to body weight ratio was increased in the 5000 ppm females.

Table 5. Selected Mean Organ Weight Data - Males

Dose (ppm)	Body Wt. (kg)	Liver		Thyroid	Parathroid
		Absolute Wt (gm)	Relative Wt.	Absolute Wt. (gm	Relative Wt.
0	10.6	263±3	2.6166	0.8720±0.2772	0.0087±0.0028
100	10.6	282±28	2.6736	1.1315±0.1662	0.0098±0.001?
500	10.2	222±38	2.5520	0.7075±0.0035	0.0081±0.0011
2500	10.3	314±45	3.1145	1.2105±0.3466	0.0119±0.0015
5000	95	361±7	3.4694	1.2405±0.2963	0.0119±0.0022

NOTE: This Table is comprised from portions of the Registrant's submission.

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Table 6. Selected Mean Organ Weight Data- Female

Dose (ppm)	Body Wt. (kg)	Liver		Ovaries	
		Absolute Wt (gm)	Relative Wt.	Absolute Wt. (gm	Relative Wt.
0	8.5	263	2.5113	0.6056±0.0233	0.0070±0.0009
100	9.5	255	2.8559	0.6150±0.2659	0.0067±0.0019
500	9.2	249	2.8049	0.7095±0.0177	0.0080±0.0005
2500	9.3	299	3.2585	0.6100±0.1400	0.0066±0.0012
5000	8.8	315	3.4032	1.1335±0.261	0.0123±0.0006

NOTE: This Table is comprised from portions of the Registrant's submission.

Gross Pathology was similar for all groups. No unusual pathology was reported.

Histopathology: No neoplastic lesions were reported in any animal. Nothing noteworthy was seen in seen in any group; those reported findings which would appear to be treatment related are in Table 7. However, with the very few animals examined (2/sex/dose) and the short duration of the study, it would be difficult to assess any significance in these findings.

Table 7. 90-Day Feeding In Dogs ---Histopathology

	Males					Females				
Dose (ppm)	0	100	500	2500	5000	0	100	500	2500	5000
Kidneys										
° Mineralization (medulla)	0/2	0/2	0/2	1/2	2/2	0/2	2/2	0/2	0/2	1/2
° Swelling of individual glomerular cells	0/2	1/2	1/2	2/2	1/2	0/2	1/2	1/2	2/2	1/2
Thyroid										
° C-Cell Hyperplasia	0/2	0/2	0/2	/2	0/2	0/2	1/2	0/2	1/2	0/2
Stomach										
° Mineralization, Mucosa	0/2	0/2	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2
Brain (cerebellum)										
° Glial Nodules	1/2	1/2	0/2	1/2	0/2	0/2	0/2	1/2	1/2	2/2

CONCLUSIONS:

5000 ppm (HDT): Decreased body weights (males and females); increased mean cholesterol values (males and females); decreased mean creatinine phosphokinase and serum glutamic pyruvic transaminase (females only); increased mean absolute and relative liver weight (males and females); increased absolute and relative thyroid/parathyroid weights (male only); increase in absolute and relative ovary weight.

2500 ppm: Increased mean cholesterol values (males and females); increased mean absolute and relative liver weights (males and females); increased mean absolute and relative thyroid/parathroid weights (males and females).

500 ppm: Similar to control values

100 ppm (LDT): Similar to control values

Classification: INVALID as 90-day feeding study because only 2 animals/sex/dose were examined; data can be used in concert with the Final 1-year feeding study. Submitted study was incomplete, i.e. urine analysis incomplete, compound stability not provided.

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Citation: 90-Day/3-Month Interim Report For 90-Day Subchronic Toxicity Dietary And 24-Month Chronic Toxicity And Oncogenicity Dietary Study In Mice Utilizing FMC 57020 Technical. Submitted To: FMC Corporation (FMC Study No. A81-651). Submitted by: Toxigenics, Inc. (Study No. 410-0817). Dated April 11, 1983.

Study Type: Subchronic Oral Toxicity - Mice

Accession Number: 072044, 072046, 72047

MRID Number: N/A

Sponsor: FMC Corporation

Contracting Lab: ToxiGenics, Inc.

Date: April 11, 1983

Test Material: FMC 57020

Protocol:

1. Test substance and purity: FMC 57020, Technical (88.8% pure). Reference EL756-146)

2. Species of animals: Weanling Charles River, CD-1 mice.

3. Dosing schedule: Groups of 120 male and 120 female mice were fed 0, 20, 100, 500, 1000, 2000, 4000, or 8000 ppm FMC 57620 in the diet. During the 90-day portion of the study, 10 males and 10 females were sacrificed after 1 and 2 months of treatment and 20 males and 20 females after 3 months of treatment. The remaining animals were maintained as part of a chronic/oncogenicity study.

4. Parameters to be examined: Diet analysis, mortality, body weight, hematology, clinical chemistry, organ weight, and pathology.

5. Statistics used: ANOVA plus Tukey's test or Scheffe's test of Multiple Comparisons. Kruskal-Wallis Multiple Comparison Test and Chi-square test.

Results:

Dietary Analysis

Fresh diets were prepared weekly. Stability tests and homogeneity

were conducted prior to study initiation. Homogenity studies were also conducted for selected dietary concentrations for each fresh preparation. Diet analyses are showed that these diets were within an acceptable range.

Mortality: Mortality was similiar for all groups.

Body Weight

Although one value for males and one for females were statistically significantly different from control values, the values were at different time periods and do not indicate a dose-response.

Hematology

No statistically significantly different values for either males or females at months 1, 2 or 3 were noted among the hematologic parameters: erythrocyte count; hematocrit, hemoglobin concentration, leucocyte count, differential leucocyte count, platelet count, reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

Clinical Chemistry

No statistically significantly different values were noted for either males or females at month 1, 2, or 3 for following clinical chemistry parameters: gamma-glutamyl transpeptidase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and albumin concentration. However there was a noted increase in SGPT for the 8000 ppm females over the 1, 2 and 3 month examinations when compared to controls as follows:

Mean SGPT Females

Time	Control	8000 ppm
Baseline	27.5 ± 11.5	-
1 month	27.5 ± 7.6	36.2 ± 16.0
2 month	30.7 ± 8.5	64.9 ± 39.4
3 month	32.6 ± 19.7	52.3 ± 17.3

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Organ Weight

Absolute and relative organ weights were reported for brain, heart, kidneys, and gonads for mice sacrificed after 90 days on test. "Tissues and organs of animals sacrificed at 1 and 2 months were discarded following gross examination." Significant differences were noted for the liver. Significant differences were seen for both males and females fed 4000 or 8000 ppm.

MEAN ABSOLUTE AND RELATIVE LIVER WEIGHT DATA FOR MICE FED FMC 57020

Treatment Level (ppm)	Fasted Body Weight (g)	Absolute Liver Weight (g)	Liver/ Body Weight Ratio (%)	Liver/ Brain Weight Ratio(%)
Males				
0	31.9	1.42 (100)	4.45 (100)	2.26 (100)
20	32.4	1.45 (102)	4.40 (99)	2.67 (118)
100	32.7	1.51 (106)	4.62 (104)	2.82 (125)
500	33.0	1.52 (107)	4.62 (104)	2.79 (123)
1000	32.4	1.53 (108)	4.74 (107)	2.74 (121)
2000	33.0	1.61 (113)	4.88 (110)	2.96 (131)
4000	32.5	1.80 (127)	5.53 (124)	3.29 (146)
8000	31.7	1.81 (127)	5.73 (129)	3.26 (144)
Females				
0	25.3	1.09 (100)	4.30 (100)	2.04 (100)
20	25.1	1.08 (99)	4.25 (99)	2.01 (99)
100	25.5	1.11 (102)	4.39 (102)	2.01 (99)
500	25.9	1.14 (105)	4.35 (101)	2.07 (101)
1000	25.5	1.20 (110)	4.85 (113)	2.17 (106)
2000	24.7	1.23 (113)	4.98 (116)	2.32 (114)
4000	26.2	1.35 (124)	5.14 (120)	2.47 (121)
8000	24.6	1.45 (133)	5.86 (136)	2.69 (132)

()= liver weights expressed as precent of control

Dose-related increased liver weight was observed in both sexes. This became apparent when liver weights were expressed as percent of control. In the males absolute liver weight was increased in a dose-related fashion at all doses, relative to body weight at 100 ppm and higher doses and relative to brain weight at all doses. In the females, liver weight, absolute and relative to body weight was increased at 100 ppm and higher doses and relative to brain weight at 500 ppm and higher doses.

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PATHOLOGY

Tissues from the 0, 20, 500, 2000 and 8000 ppm treated aniamls were examined histologically at 3 months; mice fed 100, 1000, or 4000 ppm were not examined histologically.

The incidence of liver megalocytosis was increased in the 20, 2000 and 8000 ppm treated males and the 8000 ppm treated females when compared to respective controls. This data was taken from the individual animal data, however, this effect was only noted in the Petitioner's summary table at the 8000 ppm dose in both sexes. This lesion is an indication of cell degeneration which may be an adaptive change rather than a toxic reaction and should be further examined in the final outcome of the 2-year feeding study.

Histopathology of Mice Fed FMC-57020 for 90 Days

	MALES					FEMALES				
Dose (ppm)	0	20	500	2000	8000	0	20	500	2000	8000
Liver megalocytosis	0/19	3/20	0/20	3/20	11/20	0/19	0/20	0/20	0/20	3/19

Conclusion:

The incidence of liver megalocytosis was increased in the 20, 2000 and 8000 ppm males and the 8000 ppm females. In addition, in the males, absolute liver weight was increased in a dose-related fashion at all doses, relative to body weight at 100 ppm and higher doses and relative to brain weight at all doses. In the females, liver weight, absolute and relative to body weight was increased at 100 ppm and higher doses and relative to brain weight at 500 ppm and higher doses.

NOEL = Not established

Classification: Minimum. (In the Registrant's submitted Table 18, Page 22 and page 24 have identical information, the Registrant is requested to provide the missing information on the testes.)

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John Hexter
MITRE Corporation
5-16-84

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* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not assigned

Study Type: Teratogenicity in Rabbits

Accession Number: ~~Not specified~~ 071063

MRID Number: Not assigned

Sponsor: FMC Corporation

Contracting Lab: WIL Research Laboratories Incorporated
1407 Montgomery Township Road
Ashland, Ohio 44805

Date: September 14, 1982

Test Material: FMC 57020 (technical; 88.8 % pure)

Protocol:

The following description of the materials and methods used for this study was abstracted and paraphrased from the original report.

FMC 57020 (technical grade, 88.8% pure) (hereafter referred to as FMC) was obtained from FMC Corporation, Princeton New Jersey on December 7, 1981 as a golden-colored liquid. The complete chemical composition of the remaining 11.2% of the material was not supplied with the original report. The test material was maintained under refrigerated conditions and was stated to be stable for one month.

Ninety-two virgin New Zealand white female rabbits were received from Langshaw Farms, Augusta, Michigan on December 28, 1981.

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The rabbits were approximately five months of age upon receipt at WIL Research Laboratories. They were allowed to acclimate to laboratory conditions for 56 days prior to the initiation of the experiment. The does were housed individually in stainless steel wire-mesh bottom cages which were suspended above deotized board. The ambient room temperature was maintained between 69 to 75° F and the relative humidity was maintained at approximately 42%. Filtered fresh air was changed approximately 10 times per hour throughout the entire study period. The light cycle was maintained at 12 hours of light to 12 hours of darkness. During the acclimation period the rabbits were allowed free access to municipal drinking water provided by automatic watering system and a ration of 150 grams of diet per day (Purina Certified Rabbit Chow No. 5322).

The breeding males for this study were six proven sires from whom spermatozoa were collected for use in the artificial insemination technique. Semen was collected from an individual male and diluted; the diluted semen from a particular male was used to inseminate an equal number of females from each treatment group per day. The artificial insemination procedures were carried out on five separate days.

Following the acclimation period, 72 females which appeared to be in good health were selected at the discretion of the study director for artificial insemination. The does weighed between 3.0 to 4.5 kg and were inseminated by the following method. On the day of insemination approximately 0.25-0.5 ml of diluted semen was introduced into the anterior portion of the vagina by means of a glass insemination pipet. Immediately thereafter each doe was injected intravenously with 100 USP units of human chorionic gonadotropin.

The animals were assigned to different treatment groups (18 per group) by means of a computer generated random numbers procedure which stratified the animals according to weight classes. During the period of presumed gestation the animals were allowed free access to food throughout the remaining portion of the study. Water was allowed ad libitum.

The rabbits were observed for physical signs and general appearance on a daily basis throughout the acclimation period and during gestation. FMC was administered via gastric intubation to does on gestational days 6-12 at dosages of 0, 30, 240 or 1000 mg/kg/day. Due to excessive maternal weight loss and mortality in the high dose group, the dosage was reduced from 1000 mg/kg to 700 mg/kg on gestational day 13. Thus, FMC was administered at doses of 30, 240, or 700 mg/kg/day on gestational days 13-16. The dosages

were based upon the maternal body weight on gestational day 6. All dosage solutions were comprised of suspension of FMC in 1% aqueous methylcellulose. The total volume of dosage solution was maintained at 4 ml/kg body weight. Control animals received a gavage of 4 ml/kg of 1% aqueous methylcellulose. The different dosage solutions of FMC were prepared prior to initiation of dosing and then at weekly intervals thereafter. In all cases the FMC solution provided by the sponsor was weighed into an Erlenmeyer flask with no adjustment made for purity. Then the flask was filled to the appropriate volume with the vehicle solution. The authors did not indicate whether or how often samples were withdrawn for analysis of homogeneity, stability, or proximity to the the target concentrations. The dosage solutions were maintained under refrigerated conditions.

Throughout gestation the animals were observed on a daily basis for signs of general appearance, behavior, moribundity, survival and clinical signs of toxicity once dosing had begun. In addition to general signs as described above the does were weighed on gestational days 0, 6, 12, 18, 24 and 29 just prior to sacrifice. The mean body weights and body weight changes were calculated for each group. Does which died prior to the time of scheduled sacrifice were necropsied in order to determine the possible cause of death. There were

discrepancies in the description of the methods concerning those animals which aborted. On page 6 of the original document, the authors state that all does which aborted were sacrificed and necropsied on the day on which the abortion occurred. However, on page 9 they state that females which aborted were sacrificed at the scheduled time for sacrifice (i.e. day 29) with the other animals and that they were examined for evidence as to why they may have aborted.

On gestational day 29 the does were killed by intravenous injection of T-61 euthanasia solution. The abdominal cavity of each doe was laparotomized and the uterus was exteriorized to examine its contents. The uterus was scored for the presence, sites, and numbers of implantations, resorptions (both early and late) and living and dead fetuses. The ovaries were examined with the aid of a low power microscope to determine the number of corpora lutea. Subsequently, the gravid uteri were removed and opened; each individual fetus was identified with a tag, examined for viability, weighed, and examined for any gross external anomalies. The crown rump length of each fetus was measured as was the crown rump length of all late resorptions and each fetus was examined for soft tissue anomalies of the thorax and abdomen by the Staples dissection technique. The eviscerated fetuses were skinned and the brain was removed by an

undescribed method. The brain was subsequently subjected to examination by a mid-coronal slice. The carcass of each fetus was fixed in 95% isopropyl alcohol subjected to the alizarin red S staining technique for visualization of osseous structures.

The following statistical analyses were performed on the data. All analyses used the two-tailed test for significance at a significance level of $p \leq 0.05$.

- Male/female sex ratios of live fetuses were compared by the Chi-square test with Yates's correction factor.
- The numbers of litters with malformations and/or variations were compared by Fisher's exact test.
- The incidence of early and late resorptions, dead fetuses, and post-implantation loss, were compared by the non-parametric Mann-Whitney U-test.
- Mean numbers of corpora lutea, implantations, viable fetuses, mean fetal and maternal body weights, and maternal body weight changes were analyzed by a one-way analysis of variance followed by Dunnett's test.

RESULTS AND DISCUSSION

This study appeared to be a well-designed, well-executed, and well-documented investigation. Only minor discrepancies were noted. In particular there is a discrepancy concerning when animals which aborted were necropsied. This descrepancy appears to be of a descriptive nature and can be straightened out easily by requesting clarification from the registrant. The outcome of such information should not impact on the evaluation of the data in this study.

Relatively few clinical signs were observed in the lower two treatment groups and the control group. The clinical signs which were observed in those groups were distributed relatively evenly among all treatment and control groups and included such things as nasal discharge, lacrimation, and loose feces. Some signs, however, showed a preponderance in the high dose group. These included ataxia (which occurred in two animals), red discharges of the vagina (observed in four does, three of which later aborted) and decreased or lack of defecation. The authors did not give the exact incidences of these clinical findings.

In addition there were two other findings which were not considered to be related to treatment. One rabbit in the low dose group had an abscess of the dulap (the flap of skin under the chin); one high dose animal exhibited a necrotic fallopian tube at sacrifice.

Four animals died prior to scheduled sacrifice and an additional four animals aborted during this study. Seven of those eight animals were in the high dose group. Three pregnant and one non-pregnant doe from the high dose group died prior to sacrifice and three high dose animals aborted. The last abortion was one in a control doe. The maternal deaths and abortions of the high dose are considered to be related to FMC treatment.

The body weight and weight gain data for pregnant rabbits are presented in Table 1. There were no significant differences among the mean body weights for all groups of animals at the initiation of study; at the initiation of dosing on day 6; or after the period of dosing on days 24 and 29. However, there were significant differences between the mean weights of the animals in the high dose group when compared to the other groups on gestational days 12 and 18. In particular, due to the mortality and loss of weight among animals of the high dose group, the dose was reduced from 1000 mg/kg/day to 700 mg/kg/day, on gestational day 13 . The effect on body weights can be seen by comparing the mean change in body weights for animals over the period of treatment (days 6-18) and over the entire period of gestational (days 0-29). Inspection of Table 1

TABLE 1

MEAN MATERNAL BODY WEIGHTS AND BODY WEIGHT CHANGES FOR RABBITS INTUBATED WITH VARIOUS DOSES OF FMC DURING GESTATIONAL DAYS 6-18

Treatment	No. of Pregnant Does	Mean Body Weight (g) at Gestational Day						Mean Change in Body Weight For Surviving Pregnant Does Over Period			
		0	6	12	18	24	29	Treatment (day 6-18) g	%	Gestational (day 0-29) g	%
Control	16	3713	3892	3931	4046	4071	4126[a]	154	4	434[a]	12
FMC (mg/kg/day)											
30	14	3827	3976	4115	4195	4258	4328	219	6	501	13
240	16	3816	3931	3951	4027	4094	4117	96	3	301	8
700/1000	16	3702	3838	3615[d]	3538[d]	3926[b]	4020[c]	-300[d]	-8	256[c]	7

[a] N = 15; one doe aborted on day 29.
[b] N = 11; 2 does aborted (days 21 and 24) and 2 does died (day 19 and 20).
[c] N = 10; 1 doe died on day 26.
[d] Significantly lighter than controls $p < 0.01$ (ANOVA; Duncan's Multiple Range Test) (Statistics calculated by MITRE).

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reveals that during the period of treatment the animals in the highest dose group lost approximately 8% of their intial body weights (300 g) as opposed to the animals in the other groups which all gained from 3 to 6% of their body weights (96 - 219 g). By the end of gestation, however, the body weights of animals in the high dose group had caught up to those of the other group; thus, over the entire period of gestation the high dose animals gained a total of 7% of their body weight compared to 12% gained in the control animals. Based upon the maternal body weight effects in the high dose group and based upon the increased abortions and deaths in that group the low effect level for FMC in pregnant rabbits appears to be 700 mg/kg/day. The NOEL is 240 mg/kg.

Pertinent gestational data for control and FMC treated litters are summarized in Table 2. The pregnancy rates (percent of inseminated does which were pregnant) were similar among all groups. The mean numbers of corpora lutea and implantations per litter were not adversely effected by treatment in FMC. In fact, FMC treatment was associated with an increased number of implantations per litter in all three groups. Interestingly, the implantation rates (percent of corpora lutea which were implanted) were also higher among the FMC-treated litters. It should be noted that the identification of corpora lutea in the rabbit ovaries is a difficult exercise

TABLE 2

GESTATIONAL DATA FOR RABBITS INTUBATED WITH VARIOUS DOSES OF FMC DURING GESTATIONAL DAYS 6-18

Treatment	No. Fertilized/ No. Inseminated (%)	Cases of Maternal Mortality	No. Aborted	No. Litters Evaluated	Corpora Lutea		Implantations			No. Litters Exhibiting Resorptions	
					Total	Mean/Litter	Total	Mean/ Litter	Rate[a] (%)	Partial	Complete
Control	16/18 (89)	0	1	15	191	12.7	98	6.5	51	6	0
FMC (mg/kg/day)											
30	14/18 (78)	0	0	14	173	12.4	102	7.3	59	8	1
240	16/18 (89)	0	0	16	192	12.0	117	7.3	61	5	0
700/1000	16/18 (89)	3P+1NP[b]	3	10	174	11.4	73	7.3	42	5	0

[a] Implantation Rate = $\frac{\text{No. Implantations}}{\text{No. Corpora Lutea}} \times 100$

[b] P = Pregnant; NP = not pregnant

because the follicles in rabbit ovaries are in all stages of development regardless of whether or not the animal has mated. Since Graafian follicles closely resemble the corpora lutea in the rabbit ovary it is possible that these may have been mistaken for corpora lutea. This is in distinction to the rat or the mouse in which the corpora lutea and follicles are under strict control of the estrus cycle. Thus, it is possible that some mistakes in correctly identifying corpora lutea may have been responsible for the low implantation presented in Table 2.

Similarly, FMC treatment was not associated with adverse changes in the percent of animals which were fertilized nor in the number of litters which exhibited resorptions. The low dose FMC group exhibited highest incidence of litters with resorptions, and it did exhibit one litter which was completely resorbed. It should be noted, however, that the completely resorbed litter was comprised of only a single implantation site. Thus, the finding of a completely resorbed litter in this case is not considered to be evidence of maternal toxicity.

The embyrotoxicity data for FMC are presented in Table 3. FMC caused no changes from controls in the incidence of live fetuses per litter or in mean fetal weights. The male/female sex ratios were not different from the expected values of 50% in any group. The litters with the smallest percent of male fetuses were found in the control group (42%). Some differences were noted in the distribution of

TABLE 3

EMBRYOTOXICITY DATA FOR OFFSPRING OF RABBITS INTUBATED WITH VARIOUS DOSES OF FMC DURING GESTATIONAL DAYS 6-18

Treatment	Total Implantations	Resorptions			Dead Fetuses	Percent Dead or Resorbed	Live Fetuses					Male:Female Ratio of Live Fetuses (% male)
		Early	Late	Total			Total	Mean No./ Litter	Mean Weight(g)	Malformed No.	(%)	
Control	98	4	2	6	0	6	92	6.1	40.0	2	(2)	39/53 (42)
FMC (mg/kg/day)												
30	102	8	9[a,d]	17[c,d]	1	18	84	6.0	40.4	4	(5)	36/48 (43)
240	117	4	3	7	0	6	110	6.9	37.5	5	(5)	56/53[e] (51)
700/1000	73	4	9[b,d]	13[c,d]	0	18	60	6.0	37.0	0	(0)	30/30 (50)

[a]Statistically greater than control, $p \leq 0.05$, Fisher's Exact Test (calculated by MITRE).
[b]Statistically greater than control, $p \leq 0.01$, Fisher's Exact Test (calculated by MITRE).
[c]Statistically greater than control, $p \leq 0.02$, Fisher's Exact Test (calculated by MITRE).
[d]Not significant by the Wilcoxon Rank Sum method (calculated by MITRE).
[e]Sex could not be determined for one fetus.

distribution of resorptions between control and FMC treated litters. There were no significant differences for early resorptions in any of the groups, however, the low and high dose FMC treatment groups had significantly more late resorptions then did either the mid dose or control groups. The incidence of late resorptions was statistically significant by Chi-square and Fisher's exact in both cases at $p \leq .02$. The finding of excess resorptions in the low dose group is somewhat perplexing. This is especially true in light of the fact that there were no other signs of toxicity seen in FMC treatment. For instance, the number of malformed fetuses or fetuses exhibiting anatomical variations were not different among any of the groups when compared to the control group. Similarly, there were no indications of maternal toxicity in the low dose group. Although, the number of litters which exhibited resorptions were elevated in the low dose group, incidence was not statistically significant from either control or other treated groups. This may mean that there is a litter effect. Consequently, MITRE re-analyzed the resorption data using the Wilcoxon Rank Sum method. This method is a non-parametric method which helps to correct for possible litter effects. The results of that analysis revealed no statistically significant difference between any treated group and the control group. This suggests the presence of a litter effect for resorptions.

Treatment with FMC was not associated with statistically significant increased incidences of malformations. Two control fetuses exhibited skeletal malformations, including one case of fused sternebrae and one case of fused vertebrae. There were four skeletal malformations in the low dose group, including one case each of fused vertebrae, malformed scapulae, and prematurely fused bones of the skull, and two cases of fused sternebrae. The mid-dose group exhibited four fetuses which were malformed, including one fetus with absence of the gonads, two fetuses with fused sternebrae, one fetus with bifid ribs, and one fetus with both a bifid rib and fused sternebrae. There were no malformations in the high dose group.

The anatomical variations reported by the authors were spread evenly among all groups and are typical of the variations seen among rabbits. These included variations in the number of ossified and unossified sternebrae, the number of pairs of ribs (12 or 13), accessory blood vessel, absence of the gall bladder, and variations in the ossification or alignment of the hyoid bones. Thus, no evidence of teratogenicity was observed in any dose group and FMC can be considered not teratogenic to rabbits under the conditions of the current study at doses as high as 700 mg/kg/day.

CONCLUSIONS:

In this teratology study FMC (technical grade 88.8% pure; 11.2% unidentified ingredients/impurities) was mixed with 1% aqueous methylcellouse and administered by gastric intubation to group of 18 inseminated New Zealand white rabbits at nominal dosages of 30, 240, and 700/1000 mg/kg/day on gestational days 6-18. Control does received 1% aqueous methylcellouse only. The volume of dosage solutions was maintained at 4 ml/kg body weight. Few clinical signs were observed in the lower treatment groups throughout gestation, however, treatment with the highest dose of FMC was associated with increased abortions, maternal ataxia, red vaginal discharge, decreased or absent defecation and maternal deaths. The high dose of FMC was also associated with a decreased gain in maternal body weight especially seen over the early days of treatment, (days 6-13). After day 13, and due to the evident toxicity in the high dose group, the high dose treatment was reduced to 700 mg/kg/day. The maternal body weight gain over the entire period of treatment was greatly reduced in the high dose group (-8%). Based on these data the maternal systemic low effect level for FMC in pregnant rabbits appears to 700 mg/kg/day. The NOEL is 240 mg/kg/day.

FMC treatment was associated with an increased number of late resorptions as seen in both the low and high dose treatment groups.

However, no adverse effects were seen in terms of the mean fetal weights, distribution of male and female pups, presence of excessive anatomical variations nor the presence of increased malformations. The malformations which were observed in the treated groups are the malformations which are commonly seen among rabbits and were restricted by primarily to skeletal system including fusion of the vertebrae and bifurcation of the ribs. One fetus exhibited apparent agenesis of the gonads. From these data FMC does not appear to be teratogenic to pregnant rabbits at doses at least as high as 700 mg/kg/day under the conditions of this study.

CORE Classification: Core Guidelines.

003946

EPA: 68-01-6561
TASK: 53
August 10, 1984

DATA EVALUATION RECORD

FMC 57020 Technical

Mutagenicity (Unscheduled DNA Synthesis)

CITATION: Thilager, A. 1983. Unscheduled DNA Synthesis assay of FMC 57020 in Rat Primary Hepatocytes. A report on Study A 83-1036(T210.380) prepared by Microbiological Associates for FMC Corporation. Direct [illegible]

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36/68

DATA EVALUATION RECORD 003946

STUDY TYPE: Mutagenicity (Unscheduled DNA Synthesis).

CITATION: Thilager, A. 1983. Unscheduled DNA Synthesis assay of FMC 57020 in Rat Primary Hepatocytes. A report on Study A 83-1036 (7210.380) prepared by Micrcbiological Associates for FMC Corporation.

ACCESSION NUMBER: 072067.

LABORATORY: Microbiological Associates, 1530 East Jefferson Street, Rockville, Maryland 20852.

QUALITY ASSURANCE STATEMENT: Present, signed and dated, 9/19/83.

TEST MATERIAL: The test material was identified as FMC 57020 Technical (Tox. Sample) Lot No. E-1756-146-20 and reported to be 88.8 percent pure. It was a yellow liquid at room temperature.

METHODS:

Preparation of test materials: The test material, FMC 57020 Technical, was dissolved and diluted in ethanol to the appropriate concentrations just prior to use in the assays. The compound assayed at concentrations of 7.5, 5.0, 1.0, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 and 0.0005 µl/ml in unscheduled DNA synthesis (UDS) assay and in the parallel toxicity test.

Preparation of primary cell cultures: Rat hepatocyte cell (HPC) cultures were obtained using a modification of the procedure of Williams[1]. Rats were killed by metofane inhalation, dissected and perfused with 0.5mM EGTA solution, and the perfusion continued with a collagenase solution. After removal of the liver and dissociation of the cells, the cells were counted and seeded into 35 µl dishes with coverslips. The adjusted viable cells per dish was 5.0×10^5. After seeding the cell suspension in Williams medium E (WME) supplemented with 10 percent fetal bovine serum, 10 mU insulin/µl, 2 mM L-glutamine, 100 units of penicillin and 100 µg of streptomycin, the cultures were incubated at 37 ± 1° C in a humidified 5 percent CO_2 atmosphere for 2 hours. The cultures were then washed and resuspended in serum-free medium before use in the assay.

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[1] Williams, G. 1977. In Vitro 13:809-817.

Control Chemicals: The positive control chemical was 2-acetylaminofluorene (2-AAF) of lot no. H3 from Aldrich Company. It was solubilized in ethanol. The solvent (negative) control was ethanol.

Preliminary Cytotoxicity: Ten doses of FMC 57020, ranging from 0.0005 µl/ml to 7.5 µl/ml were used to treat duplicate HPC cultures although the authors stated that "Three replicate plates were used for counting at each dose level." This treatment was administered 2 hours after seeding, and eighteen hours later the cultures were washed twice with Ca^{++} and Mg^{++} free phosphate buffered saline (PBS). The cultures were then "tryosinized, stained with trypan blue and counted in a hemacytometer." Comparisons of survival in the treated and control groups were used to determine the relative survival indices (RSI) by the following formula:

$$RSI = \frac{\text{Viable test culture cells (Ave.)}}{\text{Viable solvent control cells (Ave.)}} \times 100$$

Unscheduled DNA Repair Assay: Triplicate plates were seeded with 5.0×10^5 HPC/plate and with five decreasing dose levels of the test material ranging from 0.10 to 0.0001 µl/ml, delivered from a 100 x stock solution to the plate in serum-free WME. In addition, ethanol treated (solvent control) and 2-AAF-treated (2 µg/ml and 20 µg/ml) triplicate plates were included in the assay. The treatment medium for the UDS assay contained 10 µCi/ml of ^{3}[H]-thymidine. The plates serving as parallel to toxicity controls lacking ^{3}[H]-thymidine had 2 ml of the test material delivered directly to the plate in serum-free WME. Parallel triplicate cultures at each dilution were treated so that the RSI could be determined.

After exposure for 18 hours, the UDS assay cells were washed in serum-free WME, swelled in 1 percent sodium citrate, and fixed in ethanol-acetic acid on coverslips. The coverslips were mounted with the cells up and air dried. After coating the slides with Kodak emulsion and storage at 4° C for 10 days in light tight boxes containing desiccant, the slides were developed in Kodak D19, fixed in Kodak fixer and stained in hematoxylin-sodium acetate-eosin.

Slide Scoring: A colony counter was used to read the slides "blind"; nuclear grains were counted in 25 cells on each of the three coverslips per treatment in random areas of the slide. From each grain count was subtracted the average cytoplasmic grain count (from three adjacent nucleus-sized areas) in order to determine the net nuclear counts. If the nuclei were intensely blackened by grains, they were not counted because this was interpreted as replicative [rather than repair] chromosome synthesis. Also cells with nuclei exhibiting cytotoxic effects of treatment, such as irregular shape, disrupted membranes, or diminished size (< 4.0 mm^2) were not counted.

Data Evaluation and Statistics: If the mean net nuclear count at a given dose level was increased by at least five counts over the control, the result would be considered significant. If a dose-related increase was observed in at least two successive increasing doses, the test material was considered to induce a positive response. If only one dose level showed an increase in the mean net nuclear count, the substance was considered marginally positive. A negative response was considered to be one in which there was no "significant increase in the mean net nuclear grain counts at any dose level."

RESULTS:

In the preliminary cytotoxicty assay, the FMC 57020 sample had relative toxicities (RT) of 87.0 percent at 7.5 µl/ml and 13.0 percent at 0.0005 µl/ml. The highest dose selected for the UDS assay was 0.10 µl/ml, at which the RT was 77.2 percent. The RSI values for the test material were 2.4, 4.2 and 16.0 at doses of 7.5, 0.10 and 0.0005 µl/ml, respectively. The RT values for both the solvent control (ethanol) and the untreated control (WME) were 100 percent and the RSI values for both of these controls were 18.4.

In the parallel toxicity test for the UDS assay, the average RT values of three replicates of 5 doses of FMC 57020 ranged from 88.6 to 2.6 percent at the highest dose (0.10 µl/ml) and the lowest dose (0.001 µl/ml), respectively. The RT values for the controls were: ethanol (0.0 percent); 20 µg/ml, 2-AAF (57.9 percent); and 2 µg/ml, 2-AAF (32.46 percent). The RSI values were 22.8, 9.6 and 15.4 for ethanol, 20 µg/ml 2-AAF and 2 µg/ml 2-AAF, respectively.

The study showed that none of the test doses of FMC 57020 caused a significant increase in the mean net nuclear grain counts of 3 replicate slides for which 25 nuclei per slide were counted. The solvent control had a mean net grain count of 0.4 versus net grain counts in the test material ranging from 0.6 (at 0.001 µl/ml) to 0.9 (at 0.10 µl/ml). The mean net grain counts for the positive controls (2-AAF) at both 20 and 2 µg/ml, were 39.6 and 34.1, respectively. For the WME untreated control, the mean net grain count was -0.2 after correcting for background.

DISCUSSION:

The author concluded that technical grade FMC 57020 (Tox. Sample) at a dose range of 0.001 to 0.10 µl/ml did not induce a significant increase in unscheduled DNA synthesis under the conditions of the assay. Under the same experimental condicions, the positive control (2-AAF) at 2 and 20 µg/ml, induced a significant mean net nuclear grain count over the solvent control (ethanol). It was also reported that the solvent control values were not significantly increased over the untreated control.

Our assessment is that the test material, FMC 57020 Technical, was assayed by a system that could detect DNA damage by the UDS assay method. The positive, solvent, and negative control data were all within acceptable ranges and sufficient samples were assayed. The dose range chosen for the assays was based on a cytotoxicity assay which showed that 23 percent of the cells survived treatment with 0.1 µl/ml of the test material (the highest dose). Since the dose range included doses where approximately 37 percent of the cells survived, it was an appropriate range for testing UDS.

CONCLUSIONS:

Under the conditions of this study, FMC 57020 Technical, did not induce unscheduled DNA synthesis.

CLASSIFICATION: Acceptable.

003946

EPA: 68-01-6561
TASK: 53
August 10, 1984

DATA EVALUATION RECORD

FMC 57020 Technical

Mutagenicity (In Vivo Cytogenetics Assay)

CITATION: Putman DL and Moore WA. 1982. In vivo cytogenetic assay of FMC 57020 technical in rats. A report on FMC study A82-778 prepared by Microbiological Associates for FMC Corporation. FMC Report No. A82-778. Dated 12-

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity (in vivo cytogenetic assay).

CITATION: Putman DL and Moore WA. 1982. In vivo cytogenetic assay of FMC 57020 technical in rats. A report on FMC study A82-778 prepared by Micro- biological Associates for FMC Corporation.

ACCESSION NUMBER: 072067.

QUALITY ASSURANCE STATEMENT: Present, signed and dated 2/28/83.

LABORATORY: Microbiological Associates, 6221 River Road, Bethesda, Maryland 20816.

TEST MATERIAL: FMC 57020 Technical (88.8 percent pure) Lot No. EL 756-146-20, FMC Reference A82-778 was the material tested in this study. It was described as "an amber semi-viscous liquid with a crystalline precipitate". This material was assigned the code number T1839. "At the time of testing, T1839 was described as a viscous amber liquid (at 50° C)."

METHODS:

Preparation of Test Material

The test material, FMC 57020 Technical, was stored under refrigeration until the day before administration, "at which time it was warmed overnight at 50° C to liquify." The test material was mixed with USP grade corn oil, which served as the carrier vehicle. It was administered at dosages of 1,000, 2,000, 3,000, or 4,000 mg/kg/day in the dose range finding assay and at 200, 667, and 2,000 mg/kg/day in the cytogenetic assay.

Positive Control

The positive control was triethylenemelamine (TEM) of lot 02031 from Polysciences, Inc., Warrenton, PA. It was dissolved in sterile distilled water.

Animal Phase

Male Sprague Dawley rats, weighing 200 to 250 grams were obtained from Charles River Breeding Farms, Kingston, New York. They were monitored for general health, parasites, and various infectious microorganisms, and kept under quarantine for 10-14 days. Animals used in the study were assessed

to be in good health prior to initiation of the study. They were housed in "an AAALAC-accredited facility with a controlled environment of 74 ± 6° F, 50 ± 20 percent relative humidity, and a 12 hour light/dark cycle." There were three to four animals per cage during quarantine, but thereafter they were housed singly in autoclavable plastic cages with wire lids and contained hardwood chip bedding. Water and certified laboratory rodent chow were provided ad libitum.

Using a random number table, five animals each were assigned to five groups (three treatment groups, one positive control group, and one vehicle control group), appropriately tagged, and identified individually. Each animal received by gavage either the vehicle or a formulated test material-vehicle mixture at a volume rate of 5 ml/kg/day for five consecutive days. The positive control, TEM, was administered by a single ip injection of 0.5 mg/kg at one day prior to sacrifice. In the preliminary toxicity assay, the treatment levels of T1839 were 1000, 2000, 3000 and 4000 expressed in mg/kg/day; a corn oil vehicle control group was included. In the assay for cytogenetic activity, the treatment levels expressed in mg/kg/day were 200, 667, and 2,000 for T1839; 0.5 for TEM; and corn oil at 5 ml/kg/day.

At an interval two to four hours after the five day dosing regimen was completed, colchicine was injected ip at a dosage of 1 mg/kg to arrest bone marrow cells in the metaphase stage. The animals were then killed using carbon dioxide asphyxiation and their femurs were exposed and cut just above the knee. Their bone marrows were aspirated into syringes containing Hank's balanced salt solution (HBSS) and transferred to a capped centrifuge tube containing 5 ml of cold HBSS. After thoroughly mixing the cells in HBSS, the tubes were maintained throughout the collection period in an ice bath.

Cytogenetic Phase

The bone marrow cell-HBSS mixture was centrifuged at 100 x g for 10 min, the supernatant fluid removed and discarded, and the cells resuspended in 5 ml of 0.075M KCl (held at 37° C) and then incubated for 10 min at 37° C. The cell suspension was again centrifuged and the cell pellet was resuspended in fresh Carnoy's fixative. These cell suspensions were held for 30 min, centrifuged, the supernatant decanted, and 5 ml of fresh fixative added to each tube. Each tube was capped and stored overnight at 4° C. Next the cells were centrifuged at 100 x g for 10 min, the supernatant fluid decanted, and the cells resuspended in approximately one ml of fixative. Two or three drops of this suspension were delivered to a glass slide; the slide was air dried, stained with 4 percent Giemsa and permanently mounted.

At least three stained slides, coded without regard to treatment group, were scored for chromatid and chromosome breaks, gaps, fragments, structural rearrangements, and ploidy using a minimum of 50 metaphase cells from each animal. For a minimum of 500 cells, the proportion of mitotic cells was determined and the mitotic index (mitotic cells per total cells x 100) calculated.

Evaluation

For each animal the mitotic index, modal chromosome number, types and numbers of aberrations, and percentage of damaged cells were reported. Chi-square analysis was used to ascertain significant differences between the number of cells with aberrations in treatment groups and control groups. Although data for chromatid and chromosome gaps were presented, they were excluded from the calculations of the average number of aberrations per cell and in the total percentage with one or more aberrations. Pairwise comparisons of the number of aberrations per cell in each treatment group to the vehicle control group were made by using the t-test. The samples were treated as independent random entities with unequal variance and compared to the vehicle control groups for statistically significant differences. A positive response was a significant increase ($p < 0.05$) in the percentage of cells with chromosomal aberrations in any treatment group relative to the control by Chi-square analysis and also a significant increase ($p < 0.05$) relative to the vehicle control by the t-test.

Statistical analysis was to be performed on a valid assay in which the following criteria were met:

1. There were no more than 4 percent of the vehicle control cells with "aberrations of any type, other than gaps."

2. The positive control cells had to show a statistically significant increase ($p < 0.05$) relative to the solvent control by the Chi-square analysis.

RESULTS:

Whole Animal Responses

From the preliminary toxicity studies, a high dosage of 2,000 mg/kg/day of FMC 57020 was chosen for the cytogenetic assay because mortality was excessive at 4000 and 3000 mg/kg/day, i.e., 5/5 and 4/5 rats, respectively, died. Although there were no mortalities at dosages of 2000 mg/kg/day and lower, the weight gain of the 2000 mg/kg/day treatment group was only 90 percent of the control group at five days.

During the cytogenetic assay, one rat in the T1839 high dosage group died after two gavage administrations. However, the test material was not considered the cause of death because no gross lesions were revealed at necropsy. Excessive salivation was a toxic clinical sign observed in the 2000 and 667 mg/kg/day treatment groups.

Cytogenetic Responses

The modal chromosome number was 42 for every rat in all groups. The mitotic index averaged 4.72 in the corn oil-treated animals versus an average of 2.84 in the TEM-treated animals; in the T1839 dosed groups the

mitotic index averages were 4.72, 4.84, and 4.64 at 2000, 667, and 200 mg/kg/day, respectively. For chromatid gaps, the averages per animal in each group were: corn oil (0.2); 0.5 mg TEM (2.6); FMC 57020 treatment groups were 2,000 mg (0.75), 667 mg (0.0), and 200 mg (0.2).

The animals treated with 0.5 mg/kg TEM each averaged 8 chromatid breaks, 2 fragments, 2.6 rearrangements (all exchange figures), and 3.6 severely damaged cells per 50 bone marrow cells examined. None of these types of chromosomal aberrations were seen in the corn oil or FMC 57020 treatment groups in which the same number of bone marrow cells were examined.

DISCUSSION:

The authors concluded that FMC 57020 gavage treatment at 200, 667 and 2,000 mg/kg/day had no effect on the modal cell number on the mitotic index and did not increase the number of chromosomal aberrations over the corn oil (negative control) treatment animals. However, TEM reduced the mitotic index and "induced an average of approximately one aberration per cell with approximately 15 percent of all cells analyzed containing one or more aberrations." Hence, they concluded that the positive and negative controls fulfilled the requirement for a valid assay and that "under the conditions of the assay described in this report, T1839 [FMC 57020] was negative in the in vivo cytogenetics assay."

Our assessment is that the authors conclusions were correct and valid for the data reported. Although the data showed that FMC 57020 did not cause cytogenetic damage to the bone marrow cells and the data supported a sufficiently clear interpretation, the results of the statistical analyses were not reported. Furthermore, it is noted that although ip administration of TEM, the positive control, is a commonly accepted procedure, this route differs from the gavage route by which the test material was administered.

CONCLUSIONS:

Under the conditions of the assay, FMC 57020 Technical (88.8 percent pure) did not induce chromosomal aberrations in Sprague-Dawley rats within a dose range of 200 to 2000 mg/kg/day when administered by gavage for five consecutive days.

CLASSIFICATION: Acceptable.

003946

EPA: 68-01-6561
TASK: 53
August 10, 1984

DATA EVALUATION RECORD

FMC 57020 Technical

Mutagenicity/Salmonella

CITATION: Haworth SR, Lawlor TE, Smith JK, et al. 1980. Salmonella/ mammalian microsome plate incorporation mutagenesis assay. Report on Study No. 013-679-407-1 prepared by EG and G Mason Research Institute for FMC Corporation. [illegible]

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity (Bacterial Gene Mutation).

CITATION: Haworth SR, Lawlor TE, Smith JK, et al. 1980. Salmonella/mammalian microsome plate incorporation mutagenesis assay. Report on Study No. 013-679-407-1 prepared by EG and G Mason Research Institute for FMC Corporation.

ACCESSION NUMBER: 072067.

LABORATORY: EG and G Mason Research Institute 1530 East Jefferson Street, Rockville, Maryland 20852.

TEST MATERIAL: FMC 57020 Technical, Lot E249-1 (FMC Reference A80-403). The test material was a slightly viscous, clear, colorless liquid.

METHODS:

Bacterial Strains: The bacterial strains used in this study were histidine-requiring mutants (auxotrophs) of Salmonella typhymurium. The five strains in the test battery were TA98, TA100, TA1535, TA1537, and TA1538 which were obtained from Dr. B.N. Ames.

Preparation of Test Material: The test material, FMC 57020 Technical, was solubilized in dimethylsulfoxide (DMSO) and diluted at eight serial half-log intervals for plating in the preliminary toxicity test. The volumes of test material added to the plating media of the toxicity test were 0.003 µl, 0.01 µl, 0.03 µl, 0.1 µl, 0.3 µl, 1.0 µl, 3.1 µl, and 10.0 µl. For the reverse mutation assays, final volumes of 0.04 µl, 0.2 µl, 1.0 µl, 2.0 µl, and 4.0 µl, respectively, were delivered to the plates.

Preparation of S9 Mix: The S9 was prepared from liver slices of male Sprague-Dawley rats that had been injected with Aroclor 1254, diluted in corn oil to a concentration of 200 mg/ml, at a dosage of 500 mg/kg. The preparation of S9 fraction and the S9-Mix was done according to the procedure described by Ames et al.[1] The S9 fractions were aliquotted into small volumes, frozen in ampules and stored in liquid nitrogen. The S9 Mix consisted of the following components in the volumes listed in parenthesis: S9(0.10 ml); 0.4M $MgCl_2$(0.02 ml); 1.65M KCl(0.02 ml); 0.04M NADP (0.10 ml); 0.05M Glucose-6-phosphate(0.10 ml); 1.0M NaH_2PO_4, pH 7.4(0.10 ml) and H_2O(0.56 ml). The total volume of the mix added to the soft agar overlay was 1.0 ml.

Media: Top agar consisted of 8 g/liter of agar and 5 g/liter NaCl which was sterilized and fortified with 10 ml/100 ml of a sterile solution containing 0.5 mM biotin (1XSA), Bottom agar was Vogel-Bonner minimal medium E which was described by Ames et al.[1] Nutrient broth used for growing the overnight cultures of the tester strains contained 25 g/liter of Oxoid Nutrient Broth No. 2. The 10xSA supplement contained 5.0 mM L-histidine and 0.5 mM biotin.

Toxicity Assay: The survival of S. typhimurium strain TA100 on plates supplemented with 10xSA was determined in the presence of the solvent, DMSO, and at eight concentrations of the test material which were serially diluted by half-log factors. The range of FMC 57020 dilutions was from 0.003 to 10.0 µl per plate, administered by incorporation of the test material into the top agar with the tester bacteria. Toxicity was detected by a thinning or disappearance of the bacterial background lawn. The highest concentration chosen for testing was the one which gave a detectable reduction in spontaneous revertants on the 1xSA fortified plates and/or reduced survival on 10xSA supplemented plates.

Plate Incorporation Assay: Five doses of FMC 57020, ranging from 0.04 to 4.0 µl per plate were tested with all five S. typhimurium strains, i.e., TA98, TA100, TA1535, TA1537, and TA1538, with and without activation by rat liver S9 Mix. In the nonactivated assay, 50 µl of the positive controls, solvent controls or appropriately diluted test material were incorporated with 50 µl of the tester bacteria in 2.5 ml of molten top agar held at 45° C. In the activated assay, to 2.0 ml of top agar were added 0.5 ml of S9 Mix, 50 µl of the solvent, test article, or positive control chemical[2] and 50 µl of the bacterial tester strain. The top agar mixtures, with and without S9 Mix; were vortexed and poured onto the surface of 25 ml of bottom agar in a 15 x 100 mm Petri dish. After solidification of the top agar, the plates were inverted and incubated at 37° C for 48 hr.

Controls: The solvent control was DMSO and the positive control chemicals for the tester bacteria in the absence of S9 Mix were as follows: TA98(10.0 µg/plate 2NF); TA100(0.04 µl 1,3-PS); TA1535(0.04 µl 1,3-PS); TA1537(75 µg 9-AA); TA1538(10.0 µg 2-NF). The positive controls in the presence of S9 Mix were TA91(1.0 µg 2-AA)[3] and TA100(1.0 µg 2-AA)[3]. There were no positive controls listed for strains TA1535, TA1537 and TA1538 in the presence of S9 Mix.

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[1]Ames BN et al. 1975. Mutation Research 31:347-364.

[2]Positive controls: 2NF = 2-nitrofluorene; 1,3 PS = 1,3-Propane Sultone; 9AA = 9-Amino- acridine.

[3]2AA = 2-aminoanthracene.

RESULTS:

The preliminary cytotoxicity assay showed that FMC 57020 gave normal background lawns at doses between 0.003 µl and 3.1 µl per plate; background was greatly reduced when 10 µl/plate of the test material was applied. The TA100 revertants/plate were not appreciably affected in this same concentration range; however, at 3.1 µl/plate of the test material the viable count plate was 28 percent of viable count obtained with the solvent control, and at 10 µl/plate no survivor could be detected in the "appropriately diluted TA100 culture on 10xSA supplemented plates."

For strain TA98 in the presence of S9-Mix, the average revertants per plate were: DMSO(44 ± 3); FMC 57020 doses 0.04 µl(39 ± 0)[4]; 0.2 µl (42 ± 10), 1.0 µl(36 ± 7), 2.0 µl(44 ± 1), and 4.0 µl(17 ± 5). For strain TA98 in the absence of S9, the average revertants per plate were: DMSO (39 ± 5); FMC 57020 doses 0.04 µl(40 ± 9), and 4.0 µl(3 ± 3).

For strain TA100 in the presence of S9 Mix, the average revertants per plate were: DMSO(96 ± 3); FMC doses 0.04 µl(90 ± 7), 0.2 µl(99 ± 12), 1.0 µl(90 ± 10); 2.0 µl(74 ± 4), and 4.0 µl(27 ± 5). For strain TA100 in the absence of S9-Mix, the average revertants per plate were: DMSO (134 ± 5); FMC 57020 doses 0.04 µl(128 ± 9), 0.2 µl(113 ± 14), 1.0 µl (93 ± 3), 2.0 µl(89 ± 5) and 4.0 µl(22 ± 4).

For strain TA1535 in the presence of S9-Mix, the average revertants per plate were: DMSO(10 ± 1); FMC 57020 doses 0.04 µl(9 ± 2), 0.2 µl (10 ± 5), 1.0 µl(9 ± 1), 2.0 µl(7 ± 2), and 4.0 µl(2 ± 2). For strain TA1535 in the absence of S9-Mix, the average revertants per plate were: DMSO(19 ± 7), 2.0 µl(18 ± 6), and 4.0 µl(6 ± 3).

For strain TA1537 in the presence of S9 Mix, the average revertants per plate were DMSO(9 ± 2); FMC 57020 doses 0.04 µl(7 ± 3), 0.2 µl(8 ± 2), 1.0 µl (7 ± 4), 2.0 µl (6 ± 1), and 4.0 µl (1 ± 1). For strain TA1537 in the absence of S9 Mix, the average revertants per plate were DMSO (10 ± 2); FMC 57020 doses 0.04 µl(10 ± 3), 0.2 µl(8 ± 4), 1.0 µl(10 ± 2), 2.0 µl(11 ± 5), and 4.0 µl(2 ± 1).

For strain TA1538 in the presence of S9 Mix the average revertants per plate were DMSO(14 ± 7); FMC 57020 doses 0.04 µl(14 ± 5), 0.2 µl(15 ± 3), 1.0 µl(16 ± 3), 2.0 µl(12 ± 4), and 4.0 µl(2 ± 2). For strain TA1538 in the absence of S9 Mix the average revertants per plate were DMSO(8 ± 2); FMC 57020 doses 0.04 µl(9 ± 3), 0.2 µl(11 ± 2), 1.0 µl(9 ± 5), 2.0 µl (8 ± 1) and 4.0 µl(2 ± 1).

The positive controls were run concurrently with each assay using a mutagenic chemical selected for strain sensitivity and specificity. The results were as follows: the averages of revertants per plate in the absence of S9 Mix were: TA98(10 µg NF = 1451 ± 262); TA100(0.04 µl 1,3 PS = 891 ± 72); TA1535(0.04 µl 1,3 PS = 1159 ± 23); TA1537(75 µg 9AA = 2576 ±

[4]Only one plate.

40); and TA1538(10 µg 2NF = 2175 ± 52). The averages of revertants per plate in the presence of S9 Mix were: TA98(1.0 µg 2AA = 724 ± 110) and TA100(1.0 µg 2AA = 1124 ± 52).

DISCUSSION:

From their analyses of the data, the authors and the sponsor concluded that FMC 57020 batch E249-1 was not mutagenic either with or without S9 activation under the conditions used in the Salmonella/mammalian-microsome assay.

Our assessment is that the authors/sponsor have correctly interpreted their data; however, the authors failed to include a positive control chemical for strains TA1535, TA1537, and TA1538 in the presence of rat liver S9. This means that the assay for FMC 57020 was appropriately performed with the required sensitivity level in the absence of S9 activation for all five strains, but that the S9 activated assays using strains TA98 and TA100 were the only ones with adequate controls, i.e., the data for strains TA1535, TA1537 and TA1538 in the presence of S9 cannot be properly evaluated.

The histidine mutation common to TA1538 and TA98 is hisD3052 and the histidine mutation common to TA1535 and TA100 is hisG46. Strain TA1537 has been found to only rarely detect mutagenesis not detected by other members of the Ames tester series. A revised method[5] suggests that TA1535 and TA1538 be "removed from the recommended set but can be retained at the option of the investigator." Strain TA97 which carries an ochre mutation (hisD1242) on a multicopy plasmid and the gene mutation, hisD6610, was recommended as a replacement for strain TA1537 which carries the gene mutation, hisC3076, in order to broaden the scope of mutations detected by the battery. Therefore, complete data for strains TA98 and TA100 might provide data that adequately tests for gene mutations detectable by the entire battery.

CONCLUSIONS:

FMC 57020 was not mutagenic in the presence of S9 for S. typhimurium strains TA98 and TA100 and was not mutagenic for all five tester strains in the absence of S9.

CLASSIFICATION:

Acceptable for the data on FMC 57020 for all tester strains in the absence of S9 activation and for the data on TA98 and TA100 in the presence of S9; however, unacceptable for TA1535, TA1537, and TA1538 in the presence of S9 activation because positive control chemicals were not used to demonstrate an appropriate sensitivity level.

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[5]Maron, DM and Ames, BN. 1983. mutation Res. 113:173-215.