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

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AUG 20 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE:

SUBJECT: Request For Permanent Tolerance (4F3128) For Use Of COMMAND In/On Soybeans.

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

FROM: Carolyn Gregorio, Toxicologist CAG 8-8-85
Toxicology Branch/ HED (TS-769)

THRU: Clint Skinner, Ph.D. Clint Skinner 8-8-85
Section Head,
and
Theodore M. Farber, Ph.D. Theodore M. Farber 8/19/85
Branch Chief,
Toxicology Branch/ HED (TS-769)

Chemical: COMMAND (FMC 57020, Dimethazone)
2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone

Caswell No.: 463D

Petitioner: FMC Corporation

Petition No.: 4F3128; 279-GNLU/-GNLG/-GNLE; 279-3052/-3053/-3054 XX 462

Accession No.: 072818; 072814; 072797 thru 072812; 072824 thru 072827; 072829; 072815; 072067; 072771; 072813; 072821 thru 072823; 072830 thru 072832. 1

Background:

1.) The Petitioner was granted an Experimental Use Permit (279-EUP-0G) for use of FMC 57020 (Technical) and FMC 57020 (4EC) on soybeans (memo, Holder to Taylor, January 3, 1983).

2.) The Petitioner was granted an extension of the Experimental Use Permit (279-EUP-93), but was denied their request for a Temporary Tolerance (4G-2987) for use of COMMAND on soybeans (memo, Gregorio to Taylor, September 7, 1984). The data submitted were insufficient to establish a NOEL for subchronic feeding in dogs, rats, and mice.

a.) 90-Day Feeding - rat. The Petitioner's submitted text was incomplete, therefore, no review was conducted.

b.) 90-Day Feeding - mouse. Liver megalocytosis was observed at 20 (lowest dose tested), 2000, and 8000 ppm in the treated males and at 8000 ppm in treated females (the 100, 1000, 4000 ppm doses were not examined). This lesion was considered to be indicative of cell degeneration, which may be an adaptive change rather than a toxic reaction. Therefore, it was decided that this lesion development should be futher examined in the chronic feeding/onco study and no NOEL could be predicted based on the liver cytomegaly observation at the lowest dose tested. Statistically significant increases in liver weights (absolute, relative to body weight, relative to brain weight) were observed at the 4000 and 8000 ppm males and females.

c.) 90-Day Feeding - dog. Only two dogs/sex/dose were sacrificed and examined. Therefore, insufficient sample size did not permit proper evaluation.

3.) At the request of Mr. Doug Campt (meeting held September 20, 1984), the Toxicology Branch was requested to re-evaluate the Petitioner's Temporary Tolerance by reviewing the subsequently submitted 2-year feeding/onco study in rats and the 1-year feeding study in dogs.

The Petitioner was granted a Temporary Tolerance request for use of COMMAND on soybeans based on the review of the above mentioned studies (memo, Gregorio to Taylor, January 2, 1985). The Toxicology Branch's Peer Review Group discussed the histopathology of both the 2-year feeding studies in rats and mice and the 1-year feeding in dogs (December 21, 1984). The Peer Review group decided the following:

463

2

004628

a.) 2-Year Feeding/Onco-rat. Histological examination of the individual animal data showed a non-statistical and non-dose related increase in pheocromocytomas and hepatocellular adenomas in male rats. The submitted historical control data indicated a high degree of variability in the incidences of these types of tumors.

The Petitioner submitted additional pathological information with regard to the observed liver megalocytosis (letter, Cuirle to Akerman, dated May 31, 1985). As stated by the Toxicology Branch Patholoigist, Dr, Louis Kasza, "in the submitted FMC report, the megalocytosis did not progress to cell degeneration and necrosis. There was [a] difference in incidences but no significant differences in qualitative degrees of liver involvement between control and test groups" (memo, Kasza to Farber, July 17, 1985).

Systemic NOEL = 100 ppm (4.3 mg/kg/day)
Systemic LEL = 500 ppm (21.5 mg/kg/day)
[lower body weight in 1000 and 2000 ppm males, 2000 ppm females; increased cholesterol in 500, 1000, 2000 ppm females; decreased SGOT in 1000 and 2000 ppm females; increased liver weights (absolute and relative to body and brain weights) in 500, 1000, 2000 ppm males; increased incidence of liver cytomegaly in 500, 1000, 2000 ppm males]

No oncogenic potential observed.

b.) 2-Year Feeding/Onco-mice. In screening this study, it was deemed necessary to review the liver pathology. The Group decided that the data indicated liver toxicity as evidenced by the observed incidence of hepatocellular cytomegaly in the 1000 and 2000 ppm males. With regard to the incidence of liver tumors, the Group agreed that there was no dose response and that adding benign and malignant liver tumors do not demonstrate an oncogenic trend.

b.) 1-Year Feeding - dog. An increased in blood cholesterol and liver weights (absolute and relative to body weight) were observed in the 2500 and 7500/5000 ppm males and females when compared to concurrent controls throughout the study.

NOEL = 12.5 mg/kg/day
LEL = 62.5 mg/kg/day

464

004628

4.) As previously advised (memo, Gregorio to Akerman, May 10, 1985), it had come to the attention of the Toxicology Branch that the FMC Corporation may have had some additional pathological information with regard to the report entitled "Ninety-Day Subchronic Toxicity Dietary And Twenty-Four Month Chronic Toxicity And Oncogenicity Dietary Study In Rats Utilizing FMC 57020 Technical" (FMC Study No. A81-650, Toxigenics Study No. 410-0816; July 10, 1984). In addition, the Toxicology Branch informed the Registration Division that until this matter was satisfactorily resolved, the Toxicology Branch could not finalize the Petitioner's request for a Temporary Tolerance for soybeans.

The Toxicology Branch recieved the above described rat pathology data (June 16, 1985) and this information has been evaluated by Dr. Louis Kasza, Branch Pathologist (memo, Kasza to Farber, dated July 17, 1985; memo attached). Dr. Kasza's conclusion indicates that liver "megalocytosis is a mild and reversable cellular alteration. In the submitted FMC report, the megalocytosis did not progress to cell degeneration and necrosis. There was [a] difference in incidences but no significant differences in quantitative degrees of liver involvement between control and test groups. It is my opinion that as it was presented in FMC's report -- even if it [megalocytosis] is compound-related -- this cellular alteration does not interfere with the normal functions of the livers."

Action Requested: The Petitioner has requested a Permanent Tolerance request (4F3128) for the use of the herbicide COMMAND in/on soybeans. The Petitioner has based the request on residue levels not to exceed 0.05 ppm.

Recommendations: It is recommended that the request for a Permanent Tolerance for use of COMMAND on soybeans be denied at this time. The toxicology data base is insufficient to support the request:

1.) Teratology - rat: The rat teratology study (FMC Corp.; Study No. A83-1142; June 29, 1984) has been classified as Supplementary. The Petitioner is requested to provide the folowing additional information (see the attached Data Evaluation Review for more

465

4

specific details):

a.) historical control data, and

b.) explanation of dose selection used.

The additional information requested for clarification of this study is essential because several severe malformations (non-functional limbs and extended limbs with no flexion at the ankle; no anal opening) were observed in the 2-generation rat reproduction study (Toxigenics; Study No. 450-1095; June 12, 1984) at dose levels which were similiar to those used in the rat teratology study.

Due to uncertainities with regard to the results of this study, the Branch is requesting a Laboratory Audit of this study to insure the accuracy of the reported results (memo, Gregorio to Frick, dated August 12, 1985).

2.) 2-Year Feeding/Onco - mice: The 2-year feeding/oncogenicity study in mice (FMC Study No. A81-651; Toxogenics Study No. 410-0817; July 25, 1984) has been classified as Supplementary. In discussion of the data with Dr. Theodore M. Farber (Branch Chief, Toxicology Branch/HED), it was decided that a Maximum Tolerated Dose (MTD) had not been achieved. The Branch decision is based on the following:

a.) the absence of body weight change (at least a 10% decrement) throughout the study for any treatment group when compared to concurrent controls,

b.) the termination of the 4000 and 8000 ppm treatment groups at 3-months without sufficient reasoning that these doses levels would be expected to exceed a MTD for a 24-month study and thereby, jeapordize the completion of the study (the study Pathologist lletter, Harold W. Casey (Louisiana State University) to John R. DeProspo (FMC Corporation); dated August 3, 1985) reported that he terminated these doses at 3-months because "absolute enlargement of the liver plus histologic megalocytosis were found at 2000 and 8000 ppm dose levels that were examined microscopically (the 4000 ppm dose groups were not examined microscopically). Based on these findings coupled with those found in the previous 28 day study (Toxigenics' Study 410-0744 and FMC Study A81-612) showing the liver as the target organ, it appeared that the compound had both

004628

a time and dose dependent effect. These data were interpreted to indicate that the survival of mice in the 4000 and 8000 ppm dose levels could be significantly shortening during the course of a 24 month study and therefore, the recommendation was made that the 2000 ppm dose level should be used as the maximum tolerated dose group.")

c.) the absence of any life-threatening or consistent statistically significant changes in any of the examined parameters in any of the treatment groups throughout the 24-month study when compared to concurrent controls.

It is the Branch's position that this study should be repeated using higher doses and that the Petitioner should be advised that this study is insufficient to satisfy the data requirement for chronic feeding/oncogenicity.

3.) The Toxicology Branch requests that the Residue Chemistry Branch verify the quantity and significance of the impurity [redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Data Reviewed In This Petition (4F3128):

1.) 2-Year Feeding/Onco - mouse (FMC Study No. A81-651; Toxigenics Study No. 410-0817; July 25, 1984): Discussed in Recommendation Section.

2.) 2-Generation Reproduction - rat (Toxigenics Study No. 450-1095; June 12, 1984): Decreased body weight, decreased food consumption, increased liver absol[illegible] and/or relative to body weight ratio, kidney, and ovar[illegible]ights were observed in the 2000 and 4000 ppm parents. In addition, an increased incidence of dilated/distended kidneys in the 4000 ppm males and an increased incidence of urine-soaked and/or yellow-brown fur and a decreased fertility index was noted in the 4000 ppm females when compared to concurrent controls.

Slight decreases in the percentage of viable pups/litter, associated with slight increases in the percentage of dead and/or cannibalized pups and a statistically significant

467 6

004628

decreases in pup weights were observed at the 2000 and 4000 ppm groups. In addition, one pup in the 1000 ppm dose group and another in the 4000 ppm dose group had severe hind limb abnormalities (non-functional and extended limbs with no flexion at the ankle, respectively) and one pup in the 4000 ppm group had no anal opening. Although these malformations were not statistically significant and were not dose-related, causal relationship with the test compound cannot be ruled out.

Parental NOEL = 1000 ppm
Parental LEL = 2000 ppm

Progeny NOEL = 100 ppm
Progeny LEL = 1000 ppm

3.) Teratology - rat (FMC Study No. A83-1142): . This study has been classified as Supplementary as addressed in the Recommendation section of this memo.

Maternal toxicity was observed as demonstrated by clinical signs of toxicity (decreased locomotion, abdominogenital staining, chromorhinorrea) at 300 and 600 mg/kg (highest dose tested).

As an indication of fetotoxicity, there was a statistically significant (chi-square) increase in the incidence of delayed ossification or absence of four sternebrae in the 300 and 600 mg/kg groups when compared to concurrent controls. In addition, hydronephrosis and hydroureter were observed more frequently, but not statistically significant, in all dose groups when compared to concurrent controls. No historical control data were available to provide perspective for these fetotoxic effects.

No teratogenic reponse was observed at any dose. Again, no historical control data were available to provide perspective to these findings.

4.) Metabolism - rat (PRI Study No. Fm-124r, FMC Study No. PC-0017, March 22, 1984; FMC Study No. P-0898, June 18, 1984): Two metabolism studies were provided which indicate that COMMAND is excreted in the urine and feces (90-99%) within 72 hours of treatment for single oral dose (5 mg/kg), single I.V. dose (3 mg/kg), and multiple oral dose (5 mg/kg) for males and females. After a single oral high dose administration

(900 mg/kg), excretion was slightly different in that the test compound was excreted in urine the rate of 83% and 71% for females and males, respectively over 7 days. The excretion in the feces was 15% and 31% for females and males, respectively. No tissue retention was observed in any of the studies.

As discussed in the individual reviews, 16 metabolic products were identified in the urine and feces over 48 hours.

5.) Mutagenicity. The results of the submitted mutagenicity assays are as follows:

Study Type	Results
TECHNICAL (FMC 57020)	
Reverse Mutation - Salmonella (FMC No. A80-403)	Negative (without activation)
Reverse Mutation - Salmonella (Microbiological Assoc. No. A84-1273)	Negative (with/without activation)
Mutagenicity, CHO/HGPRT (Microbiological Assoc. No. A83-1143)	Weakly Positive (without Activation)
Mutagenicity, in vivo cytogenetics (Microbiological Assoc. No. A82-778)	Negative
Mutagenicity, Unscheduled DNA Synthesis (Microbiological Assoc. No. A83-1036)	Negative
MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED	
INTERMEDIATE [redacted]	
Mutagenicity, Reverse Mutation (Microbiological Assoc. No. A84-1189)	Positive (with/without activation)
INTERMEDIATE [redacted]	
Mutagenicity, Reverse Mutation (Microbiological Assoc. No. A84-1281)	Positive (with/without activation)

MANUFACTURING PROCESS INFORMATION IS NOT INC[illegible]

INTERMEDIATE [redacted]

Mutagenicity, Reverse Mutation (Microbiological Assoc. No. A84-1188)	Positive (with/without activation)
Mutagenicity, Reverse Mutation (Microbiological Assoc. No. A83-1111)	Positive (with/without activation)

COMMAND Technical (FMC 57020) Data Summary

A brief, executive summary of all the data reviewed for technical COMMAND (FMC 57020) is attached.

004628

COMMAND TECHNICAL (FMC 57020) DATA SUMMARY

Study	Results	Tox Cat
Acute Oral-rats	LD_{50} = 2077 mg/kg (M) LD_{50} = 1369 mg/kg (F)	III III
Acute Dermal-rabbit	LD_{50} = greater than 2000 mg/kg	III
Acute Inhalation-rat	LC_{50} = 6.25 mg/L (M) LC_{50} = 4.23 mg/L (F)	III III
3-Month Feeding-dog	NOEL not established; insufficient animals sacrificed (2/sex/dose)	
3-Month Feeding-mice	NOEL not established; liver cytomegaly seen at lowest dose tested (20 ppm)	
3-Month Feeding-rat	NOEL not established; report incomplete	
1-Year Feeding-dog [doses: 0, 100, 500, 2500, 5000 ppm for 1-year]	NOEL = 500 ppm (12.5 mg/kg/day) LEL = 2500 ppm (62.5 mg/kg/day) [increased liver weights, absolute and relative to body weight in males and females; increase in cholesterol]	
2-Year Feeding-rat [doses: 0, 20, 100 500, 1000, 2000 ppm for 2-years; 4000 and 8000 ppm for 3-months]	NOEL = 100 ppm (4.3 mg/kg/day) LEL = 500 ppm (21.5 mg/kg/day) [lower bdy wt in 1000 and 2000 ppm males, 2000 ppm females; cholesterol increased in 500, 1000 and 2000 ppm females; SGOT decreased in 1000 and 2000 ppm females; increased liver weights, absolute and relative to body and liver weights in 500, 1000, 2000 ppm females; increased incidence of liver cytomegaly in 500, 1000, 2000 ppm males.	
2-Year Feeding-mice [doses: 0, 20, 100, 500, 1000, 2000 ppm for 2-years; 4000 and 8000 ppm for 3-months]	NOEL = LEL = [increase in white blood cells in 500, 1000, 2000 ppm males; increase in SGOT and SGPT in 1000 ppm males at 24 months; increase in absolute liver weights at 1000 and 2000 ppm males; increase in liver cytomegaly in 1000 and 2000 ppm males; increase	

471

Study	Results
2-Year Feeding-mice	in lymphoid hyperplasia in 1000 and 2000 ppm females.
Teratology-rabbit [doses: 0, 30, 240, 1000 (reduced to 700 mg/kg/day from gestation days 13 thru 18) mg/kg/day]	Negative for teratogenicity at Highest Dose Tested, 700 mg/kg/day Maternal NOEL = 240 mg/kg/day Maternal LEL = 700 mg/kg/day [decreased body weight] Fetotoxic NOEL = 240 mg/kg/day Fetotoxic LEL = 700 mg/kg/day [increased number of resorptions]
Teratology-rat [doses: 0, 100, 300, 600 mg/kg/day]	Incomplete for teratogenicity [no historical control data] Maternal NOEL = 100 mg/kg/day Maternal LEL = 300 mg/kg/day [decreased locomotion, genital staining, runny eyes] Fetotoxic NOEL = 100 mg/kg/day Fetotoxic LEL = 300 mg/kg/day [increased incidence of delayed ossification of 4 sternebrae; increased increased incidence of hydroureter and hydronephrosis]
Mutagenicity-Reverse Mutation (Salmonella) [2 studies]	Negative with/without activation
Mutagenicity-Point Mutation (CHO/HGPT)	Positive without activation [Positive control:Benzopyrene; Command 3x background; "weakly positive"]
Mutagenicity-In Vivo Cytogenetics (chromosomal aberations)	Negative
Mutagenicity- Unscheduled Dna Synthesis	Negative

472

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

004628

July 17, 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Evaluation of Hepatocellular Cytomegaly in Rats. FMC 57020 Test Material 90-day/2-year Feeding Study

FROM: Louis Kasza, D.V.M., Ph.D.
Pathologist, Toxicology Branch TS-769

TO: Theodore Farber, Ph.D., Branch Chief
Toxicology Branch TS-769

From the FMC report, a compound- (but not dose-) related increase in incidence (M: 11, 26, 28, 21, 23, 19; F: 19, 34, 35, 29, 32, 26) of hepatocellular megalocytosis was recognized. The megalocytosis is a mild reversable cellular change which is manifested by the enlargement of cells and it commonly occurs in the livers of old rats. When a severe causative agent (chemicals or infectious organisms) affects the livers, the megalocytosis is present which always progresses to cellular degeneration and necrosis. This progressive process was not present in the submitted pathologic report of FMC. When the degrees of megalocytic liver involvement were compared between control and test groups and expressed by the mean* of quantitative changes of megalocytosis, no significant differences were found between the control and test groups (M: 2.7, 2.8, 2.9, 3.2, 2.7, 3.1; F: 3.3, 3.1, 3.0, 3.1, 3.4, 3.2).

CONCLUSION:

The megalocytosis is a mild and reversable cellular alteration. In the submitted FMC report, the megalocytosis did not progress to cell degeneration and necrosis. There was difference in incidences but no significant differences in quantitative degrees of liver involvement between control and test groups.

It is my opinion that as it was presented in FMC's report -- even if it is compound-related -- this cellular alteration does not interfere with the normal functions of the livers.

My opinion in principle is in agreement withthe two consultant pathologists', Drs. Newberne and Williams, interpretations.

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*The mean was calculated by assigning the following numerical values: 1: Focal megalocytosis, 2: Multifocal megalocytosis, 3: Centrilobular megalocytosis, and 4: Diffuse megalocytosis.

cc: W. Burnam, TS-769
C. Gregorio, TS-769
R. Engler, TS-769
R. Cobeily, TS-769
C. Skinner, TS-769
J. Quest, TS-769

473

12

004628

004628

Chemical: Command; FMC 57020

Caswell No.: 463D

Formulation: Technical (88.8% AI; "Reassayed as 91.4% using a new analytical method and standard")

Citation: L. D. Morrow et al. 90-day Subchronic Toxicity Dietary and 24-Month Chronic Toxicity and Oncogenicity Dietary Study in Mice Utilizing FMC 57020 Technical. (FMC Study No. A81-651, Toxigenics Study No. 410-0817). July 25, 1984. Unpublished report prepared by Toxigenics, Inc. for FMC Corpration.

Petitioner: FMC Corporation

Accession Nos.: 072797 thru 072812; 072824 thru 072827

Reviewed By: Carolyn Gregorio, Toxicologist
Toxicology Branch/HED (TS-769) 8-7-85

Secondary Review: Clint Skinner, Ph.D.
Section Head, Section III
Toxicology Branch/HED (TS-769)

Core Classification: Supplementary

Toxicity Category: N/A

Conclusion: CD-1 outbred albino mice were fed 0, 20, 100, 500, 1000, or 2000 ppm COMMAND for 24 months; 4000 or 8000 ppm COMMAND for 3 months.

At 3 months, the 4000 and 8000 ppm treatment groups were terminated. At this time, body weight, clinical chemistry, and hematology values were similiar for all treatment and control animals. A statistically significant increase in absolute liver weights, liver to body weight ratio, and liver to brain weight ratios were observed in the 4000 and 8000 ppm males and females when compared to controls and a dose related, non-statistical increase was observed in all male treatment groups and the 100 ppm and higher doses in the female treatment groups. An accompanying increased incidence of hepatocellular cytomegaly was observed at 20 (3/20), 2000 (3/20), and 8000 (9/20) ppm treatment males (100, 1000, and 4000 ppm treatment groups were not examined) when compared to controls.

Throughout the remainder of the study, body weight values were similiar for all treatment and control groups. A dose-related, non-statistically significant increase in white blood cells were observed in the 500, 1000, and 2000 ppm males when compared to concurrent controls at 24 months. A statistically significant increase in SGOT and SGPT was observed in the 1000 ppm treatment males when compared as

474 13

percent of control values at 24 months, which may be indicative of liver function alterations. A dose-related, non-statistical increase in absolute liver weights were observed in the 1000 and 2000 ppm males when compared to concurrent controls at 24-months. These data correlate with increased incidence of hepatocellular cytomegaly observed in the 1000 and 2000 ppm males. In addition, a statistically significant increase in liver to body weight ratio was seen in the 2000 ppm female treatment group at 6 months; at no other time sequence was there a significant deviation from control values for this parameter in female treatment groups. A statistically significant increase in lymphoid hyperplasia of the thymus gland was observed in the 1000 and 2000 ppm females when compared to concurrent controls.

The Toxicology Branch Peer Review Group discussed the liver pathology (memo, Gregorio to Taylor, December 21, 1984) and decided that the liver data demonstrate liver toxicity potential, but do not indicate an oncogenic potential.

In a final discussion of the body of data for this study with Dr. Theodore M. Farber (Branch Chief, Toxicology Branch; July 26, 1985), it was decided that a Maximum Tolerated Dose had not been achieved. This decision is based on the absence of weight change (at least a 10% decrement) throughout the study for any treatment group when compared to concurrent controls, the termination of the 4000 and 8000 ppm at 3-months (the study authors terminated these doses because of a statistically significant increase of absolute liver weights, liver to body weight ratio, and liver to brain weight ratio were observed at 4000 and 8000 ppm and a statistically significant increase in hepatocellular cytomegaly was observed at 8000 ppm), and the absence of any life-threatening or CONSISTENT statistically significant changes in any of the examined parameters in any of the remaining treatment groups throughout the 24-month study when compared to concurrent controls.

Therefore, the Petitioner is advised that this study is insufficient to satisfy the data requirement for a chronic feeding/oncogenicity study.

Homogeneity of Command in Mixed Diets: Homogeneity assays were reported for the 20, 1000, 2000 and 8000 ppm dose levels throughout the first 90 days of the study and the 20 and 2000 ppm doses subsequently for the remainder of the study. These values indicate acceptable homogeneity of the test compound in the tested diets throughout the study.

Stability of Command in Mixed Diets: Stability assays were reported for 7 and 14 days at ambient temperatures, refrigerated temperature (39°F), and frozen temperatures (25°F). "Diets were prepared weekly and held frozen for 7 days before the frozen diets were removed from the freezer one to two days prior to presentation to study animals." The assays show that the compound was stable in the diet mix for 14 days at frozen or refrigerated temperatures.

Materials and Methods: CD-1 outbred albino mice (28 days old) were received from Charles River Breeding Laboratories. After a two-week acclimation period, 120 animals/sex were assigned to dose groups. Command was administered in the diet at concentrations of 0, 20, 100, 500, 1000, 2000, 4000, and 8000 ppm. At 1-month, 2-month, 6-month, 12-month, and 18-month interim sacrifices, 10 animals/sex/dose were sacrificed; at 3 months, 20 animals/sex/dose were sacrificed from the 0, 20, 500, 2000 and 8000 ppm groups. "Immediately following the 90-day sacrifice and after consideration of the relative organ/body weight ratio data, the remaining animals in the 4000 and 8000 ppm groups were removed from the study. Prior to sacrifice of the 4000 and 8000 ppm animals, 15 mice/sex in each of these groups were selected to compromise the recovery study."

"Fresh diets were prepared weekly and held frozen for approximately seven days. Sufficient diet was offered at each feeding to assure one week's ad libitum feeding."

The following parameters were recorded throughout the study:

1. Clinical observations and mortality.

2. Body weights and food consumption.

3. Hematology (erythrocyte count, hematocrit, hemoglobin, total leukocyte count, differential leukocyte count, platelet count, reticulocyte count, cell indices [MCH, MCV, MCHC]).

4. Blood chemistry (glutamic pyruvic transaminase, glutamic oxaloaectic transaminase, gamma glutamyic transferase, albumin).

5. Pathology for animals sacrificed at 1 and 2 months were discarded following gross examination.

6. Pathology for animals at 3, 6, 12, 18 and 24 months:

   a. Organ weights (brain, gonads, heart, kidneys, liver).

   b. Histology (adrenals, bone and bone marrow [femur], brain, pancreas, pituitary, prostate, salivary gland (mandibular), esophagus, eyes, gonads, harderian glands, heart, intestine, gallbladder, kidneys, liver, lung and mainstem bronchi, skeletal muscle (rectus femoris) spleen, stomach, thymus, thyroid and parathyroids, trachea, urinary bladder, uterus, any lesions of uncertain nature and tissue masses with regional lymph nodes).

7. Additional tissues and organs from animals found dead, accidentally killed, sacrificed moribund at 6, 12, 18 and 24 months (lymph node [mesenteric], mammary gland, nerve (sciatic), skin, spinal cord [cervical and lumbar]).

RESULTS:

Observations: "Distended abdomens, discolored abdomens, yellow/brown staining of hair coat in the urogenital area, and on occasion, firm areas in the abdomen" were noted in a few males and females.

"For each sex, clinical observations were similar in frequency across all groups, including controls."

Mortality: This study is approaching the limits of good practice due to the small number of survivors at the termination of the study. Mortality incidence coupled with numerous intermediate sacrifices (a total of 70 animals/sex/dose were scheduled for sacrifice) reduced the number of survivors (Table 1.) Survival in any group should not fall below 25%.

004628

Table 1. Survivors Over Selected Sacrifice Times for Mice

Dose (ppm)	0	20	100	500	1000	2000	4000*	8000*
Month on Test								
Males								
0	120	119	120	120	120	121	120	120
3	79	78	80	80	80	80	80	78
6	67	68	69	70	69	69	-	-
12	55	51	51	55	54	53	-	-
18	35	31	31	33	33	32	-	-
24	21	22	21	23	23	23	-	-
Females								
0	120	121	120	120	120	119	120	120
3	80	80	79	80	80	79	79	79
6	70	69	69	70	69	69	-	-
12	57	55	57	56	56	59	-	-
18	40	33	32	32	34	42	-	-
24	27	21	22	23	25	26	-	-

*The 4000 and 8000 ppm doses were terminated at 3 months.

Body Weight and Food Consumption: Body weights and food consumption were similar for all male and female groups, including controls, throughout the study [see Tables 3 and 4.).

Hematology: From fasted animals "blood samples were collected from 10 mice/sex/dose at 1, 2, 3, 6, 12, 18 and 24 month intervals. Following sample collection, all animals bled were sacrificed." Mean hematology values were similar for all groups throughout the study, except for an increase in the number of white blood cells (WBC) in 500, 1000 and 2000 ppm males at 24 months (Table 2).

Table 2. Mean WBC (thousand/cu. mm) For Male Mice

Dose (ppm)	Time (months) 3 mo.	6 mo.	12 mo.	18 mo.	24 mo.
0	3.1400 ± 0.8669	1.7900 ± 0.7838	2.8000 ± 0.5657	3.3333 ± 0.6384	4.2800 ± 1.3037
20	3.0600 ± 0.8475	1.7800 ± 1.0229	3.0600 ± 0.7877	3.1500 ± 1.6494	2.9222 ± 0.9821
100	2.9100 ± 0.7445	2.0900 ± 0.5840	2.6100 ± 0.7824	3.2300 ± 2.0500	3.6222 ± 1.5393
500	2.9000 ± 0.8380	1.8800 ± 0.9259	4.3700 ± 3.5321	3.4000 ± 1.0121	5.4600 ± 5.1243
1000	2.9700 ± 1.4205	1.6200 ± 0.3938	3.1100 ± 0.7724	3.1200 ± 1.1961	8.3800 ± 12.0746
2000	2.9100 ± 1.1396	1.7100 ± 0.4280	3.6400 ± 3.1274	3.3200 ± 1.8177	8.1800 ± 14.5497
4000*	2.3667 ± 0.5979	-	-	-	-
8000*	2.7700 ± 1.0242	-	-	-	-

*The 4000 and 8000 ppm doses were terminated at 3 months.

This Table is abstracted from Registrant's submission.

Clinical Chemistry: Blood samples from fasted animals (10 mice/sex/dose) were collected at 1, 2, 3, 6 12, 18 and 24 months. "Following sample collection, all animals bled were sacrificed." Mean clinical chemistry values were similar for all groups throughout the study with the exception of SGOT (+273%) and SGPT (+373%) in the 1000 ppm male treatment group at 24 months.

Organ Weights:

3-Month Sacrifice: The absolute liver weights, liver to body weight ratio and liver to brain weight ratio were significantly increased for the 4000 and 8000 ppm males and females, when compared to concurrent controls (Table 3, 4, 5, 6) as performed by the Registrant. It should be noted that the the statistical test used for male animals was Scheffe's multiple comparison test and Tukey's multiple comparison test was used for females. A dose-related increase in absolute liver weight was observed in both sexes when liver weights

were expressed as percent of control for both males (at all doses) and females (at 100 ppm and higher) (Table 3 and 4).

A dose-related trend was observed in the relative liver to body weight ratio at 100 ppm and higher for males and females (Table 5 and 6). With regard to relative liver weight to brain weight ratio, there was also a dose related trend observed at all doses for males (Table 5) and at 500 ppm and higher for females (Table 6).

As noted previously, the 4000 and 8000 ppm treatment groups were terminated at 3-months.

6-Month through 24-Months:

MALES: At 24-months, a dose-related, non-statistical increase was observed in absolute liver weights in the 500 (+8%), 1000 (+12%), and 2000 (+21%) ppm treatment groups when compared as percent of control (Table 3). The 2000 ppm treatment group showed a non-statistical increase in absolute liver weight at 12 months (+13%), 18 months (+12%), and 24 months (+21%) when compared as percent of controls.

A non-statistically significant increase in liver to body weight ratio were observed in the 1000 ppm treatment group at 18 months (+18%) and 24 months (+11%) when compared as percent of control (Table 5).

A non-statistically significant increase in liver to brain weight ratio was observed in the 1000 ppm treatment group at 18 months ((+18%) and 24 months (+24%) when compared as percent of control (Table 5).

FEMALES: A dose-related, non-statistically significant increase in absolute liver weight was observed at 500 (+12%, +14%), 1000 (+8%, +14%), and 2000 (+13%, +17%) ppm treatment groups when compared as percent of controls at 6 and 18 months, respectively (Table 4).

A statistically significant increase of liver to body weight ratio was observed in the 2000 ppm group at 6 months. A non-statistically significant increase in liver to body weight ratio was observed at 500 (+13%, +16%) and 1000 (+14%, +15%) ppm females when compared as percent of control at 6 and 18 months, respectively; a non-statistically significant increase was also observed at 2000 ppm at 18 months.

A non-statistically significant increase of liver to brain weight was observed at 500 (+13%, +16%), 1000 (+9%, +14%), and 2000 (+17%, +20%) ppm females when compared as percent of controls at 6 and 18 months.

Table 3. Mean Body Weight and Mean Liver Weights For MALE Mice Fed COMMAND for 24-Months

Dose (ppm)	3 Months Body Wt (grams)	3 Months Liver Wt (grams)	6 Months Body Wt (grams)	6 Months Liver Wt (grams)	12 Months Body Wt (grams)	12 Months Liver Wt (grams)	18 Months Body Wt (grams)	18 Months Liver Wt (grams)	24 Months Body Wt (grams)	24 Months Liver Wt (grams)
0	31.9205 (1.9465) [—]	1.4208 (0.1346) [—]	35.5680 (3.1924) [—]	1.5421 (0.1190) [—]	35.6970 (5.1976) [—]	1.4684 (0.1586) [—]	36.6520 (5.1906) [—]	1.6632 (0.2994) [—]	34.4220 (3.1196) [—]	1.9976 (0.6499) [—]
20	32.4490 (1.8473) [—]	1.4547 (0.1630) [+2%]	35.3130 (2.8861) [—]	1.5134 (0.1768) [-2%]	35.6740 (3.6174) [—]	1.5485 (0.1945) [+5%]	37.8020 (1.8906) [+3%]	1.5945 (0.1315) [—]	35.2300 (4.1101) [+2%]	1.8308 (0.4266) [+5%]
100	32.7375 (2.5608) [—]	1.5088 (0.1349) [+6%]	35.7710 (2.9684) [—]	1.5760 (0.1503) [+2%]	37.6410 (3.6340) [+5%]	1.7322 (0.3642) [+7%]	35.0530 (5.5144) [-4%]	1.5889 (0.1925) [+1%]	34.6421 (4.2445) [—]	2.0297 (0.6712) [+4%]
500	32.9590 (2.2083) [—]	1.5225 (0.1458) [+7%]	37.2410 (2.5745) [+4%]	1.7183 (0.2127) [+11%]	34.5180 (2.5826) [-4%]	1.5299 (0.1257) [+4%]	37.4230 (5.9672) [+2%]	1.7011 (0.4120) [—]	35.8004 (2.4751) [+4%]	2.0728 (0.6984) [+8%]
1000	32.3840 (2.6373) [—]	1.5330 (0.1649) [+8%]	34.5400 (2.2438) [-3%]	1.6032 (0.2393) [+4%]	35.8940 (3.5066) [—]	1.5977 (0.1969) [+8%]	36.7050 (3.9019) [—]	1.9897 (0.8382) [—]	35.0765 (3.6637) [—]	2.2443 (0.8084) [+12%]
2000	33.0340 (3.2288) [—]	1.6106 (0.2000) [+13%]	34.9620 (2.8002) [-1%]	1.6322 (0.1932) [+6%]	36.4310 (3.4403) [+2%]	1.6521 (0.1534) [+13%]	36.7340 (4.5199) [—]	2.0224 (0.7905) [+12%]	34.1064 (3.7432) [—]	2.1304 (0.4048) [+21%]
4000	32.4720 (2.5851) [—]	1.7999** (0.2785) [+27%]	--- ---	--- ---	--- ---	--- ---	--- ---	--- ---	--- ---	--- ---
8000	31.6530 (2.5240) [—]	1.8084** (0.1540) [+27%]	--- ---	--- ---	--- ---	--- ---	--- ---	--- ---	--- ---	--- ---

** Statistically significant (p = 0.01) using Scheffe's Multiple Comparison test

--- Treatment groups were terminated at 3-month sacrifice

[ ] expressed as percent of controls

Table 4. Mean Body Weight and Mean Liver Weights For FEMALE Mice Fed COMMAND for 24-Months

Dose (ppm)	3 Months Body Wt (grams)	3 Months Liver Wt (grams)	6 Months Body Wt (grams)	6 Months Liver Wt (grams)	12 Months Body Wt (grams)	12 Months Liver Wt (grams)	18 Months Body Wt (grams)	18 Months Liver Wt (grams)	24 Months Body Wt (grams)	24 Months Liver Wt (grams)
0	25.3476 (2.4594) [--]	1.0896 (0.1142) [--]	28.3540 (3.0271) [--]	1.2073 (0.1445) [--]	30.1390 (3.3077) [--]	1.6206 (1.3400) [--]	30.7990 (4.5967) [--]	1.4047 (0.3111) [--]	30.0838 (3.8489) [--]	1.6388 (0.6650) [--]
20	25.0724 (1.5356) [-2%]	1.0807 (0.1597) [--]	28.5810 (2.8902) [--]	1.2605 (0.1431) [+4%]	28.0870 (2.4697) [-7%]	1.2357 (0.2416) [-23%]	29.0260 (2.1240) [-6%]	1.3902 (0.2428) [-1%]	30.4543 (3.5375) [+1%]	1.5636 (0.3048) [-5%]
100	25.4618 (1.9482) [--]	1.1061 (0.1424) [+1%]	27.4750 (2.7076) [-3%]	1.1858 (0.1274) [-2%]	27.1860 (3.3534) [-10%]	1.0719 (0.1769) [-34%]	30.2930 (3.5654) [-2%]	1.4858 (0.3619) [+5%]	28.7662 (4.4514) [-4%]	1.4320 (0.2398) [-12%]
500	25.9253 (2.3294) [+2%]	1.1355 (0.1354) [+4%]	29.9760 (2.8446) [+5%]	1.3521 (0.1415) [+12%]	29.9020 (2.0324) [-1%]	1.3942 (0.1970) [-14%]	30.7010 (1.8640) [--]	1.6093 (0.2231) [+14%]	29.7548 (3.8682) [-1%]	1.5771 (0.3453) [-4%]
1000	25.4900 (2.9827) [--]	1.1988 (0.1974) [+10%]	27.0900 (1.3632) [-4%]	1.3134 (0.1380) [+8%]	29.2720 (4.5255) [-3%]	1.3053 (0.2932) [-19%]	30.7930 (3.0781) [--]	1.6155 (0.3231) [+14%]	31.2616 (2.6960) [+4%]	1.6591 (0.2621) [+1%]
2000	24.7256 (2.2915) [-2%]	1.2316 (0.1491) [+12%]	27.8060 (2.9988) [-2%]	1.3686 (0.2274) [+13%]	27.4140 (2.4819) [-9%]	1.2480 (0.1920) [-23%]	32.3520 (4.6659) [+5%]	1.6512 (0.3941) [+17%]	30.2473 (4.5105) [—]	1.5943 (0.2011) [-3%]
4000	26.2238 (2.5811) [+3%]	1.3490** (0.2677) [+23]	---	---	---	---	---	---	---	---
8000	24.6356 (1.3709) [-3%]	1.4492** (0.1495) [+33]	---	---	---	---	---	---	---	---

** Statistically Significant (p = 0.01) using Tukey's Multiple Comparison test

--- Treatment groups terminated at 3-month sacrifice

[ ] Expressed as percent of controls

Table 5. Mean Relative Liver/Body Weight and Mean Liver/Brain Weights For MALE Mice Fed COMMAND For 24-Months

Dose (ppm)	3 Months Liver/Body Wt (%)	3 Months Liver/Brain Wt (%)	6 Months Liver/Body Wt (%)	6 Months Liver/Brain Wt (%)	12 Months Liver/Body Wt (%)	12 Months Liver/Brain Wt (%)	18 Months Liver/Body Wt (%)	18 Months Liver/Brain Wt (%)	24 Months Liver/Body Wt (%)	24 Months Liver/Brain Wt (%)
0	4.4507 (0.3135) [--]	2.5558 (0.2972) [--]	4.3459 (0.2435) [--]	2.7558 (0.2156) [--]	4.1526 (0.3940) [--]	2.6317 (0.2509) [--]	4.5387 (0.5073) [--]	2.9465 (0.4502) [--]	5.8646 (2.0109) [--]	3.6119 (1.1514) [--]
20	4.4820 (0.4175) [--]	2.6711 (0.2820) [+4%]	4.2812 (0.2643) [-2%]	2.6684 (0.2918) [-3%]	4.3370 (0.2901) [+4%]	2.7461 (0.3795) [+4%]	4.2227 (0.3540) [-7%]	2.7794 (0.2858) [-6%]	5.1904 (0.9457) [-11%]	3.1631 (0.8204) [-12%]
100	4.6166 (0.3271) [+4%]	2.7460 (0.2770) [+7%]	4.4076 (0.2414) [+1%]	2.8920 (0.2550) [+2%]	4.6060 (0.9208) [+1%]	3.0924 (0.6614) [+7%]	4.6067 (0.7627) [+1%]	2.7842 (0.5121) [-6%]	5.9466 (2.1713) [+1%]	3.6879 (1.3347) [--]
500	4.6153 (0.2409) [+4%]	2.7908 (0.3136) [+9%]	4.6018 (0.3247) [+6%]	3.1244 (0.3982) [+13%]	4.4470 (0.4317) [+7%]	2.8011 (0.2150) [+6%]	4.5065 (0.4056) [-2%]	2.95662 (0.6923) [--]	5.8188 (2.0607) [--]	3.6798 (1.2926) [--]
1000	4.7392 (0.4225) [+7%]	2.7353 (0.3147) [+7%]	4.6238 (0.4222) [+6%]	2.8804 (0.4114) [+5%]	4.4715 (0.6057) [+4%]	2.9276 (0.4382) [+8%]	5.4279 (2.2934) [+18%]	3.4926 (1.4501) [+18%]	6.5041 (2.6680) [+11%]	4.1165 (1.5230) [+14%]
2000	4.8786 (0.4031) [+10%]	2.9589 (0.3303) [+16%]	4.6626 (0.3403) [+7%]	2.8829 (0.3993) [+5%]	4.5475 (0.3641) [+9%]	3.0042 (0.2835) [+14%]	5.52114 (2.0775) [+8%]	3.6760 (1.3945) [+24%]	6.2751 (1.2115) [+7%]	3.8189 (0.6949) [+6%]
4000	5.5279** (0.5642) [+24%]	3.2909** (0.4952) [+29%]	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---
8000	5.7252** (0.4061) [+29%]	3.2640 (0.2801) [+28%]	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---

** Statistically Significant (p = 0.01) using Scheffe's Multiple Comparison test

--- Treatment groups terminated at 3-months

[ ] Expressed as percent of controls

Table 6. Mean Relative Liver/Body Weight and Mean Liver/Brain Weights For FEMALE Mice Fed COMMAND for 24-Months

Dose (ppm)	3 Months Liver/ Body Wt (%)	3 Months Liver/ Brain Wt (%)	6 Months Liver/ Body Wt (%)	6 Months Liver Brain Wt (%)	12 Months Liver/ Body Wt (%)	12 Months Liver Brain Wt (%)	18 Months Liver/ Body Wt (%)	18 Months Liver/ Brain Wt (%)	24 Months Liver/ Body Wt (%)	24 Months Liver/ Brain Wt (%)
0	4.2972 (0.4316) [--]	2.0396 (0.2006) [--]	4.2615 (0.3063) [--]	2.1111 (0.2421) [--]	5.2043 (3.7014) [--]	2.8955 (2.2354) [--]	4.5347 (0.6092) [--]	2.4395 (0.5362) [--]	5.4121 (1.8806) [--]	2.9172 (1.2925) [--]
20	4.2537 (0.4694) [--]	2.0123 (0.3308) [--]	4.4136 (0.2799) [+3%]	2.1971 (0.2716) [+4%]	4.4255 (0.9915) [-15%]	2.2460 (0.5030) [-22%]	4.7717 (0.6378) [+5%]	2.5348 (0.5121) [+4%]	5.1413 (0.8257) [-5%]	2.7646 (0.6577) [-5%]
100	4.3861 (0.3423) [+2%]	2.0130 (0.2673) [--]	4.3269 (0.3384) [+2%]	2.0996 (0.2521) [--]	3.9346 (0.3991) [-24%]	1.9174 (0.3434) [-34%]	4.9152 (1.1444) [+8%]	2.7304 (0.7674) [+19%]	5.0221 (0.7861) [-7%]	2.5560 (0.5065) [-12%]
500	4.3563 (0.2697) [+1%]	2.0707 (0.2552) [--]	4.5217 (0.3847) [+6%]	2.4430 (0.2558) [+13%]	4.6789 (0.7681) [-10%]	2.4748 (0.3821) [-14%]	5.2661 (0.8716) [+16%]	2.8237 (0.5306) [+16%]	5.2739 (0.7775) [-3%]	2.7814 (0.6143) [-5%]
1000	4.8617 (0.5301) [+13%]	2.1669 (0.3414) [+6%]	4.8511 (0.4677) [+14%]	2.2878 (0.2076) [+9%]	4.4269 (0.5217) [-15%]	2.3086 (0.5196) [-20%]	5.2437 (0.7920) [+15%]	2.7744 (0.5173) [+14%]	5.2962 (0.6229) [-2%]	2.8970 (0.4762) [-1%]
2000	4.9808 (0.3406) [+16%]	2.3240 (0.26995) [+14%]	4.9056* (0.4417) [+15%]	2.4711 (0.4830) [+17%]	4.5383 (0.4750) [-13%]	2.3224 (0.4315) [-20%]	5.1681 (1.3849) [+14%]	2.9344 (0.8700) [+20%]	5.3353 (0.7843) [-1%]	2.8673 (0.4173) [-2%]
4000	5.1442 (0.6556) [+20%]	2.4704 (0.5061) [+21%]	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---
8000	5.8583** (0.4232) [+36%]	2.6948** (0.3011) [+32%]	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---	--- --- ---

** Statistically Significant (p = 0.01) using Tukey's Multiple Comparison test

--- Treatment groups terminated at 3-month

[ ] Expressed as percent of controls

athology: The observed histopathology was similar for all roups when compared to concurrent controls, with the exception f liver pathology in male treatment groups and thymus gland yperplasia in female treatment groups. The liver data (Table 7) ere discussed and evaluated by the Toxicology Peer Review Gregorio to Taylor, January 2, 1985):

able 7. Liver Histopathology in Male Mice over 24 Months

ose (ppm)	0	20	100	500	1000	2000
epatocellular Adenoma						
6-12 month*	2/12	1/17	1/18	1/14	2/14	0/14
12-18 month*	2/19	1/20	1/20	0/19	0/20	0/19
18-24 month*	10/36	7/31	8/31	8/37	7/35	8/36
	14/67	9/68	9/69	9/70	9/69	8/69
epatocellular Carcinoma						
6-12 month*	0/12	0/17	2/18	0/14	0/14	0/14
12-18 month*	1/19	2/20	0/20	2/19	5/20	1/19
18-24 month*	2/36	1/31	7/31	5/37	5/35	2/36
	3/67	3/68	9/69	7/70	10/69	3/69
OTAL HEPATOCELLULAR ADENOMA AND CARCINOMA						
	17/67	12/68	19/69	16/70	19/69	11/69
epatocellular Cytomegaly						
3 month++	0/19	3/20	-	0/20	-	3/20
6 month	0/10	0/10	0/10	0/10	2/10	5/10
6-12 month*	0/12	0/17	0/18	0/14	2/14	0/14
12-18 month*	0/19	0/20	2/20	2/19	1/20	4/19
18-24 month*	1/36	0/31	1/31	0/37	0/35	4/36
OTAL HEPATOCELLULAR CYTOMEGALY						
	1/96	3/91	3/89	2/100	5/89	12/99

The denominator is a combination of scheduled sacrifice and arly death animals.

Not Examined

+ At 3-months, the 4000 ppm group was not examined, the 8000 pm group had an incidence of 9/20.

004625

mentioned previously, the Branch's Peer Review Group (Dr. odore Farber, Dr. Reto Engler, Mr. Bert Litt, Mr. William nam, Dr. Robert Zendzian) discussed the liver pathology a set on December 21, 1984. The Group agreed that the a indicated liver toxicity as evidonced by the increased idence of hepatocellular cytomegaly in the 1000 and 2000 ppm atment males from 18 through 24 months of the study and the 4000 and 8000 ppm treatment males at 3 months of the dy. With regard to the observed incidence of liver tumors, Group agreed that there was no dose response relationship erved and that additively (sum of hepatocellular adenoma hepatocellular carcinoma) there was no an oncogenic nd. Combination of benign and malignant tumors generally resent the histogenetic development of the tumors and ing them together minimizes the effect of pathologists ng different terminology. Therefore, based on these ts, the liver pathology observed in this study are not sidered to indicate an oncogenic potential.

authors reported an increased incidence of lymphoid erplasia in the thymus gland in the 1000 and 2000 ppm ales when compared to concurrent controls (Table 8). The hors described this non-neoplastic change "as a normal -related finding commonly seen in older animals. No arent pathophysiologic explanation is available but does ear to be test article related."

le 8. Thymus Lymphoid Hyperplasia Incidence In Female Mice

Dose (ppm)	0	20	100	500	1000	2000
3-month	0/20	0/20	0/20	0/20	0/20	0/20
6-month	0/13	0/12	0/11	0/10	0/11	1/10
12-month	1/13	0/13	0/12	0/14	0/11	0/10
18-month	0/9	0/18	0/20	0/21	0/10	0/13
24-month	2/33	4/34	2/29	3/34	13/23	11/41
	3/88	4/97	2/92	3/99	14/75*	12/94*

p = 0.01 (chi-square)

DATA EVALUATION RECORD 004625

Chemical: COMMAND; FMC 57020; Dimethazone
2-(2-chlorophenyl) methyl-4, 4-dimethyl-3-isooxazolidinone

Test Material: Technical (88.8%)
(Reference No. E1756-146; FMC-T#:206)

Citation: C. Freeman et al. Teratology Study in Rats With FMC 57020 Technical. (FMC Study No. A83-1142). June 29, 1984. Unpublished report prepared by FMC Corporation. EPA Accession No. 072829.

Reviewed By: Carolyn Gregorio, Toxicologist CAG 7-16-85
Toxicology Branch/HED

Secondary Review: Clint Skinner, Ph.D
Section Head
Toxicology Branch/HED
Clint Skinner 8-12-85

CONCLUSION: Treatment with COMMAND was associated with maternal toxicity as a function of clinical signs of toxicity (decreased locomotion, abdominogenital staining, chromorhinorrhea) at 300 and 600 mg/kg. As an indication of fetotoxicity, there was a statistically significant (Chi-square) increase in the incidence of delayed ossification or absence of four sternebrae in the 300 and 600 mg/kg groups when compared to concurrent controls. In addition, hydronephrosis and hydroureter were observed more frequently, but not statistically significant, in all dose groups when compared to concurrent controls.

No historical control data were available to provide perspective for these fetotoxic effects.

No teratogenicity was observed at any treatment doses or controls.

Core Classification: Supplementary

1.) Registrant should provide historical control data for rats of the same strain used in this study for the years 1982 through 1984;
2.) Registrant should provide a more comprehensive explanation of why the doses used in the study "appear to be more appropiate dosage levels" than those originally assigned (Protocol Amendment #1);
3.) This study is being recommended for a Laboratory Data Audit. The recommendation for a data audit is based on the lack of any reported teratological findings in this study, which is extremely unusual. In addition the 2-generation rat reproduction (Toxigenics Study No. 450-1095; June 12, 1984) did demonstrate some severe teratogenic findings (one fetus in 1000 and 4000 ppm dose groups had hind limb abnormalities; one fetus in the 4000 ppm dose group had no anal opening).

Materials and Methods

Test species: Young adult female Sprague-Dawley rats were used. Each female was mated overnight with a male and the following morning vaginal smears were examined for the presence of spermatozoa and/or stage of estrus. The day spermatozoa were found was designated day 0 of gestation. Test animals weighed approximately 233 g.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 15 of gestation. Doses of 0, 100, 300, or 600 mg test substance per kg body weight were given to groups of 25 mated dams.

Each dam was observed twice daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 6 through 15 and 20 of gestation. Food consumption was calculated weekly.

The rats were sacrificed on day 20 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and 50% of them were prepared for skeletal examination. The remainder were prepared for soft tissue examination, and abnormalities were noted.

"For the purpose of this study a late resorption is defined as one in which organogenesis has occurred. A dead fetus is one in which no autolysis is present. If autolysis is evident, it will be considered a late resorption."

Major abnormalities were characterized as life threatening and minor abnormalities were defined as those which would not be expected to directly affect the survival of the fetus. The report stated that variations in the degree of ossification were considered as minor defects when observed to occur more frequently than similar observations in control or background data. Terminology and definitions used in this study are listed in Appendix 1 (Attached).

Statistical procedures are discussed below as appropriate. The report noted that animals that died during gestation, aborted, or were not pregnant were not included in the analysis of results.

004628

RESULTS:

1. Maternal Effects: Maternal toxic effects were observed at the 300 and 600 mg/kg doses. These effects consisted of clinical signs of toxicity, e.g., abdominogenital staining, decreased locomotion, chromorhinorrhea. These clinical signs were more pronounced at the high dose (600 mg/kg) starting at day 6 of gestation. In addition, decreased mean food consumption (12% decreased) was observed in the high dose dams on days 6 through 13 of gestation when compared to concurrent controls (see Attachment 2; Registrant's Submission Table 2).

   There were no treatment related effects observed in dams with regard to mortality (authors reported that two dams in the 600 mg/kg group and one dam in the 300 mg/kg group died on test as a result of esophageal perforation) and body weight gains (see Appendix 2; Registrant's Submission Table 1).

2. Reproductive and Embryotoxic Effects: Pregnancy rates were comparable in all groups (96-100%) and resulted in 23-25 pregnant dams being examined at C-section, all with viable fetuses. One high dose dam (600 mg/kg) had total resorption.

   A non-statistical increase in mean total resorptions were observed at the 300 and 600 mg/kg dams when compared to controls. However, when comparing the number of litters for each dose group which had resorption(s), all dose groups were comparable to concurrent control (Table 1). Therefore, the increase in resorptions observed, is not considered to be biologically significant.

Table 1. Mean Resorption Data

Dose (mg/kg)	0	100	300	600
. Early (number/%)	4(1.3)	3(1.0)	7(2.4)	9*(3.4)
. Late (number/%)	0(0.0)	0(0.0)	1(0.3)	0(0.0)
. Total	4(1.3)	3(1.0)	8(2.7)	9(3.4)
. Rats with resorptions(%)	4/25(16%)	3/25(12%)	5/24(21.7%)	3/23(13%)

*Dam No. AA0456F had 7 resorptions; the entire litter.

No treatment-related effects were observed for the number of implantations, corpora-lutea implantations, litter size, and live versus dead fetuses (Appendix 2; Registrant's Submission Table 3).

3. Fetotoxic Effects: No treatment-related effects were observed for the numbers of fetuses/dam or fetal sex distributions for any group (see Appendix 2; Registrant's Submission Table 4) when compared to concurrent controls.

Fetotoxic treatment-related effects were observed in the in the 300 and 600 mg/kg groups when compared to concurrent controls. These effects were decreased mean fetal weights in the 600 mg/kg group (see Appendix 2; Registrant's Table 4), skeletal abnormalities in the 300, and 600 mg/kg groups (see Appendix 3, Registrant's Table 5; reviewer's Table 2), and visceral abnormalities in the 100, 300 and 600 mg/kg groups (see Appendix 3, Registrant's Table 6) when compared to concurrent controls.

Skeletal Variations: With regard to the skeletal changes observed, there was a statistically significant (Chi-square, as performed by the reviewer) increase in the delayed ossification or absence of at least four sternebrae in the 300 and 600 mg/kg groups when compared to concurrent controls. This skeletal variation is consistent with early generalized disturbances which suggest delayed development and is considered to be a sign of fetotoxicity when associated with toxic effects in dams.

The 100 mg/kg group had a slight, but not statistically significant, increase in the delayed ossification and or absence of two sternebrae when compared to concurrent controls (reviewer Table 2). In this group, the 4th and 5th sternebrae were affected, which are the last in the skeletal chain to ossify. This slight increase in skeletal variation is not considered to be biologically relevant because skeletal development is late in prenatal life and is not complete for several days after birth and there are no other associated effects in the dams or fetuses.

Historical control data should be submitted by the Registrant to provide a more comprehensive context for examining the skeletal variation observed in this study.

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Table 2. Delayed Ossification or Absence of More Than One Sternebrae

Dose (mg/kg)	0	100	300	600
2 Sternebrae (Delayed Ossification) or Absent				
. Fetuses	4/147(2.7)	9/149(6.0)	7/144(4.9)	21/128(16.4)**
. Litters	2/25 (8.0)	6/25 (24.0)	6/23(26.1)	11/22(50.0)**
3 Sternebrae (Delayed Ossification) or Absent				
. Fetuses	0/147(0)	0/149(0)	0/144(0)	1/128(0.1)
. Litters	0/25 (0)	0/25(0)	0/23 (0)	1/22(4.5)
4 Sternebrae (Delayed Ossification) or Absent				
. Fetuses	0/147(0)	0/149(0)	4/144(2.8)**	4/128(3.1)**
. Litters	0/25 (0)	0/25(0)	4/23 (17.4)**	4/22(18.2)**
Totals				
. Fetuses	4/147(2.7)	9/149(6.0)	11/144(7.6)*	26/128(20.3)***
. Litters	2/25 (8.0)	6/25 (24.0)	10/23(43.5)***	16/22(72.7)***

Note: Number abnormal fetuses or litter/number fetuses or litters examined.

( ) = percent (%)

* P = 0.10

** P = 0.05

*** P = 0.005

491

Visceral Abnormalities: Hydronephrosis and hydroureter were observed more frequently in all dose groups when compared to controls (see Appendix 3; Registrant's Table 6) and may be considered a fetotoxic effect.

The rate of development of the renal system is highly variable in rats and the observation of hydronephrosis and or hydroureter may be a condition which may be completely resolved by weaning. However, without the the perspective of historical control data, the significance of these visceral abnormalities cannot be adequately assessed.

4. Teratogenic Effects: No major fetal malformations were reported in this study. Again, no historical control data were available to assess whether the low spontaneous incidence reported is within the normal variation and range for this strain of rat. Therefore, as previously mentioned, historical control data are requested.

APPENDIX 1: Terminology and Definitions

Command tox review

Page _____ is not included in this copy.

Pages 33 through 49 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 product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ FIFRA registration data

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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.

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

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EPA: 68-01-6561
TASK: 113
June 27, 1985

DATA EVALUATION RECORD

FMC 57020

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Borders, C. K. and Salomon, C. M. Two-generation reproduction study in albino rats with FMC 57020 technical. (Unpublished Study No. 450-1095 by ToxiGenics, Inc., Decatur, IL for FMC Corporation; dated June 12, 1984.) Accession Nos. 072813, 072821, 072822, 072823, 072830, 072831, and 072832.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 6-26-85

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1. CHEMICAL: FMC 57020, Command.

2. TEST MATERIAL: FMC 57020 technical was amber colored and consisted of 88.8-91.4% active ingredient.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Borders, C. K. and C. M. Salomon. Two-generation reproduction study in albino rats with FMC 57020 technical. (Unpublished Study No. 450-1095 by ToxiGenics, Inc., Decatur, IL for FMC Corporation; dated June 12, 1984.) Accession Nos. 072813, 072821, 072822, 072823, 072830, 072831, and 072832.

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51

496

7. CONCLUSIONS:

A. The adult rat NOEL and LOEL were 1000 and 2000 ppm, respectively, based on compound-related effects on parental body weight, food consumption, clinical signs, organ changes and fertility indices at 2000 and 4000 ppm.

The NOEL and LOEL for the progeny were 100 and 1000 ppm, respectively, since adverse effects including decrease pup viability, reduced survival, decreased body weight, and potentially severe malformations were reported at 1000 ppm, and above.

B. This study is classified CORE Minimum.

8. RECOMMENDATIONS:

It would be useful for the testing laboratory to fully examine the malformed pups to determine the severity of their reported malformations, and to assess if these pups have any additional visceral and/or skeletal defects.

Items 9 and 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test material, dose levels, and route of administration: The test material used in this study (FMC 57020 technical) was described as an amber-colored product consisting of 88.8-91.4% active ingredient. Except for chemical safety data, no additional information on the test material was provided to the testing laboratory by the study sponsor. The test material was mixed with Purina Certified Rodent Chow #5002 to produce concentrations of 0 (control), 100, 1000, 2000, or 4000 ppm. Whenever possible, diet mixtures were prepared every week.

B. Test animals and experimental design: A total of 133 male and 151 female CD rats were obtained from Charles River Breeding Laboratories for this study. Following a 12-day acclimatization period, 25 males and 25 females were randomly assigned to each one of the 5 groups comprising the F_0 segment of this study. The F_0 parents were mated to produce the F_{1a} generation. Seven days after the completion of the F_{1a} weaning, each F_0 male was paired with another F_0 female to produce the F_{1b} generation. The process was repeated once again using 25 male and 25 female F_{1b} parents to produce the F_{2a} and F_{2b} generations. All animals received their corresponding diet

[1]Only items applicable for this review are included.

mixtures from the initiation of the study, until sacrifice. The diets and water were available ad libitum. Animals in the F_0 generation consumed their corresponding diet for eight weeks prior to their first mating, whereas the F_1 generation was first mated after 11 weeks of diet consumption. The study schedule is represented in the following flowchart.

Week	F_0	1st Generation (F_1)	2nd Generation (F_2)
1	F_0 dosing starts		
2			
3			
4	8 weeks		
5			
6			
7			
8	F_0 first mating starts		
9			
10	F_0 first mating ends ...	F_{1a} birth starts	
11			
12	.....	F_{1a} birth ends	
13	F_0 second mating starts		
14	.....	F_{1a} lactation ends	
15	F_0 second mating ends...	F_{1b} birth starts	
16			
17	.....	F_{1b} birth ends	
18			
19	.....	F_{1b} lactation ends	
20			
21			
22			
23			
24		11 weeks	
25			
26			
27			
28			
29	.....	F_{1b} first mating starts	
30			
31	.....	F_{1b} first mating ends...	F_{2a} birth starts
32			
33	.....		F_{2a} birth ends
34	.....	F_{1b} second mating starts	
35	.....		F_{2a} lactation ends
36	.....	F_{1b} second mating ends	F_{2b} birth starts
37			
38	.....		F_{2b} birth ends
39			
40	.....		F_{2b} lactation ends
41	.....		Termination of study

C. Measurements and observations: The concentration of the test article in the diet mixtures was analyzed at monthly intervals, and the percentage of active ingredient in the test article was determined by analytical assays every three months.

Body weights of all parental animals were measured and recorded once weekly during the premating period. In addition, females were weighed on gestational days 0, 6, 15, and 20, and on lactation days 0, 4, 7, 14, and 21. Terminal body weights were obtained for all animals prior to sacrifice.

The food consumption of parental animals was determined at weekly intervals during the premating period only. Parental animals were observed at least twice daily for overt signs of toxicity and to determine their health status. Females were allowed to give birth, and to nurse their litters for 21 days. Mothers and their pups were observed daily.

On the day of birth, litters were examined to determine the viability, number and sex of the pups. The number of live pups was subsequently determined on lactation days 1, 4, 7, 14, and 21. On lactation day 4, litters in excess of eight pups were randomly reduced to four males and four females, whenever possible. Body weights and sexes were recorded for all live pups on lactation days 0, 4, 7, 14, and 21. Litters were examined daily for mortalities and signs of abnormal behavior. Developmental abnormalities were assessed at birth and on lactation day 21.

Progeny from the F_{1a} and F_{2a} generations were sacrificed and discarded at weaning, except for animals exhibiting developmental abnormalities. Abnormal animals were subjected to pathologic examination. One male and one female were selected at random from each F_{1b} and F_{2b} litter at weaning and examined grossly for pathologic changes. A total of 25 males and 25 females were randomly selected from each treatment group at the completion of the F_{1b} litter weaning . These animals were later mated to produce the F_2 generation. All animals which appeared normal and were no longer needed for the study were sacrificed and discarded. Animals exhibiting abnormalities were retained and examined.

At necropsy, the weights of brains, hearts, kidneys, livers, and ovaries or testes were obtained. Samples from vaginas, uteri, ovaries, testes, seminal vesicles, prostates, kidneys, livers, and other organs appearing to be abnormal were retained. In addition, histopathologic examinations were conducted on samples of ovaries, uteri, vaginas, testes, prostates, seminal vesicles, and other organs appearing abnormal.

The materials and methods section of the study report is included in Appendix A of this review.

12. REPORTED RESULTS:

A. Test Material: Analytical determinations of test material concentrations in the test diets indicated that the diet mixtures were within ±10% of the target concentrations.

B. Parental Effects:

1. Mortality: The test material was not associated with increased incidences of parental mortality.

2. Body Weights: The study authors indicated that no compound-related reductions in body weights were noted in either F_0 or F_1 males during the pre-mating period. However, F_0 and F_1 females from the 2000 and 4000 ppm dosage groups had statistically significant reductions in premating body weights and body weight gains compared to controls (Table 1). These females also had statistically significant reductions in body weights compared to controls during the gestation and lactation periods of the F_{1a}, F_{1b}, F_{2a}, and F_{2b} litters (Table 2).

3. Food Consumption: The pre-mating dietary intake of males in the dosage groups was similar to that of concurrent controls except for the statistically significant reductions noted in F_0 males from the 4000 ppm group during week one, and in F_1 males from the 2000 ppm group during week eight; however, the study authors did not consider the food intake reduction among F_1 males to be compound related. Statistically significant reductions in food consumption were noted during several pre-mating weeks among F_0 and F_1 females from the 2000 and 4000 ppm groups (Table 3).

4. Test Material Intake: The data from the study report indicate that the calculated intake of test material for all groups of F_1 males and females was roughly twice that of the corresponding F_0 groups during the first two weeks of dosing. The consumption of test material was similar among corresponding F_0 and F_1 groups in the latter part of the pre-mating period (Table 4).

5. Clinical Observations: Increases in the incidence of urine-soaked and/or yellow-brown stained fur among the F_0 and F_1 females from the 4000 ppm groups were the only antemortem findings noted.

C. Reproductive Parameters

1. Fertility Indices: The percentage of mated females which were identified as pregnant was decreased only in the 4000 ppm group of the F_{1b} generation. The fertility indices of all other groups and generations were not affected (Table 5).

TABLE 1. Group Mean Values for Parental Body Weight (g) in Rats During the Pre-mating Period

	Dose (ppm)	Pre-mating Week												Pre-mating Gain	Final Weight	Total Change
		Initial Weight	1	2	3	4	5	6	7	8	9	10	11			
F_0 Males	0	277.4	309.4	343.2	370.3	395.9	415.8	436.6	453.3	471.6	--	--	--	194.2	583.9	306.5
	100	275.9	308.0	343.5	371.1	395.1	414.9	435.5	455.5	472.9	--	--	--	197.1	609.5	333.6
	1000	277.4	312.4	348.6	375.7	399.2	421.4	441.4	463.0	479.3	--	--	--	201.9	619.1	341.7
	2000	277.0	307.4	340.4	367.0	388.9	407.5	426.3	443.8	460.5	--	--	--	183.4	586.1	309.1
	4000	277.2	307.7	343.1	369.0	391.2	410.4	428.6	448.8	464.1	--	--	--	186.9	599.1	321.9
F_0 Females	0	177.3	194.4	212.9	225.0	236.3	246.6	255.0	263.0	271.6	--	--	--	94.3	356.7	179.4
	100	177.7	195.5	214.7	228.5	238.5	247.6	258.0	267.4	274.5	--	--	--	96.8	360.5	182.8
	1000	177.8	194.2	212.2	222.8	232.2	242.7	252.4	260.3	268.0	--	--	--	90.2	350.4	173.4
	2000	177.5	187.4	202.3	212.7	221.1*	227.8**	234.4**	241.3**	247.1*	--	--	--	69.6**	339.6	162.1
	4000	177.3	184.6	199.9*	211.6*	219.2**	225.8**	233.6**	241.6**	247.1*	--	--	--	69.8**	332.6	155.3*
F_1 Males	0	47.7	139.3	196.4	251.4	299.9	339.5	374.1	404.7	438.6	453.7	473.0	487.2	439.5	627.6	579.9
	100	49.3	157.8	215.7	269.0	315.3	350.5	378.4	403.0	432.6	445.4	463.6	476.8	427.5	608.4	559.1
	1000	50.8	153.2	210.0	263.1	307.1	341.1	369.8	394.3	426.7	438.5	455.8	469.4	418.5	592.0	541.2
	2000	48.2	145.3	198.8	249.0	292.9	326.8	357.8	379.0	410.6	422.3	439.6	454.4	406.1	571.2	522.9*
	4000	47.7	140.3	195.1	247.5	293.4	330.3	360.2	386.7	417.0	428.2	448.0	463.8	416.1	590.5	542.8
F_1 Females	0	43.4	120.2	149.9	172.9	196.3	212.7	226.1	239.0	254.2	260.8	267.7	274.4	230.9	351.3	308.1
	100	47.9	131.6	158.8	183.0	203.6	220.5	231.9	243.1	257.9	264.6	269.0	275.2	227.3	358.6	311.1
	1000	48.7*	129.0	156.1	178.7	197.5	212.7	225.4	237.1	249.3	255.7	263.1	267.7	219.0	356.9	308.3
	2000	44.6	118.1	145.6	167.4	185.2	199.3	212.2	222.5	235.2*	237.7**	243.9**	249.4**	204.9**	335.8	291.2
	4000	46.2	116.5	146.3	166.7	181.8	196.7*	209.6*	219.2*	231.4**	234.7**	240.9**	245.6**	199.5**	336.9	290.8

* Statistically different from control value ($p \leq 0.05$).
** Statistically different from control value ($p \leq 0.01$)

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TABLE 2. Group Mean Values for Maternal Body Weight (g) in Rats During Gestation and Lactation Periods

Litter Generation	Dose (ppm)	Gestation Day 0	6	15	20	Lactation Day 0	4	7	14	21
F_{1a}	0	277	303	336	391	307	309	319	344	332
	100	276	300	335	392	304	310	316	344	326
	1000	271	297	332	387	295	304	313	338	329
	2000	254*	275**	308*	362	281**	291	300	323*	313
	4000	251**	273**	309*	360	280**	286*	297*	308**	311
F_{1b}	0	324	347	384	435	352	357	359	376	361
	100	314	337	372	437	344	355	359	373	357
	1000	307	329	364	418	328	346	352	372	354
	2000	290**	310**	338**	396*	310**	324**	332*	346**	339
	4000	288**	307**	342**	396*	315**	327*	335	352	340
F_{2a}	0	275	297	332	395	307	319	320	333	319
	100	275	294	327	388	304	315	319	338	326
	1000	269	287	320	380	298	306	312	329	329
	2000	252*	268**	299**	360**	276**	287**	294*	311	311
	4000	246**	264**	297**	354**	275**	282**	291**	309*	313
F_{2b}	0	319	340	373	434	346	346	348	369	355
	100	317	336	368	433	346	345	346	370	354
	1000	310	331	365	431	344	346	350	364	362
	2000	288*	305*	339	401	314	320	326	345	342
	4000	285*	300**	333**	393*	316	316*	325	342	341

* Statistically different from control value ($p \leq 0.05$).
** Statistically different from control value ($p \leq 0.01$).

502

57

TABLE 3. Group Mean Values for Parental Food Consumption (mg/animal/day) in Rats During the Pre-mating Period

Parental Generation	Dose (ppm)	Pre-mating Week Interval 1	2	3	4	5	6	7	8	9	10	11
F_0 Males	0	23.7	23.7	25.7	26.2	28.5	28.3	26.6	27.1	27.4	--	—
	100	23.9	23.2	27.1	26.4	28.3	28.5	28.1	27.0	27.7	--	—
	1000	23.8	24.4	26.4	25.3	28.8	28.2	27.1	27.1	27.7	--	—
	2000	22 6	24.3	25.4	25.3	28.3	27.9	25.9	26.4	25.9	—	—
	4000	19.9**	24.4	25.8	26.7	28.6	28.0	26.4	26.5	26.5	—	—
F_0 Females	0	16.6	18.4	20.0	18.8	20.1	21.6	20.0	19.1	18.4	—	—
	100	17.6	18.5	21.3	20.2	22.5	22.2	20.8	20.8	19.9	—	—
	1000	15.4	17.6	19.4	19.3	20.7	20.1	20.0	19.9	19.5	—	—
	2000	11.5**	17.5	17.0*	17.8	19.0	18.8*	17.5	17.0	16.9	--	—
	4000	7.0**	17.0	15.9**	18.0	18.3	19.5	17.2	16.9	17.7	—	—
F_1 Males	0	19.2	24.8	25.7	26.2	26.9	27.6	27.1	28.6	28.5	29.4	28.0
	100	21.0	26.0	26.7	28.2	26.8	28.4	27.3	28.7	28.9	29.3	27.6
	1000	20.0	25.2	25.7	25.3	26.1	27.7	27.0	28.2	28.6	28.5	27.3
	2000	17.8	23.8	24.8	25.7	26.0	25.3	25.8	25.8*	26.6	27.2	26.1
	4000	17.9	23.5	24.6	26.2	25.9	27.1	26.1	26.3	27.9	27.4	26.5
F_1 Females	0	15.6	18.4	17.6	19.1	18.8	19.0	18.0	19.5	20.2	19.4	18.2
	100	16.8	19.2	19.2	21.0	19.6	20.3	19.2	20.4	20.6	19.9	19.6
	1000	15.4	18.1	17.4	18.2	18.6	19.2	19.1	19.5	19.2	18.7	18.6
	2000	13.7*	17.4	16.1	17.2	17.0	16.6**	17.0	16.8*	17.1**	18.0	16.8
	4000	14.2	17.5	16.3	17.6	16.8	17.0**	16.7	16.8*	16.5**	17.8	16.5

* Statistically different from control value ($p \leq 0.05$).
** Statistically different from control value ($p \leq 0.01$).

503

53

TABLE 4. Calculated Group Mean Values for Parental Test Material Consumption (mg/kg/day) in Rats During the Pre-mating Period

Parental Generation	Dose (ppm)	Pre-mating Week 1	2	3	4	5	6	7	8	9	10	11
F_0 Males	0	—	—	—	—	—	—	—	—	—	—	—
	100	8.6	7.6	7.9	7.1	7.2	6.9	6.5	6.0	5.9	—	—
	1000	85.8	78.0	75.7	67.4	72.2	67.0	61.6	58.6	57.6	—	—
	2000	163.5	158.3	149.7	138.1	145.5	136.2	121.6	119.1	113.1	—	—
	4000	287.9	317.8	301.3	289.5	292.4	271.9	246.4	236.0	229.1	—	—
F_0 Females	0	—	—	—	—	—	—	—	—	—	—	—
	100	9.9	9.5	9.9	8.9	9.4	8.9	8.0	7.8	7.3	—	—
	1000	86.7	90.4	91.1	86.4	89.3	82.9	79.2	76.6	72.8	—	—
	2000	130.8	187.6	169.8	168.1	173.4	165.5	149.6	141.2	135.5	—	—
	4000	158.2	372.4	318.2	339.7	333.2	348.1	293.0	279.5	286.8	—	—
F_1 Males	0	—	—	—	—	—	—	—	—	—	—	—
	100	13.5	12.2	10.0	9.0	7.6	7.5	6.8	6.7	6.5	6.4	5.8
	1000	131.0	120.2	97.8	81.9	76.7	75.0	68.6	66.3	65.4	62.6	58.1
	2000	248.3	241.1	203.0	176.8	159.6	141.7	136.3	126.5	126.6	123.9	115.1
	4000	537.4	498.4	404.4	361.6	318.9	301.9	272.7	251.3	260.3	247.4	228.4
F_1 Females	0	—	—	—	—	—	—	—	—	—	—	—
	100	12.9	12.1	10.5	10.3	8.9	8.8	7.9	7.9	7.8	7.4	7.2
	1000	119.6	115.9	97.5	92.0	87.3	85.3	80.7	78.0	75.0	70.7	69.2
	2000	236.7	242.9	194.7	186.3	171.7	157.0	153.1	143.5	145.7	146.7	135.7
	4000	500.8	491.8	395.2	388.2	345.2	324.8	305.3	292.1	281.1	296.8	269.1

2. Gestation Indices: The percentage of pregnant females that delivered pups was similar for all groups and generations (Table 5).

3. Progeny: No statistically significant differences were found in the mean number of pups delivered per litter, or in the mean number of viable, dead, or cannibalized pups among all groups and generations. No differences were reported in pup survival at days 4 and 21 of lactation for any group or generation. Compound-related reductions in pup body weight were noted only in the 2000 ppm group of the F_{2a} generation, and in the 4000 ppm groups of the F_{2a} and F_{2b} generations (Table 6).

   A few developmental abnormalities were noted among pups in this study. One F_{1b} pup from the 100 ppm group had macrophthalmia. Two F_{1b} pups from different litters in the 4000 ppm group were abnormal; one had no anal opening, and the other had a short, kinked tail. One control pup in the F_{2a} generation had a short, hair-like tail, and one pup from the 1000 ppm group in the F_{2a} generation had a malpositioned tail and dysfunctional hind legs. In the F_{2b} generation, one pup from the 100 ppm group had anophthalmia; the same finding was observed in a pup from the 4000 ppm group. In a different litter from the 4000 ppm group one pup had extended hind legs which did not bend at the ankles.

D. Necropsy Findings: The only compound-related effects reported for the F_0 generation were statistically significant increases in liver-to-body weight ratios in both males and females from the 4000 ppm groups, and increases in liver-to-brain weight ratios in males only. In addition, the study authors reported a statistically significant, but not biologically significant, increase in brain-to-body weight ratios for F_0 females in the 2000 ppm group.

   An increase in the incidence of kidneys with dilated/distended pelvis was noted in F_1 males from the 2000 and 4000 ppm groups. The mean kidney-to-body weight ratio of F_1 males from the 2000 ppm group was decreased, and the-liver-to body weight ratio for F_1 males from the 4000 ppm group was increased. A statistically significant increase in liver-to-body weight ratios was noted for F_1 females from the 2000 and 4000 ppm groups. In addition, female progeny in the F_{2b} generation from the 4000 ppm group had decreased ovarian weights.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The only conclusion presented by the study authors is that 1000 ppm of FMC 57020 in the diet is the NOEL for rats under the present study conditions.

TABLE 5. Summary of Reproductive Parameters for Two Generations of Rats Dosed with FMC 57020

Litter Generation	Dose (ppm)	No. Fem. Mated	No. Fem. Pregnant	Fertility Index	No. Litters Born	Gestation Index	Mean No. Pups/Litter	Born Viable	Born Dead
F_{1a}	0	25	22	88%	21	96%	13	97%	3%
	100	25	24	96%	23	96%	13	98%	2%
	1000	24	22	92%	22	100%	14	95%	5%
	2000	26	25	96%	24	96%	13	99%	1%
	4000	24	24	100%	24	100%	13	99%	1%
F_{1b}	0	27	21	96%	20	95%	12	99%	1%
	100	22	22	100%	22	100%	14	98%	2%
	1000	21	21	100%	20	95%	14	94%	6%
	2000	27	24	89%	23	96%	13	99%	1%
	4000	32	21	66%*	20	95%	13	97%[a]	3%[a]
F_{2a}	0	24	23	96%	23	100%	13	99%	1%
	100	25	24	96%	24	100%	13	96%	4%
	1000	25	23	92%	23	100%	13	99%	1%
	2000	24	24	100%	24	100%	14	99%	1%
	4000	24	24	100%	24	100%	13	98%	2%
F_{2b}	0	22	22	100%	20	95%	14	100%	0%
	100	23	22	96%	21	100%	13	99%	1%
	1000	23	23	100%	23	100%	14	97%	3%
	2000	22	22	100%	22	100%	14	97%	3%
	4000	25	24	96%	24	100%	13	97%[b]	3%[b]

[a] Reviewers' calculations indicate 94% viable, and 6% dead.

[b] Reviewers' calculations indicate 93% viable, 3% dead, and 4% cannibalized when litter No. AE 4907 (which was completely cannibalized) is included.

* Statistical different from control value ($p \leq 0.05$).

TABLE 6. Summary of Group Pup Survival and Body Weight Values During the Lactation Period in Two Generations of Rats Dosed with FMC 57020

Generation By Litter	Dose (ppm)	Pup Survival		Pup Body Weight		
				Day 4	Day 21	
		Days 0-4	Days 4-21	Combined	Male	Female
F_{1a}	0	90%	99%	9.5	41.5	39.1
	100	99%	99%	9.9	45.7**	43.0**
	1000	93%	93%	9.1	42.8	39.8
	2000	95%	90%	8.7	42.0	39.3
	4000	99%	97%	9.4	40.0	38.0
F_{1b}	0	99%	100%	9.5	47.0	43.9
	100	99%	100%	9.9	50.8*	47.3*
	1000	99%	99%	9.6	51.5**	48.2**
	2000	99%	99%	9.4	47.9	45.0
	4000	99%	100%	9.9	48.8	45.7
F_{2a}	0	98%	98%	8.8	46.1	42.8
	100	97%	98%	9.2	48.1	45.0
	1000	100%	100%	8.5	47.2	44.7
	2000	98%	98%	8.1**	44.5	41.1
	4000	96%	98%	8.7	42.2**	39.5**
F_{2b}	0	99%	98%	8.8	42.8	40.7
	100	99%	98%	8.9	45.5	43.2
	1000	99%	99%	8.4	44.5	41.3
	2000	92%	97%	8.4	42.8	41.0
	4000	99%[a]	97%	8.2**	40.7	38.1

[a]Reviewers' calculations indicate 95% when litter No. AE 4907 which was fully cannibalized is included.

*Statistically different from control value ($p \leq 0.05$).

**Statistically different from control value ($p \leq 0.01$).

B. A statement from the testing laboratory's Quality Assurance Office was signed and dated on June 12, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Parental Effects: No compound-related effects were noted in either male or female parents at dose levels of 100 or 1000 ppm; however, animals in the 2000 and 4000 ppm groups showed compound-related effects which included decreased body weight and food consumption, and alterations in absolute and/or relative liver, kidney, and ovary weights. The study results indicated that there was an increase in the incidence of dilated/distended kidneys which were noted during necropsies of males from the 4000 ppm group, and that females in this group had increased incidences of urine-soaked and/or yellow-brown stained fur.

The only reproductive index affected was the percentage of mated females that were pregnant (fertility index) in the 4000 ppm level. A summary of these effects is presented in Table 7.

Progeny Effects: No compound-related effects were noted in the 100 ppm dosage groups. Slight decreases in the percentage of viable pups born per litter, associated with slight increases in the percentage of dead and/or cannibalized pups, and statistically significant decreases in pup body weights were observed at the 2000 and 4000 ppm levels.

Several of the malformations observed throughout the various groups (short, kinked, and hair-like tails, macrophthalmia, anophthalmia, etc.) are commonly reported in reproductive studies on rats, and therefore may be considered spontaneous. However, one pup in the 1000 ppm level and another in the 4000 ppm level had limb abnormalities (non-functional limbs and extended limbs with [illegible] at the ankle, respectively); in addition, one pup from the 4000 ppm level had no anal opening. Although these malformations were not statistically significant and did not occur in dose-related patterns, causal relationships with the test compound cannot be completely ruled out. A summary of these effects is presented in Table 7.

B. The only major differences between the interpretations of compound-related effects presented by the study authors and by the reviewers are the following:

1. The decreases in viable fetuses in the high-dose group presented by the reviewers in Table 6 were based on our calculations of individual data from the study report. We included data from F_{2b} litter No. AE 4907 which was fully cannibalized before the end of lactation day one. The study authors did not include this litter in their calculations, nor did they consider a similar reduction in viable F_{1b}

TABLE 7. Reviewers' Summary of Compound-Related Effects in Rat Parents and Progeny

	Dose (ppm)				
	0	100	1000	2000	4000
I. PARENTS					
Increased incidence of mortality	—	—	—	--	—
Decreased body weight (1,2)[a]	—	--	—	X	X
Decreased food consumption (3)	--	--	—	X	X
Decreased fertility index (5)	—	--	--	—	X
Decreased gestation index (5)	—	—	--	--	—
Increased incidence of abortion	—	—	—	--	—
Prolonged gestation period	—	—	--	--	—
Increased incidence of dystocia	—	—	—	--	—
Increased incidence of urine-soaked and/or yellow-brown stained fur	—	—	--	—	X
II PROGENY					
Decreased no. pups per litter (5)	—	—	—	--	--
Decreased no. pups born alive (5)	—	—	--	—	X[c]
Increased no. pups born dead or cannibalized (5)	—	—	--	—	X[c]
Decreased pup survival (6)	—	—	—	X[c]	X[c]
Decreased pup body weight (6)	—	—	—	X	X
III. PATHOLOGY					
Development abnormalities in pups[b]	—	—	X[c]	—	X[c]
Increased incidence of dilated/distended kidneys in adults	—	—	--	—	X
Increased liver to body weight ratio in adults	—	--	—	X	X
Increased liver to brain weight ratio in adults	—	—	--	—	X
Decreased kidney to body weight ratio in adults	--	—	--	X	—
Decreased ovarian weight	—	--	--	—	X

[a] For details see table (from this review) indicated in parentheses.

[b] Includes only malformation that the reviewers considered potentially related to the test material.

[c] Effects considered as biologically significant by the reviewers, but not by the study authors.

64

509

fetuses from the high-dose group to be meaningful. As we indicated in Table 5, these data did not agree with the study authors' summary data, or with their conclusions.

2. Since limb and anal malformations were present only in dosage groups, the reviewers could not rule out the possibility that the test material was teratogenic at levels of 1000 ppm or above; however, the study authors indicated that the test material did not have teratogenic effects in this study.

   Based on the above, we differed with the study authors with respect to the NOEL for this study (see reviewers' conclusions).

C. The following study deficiencies were noted by the reviewers:

1. The first pre-mating period of the F_0 generation consisted of eight weeks of compound exposure. This period is considered to be marginal for male rats. Pre-mating exposures of 11 weeks, such as those implemented in this study for the F_1 generation are more acceptable since they are believed to encompass most of the spermatogenic cycle in rats. However, we assess that this deficiency did not severely compromise the validity of the study since animals of the F_1 generation were more properly exposed.

2. The study authors indicated that female No. AE 4856 (from the 1000 ppm group) delivered the F_{2b} litter on gestational day 30. Since this female's first litter (F_{2a} generation) was born within the normal range of gestational days (21-22 days), we did not concur with the authors' explanation that the 30-day gestation was due to strain variability. Instead, we concluded that the copulation record for this female for the F_{2b} generation was missed due to technical error. This was considered a minor deficiency which did not negatively affect the outcome of this study.

3. No method (such as ammonium sulfide staining) was used to establish the pregnancy status of animals appearing to be not pregnant during necropsies. This deficiency may have negatively affected the validity of fertility indices.

4. Comprehensive examinations were not conducted on pups appearing to be developmentally abnormal. In the absence of acceptable skeletal and visceral examinations it is not possible for us to determine the severity of the reported malformations. This deficiency is particularly significant since malformations, which may be severe, were reported (but were not well described) in the compound-treated groups. In addition, the absence of thorough pup examinations makes us question if additional congenital malformations were present but not detected. However, the reviewers accept that the

major purpose of this study was not to assess for potential teratogenic effects and, therefore, this deficiency did not invalidate this study.

Item 15 - see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp 8-21.

511

APPENDIX A

Materials and Methods

Command tox review

Page _____ is not included in this copy.

Pages 68 through 81 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 product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ 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.

513

001628

Data Evaluation Record

nical: CONTROL

Well No: 4636

t Material: Metabolic [14C]-labeled PMC 57020 (99.8% purity; Specific Activity 20.81 mCi/mmol) and Non-labeled PMC 57020 (9.6 Purity).

ation: Seiler, S. Mat Balance Study and Tissue Distribution of Methylene [14C]-labeled PMC 57020. (PMC Report No. PC-C017, PMC Study No. PM-134r). March 27, 1984. Unpublished report prepared by Private Research Institute (PRI) for PMC Corporation.

master No.: 072818 (Duplicate in 070814)

iewed by: Carolyn Gregorio, Toxicologist -CAG 7-18-85
Toxicology Branch/HED (TS-769)

ondary Review: Clint Skinner, Ph.D. Clint Skinner 7-25-85
Section Head
Toxicology Branch/HED (TS-769)

re Classification: Acceptable

clusion: The test compound was excreted, via the urine and es, at 90-99% within 72 hours of treatment in a similar ner for the single oral low dose (5 mg/kg), single intravaneous e (3 mg/kg) and for the multiple oral dose for males and ales. In the same studies, there was no significant retention tissues.

the single oral high dose (900 mg/kg) study, females excreted re of radiolatel in urine (83%) than males (71%) over 7 days. l males excreted more in the feces (31%) when compared to ales (15%). Again, no significant retention in the tissues observed.

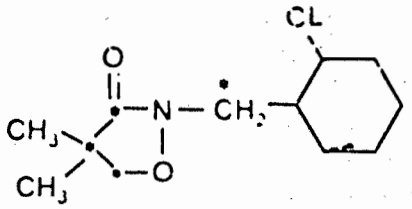
82

514

Materials and Methods:

Test Species: "All experiments were performed on adult male and female Sprague-Dawley rats (Charles River CD). CRL CD(SD)(Br). All animal body weights were between 130 to 150 grams when purchased."

Dosage: Unlabeled FMC 57020 (2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone) was mixed with ^{14}C-labeled FMC 57020. (Specific Activity = 26.81 mCi/mmol.) "Mazola corn oil was used as the carrier for all oral dosages of radiolabeled and nonlabeled compound. Distilled ionized water was used as the carrier for the intravenous (IV) dose."



*Denotes Methylene ^{14}C-labeled

Methods: Urine and core washings sampled were added directly to liquid scintillant (Instagel) for counting.

Fecal samples, lance tissues and organs were homogenized in water. Measured aliquots of hemogenate were transferred directly into combustion cores and processed for combustion. "Other tissues (gonads, muscles, adipose, spleen, bone, skin) were combusted directly. Resulting carbon dioxide was automatically trapped in Oxifluor."

"The results of the $^{14}CO_2$ study showed that less than 0.01% of the dosed radioactivity was expired as $^{14}CO_2$. All studies were thus performed in metal metabolism cages."

Experimental Procedure For Single Oral Low Dose: Five male and five female rats were given a single dose of 5 mg/kg of ^{14}C-methylene labeled FMC 57020 (in corn oil) by oral intubation. Urine, feces and cage washing debris were collected for 7 days following the dosing. Seven days after dosing, the animals were sacrificed and samples of brain, heart, pancreas, leg muscles, lungs, adipose, spleen, bone, skin, (washed and shaven) kidneys, liver, gonads and carcass were analyzed for radioactivity."

Reported Results For Single Oral Low Dose: Reported mean percentages of the administered dose recovered in urine and feces are as follows (Table 1):

Table 1: Mean Percentage Recovery of Radioactivity In Urine and Feces Following Single Oral Low Dose (5 mg/kg).

Time of Sample (hr)	Urine		Feces	
	Males	Females	Males	Females
0-24	52.52	54.43	28.02	27.94
24-48	7.93	10.72	8.45	9.65
48-72	1.63	1.44	0.95	1.01
72-168	1.61	1.80	1.29	1.02
Total	63.70	68.39	38.71	39.62

With regard to tissue residues "residue values higher than twice background (0.9 ppb) were detected in the lungs, spleen, kidney, liver, blood, skin, hair and carcass." "Total residual radioactivity varied between 0.083% and 0.179% of dosed radioactivity for the females and 0.099% to 0.283% for the males."

Discussion For Single Oral Low Dose: The data reported indicate "that there is no sex differences in the excretion pattern of radioactivity at the low dose level" (Table 1) over 7 days. In addition no significant tissue residue was reported.

001620

Experimental Procedure For Single Oral High Dose: "Five male and five female rats were given a single dose of 900 mg/kg ^{14}C-methylene labeled FMC 57020 (in corn oil) by oral intubation. Urine, feces and cage washing debris were collected for 7 days following the dosing. Seven days after dosing, the animals were sacrificed and samples of brain, heart, pancreas, leg muscles, lungs, adipose, spleen, bone, skin (washed and shaven), kidneys, liver, gonads and carcass were analyzed for radioactivity."

Reported Results For Single Oral High Dose: Reported mean percentages of the administered dose recovered in urine and feces are as follows (Table 2):

Table 2. Mean Percentage Recovery of Radioactivity in Urine and Feces Following Single Oral High Dose (900 mg/kg).

Time of Sample (hr)	Urine		Feces	
	Males	Females	Males	Females
0-24	56.83	43.44	22.03	4.55
24-48	12.23	36.23	7.19	9.23
48-72	0.85	1.57	0.54	0.55
72-168	0.73	2.26	0.71	0.71
Total	70.64	83.17	30.47	15.04

With regard to tissue residue, "residue values higher than twice background (165.6 ppb) were detected in the same organs as the low dose, namely in the lungs, kidneys, liver, blood, skin, hair and carcass." "Total residual radioactivity ranged between 0.108% to 2.432% of dosed radioactivity for the females and 0.017% to 0.232% for males."

Discussion For Single Oral High Dose: The data indicate that females excrete more of the radioactivity in the urine than males after a single high oral dose (900 mg/kg). This does contrast the results seen in the single oral low dose (5 mg/kg), where excretion of radioactivity is similar for males and females.

As seen in the single oral dose study, no significant tissue residue was observed. [redacted] female (Animal No. 1706) had 2.42% of dosed radioactivity in the carcass", which accounted for the range of 0.108% to 2.432% of dosed radioactivity in females. However, based on the residue data for the other females, this animal appears to be an outlier and of no particular significance.

Experimental Procedure For Intravenous Dose: "Five male and five female rats were given a single IV dose of 3 mg/kg of ^{14}C-methylene labeled FMC 57020 (in distilled water). Urine, feces and cage washing debris were collected for 7 days following the dosing. Seven days after dosing, the animals were sacrificed and samples of brain, heart, pancreas, leg muscles, lungs, adipose, spleen, bone, skin (washed and shaven), kidneys, liver, gonads and carcass were analyzed for radioactivity."

Reported Results For Intravenous Dose: Reported mean percentages of the administered dose recovered in urine and feces are as follows (Table 3):

Table 3. Mean Percentage Recovery of Radioactivity in Urine and Feces Following Single Intravenous Dose (3 mg/kg).

Time of Sample (hr)	Urine		Feces	
	Males	Females	Males	Females
0-24	65.49	71.37	18.96	14.49
24-48	4.26	3.65	2.61	2.04
48-72	0.59	0.50	0.27	0.22
72-168	0.70	0.86	0.25	0.31
Total	71.04	76.38	22.09	17.06

With regard to tissue residues, "residue values higher than twice the background (1 ppb) were detected in the same tissues as for the oral administration."

Discussion For Intravenous Dose: The reported data indicate that there is no sex difference in the excretion pattern of radioactivity at the single intravenous dose over 7 days. In addition, sc significant tissue residue was observed.

Experimental Procedure For Multiple Oral Dose: "Five male and five female rats were given 14 daily doses of 5 mg/kg of non-labeled FMC 57020 (in corn oil) followed by a single dose ^{14}C-methylene labeled FMC 57020 by oral intubation. Urine, feces and cage washing debris were collected for 7 days following the dosing. Seven days after dosing, the animals were sacrificed and samples of brain, heart, pancreas, leg muscles, lungs, adipose, spleen, bone, skin (washed and shaven), kidneys, liver, gonads and carcass were analyzed for radioactivity."

Reported Results For Multiple Oral Dose: Reported mean percentages of the administered dose recovered in urine and feces are as follows (Table 4):

Table 4: Mean Percentage Recovery of Radioactivity In Urine and Feces Following Multiple Doses (5 mg/kg):

Time of Sample (hr)	Urine		Feces	
	Males	Females	Males	Females
0-24	65.12	72.03	15.12	14.45
24-48	10.58	5.05	9.72	5.31
48-72	1.06	0.87	1.02	0.45
72-168	0.56	1.21	0.59	0.37
Total	77.32	79.16	26.45	20.95

Discussion For Multiple Oral Dose: The reported data indicate that there is no sex difference in the excretion pattern of radioactivity for the multiple oral dose over 7 days. In addition, no significant tissue residue was observed.

Data Evaluation Record

Chemical: Command

Caswell No: 463D

Test Material: Methylene ^{14}C-labeled FMC 57020 (99.8% purity; Specific Activity 26.81 m Ci/mmol) and Non-labeled FMC 57020 (99% Purity).

Citation: Wu, J. Metabolism of Methylene ^{14}C-labeled FMC 57020 in Rats. (FMC Report No. P.0898). June 16, 1984. Unpublished report prepared by FMC Corporation.

Accession No.: 072319 (Duplicate in 072814)

Reviewed by: Carolyn Crepario, Toxicologist CMC 7-18-85
Toxicology Branch/HED (TS-769)

Secondary Review: Clint Skinner, Ph.D.
Section Head
Toxicology Branch/HED (TS-769)

Core Classification: Acceptable

Conclusion: There are adequate data presented in this report to support the conclusions of the investigators. These data show that "FMC 57020 is readily absorbed, metabolized and eliminated following administration to rats. Some variance in rate and extent of absorption, biotransformation and excretion may occur which may be attributed to sex and/or size of administered dose."

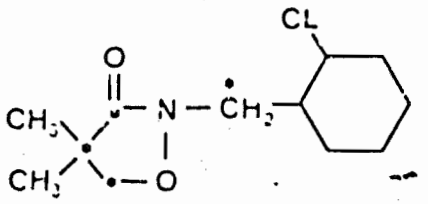
88
520

004625

Materials and Methods:

Test Species: "All experiments were performed on adult male and female Sprague-Dawley rats (Charles River CD). CRL CD(SD)(Br). All animal body weights were between 130 to 150 grams when purchased."

Dosage: Unlabeled FMC 57020 (2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone) was mixed with ^{14}C-labeled FMC 57020. (Specific Activity = 26.81 mCi/nmol.) "Mazola corn oil was used as the carrier for all oral dosages of radiolabeled and nonlabeled compound. Distilled ionized water was used as the carrier for the intravenous (IV) dose."



*Denotes methylene ^{14}C-labeled

Dose Regime

All doses of radiolabeled and unlabeled compound were dissolved in Mazola corn oil, except the intravenous dose which was dissolved in deionized water.

1. Single Oral Low Dose - Ten adult rats (5 males and 5 females) were starved for 18 hours before oral administration of radiolabeled compound by gavage with an intubation needle and a syringe. Dosing preparation L (Table 1) was used and the amount of FMC 57020 dosed was approximately 5 mg/kg of rat body weight and the total radioactivity dosed was 20 uCi/test animal.

2. Single Oral High Dose - Ten rats (5 males and 5 females) were fasted for 18 hours and dosed with preparation H (Table 1). The amount of FMC 57020 administered was about 900 mg/kg of rat body weight and the total radioactivity dosed was ~20 μCi. The rats were treated the same as those in low dose.

3. Intravenous Dose - Ten rats (5 males and 5 females) were fasted for 18 hours and anesthetized with ether. Their rear legs (one for each rat) were shaved and methylene-^{14}C FMC 57020 which was previously dissolved in distilled deionized water (Preparation I, Table 1) was injected into the safenous vein. The amount of dose was about 3-4 mg/kg of rat body weight and the total radioactivity dosed was ~20 μCi.

4. Multiple Oral Low Dose - Ten rats (5 males and 5 females) were also fasted for 18 hours before administration of unlabeled FMC 57020 in corn oil by gavage. The animals were returned to their cages and fed a normal diet 6 hours later. The rats were given food and water ad libitum for 14 days and were dosed daily with the unlabeled compound in corn oil. Rats were again fasted for 18 hours before administration of the ^{14}C-labeled FMC 57020. Preparation M (Table 1) was used and the amount of dose was about 5 mg/kg of rat body weight and the total radioactivity dosed was about ~20 μCi. The rats were treated the same as those in low dose.

SUMMARY OF SPECIFIC ACTIVITIES FOR VARIOUS DOSE GROUPS*

Dose	Rat. No.	Specific Activity mC/mM	Specific Activity DPM/ μg
L	1-10	6.03	55783
H	11-20	0.03	302
I	21-30	5.44	50400
M	31-40	4.60	42548

L: Single oral low dose
H: Single oral high dose
I: Single intravenous dose
M: Multiple oral low dose

*This table reproduced from registrant's submission.

522

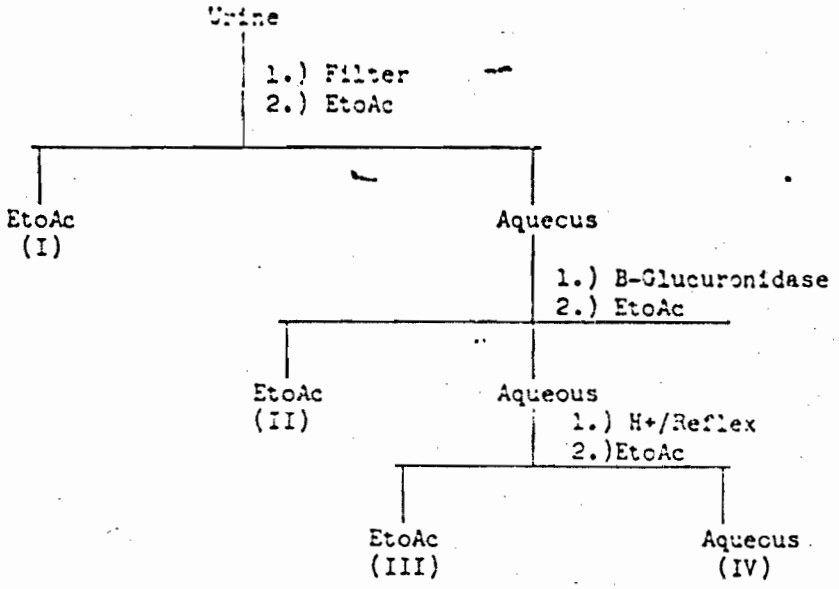
90

Methods: Urine and cage washings sampled were added directly to liquid scintillant (Instagel) for counting.

Fecal samples, large tissues and organs were homogenized in water. Measured aliquots of homogenate were transferred directly into combustion cores and processed for combustion. "Other tissues (gonads, muscles, adipose, spleen, bone, skin) were combusted directly."

"First day (0-24 hr) and second day (24-48 hr) urine and feces were pooled according to dosing level, sampling time and sex." Extraction of metabolities found in urine (Table 2) and feces (Table 3) was conducted.

Table 2: Extraction Scheme For Rat Urine*



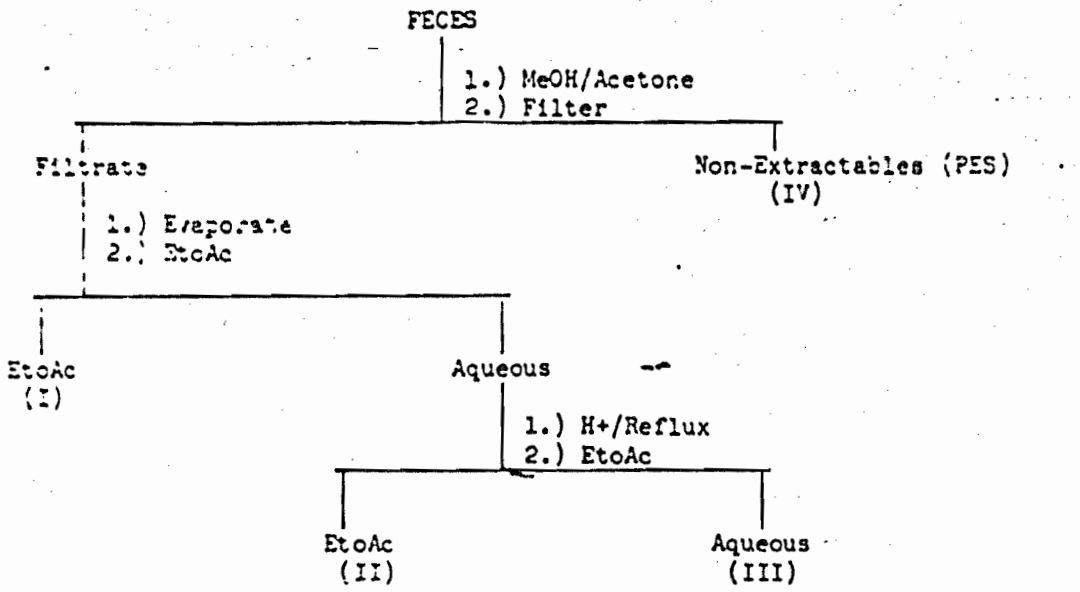
I: Free (nonconjugated) metabolities
II: Enzyeme released aglycones
III: AGD released aglycones
IV: Aqueous polar metabolities

* This Table is reproduced from Registrant's submission.

523

Urine fractions I, II, III, were analyzed by HPLC as TLC using isotopic dilution or con-chromatography with known metabolite standards.

Table 3: Extraction Scheme For Rat Feces*



I: Organo soluble free metabolites
II: Acid hydrolyzed aglycones
III: Non-organo extractable residues
IV: Post extraction solids (PES)

* This Table is reproduced from Registrant's submission.

524

92

Reported Results: Reported mean percentages of the administered dose recovered in the urine and feces after 2 days (Table 5):

Table 5: Mean Percentages of Excreted Dose in Urine and Feces After 2 Days*

Testing Regime	Sex	Day - 1 Urine	Day - 1 Feces	Day - 1 Total	Day - 2 Urine	Day - 2 Feces	Day - 2 Total	TOTAL IN Day 1 & 2
Single Oral Low Dose (5 mg/kg)	F	54.43	20.88	75.31	10.74	9.63	20.37	95.68
	M	58.55	27.58	86.13	7.33	5.35	12.68	98.81
Single Oral High Dose (500 mg/kg)	F	43.11	4.55	47.66	34.38	9.23	43.55	90.21
	M	56.83	18.35	75.18	10.80	6.91	19.14	94.32
IV Low Dose (3 mg/kg)	F	71.37	14.49	85.86	3.65	2.04	5.69	91.55
	M	65.49	18.96	84.45	4.26	2.61	6.87	91.32
Multiple Oral Low dose (5 mg/kg)	F	78.03	14.45	96.48	5.05	5.31	10.36	96.84
	M	69.12	11.12	80.24	10.58	9.62	20.20	100.44

* This Table is reproduced from registrant's submission

525

A total of 16 metabolic products were identified in the urine and feces over 48 hours (Table 6)

Table 6: Mean-Percentage of Excreted Dose As Identified Metabolities In Urine and Feces Over 48 Hours.**

Metabolite*	Single Oral Low Dose		Single Oral High Dose		Intravenous		Multiple Oral Low Dose	
	Male	Female	Male	Female	Male	Female	Male	Female
Polar Products 2/	5.1	5.9	2.4	2.2	5.7	4.0	2.5	2.6
FMC 87002	8.3	3.9	11.5	3.7	6.0	4.1	6.3	3.7
FMC 87010	10.2	12.1	10.7	7.0	9.5	14.6	7.2	17.6
FMC 87009	6.2	7.3	11.1	14.6	14.1	10.7	21.5	11.9
FMC 87011	7.0	7.3	7.3	10.2	6.9	9.5	11.5	8.6
FMC 89915	13.4	1.5	3.0	8.8	3.4	14.9	5.3	13.6
FMC 87012	2.6	2.1	2.6	2.8	2.5	1.5	3.0	2.6
FMC 87013	1.6	1.7	2.9	2.0	1.8	1.8	2.9	2.6
FMC 87014	0.6	0.7	1.6	1.5	1.2	0.9	0.8	1.1
FMC 65317	1.6	2.4	1.5	2.3	1.3	1.8	1.3	1.8
FMC 87006	1.3	1.8	1.2	1.3	1.5	1.7	1.4	2.1
FMC 62667	1.4	1.8	0.8	0.6	0.8	1.0	1.2	1.5
FMC 60217	17.5	17.9	11.9	10.4	6.2	8.4	7.6	10.2
FMC 77039	0.8	0.7	0.6	0.8	0.8	0.6	1.0	0.7
Unknown(s) 3/	2.1	2.0	2.2	2.5	2.7	2.5	2.9	2.5
FMC 57020	0.2	0.8	0.4	0.6	0.4	0.5	0.6	0.3
FMC 55626	0.9	1.0	1.7	2.2	1.7	1.6	2.2	2.2
Non-polar Products 4/	0.8	1.0	0.7	1.8	1.1	1.1	1.2	1.3
TOTAL ORGANOSOLUBLES 1/	81.5	82.3	81.9	84.2	77.8	79.4	88.7	86.1
Aqueous	9.5	9.3	9.0	6.8	6.9	7.4	6.4	7.1
FES	4.6	4.0	3.3	2.7	5.3	4.2	6.3	4.1
TOTAL	95.6	95.6	94.2	93.7	90.0	91.0	101.4	97.3

1/ Total percent distribution in Fractions I-III
2/ Include FMC 14791 and FMC 87007
3/ At least 4-6 minor unknowns, none exceeding 1%
4/ Radiocarbon recovered from HLPC following elution of FMC 55626

* Structure identification of metabolites, see Appendix A

** This table is reproduced from registrant's submission.

526

The proposed metabolic pathway may be found in Attachment B.

Discussion (By Author)

"The observed metabolites are the same qualitatively both in urine and feces in all four dosing groups. However, the observed quantitative differences between sexes (high dosed rats) and routes of administration can be summarized as follows:

1. At high dose (>900 mg/kg), excretion (48.4%) was lowest in female rats in the 24-hour period. Examining the total weight of excreta revealed that the amount of feces from females was substantially supressed in the first day.

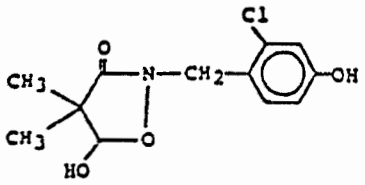
2. Total amount of 5-hydroxy FMC 57020 was highest in single oral low dose group but not in multiple oral low dosed group indicating the possibility of an enzyme induction effect.

3. Both single oral low and high dosed groups gave a high percentage of 5-hydroxy FMC 57020 in their feces samples especially in the first day's feces indicating the possibility of enterohepatic circulation. This possibility was further enhanced by the fact that less than 1% of the fecal and urinary metabolites was unmetabolized parent molecule.

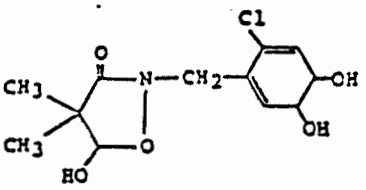
4. It is reasonable to assume that 5-OH FMC 57020-conjugate formed in the liver was excreted to gastrointestinal tract via bile duct and this conjugate was readily cleaved to 5-OH FMC 57020 and the excreted in the feces.

5. In the multiple oral low dosed group, the amounts of dihydroxylated, trihydroxylated and dihydrodiol metabolites were increased significantly indicating that an induction effect on the mixed function oxidase (MFO) (possibility cytochrome p-450 system) had developed.

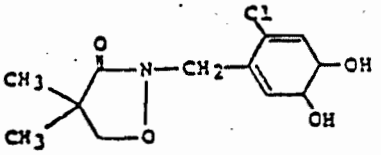
ATTACHMENT "A" (Identification of Metabolites)



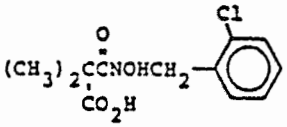
FMC 8391[illegible]
(4',5 -dihydroxy-
FMC 57020)



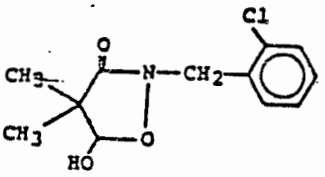
FMC 87010
(4',5'-dihydrodiol-
5-hydroxy-FMC 57020)



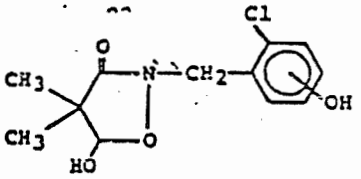
FMC 87009
(4',5'-dihydrodiol-
FMC 57020)



FMC 87008
(N-hydroxy-carboxylic
acid)

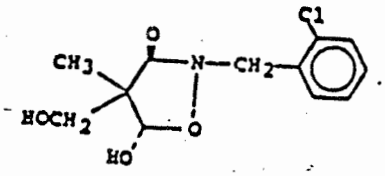


FMC 60217
(5-hydroxy
FMC 57020)

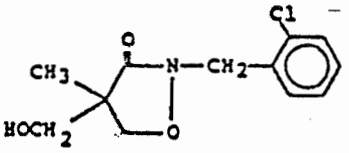


FMC 87011
(dihydroxy-
FMC 57020)

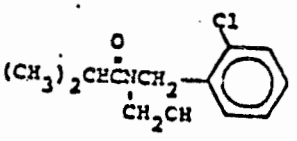
529



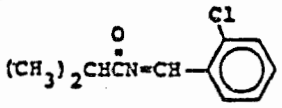
FMC 87012
(4-Hydroxymethyl-
5-hydroxy FMC 57020)



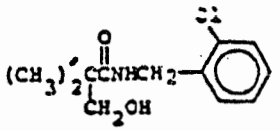
FMC 87006
(4-Hydroxymethyl
FMC 57020)



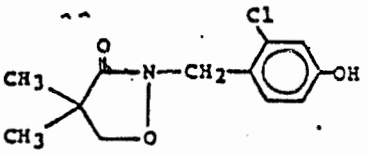
FMC 87013
(N-Hydroxymethyl-
benzyl-isobutyramide)



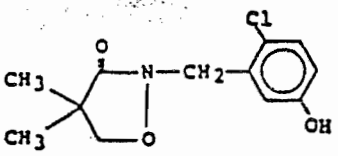
FMC 87014
(Benzylidinamide)



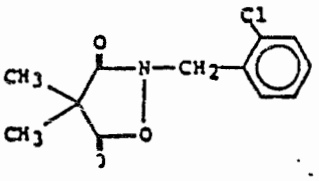
FMC 65317
(Seco- FMC 3517)



FMC 62667
(4'-hydroxy FMC 57020)

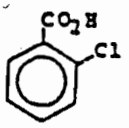


FMC 77039
(5'-hydroxy
FMC 57020)

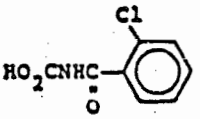


FMC 55626
(5-keto FMC 57020)

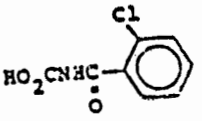
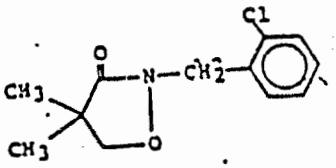
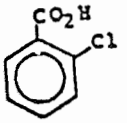
530



FMC 14791
(2-chlorobenzoic acid)



FMC 87007
(Carboxy benzamide)

FMC Number	Name	Structure
FMC 87007	Carboxybenzamide	
FMC 57020	2-[(2'-Chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone	
FMC 14791	2-Chlorobenzoic acid	

FMC 14791
FMC 87006
FMC 87007
FMC 65317
FMC 62667
FMC 77039
FMC 87012
FMC 57020
FMC 87009
FMC 87010
FMC 83918
FMC 67014
FMC 55625
FMC 60217
FMC 87008
FMC 87013
FMC 87011
Conjugate
Cytochrome p-450(E.R.)
NADPH
Reductase
Epoxide Hydrase
H_2O
$-H_2O$
[O]
P

532

004628

Data Evaluation Record

Chemical: Command

Caswell No: 463D

Test Material: Methylene ^{14}C-labeled FMC 57020 (99.8% purity; Specific Activity 26.85 mCi/nmol) and Carbonyl ^{14}C-labeled FMC 57020 (99.3% Purity; Specific Activity = 27.98 mCi/nmol)

Citation: Wu, J. Identification of Metabolites In Urine and Feces of Rats Dosed With ^{14}C-FMC 57020. FMC Report No. P-0897, June 14, 1984. Unpublished report prepared by FMC Corporation.

Accession No.: 072818 (Duplicate in 072814)

Reviewed by: Carolyn Gregorio, Toxicologist CAG
Toxicology Branch/HED (TS-769) 7-18-85

Secondary Review: Clint Skinner, Ph.D.
Section Head
Toxicology Branch/HED (TS-769)

Core Classification: Incomplete Study

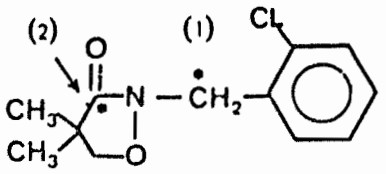
Conclusion: Although this report is incomplete (quantification of metabolities "will appear in a subsequent report)," the data presented are adequate to support the conclusions of the investigations. The data indicated that "FMC-57020 is extensively metabolized in the rat by a number of hydroxylated derivatives of the parent chemical. Mono- di- and trihydroxylated metabolites are formed at various sites on the molecule." Additional metabolites are formed by oxidation and opening of the heterocyclic ring."

533

Material and Methods:

Test Species: Young adult male and female Sprague-Dawley Rats (Charles River CD) weighing between 70-90 grams were used in this study.

Dosage: "FMC 57020, labeled with carbon-14 in the benzylic (methylene-^{14}C FMC 57020, 1) and carbon (carbonyl-^{14}C-FMC 57020, 2) groups of the molecule were used in this work."



* Denotes Position of ^{14}C-Label.

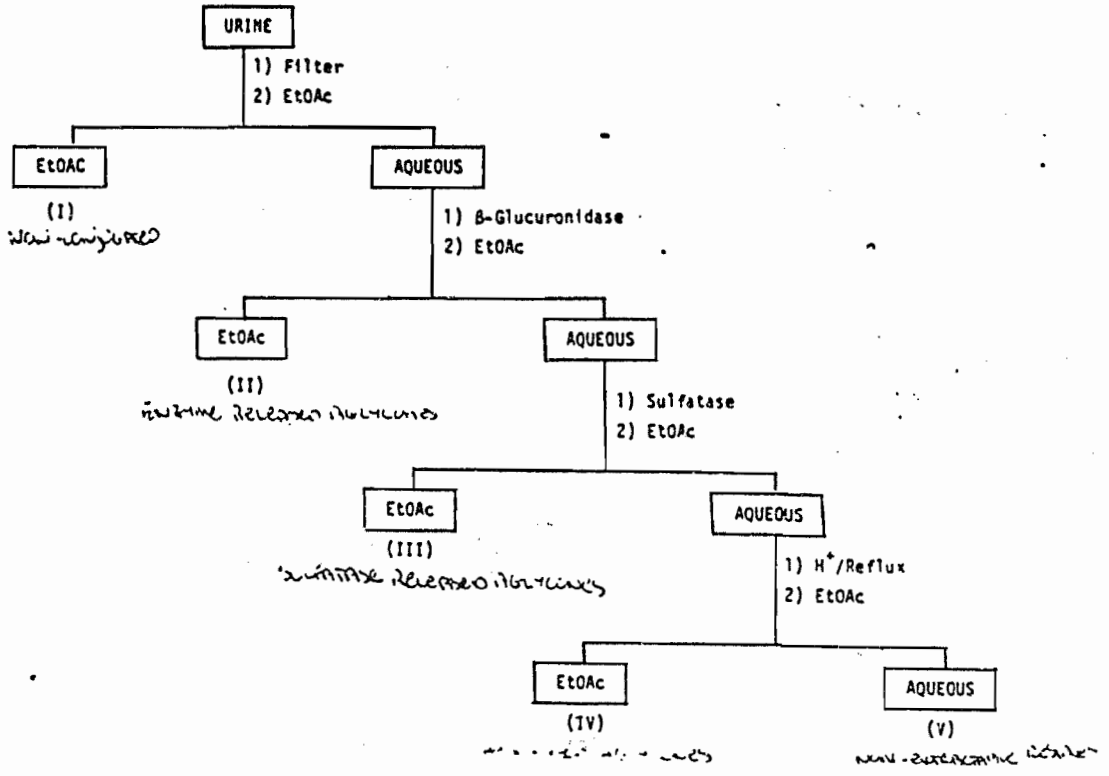
Dose Regime: "Eight rats (4 males and 4 females) were administered with a single oral dose of isotopically diluted ^{14}C-labeled FMC 57020 at a 50 mg/kg level. Each rat received about 20 µCi of radioactivity yielding specific a ities of 7196 dpm/µg for methylene-^{14}C FMC 57020 and 6[illegible] dpm/µg for carbonyl-^{14}C FMC 57020. Immediately after dosing, rats were transferred to individual metal cages. Six hours after dosing, animals were fed with a normal diet of Purina Rat Chow."

004628

Methods: "Urine and feces were collected separately. Urine was immediately freeze trapped while feces samples were frozen upon collection. Samples were subjected to ^{14}C assay and ultimately were combined into 24 hour composites according to excreta type and sex and stored frozen (-20°C) prior to further assay."

Extraction of metabolites found in urine (Table 1) and feces (Table 2) were conducted.

Table 1. Extraction Scheme For Rat Urine*



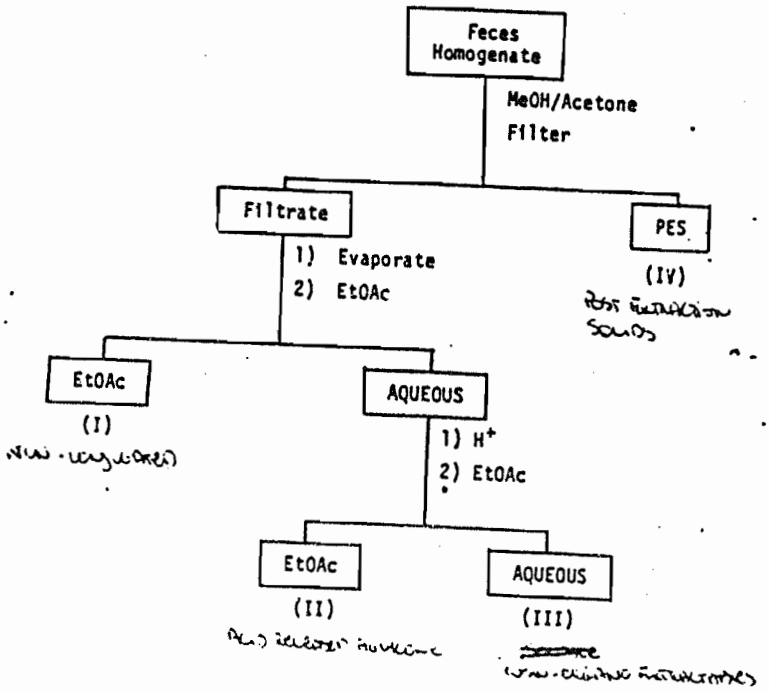
* This Table is reproduced from the Registrant's submission.

535

004628

Urine fractions I, II, III, IV were analyzed chromatographically (TLC, GC, HPLC) and spectroscopically (NMR, MS). Urine Fraction V was assayed by LSC.

Table 2. Extraction Scheme for Feces*



* This Table is reproduced from registrant's submission

536

Feces Fractions I and II were analyzed chromatographically by TLC. Post extraction solids (PES) on Fraction IV were analyzed by combustion. Liquid scintillation was used for feces Fraction III.

Reported Results: A number of products (ca. 12-15) were detected in excreta of rats administered ^{14}C-FMC 57020" (See Appendix A for identification and discussion). "Further delineation on the magnitude of each product will appear in a subsequent report." This subsequent report has not been received at this time.

Discussion (By Author): Please see Attachment A

004628

ATTACHMENT "A"

Command toxicology review

Page _____ is not included in this copy.

Pages 107 through 118 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 product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ 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.

539

004628

EPA: 68-01-6561
TASK: 53
May 29, 1985

DATA EVALUATION RECORD

FMC 57090 (Command)

Mutagenicity - Reverse Mutation in Salmonella

STUDY IDENTIFICATION: Farrow, M. G. and McCarroll, N. E. Salmonella typhimurium/mammalian microsome plate incorporation assay with compound FMC 57090. (Unpublished study No. A83-864 by Hazelton Laboratories, Vienna, VA for FMC Corporation, Princeton, NJ; dated April 28, 1983.) Accession No. 072815.

APPROVED BY:

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

Signature: [signature]

Date: 5-27-85

004623

1. CHEMICAL: FMC 57090 (Command).

2. TEST MATERIAL: FMC 57090 (Command).

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

4. STUDY IDENTIFICATION: Farrow, M. G. and McCarroll, N. E. Salmonella typhimurium/mammalian microsome plate incorporation assay with compound FMC 57090. (Unpublished study No. A83-864 by Hazelton Laboratories, Vienna, VA for FMC Corporation, Princeton, NJ; dated April 28, 1983.) Accession No. 072915.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Author
Dynamac Corporation

Signature: Brenda Worthy
Date: 5-29-85

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

Signature: William L McLellan
Date: May 29, 1985

6. APPROVED BY:

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

Signature: I Cecil Felkner
Date: 5-29-85

C. Gregorio, Ph.D.
EPA Reviewer

Signature: [illegible]
Date: 7-18-85

Clint Skinner, Ph.D.
EPA Section Head

Signature: [illegible]
Date: 8-12-85

541

7. CONCLUSIONS:

Under the conditions of the assay, FMC 57090 induced greater than a two-fold increase in revertant colonies in S. typhimurium strains TA1535 and TA100 with and without metabolic activation; however, this increase was only at the highest dose tested (15,000 µg/plate). Since this two-fold increase was not accompanied by a dose-response effect, the mutagenicity of FMC 57090 is assessed as a presumptive positive. Therefore, the study is inconclusive.

8. RECOMMENDATIONS:

We recommend that the assay be repeated at 5 or 6 dose levels between 1,000 and 15,000 µg/plate to verify the cytotoxicity range and to validate a potential linear mutagenicity dose response.

9. BACKGROUND: Not applicable.

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

11. MATERIALS AND METHODS (PROTOCOLS): See Appendix A.

A. Materials and Methods:

1. FMC 57090, Lot No. E2383-65, a crystalline compound, stable at room temperature, with a purity of 99%. The test material was diluted in dimethylsulfoxide to final concentrations of 185.2, 555.6, 1667, 5000, and 15,000 µg/plate.

2. The following strains of Salmonella typhimurium were used: TA1535, TA1537, TA1538, TA98, and TA100. The method employed was the Ames mutagenicity assay[1], with and without metabolic activation.

B. Protocol:

See Appendix A.

12. REPORTED RESULTS:

A. Toxicity Assay: The test material was solubilized in dimethylsulfoxide (DMSO) to yield a stock solution of 100 mg/ml. The stock solution and five ten-fold dilutions (0.1, 1.0, 10, 100, and 1000 µg per 0.1 ml) were used without metabolic activation by rat liver S9 in the cytotoxicity assay.

---

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

542

The authors reported a 2.3 fold increase in revertant colonies after a 48 hr incubation with TA100 at the highest dose (1000 µg/plate). Since no toxicity was exhibited at this dose, the highest dose selected for the mutagenesis assay was increased to 15,000 µg/plate.

B. Mutagenesis Assay: Five doses, 0.1 ml containing 185.2, 555.6, 1667, 5000, or 15,000 µg of the test material were plated in triplicate with each of the five Salmonella tester strains. After a two day incubation, there was a significant increase in the number of revertant colonies over the solvent control at 15,000 µg/plate in strains TA1535 and TA100 with and without metabolic activation (Table 1). The test material did not induce a significant increase in the number of revertant colonies over the solvent control in strains TA1537, TA1538 or TA98 at any dose level assayed with or without S9 activation.

TABLE 1. Salmonella/Microsomal Assay of FMC 57090

Substance	Dose/ Plate	S9 Activation	Strains/Revertants/plate[a] TA1535	TA100
DMSO	100 µl	-	22	105
		+	15	99
FMC 57090	15,000 µg	-	76	221
		+	43	330

[a]Average of triplicate plates.

The numbers of revertants of the negative and solvent controls for each tester strain were within an acceptable range relative to published data[1], and that the positive controls demonstrated that the test system had an appropriate level of sensitivity (Table 2).

12 STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that while the test material caused a significant increase in revertant colonies of strains TA1535 and TA100 with and without metabolic activation, "this response was limited to the highest concentration, therefore the mutagenic potential, if any, of the test material could not be fully characterized."

B. A quality assurance statement was present, signed, and dated October 21, 1983.

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

543

TABLE 2. Control Results Used in the Assay of FMC 57090

Substance	Dose/ plate	S9 Activation	Strains/Revertants/plate[a] TA1535	TA1537	TA1538	TA100	TA98
gative (Bacteria) Control	100 µl	-	23	6	12	106	19
gative (S9) Control	100 µl	+	22	8	32	120	37
lvent (DMSO Control)	100 µl	-	22	5	11	105	25
		+	15	11	28	99	33
sitive Controls							
MNNG[b]	5 µg	-	1789			2321	
2-AA[c]	5 µg	+	502			2228	
9-AA[d]	75 µg	-		638			
2-AA	5 µg	+		210			
2-NF[e]	50 µg	-			1415		1942
2-AA	5 µg	+			1937		2108

verage of triplicate plates

ethylnitronitrosoguanidine

-aminoanthracine

-aminoacridine

-nitrofluorene

544

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

A. The results indicated that all the tester strains were capable of giving a positive response with and without S9 activation. The number of revertant colonies in the negative and positive controls were within the expected range relative to published values[1]. Although the test material induced a two-fold increase in revertant colonies in strains TA1535 and TA100, this positive response was reported only at the highest dose tested. According to the authors' evaluation criteria, for a test material to be considered mutagenic it must give a positive dose response over three concentrations, and the smallest of these increases in revertants should be equal to twice or more than the number present in the solvent control. Hence, the potential mutagenicity of FMC 57090 was inconclusive (equivocal).

B. By our assessment, a two-fold or greater increase in revertant colonies was demonstrated at the highest concentration in both strains TA1535 and TA100 (testers are derived from the same parental strain-G46) with and without S9 activation. This indicates the potential of a mutagenic effect. Data from a dose range between 1,000 and 15,000 µg/plate could verify the results obtained for the toxicity and mutagenesis assays. From this data we could better assess if the test material induced mutagenicity within a linear dose response range.

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

16. CBI APPENDIX: Appendix A, Experimental Procedures (Materials and Methods/Protocol), CBI pp 2-6.

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

Appendix A

Experimental Procedures (Materials and Methods/Protocol)

Command toxicology review

Page _____ is not included in this copy.

Pages 126 through 130 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 product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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204625

EPA: 68-01-6561
TASK: 53
June 7, 1985

DATA EVALUATION RECORD

FMC 57020 (Command)

CHO/HGPRT Point Mutation Assay

STUDY IDENTIFICATION: Thilagar, A., Brauninger, R., and Pant, K.J. CHO/HGPRT mutation assay in the presence and absence of exogeneous metabolic activation. (Unpublished study No. FMC A83-1143 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated June 6, 1984.) Accession No. ~~not available~~ 071814.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 6-7-85

548

004628

1. CHEMICAL: FMC 57020 (Command).

2. TEST MATERIAL: FMC 57020, Lot No. E1756-146, was described as a light yellow viscous liquid of 88.8% purity.

3. STUDY/ACTION TYPE: Mutagenicity: Chinese hamster ovary (CHO)/hypoxanthine-guanine phosphoribosyl transference (HGPRT) point mutation assay.

4. STUDY IDENTIFICATION: Thilagar, A., Brauninger, R., and Pant, K.J. CHO/HGPRT mutation assay in the presence and absence of exogeneous metabolic activation. (Unpublished study No. FMC A83-1143 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated June 6, 1984.) Accession No. ~~not available~~ 072814.

5. REVIEWED BY:

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Principal Author
Dynamac Corporation

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Date: June 7, 1985

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Date: June 7, 1985

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Genetic Toxicology
Technical Quality Control
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Date: 6-7-85

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EPA Reviewer

Signature: CG
Date: 6-11-85

Clint Skinner, Ph.D.
EPA Section Head

Signature: Clint Skinner
Date: 7-25-85

549
132

7. CONCLUSIONS:

A. Our conclusion is that FMC 57020 induced weak mutagenic activity as detected in the CHO/HGPRT point mutation assay in the non-activated system. While there was no dose response associated with exposure to FMC 57020 in the concentration range of 200 to 600 µg/ml, there was a statistically significant increase in mutation frequency observed at the 600 µg/ml dose in the non-activated system. It should be noted that this conclusion is in disagreement with the authors' conclusion.

8. RECOMMENDATIONS: A repeat assay should be performed using five doses ranging from 500 to 700 µg/ml of the test material.

9. BACKGROUND: Not applicable.

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material FMC 57020, Lot No. E1756-146, was described as a light yellow viscous liquid of 88.8 percent purity. The stability of the test material was not determined in the conduct of this study.

   Dimethylsulfoxide (DMSO) was used as the test material solvent and the solvent control.

2. The Chinese hamster ovary cells, CHO-K_1-BH_4 Lot Nos. 3-26-82 and 1-23-84, used in this study were originally obtained from Dr. Abraham Hsie, Oak Ridge National Laboratories.

3. The S9 fraction used for this study was prepared from the livers of Fischer 344 rats induced with Aroclor 1254.

4. Cytotoxicity Assay: Duplicate cultures of cells, seeded at 5×10^5 cells/T-25 flask were treated for 5 hours with dilutions of the test material, with or without S9 activation. Colonies were counted after 7-12 days incubation, and cytotoxicity determined relative to the solvent control.

5. Mutagenicity Assay: Duplicate flasks were treated with appropriate levels of test material, positive or solvent control, and with or without S9 activation. The cells were then washed and incubated in complete medium for 18 hours. For

550

133

mutant selection, the cells were plated at a density of 2×10^5/100 mm plate (5 plates) in hypoxanthine-free medium containing 10 µM 6-thioguanine. Plates were incubated for 11 days and colonies counted. Cloning efficiency was assessed in hypoxanthine-free medium without 6-thioguanine.

6. Evaluation Criteria: The assay is considered positive if there is a dose-dependent and statistically significant increase in the mutation frequency relative to the solvent. The assay is considered weakly positive if there is no dose response but one or more doses causes a statistically increased incidence of mutation frequency.

7. The test method employed was that of Hsie, A.W. et al. Mutation Research 86:193-214, 1981.

B. Protocol: Appendix A.

12. REPORTED RESULTS:

Cytotoxicity: Three preliminary cytotoxicity tests on FMC 57020 were performed. However, the results of only the first and third tests were reported. The first cytotoxicity test at concentrations from 0.5 to 4991 µg/ml was repeated to include higher dose levels and to narrow the dose range tested. In the repeat cytotoxicity assay, which tested 9 doses in the concentration range of 40 to 10,250 µg /ml, it was observed that test doses at 1281 µg/ml and above killed all of the cells. At the 641 µg/ml dose level, the relative cell survival was 76.7 percent in the nonactivated system and 86.8 percent in the activated system.

In the cytotoxicity test performed in parallel with the mutation assay, the relative cell survival in the nonactivated system was 8.8, 45.6, 80.0, and 74.4 percent at FMC 57020 doses of 600, 500, 300, and 200 µg/ml, respectively. In the S9 activated assay, relative survival was 36.5, 76.5, 93.9, and 91.3 percent at test material doses of 600, 500, 300, and 200 µg/ml, respectively. Survival in the positive controls was 19.1 and 40.9 percent with 0.5 µl and 0.25 µl/ml EMS (without S9 activation), respectively and 7.0, 35.1, and 75.4 percent at 4.0, 3.0, and 2.0 µg/ml B(a)P (with S9 activation), respectively.

Mutagenicity Assay: The mutation frequency for the solvent control was 22.7 mutants/10^6 survivors in the nonactivated system and 45.5 mutants/10^6 survivors in the activated system. In the nonactivated system, 0.25 µl/ml of EMS, the positive control, induced 700 mutants/10^6 survivors (0.5 µg/ml test result lost due to contamination). The negative control (untreated cells) and the 500, 300, and 200 µg/ml dose levels of FMC 57020 had mutation frequencies which ranged from 23.4 to 29.8 mutants/10^6 survivors comparable to that of the solvent control. The 600 µg/ml dose of FMC 57020 in the nonactivated system had a mutation frequency of 76.2 mutants/10^6 survivors (Table 1).

551

134

In the S9 activated system, B(a)P induced 254.5, 152.2, and 70.8 mutants/10^6 survivors at the 4.0, 3.0, and 2.0 µg/ml dose levels, respectively. The untreated controls and all test doses of FMC 57020 had mutation frequencies ranging from 19.1 to 55.1 mutants/10^6 survivors comparable to that of the solvent control (45.5 mutants/10^6 survivors) (Table 1).

TABLE 1. Cloning Efficiency (CE) and Mutant Frequency in the CHO/HGPRT Mutation Assay

	Nonactivated		Activated	
	CE (%)	Mutants/10^6 Survivors	CE (%)	Mutants/10^6 Suvivors
Untreated control	64	23.4	50	26.0
FMC 57020				
200 µg/ml	58	29.3	54	35.2
300 µg/ml	57	29.8	45	22.2
500 µg/ml	57	28.1	49	55.1
600 µg/ml	42	76.2	47	19.1
EMS (0.25 µl/ml)	41	700	-	-
EMS (6.5 µl/ml)	[a]	-		
DMSO	66	22.7	44	45.5
B(a)P				
2 µg/ml	-	-	48	70.8
3 µg/ml	-	-	46	152.2
4 µg/ml	-	-	44	254.5

[a]Culture lost.
EMS = Ethyl methane sulphonate.
B(a)P = Benzo(a)pyrene.

552

135

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The study authors' concluded:

"The results of this CHO/HGPRT point mutation assay indicate that FMC 57020 did not cause a significant response in the frequencies of mutations in the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus of Chinese Hamster Ovary Cells. It is therefore concluded that, under conditions of the assay, FMC 57020 is negative in the CHO/HGPRT Point Mutation Assay."

A quality assurance statement dated June 21, 1984 was present with the report. Protocol Amendments that were associated with amendments/corrections to the study report were attached in an appendix to the study report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Our assessment of the results is that the authors' conclusion is not supported by the data. It was noted that the 600 µg/ml dose of FMC 57020 induced 76.2 mutants/10^6 survivors which represents a greater than three-fold increase over the spontaneous mutation frequency of 22.7 mutants/10^6 survivors observed with the corresponding solvent control. This increase was evaluated for statistical significance by our reviewers using the analytical methods described by the authors (see Appendix A) and was found to be statistically significant (calculated $p = 0.00012$). While the 600 µg/ml dose, the highest dose tested, was associated with a relative cell survival of 8.8 percent, it may be feasible to test a somewhat higher dose range of FMC 57020. However, by the authors' own evaluation criteria, FMC 57020 should be considered weakly positive or equivocal, since the highest dose of the test material induced a statistically significant increased mutation frequency.

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

16. CBI APPENDIX: Appendix A, Methods and Materials, CBI pp. 4-7.

553

139

APPENDIX A

(Methods and Materials)

Command tox review

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Pages 138 through 141 are not included in this copy.

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___ Identity of product inert ingredients

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___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

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___ Information about a pending registration action

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APPENDIX A

(Methods and Materials)

Command tox review

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001628

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NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 113
June 3, 1985

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.

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Date: 7-25-85

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: FG 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 of unspecified purity.

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 TA98(1.0 µg 2-AA)[3] and TA100(1.0 µg 2-AA)[3]. There were no positive controls used concurrently 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-Aminoacridine.

[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 2NF = 1451 ± 262); TA100(0.04 µl 1,3 PS = 891 ± 72); TA1535(0.04 µl 1,3 PS = 1159 ± 23); TA1537

[4]Only one plate.

(75 µg 9AA = 2576 ± 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 = 1124 ± 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 at concentrations between 0.04 to 4.0 µg/plate in the presence of S9 for S. typhimurium strains TA98 and TA100 (inconclusive for all other tester strains) 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] Mortelmans, DM and Ames, BN. 1983. mutation Res. 113:173-215.

004628

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

EPA: 68-01-6561
TASK: 113
June 3, 1985

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.

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152 563

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity (*In vivo* cytogenetics assay).

CITATION: Putman DI 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.

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 (not significant) 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 (MI) averages were 4.72, 4.84, and 4.64 i.e., not different from each other or the control, 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 marrrow 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. The 10% reduction in weight at the highest dose selected did not prove that the animals were dosed at the maximum tolerated level, and the MIs did not show that the test material had reached the target cells. However, the range-finding experiment showed that 3000 mg/kg would have been an excessive dose. Hence, there would be little information gained from repeating the study using higher doses.

CLASSIFICATION: Acceptable for male rats only; females were not assayed.

EPA: 68-01-6561
TASK: 113
June 3, 1985

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.

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DATA EVALUATION RECORD

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 (T210.380) prepared by Microbiological 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: Adult male rat hepatocyte cell (HPC) cultures were obtained using a modification of the procedure of Williams[1]. Rats were killed by metofane inhalation, dissected and individually 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.

569

159

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 "trypsinized, 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 exp[illegible] 18 hours, the UDS assay cells were washed in serum-free WME, swe[illegible]ercent sodium citrate, and fixed in ethanol-acetic acid on cov[illegible]he coverslips were mounted with the cells up and air dried. [illegible]cing the slides with Kodak emulsion and storage at 4° C for 10 [illegible]ight tight boxes containing desiccant, the slides were developed i[illegible]ak 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.

570

153

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 cytotoxicity assay, the FMC 57020, it was reported that the 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 relative survival index (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 conditions, 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.

572

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

EPA: 68-01-6561
TASK: 53
June 7, 1985

DATA EVALUATION RECORD

FMC-57020 (Command)

Mutagenicity (reverse mutation in Salmonella)

CITATION: Haworth, S.R., Lawlor, T.E., Olewine, S.M., et al. Salmonella/mammalian-microsome plate incorporation mutagenicity assay. (Unpublished report No. A84-1273 prepared by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated June 14, 1984.)

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513

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity (reverse mutation in Salmonella).

CITATION: Haworth, S.R., Lawlor, T.E., Olewine, S.M., et al. Salmonella/mammalian-microsome plate incorporation mutagenicity assay. (Unpublished report No. A84-1273 prepared by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated June 14, 1984.)

ACCESSION NUMBER: Not available.

LABORATORY: Microbiological Associates, 5221 River Rd, Bethesda, MD 20916.

QUALITY ASSURANCE STATEMENT: Present, signed and dated June 14, 1984.

TEST MATERIAL: FMC 57020, Lot No. E3376-112, a clear viscous liquid with a purity of 93.4%.

METHOD:

Bacterial Strain: The following strains of Salmonella typhimurium were used: TA98, TA100, TA1535, TA1537, and TA1538. The strains were obtained from Dr. Bruce Ames (University of California, Berkeley). Fresh broth cultures of the tester strains, grown overnight at 37° C with shaking, were prepared from frozen stocks stored in liquid nitrogen. On the day of the assay all tester strain cultures were checked for the presence of their respective specific genetic markers.

Preparation of S9: The S9 fraction was prepared from the livers of male Sprague-Dawley rats injected i.p. with 500 mg/kg of Aroclor 1254. The S9 mix contained the following:

S9 Mix	
S9 fraction	0.10 ml
0.2 M $MgCl_2$/0.825 M KCl	0.04 ml
0.04 M NADP	0.10 ml
0.05 M Glucose-6-phosphate	0.10 ml
1.0 M NaH_2PO_4/K_2HPO_4, pH.7.4	0.10 ml
H_2O	0.56 ml

574

163

Preparation of Test Material: The test material was diluted in dimethylsulfoxide (DMSO) to final concentrations of 10, 33, 67, 100, 333, 667, 1,000, 3,333, 6,667, and 10,000 µg/plate in the cytotoxicity assay. The concentrations used in the mutagenesis assay were 50, 250, 1,250, 2,500, and 5,000 µg/plate.

Controls: The negative (solvent) control was DMSO. The positive controls are listed in Table 1.

TABLE 1. Positive Control Substances

Strain	S9 Activation	Substances	Concentration (µg/plate)
TA98	+	2-Aminoanthracene (2AA)	4
	-	2-Nitrofluorene (2-NF)	5
TA100	+	2-AA	4
	-	Sodium azide	5
TA1535	+	2-AA	4
	-	Sodium azide	5
TA1537	+	2-AA	4
	-	9-Aminoacridine (9-AA)	75
TA1538	+	2-AA	4
	-	2-NF	5

Cytotoxicity Assay: An overnight broth culture of S. typhimurium TA100 and the test material were plated on selective minimal agar plates with and without S9 activation. The degree of toxicity was determined by a decrease in the number of revertant colonies per plate and/or by the reduction of microcolonies in the background lawn.

Mutagenesis Assay: Five dose levels of the test material were plated in triplicate with each of the five tester strains, with and without metabolic activation. The controls were also plated in triplicate.

In the nonactivated system 50 µl of each tester strain and 50 µl of solvent, test material (at the appropriate concentrations), or control substances were added to 2.5 ml of molten top agar. In the activated system, 0.5 ml of S9 mix plus the above components were added to 2.0 ml of molten top agar. The tubes were mixed then poured onto minimal agar plates. The plates were allowed to solidify then incubated at 37° C for 48 hr.

Mutation Scoring: In the nonactivated and activated systems 1.2 - 1.6 x 10^8 cells were seeded per plate, with the exception of tester strains TA98 and TA100, with S9 mix, which were seeded 2.4 and 2.5 x 10^8 cells per plate, respectively.

575

After incubation the number of revertant colonies were scored, either by automatic colony counter or manually. Those plates which were not scored immediately following incubation were stored at 4° C until colony scoring was conducted.

Evaluation Criteria: For each dose level, an average for triplicate plates and standard deviation were calculated. For the test material to be considered mutagenic, at least a doubling of the mean number of revertants. plate had to be observed in at least one strain. In addition, the increase had to be associated with a positive dose response. In those instances where a positive dose response in TA1537 or TA1538 was less than three fold, the assay had to be repeated and reproducible results obtained.

RESULTS:

Toxicity Assays: The preliminary toxicity determination indicated a toxic response without S9 activation at the two highest dose levels (6,667 and 10,000 µg/plate) tested. There was a reduction in the number of revertant colonies with an extremely reduced background lawn. In the presence of S9 there was a reduction in the number of revertant colonies at the three highest dose levels (3,333, 6,667 and 10,000 µg/plate) and a slight to moderate reduction in the background lawn for the two highest dose levels. Based on these findings the maximum dose level to be tested in the mutagenesis assay was 5,000 µg/plate.

Mutagenicity Assays: In the mutagenesis assay, the solvent control was within an acceptable range and the results of the positive controls confirmed that the assay had an appropriate level of sensitivity (Table 2).

The S9 activation experiment was repeated because of bacterial contamination of the S9 mix.

Tester strains TA98 and TA100 had viable counts which were outside the expected titer range of 4×10^9; the values were 4.8 and 4.9×10^9 respectively. However, because all other conditions used to monitor the tester strains were within the appropriate ranges, the data generated from the two strains were accepted.

Toxicity was observed with the test material at the 5,000 µg/plate level, since the number of revertant colonies in strain TA98, TA100, TA1535 and TA1537 with and without metabolic activation was lower than in controls. There was also a reduction in revertants in strain TA1538 without S9 activation when compared to solvent controls. In addition, there was moderate and slight decrease in the background lawn of microcolonies in strains TA98 and TA1537, respectively, in the presence of S9 activation.

TABLE 2. Control Results - Average of Triplicate Plates

Controls	Concen. µg/Plate	S9 Activation	TA98	TA100	TA1535	TA1537	TA1538
DMSO	50 µl	+	28	108	11	10	23
		-	23	118	23	7	16
Positive							
2-AA	4	+	3170				
2-NF	5	-	427				
2-AA	4	+		3087			
Sodium Azide	5	-		2100			
2-AA	4	+			162		
Sodium Azide	5	-			1238		
2-AA	4	+				372	
9-AA	75	-				516	
2-AA	4	+					2839
2-NF	5	-					1093

The test material did not cause an increase in revertant colonies over the solvent controls at doses ranging from 50 to 5,000 µg/plate; therefore, FMC 57020 (Command) was not considered mutagenic.

DISCUSSION:

The investigators concluded that FMC 57020 was not mutagenic with or without metabolic activation. All the tester strains including TA98 and TA100, which were seeded outside the acceptable range, were capable of giving a positive response with and without S9 activation. The number of revertant colonies in the solvent and positive controls was within an acceptable range relative to historical data.

Our assessment is that the investigators interpreted their data correctly. Strains TA98 and TA100 were seeded for viable counts at concentrations higher than the accepted titer limit; however, this deviation from the protocol did not affect the validity of the results.

CONCLUSIONS:

Under the conditions of the assay, FMC 57020 (Command) was not mutagenic with or without metabolic activation at dose levels ranging from 50 to 5,000 µg/plate.

CLASSIFICATION: Acceptable.

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

EPA: 68-01-6561
TASK 53
May 29, 1985

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

DATA EVALUATION RECORD

FMC [redacted] (Command)

Mutagenicity-Reverse Mutation in Salmonella

STUDY IDENTIFICATION: Haworth, S.R., Lawlor, T.E., Olevine, S.M., et al Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Unpublished study No. A84-1189 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated May 3, 1984.) Accession No ~~not available~~.

APPROVED BY:

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Dynamac Corporation

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Date: _____

168 579

1 TEST MATERIAL: FMC [redacted] Chemical

2 TEST MATERIAL: FMC [redacted], Lot No. 631-6-134-6, a clear liquid with a purity > 94%

3 STUDY TYPE: Mutagenicity (reverse mutation) in Salmonella

4 STUDY IDENTIFICATION: Haworth, S.R., Lawlor, T.E., Olewine, S.W., et al. Salmonella mutagenicity plate incorporation mutagenicity assay. (unpublished study, No. 284-0189 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated May 3, 1984.) Accession No.: [redacted]

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580

CONCLUSIONS:

A. Under the conditions of the assay, FMC [redacted] was mutagenic for Salmonella typhimurium tester strain TA100. FMC [redacted] induced an increase in revertant colonies over the solvent control at dose levels of 340 and 680 ug/plate, with and without S9 activation.

B. The results indicated that all the tester strains were capable of giving a reproducible positive response with and without metabolic activation. The number of revertant colonies in the solvent control was within an acceptable published[1] range. Therefore, the study is acceptable.

RECOMMENDATIONS: Not applicable.

BACKGROUND: Not applicable

DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for complete details.

1. The test material, FMC [redacted], lot E3177-104-6 was described as a clear liquid of greater than 98% purity. It was stored at room temperature and protected from light. The test compound was solubilized and serially diluted in DMSO immediately before use in the assay. Sterility was determined by plating test material on selective medium. The stability under actual experimental conditions was not determined.

2. The Salmonella typhimurium strains used were TA98, TA100, TA1535, TA1537, and TA1538. On the day of the assay, all strains were tested for the rfa mutation, and the presence of pkm101 plasmid was confirmed for strains TA98 and TA100.

3. Cytotoxicity of the test material was assessed with strain TA100 with and without S9 activation at concentration levels from 10 to 10,000 ug/plate.

4. Mutagenicity was determined by the method of Ames[1] with and without S9 activation at levels of test compound from 17 to 680 ug/plate.

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

5. The evaluation criteria used to assess the test material as mutagenic included a doubling in the mean revertants per plate in at least one tester strain, and this increase in revertants must show a dose response increase. For strains TA1537 or TA1538 if the dose-responsive increase is less than 3-fold the response must be reproducible to consider the test compound a mutagen.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity Assay: The preliminary toxicity assay in strain TA100 showed a cytotoxic response with and without S9 activation at the four highest dose levels (1000, 3333, 6667, and 10,000 µg/plate) tested. There was reduction in the number of revertant colonies and a moderate to extreme reduction in bacterial background lawns. Based on these findings the maximum dose level selected for the mutagenicity assay was 680 µg/plate (Table 1).

Mutagenicity Assay: In the mutagenicity assay, the number of revertant colonies in the solvent control for each tester strain was within the acceptable published range; and the positive control chemicals were mutagenic at concentration levels below those used to assay the test material. These results confirmed that the assay had an appropriate level of sensitivity (Table 2).

The test material gave a positive response, causing a two-fold increase in the mean number of revertants in tester strain TA100 at 340 and 680 µg/plate with and without S9 activation (Table 3). The test material was not mutagenic for strains TA98, TA1535, TA1537, or TA1538.

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

A. The authors concluded from the results that "the Salmonella/Mammalian-Microsome Mutagenicity Assay indicates that under the conditions of this study, FMC [redacted] Lot No. E3175-104-5 (MA #72423) did cause a positive response in tester strain TA100 both in the presence and absence of liver microsomes."

B. The quality assurance statement is present, signed, and dated May 1, 1984. The study was monitored at various phases during the course of this study (protocol review, treatment and plating of the cultures, draft report, and draft to final report).

TABLE 1 Preliminary Toxicity Assay with FMC [redacted]

Test Substance	S9 Activation	TA100 Revertants/Plate[a]	TA100 Background Lawn
Solvent Control			
DMSO (50 µl/plate)	+	100	Normal
	-	74	"
FMC [redacted]			
(µg/plate)			
10	+	89	Normal
	-	89	"
33	+	100	"
	-	62	"
67	+	131	"
	-	95	"
100	+	144	"
	-	92	"
333	+	194	Slightly reduced
	-	142	Normal
667	+	285	Slightly reduced
	-	153	"
1000	+	86	Moderately reduced
	-	25	"
3333	+	0	Extremely reduced
	-	0	"
6667	+	0	"
	-	0	"
10,000	+	0	"
	-	0	"

[a]Average of duplicate plates.

TABLE 2. Solvent and Positive Control Results in Assay with FMC [redacted]

Test Substance	Conc. µg/plate	S9 Activation	TA98	TA100	TA1535	TA1537	TA1538
Solvent Control:							
DMSO (50 µl/plate)		+	41	96	12	7	24
		-	32	101	24	8	17
Positive Control:							
2-Aminoanthracene (AA)	4	+	2436				
2-Nitrofluorene (NF)	5	-	672				
2-AA	4	+		2564			
Sodium Azide (SA)	5	-		1754			
2-AA	4	+			146		
SA	5	-			1310		
2-AA	4	+				303	
9-Aminoacridine	75	-				743	
2-AA	4	+					2483
2-NF	5	-					993

[a]Average of triplicate plates.

TABLE 3. Salmonella Mutagenicity Assay with FMC [redacted]

Test Substances	S9 Activation	TA100[a] Revertants/Plate[b]
Solvent Control DMSO (50 µl/plate)	+	96
	-	101
FMC [redacted][b] (µg/plate)		
17	+	95
	-	124
34	+	106
	-	111
170	+	155
	-	173
340	+	208
	-	230
680	+	301
	-	305

[a]Cells density was at 0.6 x 10^8 per plate.
[b]Average of triplicate plates.

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

Our assessment is that the investigators interpreted their data correctly, and that under the conditions of the assay, FMC [redacted] was mutagenic for Salmonella typhimurium TA100 at 340 and 680 ug/plate with and without S9 activation.

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

16. CBI APPENDIX: Appendix A, Materials and Method (Protocols), CBI pp 2-9, 23-32.

Appendix A

Materials and Methods (Protocols)

Command toxicology review

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Pages 177 through 194 are not included in this copy.

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___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ FIFRA registration data

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588

EPA: 68-01-6561
TASK: 53
May 29, 1985

DATA EVALUATION RECORD

FMC [redacted] (Command)

Mutagenicity - Reverse Mutation in Salmonella

STUDY IDENTIFICATION: Haworth, S.R., Lawlor, T.E., Olewine, S.M., et al. Salmonella/mammalian-microsome plate incorporation mutagenicity assay. (Unpublished report No. A84-1281 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated June 12, 1984.) Accession No. 072815.

APPROVED BY:

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

Signature: _____

Date: _____

1. CHEMICAL: FMC [redacted] (Com

2. TEST MATERIAL: FMC [redacted] (Command), Lot No. [illegible], [illegible] solid, with a purity of > 98%.

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

4. STUDY IDENTIFICATION: Haworth, S.R., Lawlor, T.E., Olewine, S.M., et al. Salmonella/mammalian-microsome plate incorporation mutagenicity assay. (Unpublished report No. A84-1281 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated June 12, 1984.) Accession No. 072815.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Author
Dynamac Corporation

Signature: Brenda Worthy
Date: 5-29-84

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

Signature: William L. McLellan
Date: May 29, 1985

6. APPROVED BY:

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

Signature: [illegible]
Date: [illegible]

Carolyn Gregorio, Ph.D.
EPA Reviewer

Signature: CG
Date: 6-11-85

Clint Skinner, Ph.D.
EPA Section Head

Signature: Clint Skinner
Date: 2-25-85

7. CONCLUSIONS:

A. Under the conditions of the assay, FMC [redacted] was mutagenic in Salmonella typhimurium strains TA1535 and TA100 with and without metabolic activation at dose levels ranging from 500 to 10,000 µg/plate.

B. The results indicated that all tester strains were capable of giving a reproducible positive response with and without metabolic activation. The number of revertants in the solvent control was within an acceptable range. Therefore, the study is acceptable.

8. RECOMMENDATIONS: Not applicable.

9. BACKGROUND: Not applicable.

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

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for complete details.

1. The test material, FMC [redacted] Lot No. E3175-1401 was described as a crystalline solid with a purity of > 98%. It was stored at room temperature with desiccation, and protected from light. The test material was diluted in dimethylsulfoxide (solvent control) to final concentrations of 100, 500, 2500, 5000, and 10,000 µg/plate.

2. The following strains of Salmonella typhimurium were used: TA98, TA100, TA1535, TA1537, and TA1538. On the day of their use all strains were tested for their specific genotype characterization. Strains TA98 and TA100 were tested for the presence of the pkm 101 plasmid.

3. Toxicity of the test material was determined with strain TA100 with and without metabolic (S9) activation at 10 dose levels between 10 and 10,000 µg/plate.

4. Mutagenicity was determined by the method of Ames[1] with and without metabolic activation.

---

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

5. Evaluation criteria: To be considered positive a test material must cause a doubling in the mean revertants/plate in at least one tester strain and this increase in revertants must be dose responsive. For strains TA1537 and TA1538 if the dose-response increase is less than 3-fold the response must be reproducible by a repeat assay to consider the test material a mutagen.

B. Protocol:

See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity Assay: The test material was diluted in dimethylsulfoxide (DMSO) to final concentrations of 10, 33, 67, 100, 333, 667, 1000, 2333, 6667, and 10,000 µg/plate with and without metabolic activation.

After incubation with strain TA100, there was a two-fold or greater increase in the number of revertant colonies over the solvent control with metabolic activation at dose levels of 333, 667, 1000, 3333, 6667, and 10,000 µg/plate; however, there was no reduction in the bacterial background lawn. Without metabolic activation, cytotoxicity (a decrease in revertant colonies) was observed, with an extreme reduction in the bacterial background lawn at the highest dose level (10,000 µg/plate). There was also a two-fold or greater increase in the number of revertant colonies over the solvent control at dose levels of 333, 667, 1000, 3333, and 6667 µg/plate.

Based on the results of this cytotoxicity assay, the maximum dose levels to be tested with and without metabolic activation were set at 10,000 and 5000 µg/plate, respectively.

Mutagenesis Assay: Five dose levels of the test material, 100, 500, 2500, 5000, and 10,000 µg/plate with metabolic activation and 50, 250, 1250, 2500, and 5000 µg/plate without metabolic activation were plated in triplicate with each of the 5 tester strains.

The following results were noted after a 48-hr incubation: after S9 activation, there was a two-fold or greater increase in revertant colonies over the solvent control, in strains TA100 and TA1535 at dose levels of 2500, 5000, and 10,000 µg/plate and also in strain TA100 at 500 µg/plate. The bacterial background lawns for all plates were normal. A two-fold increase in revertant colonies, over the solvent control was observed in strains TA100 and TA1535 at dose levels of 1250, 2500, and 5000 µg/plate in the nonactivated assay. In strain TA100 at 5000 µg/plate there was a slight decrease in revertants (892) when compared to the number of revertants (918) at the 2500 µg/plate dose level. There was a slight reduction in the bacterial background lawn at the 1250 and 2500 µg/plate dose levels in both strains, and a moderate/slight reduction at the 5000 µg/plate in strain TA100 and TA1535, respectively (Table 1).

TABLE 1. Results of Salmonella/Microsome Assay with FMC [redacted]

Substance	S9 Activation	Strains — [a]Revertant/Plate (Background Lawn) TA100	TA1535
DMSO (50 µl)	+	99(N)	11(N)
	-	122(N)	15(N)
FMC [redacted] (µg/plate)			
500	+	212(N)	N.R.
2500	+	905(N)	26(N)
5000	+	1697(N)	39(N)
10,000	+	2659(N)	77(N)
1250	-	511(slight)	41(slight)
2500	-	918(slight)	44(slight)
5000	-	892(moderate)	57(slight)

[a]Average of triplicate plate
N = normal bacterial background lawn.
N.R. = negative response; no two-fold increase over solvent control.

The test material did not induce, at any dose level, a significant increase in revertant colonies over the solvent control in strains TA98, TA1537, or TA1538 with or without metabolic activation. In the solvent control, the number of revertants were similar to the values published by Ames et al.[1] The positive controls induced high numbers of mutants at levels below the test material concentrations showing that the test system had an appropriate level of sensitivity (Table 2).

Strains TA98 and TA100 had higher viable counts than the expected cell density of 4×10^9; the values were 4.8 and 4.9×10^9, respectively. However, because all other conditions used to monitor the tester strains were within the appropriate ranges, the data generated from the two strains were accepted by the study director.

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

A. The authors concluded that, "FMC [redacted] Lot No. E3175-140-1 (MA#T2476), did cause a positive response on tester strains TA100 and TA1535 both in the presence and absence of metabolic activation."

B. A quality assurance statement was present, signed, and dated June 12, 1984.

Phases Inspected	Date of Inspection
Protocol Review	May 17, 1984
Preparation of S9 Mix	June 1, 1984
Draft Report	June 6, 1984
Draft to Final Report	June 12, 1984

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

A. The results indicated that all the tester strains including TA98 and TA100, which had cell densities higher than the acceptable range specified in the protocol, were capable of giving a positive response with and without S9 activation. The number of colonies in the negative control was within the expected range relative to data published by Ames et al.[1]

B. It is our assessment that FMC [redacted] induced a mutagenic response in strain TA100 and TA1535 with and without S9 activatior at dose levels of 500, 2500, 5000, and 10,000 µg/plate. This assessment is based on the criteria that a test material is to be considered positive, if it induces a doubling in revertants in at least one tester strain, and that the increase is accompanied by a dose response to increasing concentrations of the test material.

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

TABLE 2. Positive Control Results in the Salmonella/ Microsome Assay Used with FMC [redacted]

Substance	Conc. µg/plate	S9 Activation	Strains Revertants/Plate[a] TA98	TA100	TA1535	TA1537	TA1538
2-AA	4.0	+	3153				
2-NF	5.0	-	497				
2-AA	4.0	+		2956			
SA	5.0	-		1150			
2-AA	4.0	+			149		
SA	5.0	-			1054		
2-AA	4.0	+				479	
9-AA	75	-				944	
2-AA	4.0	+					2883
2-NF	5.0	-					1126

[a]Average of triplicate plates.

2-AA = 2 - Aminoanthracene
2-NF = 2 - Nitrofluorene
9-AA = 9 - Aminoacridine
SA = Sodium Azide

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

16. CBI APPENDIX: Appendix A, Materials and Methods (Protocols), CBI pp. 2-9, 23-32.

Appendix A

Materials and Methods (Protocols)

Command toxicology review

Page _____ is not included in this copy.

Pages 204 through 221 are not included in this copy.

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___ Identity of the source of product ingredients

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___ The product confidential statement of formula

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EPA: 68-01-6561
TASK: 53
May 28, 1985

DATA EVALUATION RECORD

FMC 57090 (Command)

CHO/HGPRT Point Mutation Assay

STUDY IDENTIFICATION: Thilagar, A., Brauninger, R., and Holley, M.N. CHO/HGPRT mutation assay in the presence and absence of exogeneous metabolic activation. (Unpublished study No. FMC A84-1188 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated 7-5-84.) Accession No. 072815.

APPROVED BY:

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

Signature: _____

Date: _____

1. CHEMICAL: FMC 57090 (Common Name: Command).

2. TEST MATERIAL: FMC 57090, Lot No. E3175-104-5, a light yellow crystalline powder with a 98 percent purity.

3. STUDY/ACTION TYPE: Mutagenicity: Chinese hamster ovary (CHO)/ hypoxanthine guanine phosphoribosyl transferase (HGPRT) point mutation assay.

4. STUDY IDENTIFICATION: Thilagar, A., Brauninger, R., and Holley, M.N. CHO/HGPRT mutation assay in the presence and absence of exogeneous metabolic activation. (Unpublished study No. FMC A84-1188 by Microbiological Associates, Bethesda, MD for FMC Corporation, Princeton, NJ; dated 7-5-84.) Accession No. 072815.

5. REVIEWED BY:

Brenda Worthy, M.T.
Project Scientist
Dynamac Corporation

Signature: Brenda Worthy
Date: 5-29-85

William L. McLellan, Ph.D.
Senior Scientist
Dynamac Corporation

Signature: William L. McLellan
Date: May 29, 1985

6. APPROVED BY:

J. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Assurance
Dynamac Corporation

Signature:
Date: 5-30-85

Carolyn Gregorio, Ph.D.
EPA Reviewer

Signature:
Date: 6-11-85

Clint Skinner, Ph.D.
EPA Section Head

Signature:
Date: 7-25-85

7. CONCLUSIONS:

Our conclusion is that FMC 57090 did not induce mutagenicity at dose ranges between 400 to 700 µg/ml without S9 activation or 400 to 800 µg/ml with S9 activation in the CHO/HGPRT point mutation assay. Although a higher dose (800 µg/ml) in the nonactivated assay could have been used, the study is acceptable.

8. RECOMMENDATIONS: Not applicable.

9. BACKGROUND: Not applicable.

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

11. MATERIALS AND METHODS (PROTOCOLS): (See Appendix A)

A. Materials and Methods:

1. The test material was identified as FMC 57090, Lot No. E3175-104-5 and its purity was unspecified. The test material was dissolved in acetone at concentrations of 400, 500, 600, and 700 µg/ml without metabolic activation and at concentrations of 500, 600, 700, and 800 µg/ml with metabolic activation. Acetone was used as the solvent controls for the test material.

2. The Chinese hamster ovary cells (Lot No. 6-6-83) used in this study were of the line originally obtained from Dr. Abraham Hsie, Oak Ridge National Laboratories.

3. The S9 fraction used was prepared from the livers of Fischer 344 rats induced with Aroclor 1254.

4. Cytotoxicity Assay: Duplicate cultures of cells, seeded at $5x10^5$ cells/T-25 flask were treated for 5 hours with dilutions of the test material with and without S9 activation. Colonies were counted after 7-12 days incubation, and cytotoxicity of the test material determined relative to the solvent control.

5. Mutagenesis Assay: Duplicate flasks were prepared and treated with appropriate levels of test compound, positive or solvent controls, with and without S9 activation. The cells were then washed and incubated in complete medium for 18 hr. For mutant selection, the cells were plated at a density of $2x10^5$/100mm plate (5 plates) in hypoxanthine-free medium containing 10 µM 6-thioguanine. Cloning efficiency was assessed in hypoxanthine-free medium without 6-thioguanine.

6. Evaluation criteria: The test material was considered positive if there was a dose-dependent and statistically significant increase in the mutation frequency relative to the solvent control.

7. The test method employed was that of Hsie, A. W. et al. Mutation Research 86: 193-214, 1981.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

Cytotoxicity: In the initial cytotoxicity test with S9 activation, the relative cell survival was 92 percent at 500 µg/ml, 91 percent at 250 µg/ml and higher at the lower dose levels. In the non-activated system, the relative cell survival was 77 percent at 500 µg/ml, 86% at 250 µg/ml, and higher at the lower dose levels.

In the cytotoxicity test performed in parallel with the mutagenicity assay, relative cell survival in the S9 activated system was 51, 61, 81, and 97 percent at FMC 57090 doses of 800, 700, 600, and 500 µg/ml, respectively. Relative cell survival in the non-activated system was 57, 69, 59, and 87 percent at test material doses of 700, 600, 500, and 400 µg/ml, respectively. Survival in the positive controls was 86 and 95 percent following treatment with 4.0 and 2.0 µg/ml, respectively, of benzo(a)pyrene [B(a)P] (with S9 activation) and 17 and 81 percent after treatment with 0.5 and 0.25 µg/ml, respectively, of ethylmethanesulfonate [EMS] (without S9 activation).

Mutation Assay: In the S9 activated system, the reversion frequency in the solvent control was 10.1 mutants/10^6 survivors. All of the FMC 57090 dose groups gave mutation frequencies approximately equal to the solvent control frequency. The frequency of mutants ranged from 9.4 to 15.9 mutants/10^6 survivors after treatment concentrations of FMC 57090 ranging from 500 to 800 µg/ml. The authors reported that the positive controls with S9 activation induced 37.7 and 103.0 mutants/10^6 survivors following treatment with 4.0 and 2.0 µg/ml, respectively, of B(a)P.

In the nonactivated system, the solvent control reversion frequency was 7.5 mutants/10^6 survivors. All of the FMC 57090 dose groups had mutation frequencies approximately equal to the solvent control reversion frequency. The frequency of mutants ranged from 8.7 to 12.1 mutants/10^6 survivors after treatment with 400 to 700 µg/ml of FMC 57090. The EMS positive controls in the nonactivated system induced 982.5 and 465 mutants/10^6 survivors at doses of 0.5 and 0.25 µg/ml, respectively.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The study authors' conclusion was:

"The results of the study indicate that under the conditions of the test, the test article did not cause a significant increase in the frequencies of mutants."

A quality assurance statement, signed and dated 7/5/84, was present with the report. Protocol amendments associated with amendments/ correction to the study report were attached as an appendix to the study report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The authors concluded that there was no statistically significant increase in mutant frequency over the solvent control after dosing cells with FMC 57090 up to 800 µg/ml in the S9 activated system and with FMC 57090 concentrations up to 700 µg/ml in the non-activated system by the Poisson-based test included in the authors' method section.

Our assessment is that the authors' conclusions are supported by the data. A higher dose could have been used in the nonactivated system since cell survival at 700 µg/ml was 57 percent and FMC 57090 was soluble to at least 800 µg/ml (the highest dose used in the S9 activated system). The positive controls data showed that the assay was sensitive in detecting mutants at 2.0 µg/ml of S9 activated B(a)P and at 0.25 µg/ml of nonactivated EMS. Cloning efficiencies in the solvent controls were greater than 90 percent and their mutation frequencies were within an acceptable range.

Under the condition of this study, FMC 57090 was not mutagenic for the HGPRT locus of CHO cells in the presence or absence of S9 activation at doses resulting in survival ranges from 77 to 92 percent.

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

16. CBI APPENDIX: Appendix A, Methods and Materials (Protocols), CBI pp 4-7, 15-26, 29.

Appendix A

Materials and Methods (Protocols)

Command toxicology review

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Pages 228 through 244 are not included in this copy.

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004628

EPA: 68-01-6561
TASK: 53
May 29, 1985

DATA EVALUATION RECORD

FMC 57090 (Command)

Mutagenicity-Reverse Mutation in Salmonella

STUDY IDENTIFICATION: Haworth, S.R., Lawlor, T.E., Plunkett, R.J., et al. Salmonella/mammalian-microsome plate incorporation mutagenicity assay. (Unpublished Study No. A83-1111 by Microbiological Associates, Rockville, MD for FMC Corporation, Princeton, NJ; dated January 24, 1984.) Accession No. 072815.

APPROVED BY:

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

Signature: _____

Date: _____

245

1. CHEMICAL: FMC 57090 (Command).

2. TEST MATERIAL: FMC 57090 (Command).

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

4. STUDY IDENTIFICATION: Haworth, S.R., Lawlor, T.E., Plunkett, R.J., et al. Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Unpublished Study No. A83-1111 by Microbiological Associates, Rockville, MD for FMC Corporation, Princeton, NJ; dated January 24, 1984.) Accession No. 072815.

5 REVIEWED BY:

Brenda Worthy, M.T.
Principal Author
Dynamac Corporation

Signature: Brenda Worthy
Date: 5-29-85

William McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L McLellan
Date: May 29, 1985

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
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Dynamac Corporation

Signature: [illegible]
Date: [illegible]

C. Gregorio, Ph.D.
EPA Reviewer

Signature: CAG
Date: 6-11-85

Clint Skinner, Ph.D
EPA Section Head

Signature: Clint Skinner
Date: 7-25-85

7. CONCLUSIONS:

A. Under the conditions of the assay, FMC 57090 was mutagenic with and without S9 activation in Salmonella strains TA100 and TA1535 at dose levels of 5000 and 10,000 ug/plate. The test material, therefore, induces base pair substitutions in the Salmonella/microsome assay.

The study is acceptable.

8 RECOMMENDATIONS: Not applicable.

9 BACKGROUND: Not applicable.

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

11. MATERIALS AND METHODS (PROTOCOLS): See Appendix A.

A. Materials and Methods:

1. FMC 57090, Lot No: E2383-139A, a beige powder. Purity not specified. The test material was diluted in dimethylsulfoxide to final concentrations of 100, 500, 2500, 5000 and 10,000 µg/plate.

2. The following strains of Salmonella typhimurium were used: TA98, TA100, TA1535, TA1537, and TA1538. The Ames Mutagenicity Assay, with and without S9 activation was employed.

B Protocol: See Appendix A.

12. REPORTED RESULTS:

A Toxicity Assay: The test material was solubilized in dimethyl sulfoxide (DMSO) and assayed at concentrations of 10, 33, 67, 100, 333, 667, 1000, 3333, 6667, and 10,000 µg/plate with and without S9 activation. After incubation with TA100, there was a two-fold or greater increase in revertant colonies over the solvent control at dose levels of 6667 and 10,000 µg/plate with and without S9 activation. No toxicity was observed at the highest level tested. Based on the results, the maximum dose level selected for the mutagenesis assay was 10,000 µg/plate.

B. Mutagenesis Assay: Five doses (100, 500, 2500, 5000, and 10,000 µg/plate) of the test material were plated in triplicate with each of the five tester strains.

Following a 48-hr incubation, there was a significant two-fold or greater increase in the mean number of revertant colonies over the solvent control in strain TA1535 with and without S9 activation at 5000 and 10,000 µg/plate. A two-fold increase in the mean number of revertant colonies was noted in strain TA100 with and without S9 activation at 10,000 µg/plate and without S9 activation at 5000 µg/plate (Table 1).

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

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608

TABLE 1. Results of Salmonella/Microsome Assay with FMC 57090

Substance	Dose/ plate	S9 Activation	Strains/Revertants/plate[a] TA1535	TA100
DMSO	50 µl	+	15	97
		-	36	84
FMC 5790	10,000 µg	+	67	247
		-	100	377
	5000 µg	+	43	185
		-	78	198

[a] Average of triplicate plates.

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The test material did not induce, at any dose level, a significant increase in the mean number of revertant colonies over the solvent control in strains TA1537, TA1538, or TA98 with or without S9 activation

The solvent control plates had spontaneous revertants within an acceptable range relative to published data.[1] The positive controls induced high numbers of mutants at concentrations lower than the test materials, therefore, the test system had an appropriate level of sensitivity (Table 2).

The authors reported that cell density of the TA1538 culture was calculated to be 8.0×10^8. The minimum cell concentration required by the protocol was 1×10^9, but the solvent and positive control [revertant values][1] were well within the normal range. The authors judged that this discrepancy in no way invalidated the conclusions drawn from the study.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that, "FMC 57090, Lot No. E2383-139A (MA #T2176) did cause a positive response on TA100 and TA1535 in both the presence and absence of rat liver microsomes".

B. A quality assurance statement was present, signed, and dated January 31, 1984.

Phase-Inspected	Date of Inspection
Protocol review	10-19-83
Initial toxicity:	
Strain characterization	10-19-83
Weighing of test article	10-21-83
Draft report	11-21-83
Final report	1-24-84

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

A. The results indicated that all the tester strains, including TA1538 which the authors considered to be at a lower than acceptable cell density, were capable of giving a positive response with and without S9 activation. The number of colonies in the negative and positive controls were within an acceptable range relative to historical data.

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[1] Implied.

TABLE 2. Control Results Used in the Salmonella/Microsome Assay with FMC 57090

Substance	Dose/ plate	S9 Activation	Strains/Revertants/plate[a] TA98	TA100	TA1535	TA1537	TA1538
DMSO	50 µl	+	36	97	15	8	25
		-	20	84	36	9	19
Positive Controls							
2-AA[b]	4 µg	+	3247				
2-NF[c]	5 µg	-	744				
2-AA	4 µg	+		3323			
Sodium Azide(SA)	5 µg	-		1356			
2-AA	4 µg	+			208		
SA	5 µg	-			1517		
2-AA	4 µg	+				509	
9-AA[d]	75 µg	-				977	
2-AA	4 µg	+					3117
2-NF	5 µg	-					1496

[a]Average of triplicate plates.

[b]2-aminoanthracene.

[c]2-nitrofluorene.

[d]9-aminoacridine.

B. Based on the evaluation criteria that for a test material to be considered positive, it must cause at least a doubling in the mean revertants of at least one tester strain and that increase must be accompanied by a dose response to increasing concentrations of the test material, it is our assessment that FMC 57090 induced a mutagenic response in strains TA1535 and TA100 (base pair substitutions) with and without S9 activation at levels of 5,000 and 10,000 µg/plate.

Strain TA1538 was seeded at a concentration slightly lower than accepted cell density; however, this deviation from the protocol did not affect the validity of the results.

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

16. CBI APPENDIX: Appendix A, Materials and Methods (Protocol), CBI pp 2-9, 24-33.

Appendix A

Materials and Methods (Protocols)

Command toxicology review

Page _____ is not included in this copy.

Pages 253 through 270 are not included in this copy.

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