8-15-94

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

011176

AUG 15 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: F6285 Herbicide - Experimental Use Permit

TO: Joanne Miller/Jesse Mayes (PM-23)
Fungicide/Herbicide Branch
Registration Division (7505C)

FROM: Susan L. Makris, M.S. Susan L Makris 8/10/94
Review Section IV, Toxicology Branch II
Health Effects Division (7509C)

THRU: Marcia van Gemert, Ph.D., Chief Marcia van Gemert 8/12/94
Toxicology Branch II
Health Effects Division (7509C)

Chemical:	F6285 4F Herbicide (39.6% a.i.)
Caswell No.:	951
PC Code:	129081
DP Barcode Nos.:	D198408, D202046, D203929
Submission No.:	S456588
Case No.:	034954
ID No.:	000279-EUP-RGR

Registrant: FMC Corporation
Agricultural Chemical Group
Research and Development Department
Product Development
1735 Market Street, Room 2232
Philadelphia, Pennsylvania 19103

Action requested: The petitioner, FMC, has requested an Experimental Use Permit for the purpose of evaluating the performance of F6285 4F Herbicide (39.6% a.i.) in/on soybeans. In this proposed experimental program, F6285 4F will be applied to or incorporated into the soil, preemergence at a rate of 0.25-0.5 pound active ingredient per acre, for the control of broadleaf weeds and some annual grasses in soybeans. After harvest, the first rotational crop is to be destroyed; livestock are not to graze on treated plants, nor are livestock to be fed treated plants

(1)

or plant trash. In support of this application, a temporary tolerance petition for residues of the metabolite, 3-hydroxymethyl F6285, in or on soybeans was submitted (D198715, Case 285252, S457101, ID# 3G04272); the proposed tolerance is 0.025 ppm.

Toxicology data submitted:

The registrant (FMC Corporation) has submitted 8 studies in support of an Experimental Use Permit for preemergence application to soybeans. These studies have been evaluated, and the Data Evaluation Reports are attached. The conclusions are as follows:

1. Oral Developmental Toxicity Study in Rats (§83-3) - MRID Nos. 429321-03 and -04

The test substance, F6285, was administered by gavage to pregnant female Crl:CD®BR (Sprague-Dawley) rats on days 6-15 of gestation at dose levels of 1.0, 10.0, 25.0, and 50.0 mg/kg/day. The rats were observed for signs of toxicity; body weight and food consumption values were recorded. On day 20 of gestation, the rats were sacrificed and necropsied; spleen and uterine weights were recorded; spleens were examined histopathologically. The uteri were examined, implantation sites were counted, and the numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external anomalies. They were then processed for visceral and skeletal evaluation.

Evidence of treatment-related maternal toxicity at the 50.0 mg/kg/day dose level consisted of significantly increased mean spleen-to-brain weight ratio and a moderate increase in splenic extramedullary hematopoiesis, which was interpreted as being related to an increased physiological demand for erythrocyte production over and above that in the bone marrow. Clinical observations (fresh or dried blood observed around the vagina) and significant decreases in mean maternal body weight change values on days 15-20 and 0-20 were considered to result from treatment-related fetal loss.

Maternal LOEL = 50.0 mg/kg/day
(based upon increased relative spleen weight and splenic extramedullary hematopoiesis)
Maternal NOEL = 25.0 mg/kg/day

Evidence of treatment-related developmental toxicity consisted of decreased fetal viability, decreased fetal body weight, and increased incidences of fetal alterations, comprised, for the most part, of skeletal malformations and variations.

Fetal viability: At the 50.0 mg/kg/day dose level, treatment-related decreases in mean litter size and in the percent of total fetuses and live fetuses were noted. In addition, treatment-related increases were noted for the percent of dead fetuses; mean number of resorptions; percent of early, late, and total resorptions; and percent of rats with any resorption.

Fetal body weight: Treatment-related decreases in mean fetal weight values (total and by sex) were observed for the 25.0 and 50.0 mg/kg/day dose groups.

Fetal alterations: In the high-dose group (50.0 mg/kg/day), the percent of litters with fetuses

with any alteration was significantly increased (91.3%). At the same dose, significant increases occurred for the percent of fetuses with any alteration (25.8%) and the average percentage of fetuses with any alteration (30.24 per litter). The increased incidences of alterations at the high-dose were attributed to significant increases in the fetal and litter incidences of both malformations and variations at that dose. The percent of litters with fetuses with any malformation (30.4%) or variation (87.0%), the percent of fetuses with any malformation (4.8%) or variation (23.1%), and the mean percent of fetuses with any malformation (6.63) or variation (27.52) per litter were increased ($p \leq 0.01$). In addition, at the 25.0 mg/kg/day level, a significant increase ($p \leq 0.05$) in the percentage of litters with any variation was noted.

Treatment-related malformations (only at the 50.0 mg/kg/day dose level) included the following: 1) The fetal and litter incidences of edema (anasarca) were increased. Four fetuses (from four litters) were observed with anasarca at this dose, whereas no edematous fetuses were observed in the control or other treated groups. 2) The fetal incidence of short ribs was increased. Since this malformation was believed to be related to significantly increased skeletal variations of the ribs (hypoplasia and/or wavy ribs), it was attributed to treatment. 3) An increase in the number of fetuses with bent radius and ulna was noted, and an observation of bent fibula was noted in one fetus at that same dose level. These observations were not present in the control or other treated groups for this study, nor were they present in the historical control data from the performing laboratory (included with the study report).

Treatment-related variations included the following: 1) Increases in the fetal and/or litter incidences of skeletal variations occurred at the 50.0 mg/kg/day dose level in the vertebral arches (incompletely ossified), ribs (hypoplastic or wavy), sternebrae (incompletely ossified or unossified) and pelvis (incompletely ossified ischia or pubis). 2) A significant reduction in the mean numbers of caudal vertebral and metacarpal ossification sites was noted for both the 25.0 and 50.0 mg/kg/day dose groups. At 50.0 mg/kg/day, the ossification site averages were also significantly reduced for sternal centers, metatarsals, and hindpaw phalanges.

Developmental LOEL = 25.0 mg/kg/day
[based upon 1) decreased mean fetal weight and 2) retardation in skeletal development as evidenced by an increased number of litters with any variation and by decreased numbers of caudal vertebral and metacarpal ossification sites]
Developmental NOEL = 10.0 mg/kg/day

CORE Classification: **Guideline**; this study satisfies the guideline recommendations for a §83-3(a) developmental toxicity (teratology) study in rats.

2. Dermal Developmental Toxicity Study in Rats (§83-3) - MRID Nos. 430046-03 and 429321-05

F6285 was administered by 6-hour dermal application to pregnant female Crl:CD®BR (Sprague-Dawley) rats on days 6-15 of gestation at dose levels of 5, 25, 50, 100, and 250 mg/kg/day. The rats were observed for signs of toxicity; body weight and food consumption values were recorded. On day 20 of gestation, the rats were sacrificed and necropsied; spleen and uterine weights were recorded. The uteri were examined, implantation sites were counted, and the

numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external anomalies. They were then processed for visceral and skeletal evaluation.

There was no evidence of treatment-related maternal toxicity. All rats survived to cesarean section. Maternal body weight change, food consumption, gross pathological findings, and absolute and relative (to brain) spleen weight values were comparable between control and treated groups. Vaginal bleeding between gestation days 13 and 17 was observed in rats of all groups (including control) and was judged by the study author to be related to the extrusion of Reichert's membrane, which has been shown to occur during this stage of pregnancy and is frequently observed in dermal studies because the rats cannot groom themselves (Long and Evans, 1920). This finding, although attributed to treatment, was not considered a toxic effect, since the incidence of this finding in the control animals was high (14/24), and no correlation to fetal loss was observed in any group.

Maternal LOAEL = Not determined
Maternal NOAEL $\geq$ 250 mg/kg/day

Evidence of treatment-related developmental toxicity consisted of decreased fetal body weight and increased incidences of fetal alterations, comprised primarily of skeletal variations and reductions in mean numbers of ossification sites.

At the high-dose level (250 mg/kg/day), significant treatment-related decreases in mean fetal body weight (males, females, and combined) were observed. In addition, the percent of fetuses with any alteration observed (9.8%) was increased ($p \leq 0.01$) from the control incidence (3.2%). The percent of litters containing fetuses with any alteration (68.0%) was also significantly increased as compared to the control (37.5%) at the high dose, and was primarily attributable to increased incidences of skeletal variations.

Fetal malformations noted were sporadic and not attributed to treatment. No external or visceral variations of concern were observed. Significant treatment-related increases in the fetal and litter incidences of incompletely ossified lumbar vertebral arches, hypoplastic or wavy ribs, and incompletely ossified or nonossified ischia or pubes occurred at the high-dose (250 mg/kg/day). An additional significant increase in the high-dose fetal incidence of variations in the sternebrae (incompletely ossified or unossified) was not judged to be treatment-related. At 250 mg/kg/day, the mean numbers of thoracic vertebral and rib ossification sites were significantly decreased, a high-dose effect of treatment with F6285, consistent with the significant treatment-related hypoplasia observed in the skeletal evaluation of the ribs.

Developmental LOEL = 250 mg/kg/day
(based on decreased fetal body weight; increased incidences of fetal variations: hypoplastic or wavy ribs, incompletely ossified lumbar vertebral arches, and incompletely ossified ischia or pubes; and reduced number of thoracic vertebral and rib ossification sites)
Developmental NOEL = 100 mg/kg/day

CORE Classification: **Guideline**; this study satisfies the guideline recommendations for a §83-

3(a) developmental toxicity (teratology) study in rats.

3. Oral Developmental Toxicity Study in Rabbits (§83-3) - MRID No. 429321-06

F6285 was administered by gavage to pregnant female New Zealand White rabbits (20/group) on days 7-19 of gestation (with the day of mating defined as gestation Day 0) at dose levels of 100, 250, and 375 mg/kg/day. The rabbits were observed for signs of toxicity; body weight and food consumption values were recorded. Cesarean section was performed on Day 28 of gestation; the does were necropsied, uterine weights were recorded, and uterine contents were examined. Fetal specimens were evaluated for external, visceral, and skeletal abnormalities by standard methodologies.

Maternal toxicity: In the does, treatment-related incidences of decreased feces and hematuria were noted at the 250 mg/kg/day or greater. In addition, at the 375 mg/kg/day dose level, five rabbits aborted. Significant reductions in mean body weight change were observed for the dosing period (GD 7-19) and for the study duration (GD 0-29, both before and after adjustment for gravid uterine weight) at the 250 and 375 mg/kg/day dose levels.

Maternal LOEL = 250 mg/kg/day, based upon increased abortions, clinical signs (hematuria and decreased feces), and reduced body weight gain
Maternal NOEL = 100 mg/kg/day

Developmental toxicity: At the 250 and 375 mg/kg/day dose levels, significant decreases in the percent live fetuses per litter, significant increases in the percent early resorptions per litter, and significantly decreased fetal body weight (8 and 15% below control, respectively) were observed. These decrements in litter size, survival, and weight were also observed as a significantly decreased mean gravid uterine weight value in does at the 375 mg/kg/day dose level.

No external or visceral findings in fetuses suggested a response to treatment; however, skeletal evaluation revealed dose- and treatment-related findings at the 375 mg/kg/day dose level. These included significant increases in both the fetal and litter incidences of fused caudal vertebrae (a malformation) and of partially fused nasal bones (a variation). In addition, at 375 mg/kg/day, significant treatment-related reductions in ossification site averages were observed for metacarpals and both fore- and hindpaw phalanges.

Developmental LOEL = 250 mg/kg/day, based upon increased resorptions, decreased live fetuses per litter, and decreased fetal weight
Developmental NOEL = 100 mg/kg/day

CORE Classification: **Guideline**; this study satisfies the guideline recommendations for a §83-3(b) developmental toxicity (teratology) study in rabbits

4. Subchronic Dietary Toxicity in Rats (§82-1) - MRID No. 430046-01

The test substance, F6285, was administered to Fischer 344 rats (10/sex/group) for 90 days at dietary levels of 0, 50, 100, 300, 1000, 3000, and 7000 ppm. Ten additional rats per sex per

5 c

group at the control, 1000, and 3000 ppm levels were maintained an additional 4 weeks to assess recovery. The rats were observed for signs of toxicity; body weight and food consumption values were recorded weekly. Ophthalmoscopic examinations were conducted prior to treatment and at study termination. Blood samples were collected from all animals at termination, and hematology and clinical chemistry evaluations were performed. After 90-days of treatment, 10 rats/sex/group were sacrificed and necropsied. Recovery animals were sacrificed and necropsied after 4-weeks on control feed. Organ weight data were recorded, and tissues were processed for subsequent specified histopathological examination.

Administration of the test substance caused severe anemia complicated by inanition. The anemia was postulated to result from the interference of heme biosynthesis through the inhibition of protoporphyrin oxidase and the accumulation of protoporphyrin IX. As a result of the anemia and inanition, all high-dose (7000 ppm) rats died before Week 6 of study and one 3000 ppm female died during Week 2. Treatment-related clinical observations in all 7000 ppm animals and all 3000 ppm females included decreased or brownish-red feces, red abdominogenital staining, decreased locomotion, hypersensitivity to touch, dehydration, pale eyes and ears, shedding fur, unthriftiness, and walking on toes. At 3000 and 7000 ppm, mean body weight values for both sexes were significantly decreased through the periods of treatment and recovery. Decreased body weight gain was also noted for males at 1000 ppm during recovery. Food consumption was decreased for all animals that died on study and at 3000 ppm for some measured intervals.

During the treatment phase, increased white blood cell counts, decreased hemoglobin and hematocrit, decreased mean corpuscular volume and mean corpuscular hemoglobin measurements in 1000 and 3000 ppm rats were attributed to treatment; some of these effects remained through the recovery period. Treatment-related decreases in platelet counts were noted in 1000 ppm males after treatment, and in 1000 and 3000 ppm females at both bleeding intervals. The number of nucleated red blood cells was significantly higher in 3000 ppm rats after treatment, and in 1000 and 3000 ppm females after the recovery period; red blood cell counts were significantly elevated at 1000 and 3000 ppm after the recovery period. ALT levels were significantly decreased for 1000 and 3000 ppm males after treatment, and for 1000 ppm males following the recovery period.

Gross and microscopic pathological treatment-related findings in the spleen were attributed to the observed anemia. Enlarged spleen was observed in 1/10 males and 9/10 females at 3000 ppm, and in 8/10 males and 1/10 females at 7000 ppm. At 3000 ppm, absolute and relative spleen weights were increased for both sexes, with the effects more pronounced in the females. After 4-weeks of recovery, enlarged spleen was noted for 1/10 females at 3000 ppm, and although spleen weights remained increased, some recovery was evident.

Significantly increased liver weight (relative to body weight) for males and females following treatment and significantly decreased liver weight (relative to brain weight) for females following the recovery period were noted at 3000 ppm. These changes may be related to treatment. Other significant organ weight findings, in the adrenals, heart, brain, kidneys, and testes of animals in the 3000 ppm treatment group and in the heart and testes of males at 1000 ppm were considered to be secondary toxic effects resulting from the treatment-related body weight depression and anemia.

Following the treatment period, histopathological findings were observed in both sexes at the 3000 and 7000 ppm levels; no treatment-related lesions were observed following the recovery period. For both the 7000 and 3000 ppm treatment groups, anemia was directly associated with microscopic alterations in the bone marrow and spleen and with related lesions in the heart, liver, lungs, and kidneys. Effects were more severe in females, resulting in earlier mortality at 7000 ppm (average time to mortality was 18 days for females, as compared to 32 days for males); in the males, the longer exposure time prior to death resulted in increased severity of erythroid hyperplasia in the bone marrow. The anemia resulted in a loss of mature erythrocytes and accumulations of large numbers of nucleated erythrocyte precursors (reticulocytes) in the bone marrow and spleen. Splenic effects also included increased extramedullary hematopoiesis. Other microscopic changes noted in both sexes at 3000 and 7000 ppm were associated with inanition.

Based upon findings following a 4-week recovery period, the effects of dietary administration of F6285 appear to be reversible.

NOEL = 300 ppm (19.9 mg/kg/day in males; 23.1 mg/kg/day in females)
LOEL = 1000 ppm (65.8 mg/kg/day in males; 78.1 mg/kg/day in females)
based on clinical anemia (reduced hematocrit, hemoglobin, mean cell volume, and mean cell hemoglobin values during treatment; increased red blood cell count during recovery)

CORE Classification: **Guideline**; this study satisfies the requirements for a §82-1 subchronic toxicity study in rats and is acceptable for regulatory purposes.

5. Subchronic Dietary Toxicity in Mice (§82-1) - MRID No. 430046-02

In a subchronic toxicity study, F6285 was administered by dietary admix to Charles River B6C3F1 mice (10/sex/group) at doses of 0, 50, 100, 300, 550, 1,000 and 3,000 ppm (mg/kg/day: males = 0, 10.3, 17.8, 60.0, 108.4, 194.4 and all dead by day 9; females = 0, 13.9, 29.0, 79.8, 143.6, 257.0 and all dead by day 9). There was a 4-week recovery period (10/sex/group) for 0, 550 and 1,000 ppm animals of both sexes. The following parameters were examined: mortality, clinical signs, body weights, food consumption, hematology, macroscopic pathology, organ weights and microscopic pathology.

All 3,000 ppm mice died by study day 9; histopathological evaluation revealed erythroid hypoplasia of the bone marrow and evidence of inanition. The following test article effects were observed at 550 and 1,000 ppm: decreases in body weights and/or gains; decreased erythrocytes, hemoglobin and hematocrit values; and splenic microscopic pathology (increased incidence and severity of extramedullary hematopoiesis). A 4-week recovery period reversed all of the test-article effects with the exception of the splenic hematopoietic findings in females only; however, the post-recovery splenic alterations in 1,000 ppm females were reduced in severity. **The NOEL is 300 ppm (60.0 mg/kg/day for males; 79.8 mg/kg/day for females) and the LOEL is 550 ppm (108.4 mg/kg/day for males; 143.6 mg/kg/day for females).**

CORE Classification: **Minimum**; this study satisfies the data requirement (§82-1) for a 13-week subchronic toxicity study in mice.

6. Subchronic Dietary Toxicity in Dogs (§82-1) - MRID No. 429321-02

In a subchronic toxicity study, F6285 was administered by dietary admix to Marshall Farms beagle dogs (4/sex/group) at doses of 0, 300, 800 and 2,000 ppm (mg/kg body weight/day: males = 0, 10, 28 and 57; females = 0, 10, 28 and 73) for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, macroscopic pathology, organ weights and microscopic pathology.

The highest dose tested (2,000 ppm) caused: lower body weights (7-10%) and weight gains in males and females mostly during the first 5 weeks of the study; decreases in hemoglobin and hematocrit (as well as MCV, MCH and MCHC); elevated alkaline phosphatase levels; increased liver weights; and microscopic liver as well as splenic changes. **The NOEL is 800 ppm (28 mg/kg/day both sexes) and the LOEL is 2,000 ppm (57 and 73 mg/kg/day, males and females).**

CORE classification: **Guideline**; this study satisfies the data requirement (§82-1) for a 13-week subchronic toxicity study in dogs.

7. Mutagenicity: In Vitro Mouse Lymphoma Assay (§84-2) - MRID No. 430046-04

In two independently performed mouse lymphoma L5178Y $TK^{+/-}$ forward mutation assays, F6285 nonactivated doses of 424, 522, 620, 718, 817, 915, 1013, 1112, 1210, and 1308 μg/mL (Trial 1) and 1308, 1407, 1505, 1603, 1702, 1800, 2000, 2400, 2700, and 3000 μg/mL (Trial 2) were evaluated. In the S9-activated phase of testing, F6285 doses of 424, 620, 817, 1013, 1112, 1210, 1308, and 1407 μg/mL (Trial 1) and 915, 1013, 1112, 1210, 1308, 1407, 1505, 1603, 1702, and 1800 μg/mL (Trial 2) were assayed. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1242/1254 (2:1), and F6285 was delivered to the test system in dimethyl sulfoxide.

In the presence of S9 activation, the test material was cytotoxic (≈1800 μg/mL) but did not induce a mutagenic response. Results from the first nonactivated trial also indicated that F6285 at levels up to 1308 μg/mL was not mutagenic. However, dose-related increases in the mutation frequencies (MFs) occurred at precipitating levels (≤2400 μg/mL) in the absence of S9 activation in the second trial. Although the results are not sufficient to classify F6285 as mutagenic, the findings are considered to be equivocal.

CORE Classification: **Acceptable**; this study satisfies the guideline requirements for an in vitro mammalian cell mutation assay (§84-2).

8. Mutagenicity: In Vivo Micronucleus Assay in Mice (§84-2) - MRID No. 430046-05

In an in vivo mouse micronucleus assay, groups of five male and five female ICR mice were administered single intraperitoneal injections of 85, 170, or 340 mg/kg F6285. The test material was delivered to the animals in corn oil, and bone marrow cells were harvested 24, 48, and 72 hours posttreatment.

Based on preliminary testing, 340 mg/kg was estimated to be approximately 80% of the $LD_{50/7}$. Lethargy was noted in the high-dose animals; however, no evidence of a cytotoxic effect on the target organ was seen. Similarly, no significant increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow cells harvested for either sex at any dose or sacrifice time occurred.

CORE Classification: **Acceptable**; this study satisfies the guideline requirements for an in vivo mouse micronucleus assay (§84-2).

Data requirements: Tables 1a and 1b summarize the current toxicology profile of F6285 technical and 39.6% formulation, respectively; Table 2 presents the EUP data requirements.

Data gaps:

1. Chronic and reproductive toxicity data: According to the Registrant, a chronic toxicity/oncogenicity study in the rat, an oncogenicity study in the mouse, and a one-year toxicity study in the dog have all been completed but not yet submitted to the Agency. In addition, the Registrant has completed a two-generation reproductive toxicity study (meeting with Registrant; May 17, 1994) and is doing additional research to clarify effects observed. For the purposes of this EUP, the Registrant states that in crop field trials, the magnitude of the residue found in/on soybeans suggests that the theoretical maximum residue concentration (TMRC) will not exceed 50% of the maximum permitted intake (MPI) based on the developmental toxicity studies. Therefore, in accordance with 40 CFR 158.340, the requirements for a one-year interim report on a chronic dietary study and a one-generation reproductive toxicity study to support a temporary tolerance are waived at this time.

   These calculations were performed as follows: the Acceptable Daily Intake (ADI), based on the developmental NOEL from the oral developmental toxicity study in rats (10 mg/kg/day) and using a safety factor of 100, is calculated to be 0.1 mg/kg/day. The maximum permissible intake (MPI) is calculated to be 6 mg/day for a 60 kg person. The establishment of the 0.025 ppm proposed temporary tolerance for the 3-hydroxymethyl F6285 metabolite will result in a theoretical maximum contribution (TMRC) of 0.0085 μg/kg/day, based on soybean consumption of 0.340 g/kg/day and will utilize 0.0085% of the ADI.

2. Inhalation study: A data gap exists for the acute inhalation toxicity study with the 39.4% formulation of F6285. Since, based upon the results of other acute studies conducted on the technical material and the formulation (Tables 1a and 1b), the toxicity of the formulated product does not appear to be enhanced by the inert ingredients, an increased risk to those involved in the experimental program is not considered to be likely. Therefore, the requirement for an inhalation study on the end-use product can be deferred at this time. However, this remains a data gap, and the Registrant should be reminded of a commitment made in relation to a previous EUP application: FMC has stated that the company fully intends to perform an acute inhalation toxicity study on whatever formulation is ultimately selected for registration (279-EUP-RGN; HED Doc. No. 009956).

Table 1a. F6285 (Technical): Toxicology Profile

Guideline	Study	Species	MRID(s)	Toxicity	CORE Grade/Comments
81-1	Acute oral	Rat	419116-05	Tox Cat III; LD_{50} (M) 3034 mg/kg; (F) 2689 mg/kg	Guideline
81-2	Acute dermal	Rabbit	419116-07 422864-00	Tox Cat III; LD_{50} >2000 mg/kg	Guideline
81-3	Acute inhalation	Rat	424710-02	Tox Cat III; LC_{50}(4 hr) > 4.13 mg/L	Minimum
81-4	Primary eye irritation	Rabbit	419116-08 422864-00	Tox Cat III; corneal opacity and iritis, cleared by day 4	Guideline
81-5	Primary dermal irritation	Rabbit	419116-09 422864-00	Tox Cat IV; no erythema or edema	Guideline
81-6	Dermal sensitization	Guinea pig	419116-10 422864-00 422864-01	Not a sensitizer	Guideline
82-1	90-day subchronic	Rat	430046-01	NOEL = 300 ppm (males: 19.9 mg/kg/day; females: 23.1 mg/kg/day) LOEL = 1000 ppm (males: 65.8 mg/kg/day; females: 78.1 mg/kg/day), based on increased WBC; decreased HGB, HCT, MCV, MCH; increased RBC during recovery	Guideline
82-1	90-day subchronic	Mouse	430046-02	NOEL = 300 ppm (males: 60.0 mg/kg/day; females: 79.8 mg/kg/day) LOEL = 550 ppm (males: 108.4 mg/kg/day); females: 143.6 mg/kg/day), based on decreased BW, BW gain; decreased RBC, HgB, HCT; splenic extramedullary hematopoiesis	Minimum
82-1	90-day subchronic	Dog	429321-02	NOEL = 800 ppm (males and females: 28 mg/kg/day) LOEL = 2000 ppm (males: 57 mg/kg/day; females: 73 mg/kg/day), based on decreased BW, BW gain; decreased HgB, HCT, MCV, MCH, MCHC; increased ALKPHOS; increased liver weights; microscopic changes to the liver and spleen	Guideline

Table 1a. F6285 (Technical): Toxicology Profile

Guideline	Study	Species	MRID(s)	Toxicity	CORE Grade/Comments
83-3	Developmental toxicity (oral)	Rat	429321-03, 429321-04	Maternal NOEL = 25.0 mg/kg/day Maternal LOEL = 50.0 mg/kg/day, based on vaginal bleeding, increased relative spleen weight, increased splenic extramedullary hematopoiesis Developmental NOEL = 10.0 mg/kg/day; Developmental LOEL = 25.0 mg/kg/day, based on decreased fetal BW, delayed skeletal ossification (increased number of litters with any variation; decreased numbers of caudal vertebral and metacarpal ossification sites)	Guideline
83-3	Developmental toxicity (dermal)	Rat	430046-03, 429321-05	Maternal NOEL > 250 mg/kg/day Maternal LOEL = Not determined Developmental NOEL = 100 mg/kg/day; Developmental LOEL = 250 mg/kg/day, based on decreased fetal BW, increased fetal variations (hypoplastic/wavy ribs; incompletely ossified vertebral arches, ischia, pubes; reduced number of thoracic vertebral and rib ossification sites	Guideline
83-3	Developmental toxicity	Rabbit	429321-06	Maternal NOEL = 100 mg/kg/day Maternal LOEL = 250 mg/kg/day, based on decreased BW gains, increased abortions and clinical signs (hematuria and decreased feces) Developmental NOEL = 100 mg/kg/day; Developmental LOEL = 250 mg/kg/day, based on increased resorptions, decreased live fetuses, decreased fetal BW	Guideline
84-2(a)	Salmonella mutagenicity (Ames)	In vitro Salmonella	419116-11	Not cytotoxic or mutagenic, with and without activation	Acceptable
84-2(b)	Gene mutation in cultured mammalian cells	Mouse lymphoma	430046-04	Cytotoxic in presence of S9; findings of mutagenicity were equivocal, but were not sufficient to classify material as mutagenic	Acceptable
84-2(c)	In vivo cytogenetics: micronucleus	Mouse	430046-05	No evidence of cytotoxic effect; no increase in micronucleated PCEs; not mutagenic	Acceptable

Table 1b. F6285 (39.6% a.i.), Toxicology Profile

Guideline	Study	Species	MRID(s)	Toxicity	CORE Grade/Comments
81-1	Acute oral	Rat	419116-12	Tox Cat III; LD_{50} (F) = 2084 mg/kg	Minimum
81-2	Acute dermal	Rat	419116-13	Tox Cat III; LD_{50} > 2000 mg/kg	Guideline
81-3	Acute inhalation	Rat			
81-4	Primary eye irritation	Rabbit	419116-14	Tox Cat IV	Guideline
81-5	Primary dermal irritation	Rabbit	419116-15	Tox Cat IV	Guideline
81-6	Dermal sensitization	Guinea pig	419116-16 422864-02	Not a sensitizer	Guideline

Table 2. EUP Data Requirements for F6285

Study Type		Required	Satisfied
Required for all uses a			
81-1	Acute oral (rat)	YES	YES
81-2	Acute dermal (rabbit)	YES	YES
81-4	Primary eye irritation	YES	YES
81-5	Primary dermal irritation	YES	YES
Required for food/feed use b			
82-1	Subchronic feeding (rodent)	YES	YES
82-1	Subchronic feeding (dog)	YES	YES
83-3	Developmental toxicity (one species)	YES	YES
84-2(a)	Mutagenicity: Salmonella reverse mutation	YES	YES
84-2(b)	Mutagenicity: mammalian cells in vitro	YES	YES
84-2(c)	Mutagenicity: in vivo cytogenetics	YES	YES
83-1	Chronic feeding (rodent, one-year interim) c	YES e	NO
83-4	Reproduction (rat, one generation) c	YES e	NO
Conditional test requirements d			
81-3	Acute inhalation (rat) a	YES e	NO
81-6	Dermal sensitization a	YES	YES
81-7	Acute neurotoxicity b	NO	NO

a End use product.
b Technical grade active ingredient.
c Required if the TMRC exceeds 50% of the MPI.
d Required if needed to support registration.
e Deferred for purposes of this EUP; see discussion on data gaps, above.

Temporary tolerance petition:

A temporary tolerance of 0.025 ppm for residues of 3-hydroxymethyl F6285 is proposed, based upon analytical method sensitivity levels of 0.025 ppm and method detectability levels of 0.005 ppm for F6285 and the 3-hydroxymethyl metabolite. The 3-hydroxymethyl metabolite of F6285 was selected as an adequate representative or marker residue, based upon a metabolism study in soybeans, in which labelled F6285 was completely metabolized and 3-hydroxymethyl F6285 represented the major radioactive residue component. The Registrant reports that in 21 residue trials conducted with F6285 on soybeans, no detectable residues of F6285 were found in any of the treated soybeans and no residues of 3-hydroxymethyl F6285 were found at the limit of quantitation (0.025 ppm). The Registrant states that since no residues were found in soybean hulls, meal, oil, and soapstock treated at an exaggerated rate of 1.5 lb. a.i./A, no food additive tolerances are being proposed for hulls, meal, oil, and soapstock; and since no residues were found in the raw agricultural commodity, no animals studies are needed at this time.

Recommendations:

1. Toxicology Branch II has no objection to granting an Experimental Use Permit and temporary tolerance for the evaluation of F6285 on soybeans under the conditions specified in the application.

2. The low NOELs on the developmental toxicity studies, and the results indicating fetal (developmental) effects at doses lower than those at which maternal toxicity is observed, are a cause for concern. It is recommended that F6285 be forwarded for Developmental Peer Review following submission and review of the two-generation reproduction study and other supplementary developmental or reproductive toxicity studies currently in progress by FMC.

Reviewed by: Susan L. Makris, M.S. Susan L Makris 7/5/94
Section IV, Toxicology Branch II (7509C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 7/12/94
Section III, Toxicology Branch II (7509C)

011176

DATA EVALUATION REPORT

STUDY TYPE: Oral Developmental Toxicity Study in Rats (§83-3)

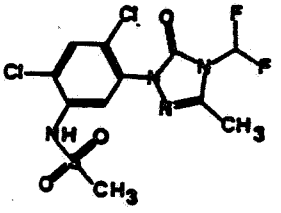
EPA NOS.: MRID NOS.: 429321-03 (Range-finding), 429321-04
PC CODE: 129081

DP BARCODE NOS.: D198408 and D198715
SUBMISSION NOS.: S456588 and S457101
CASE NOS.: 034954 and 285252
ID NOS.: 00279-EUP-RGR and 3G04272

TEST MATERIAL: F6285 Technical

SYNONYMS: 2-(2,4-dichloro-5-methylsulfonylamidophenyl)-4-difluoromethyl-2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one
FMC 97285
Sulfentrazone
Methanesulfonam

CHEMICAL STRUCTURE:



STUDY NUMBER: FMC No.: A91-3410
Argus No.: 106-009

SPONSOR: FMC Corporation, Agricultural Chemical Group
1735 Market Street, Philadelphia, PA 19103

TESTING FACILITY: FMC Corporation Toxicology Laboratory (in-life)
Box 8, Princeton, NJ 08543

Argus Research Laboratories, Inc. (fetal exams)
905 Sheehy Drive, Horsham, PA 19044

TITLE OF REPORT: F6285 Technical, Teratology Study in Rats (Oral)

AUTHOR: Christine Freeman

STUDY COMPLETION DATE: June 11, 1992

15

EXECUTIVE SUMMARY: The test substance, F6285, was administered by gavage to pregnant female Crl:CD®BR (Sprague-Dawley) rats on days 6-15 of gestation at dose levels of 1.0, 10.0, 25.0, and 50.0 mg/kg/day. The rats were observed for signs of toxicity; body weight and food consumption values were recorded. On day 20 of gestation, the rats were sacrificed and necropsied; spleen and uterine weights were recorded; spleens were examined histopathologically. The uteri were examined, implantation sites were counted, and the numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external anomalies. They were then processed for visceral and skeletal evaluation.

Evidence of treatment-related maternal toxicity at the 50.0 mg/kg/day dose level consisted of significantly increased mean spleen-to-brain weight ratio and a moderate increase in splenic extramedullary hematopoiesis, which was interpreted as being related to an increased physiological demand for erythrocyte production over and above that in the bone marrow. Clinical observations (fresh or dried blood observed around the vagina) and significant decreases in mean maternal body weight change values on days 15-20 and 0-20 were considered to result from treatment-related fetal loss.

Maternal LOEL = 50.0 mg/kg/day
(based upon increased relative spleen weight and splenic extramedullary hematopoiesis)
Maternal NOEL = 25.0 mg/kg/day

Evidence of treatment-related developmental toxicity consisted of decreased fetal viability, decreased fetal body weight, and increased incidences of fetal alterations, comprised, for the most part, of skeletal malformations and variations.

Fetal viability: At the 50.0 mg/kg/day dose level, treatment-related decreases in mean litter size and in the percent of total fetuses and live fetuses were noted. In addition, treatment-related increases were noted for the percent of dead fetuses; mean number of resorptions; percent of early, late, and total resorptions; and percent of rats with any resorption.

Fetal body weight: Treatment-related decreases in mean fetal weight values (total and by sex) were observed for the 25.0 and 50.0 mg/kg/day dose groups.

Fetal alterations: In the high-dose group (50.0 mg/kg/day), the percent of litters with fetuses with any alteration was significantly increased (91.3%). At the same dose, significant increases occurred for the percent of fetuses with any alteration (25.8%) and the average percentage of fetuses with any alteration (30.24 per litter). The increased incidences of alterations at the high-dose were attributed to significant increases in the fetal and litter incidences of both malformations and variations at that dose. The percent of litters with fetuses with any malformation (30.4%) or variation (87.0%), the percent of fetuses with any malformation (4.8%) or variation (23.1%), and the mean percent of fetuses with any malformation (6.63) or variation (27.52) per litter were increased ($p \leq 0.01$). In addition, at the 25.0 mg/kg/day level, a significant increase ($p \leq 0.05$) in the percentage of litters with any variation was noted.

Treatment-related malformations (only at the 50.0 mg/kg/day dose level) included the following: 1) The fetal and litter incidences of edema (anasarca) were increased. Four fetuses (from four

16

litters) were observed with anasarca at this dose, whereas no edematous fetuses were observed in the control or other treated groups. 2) The fetal incidence of short ribs was increased. Since this malformation was believed to be related to significantly increased skeletal variations of the ribs (hypoplasia and/or wavy ribs), it was attributed to treatment. 3) An increase in the number of fetuses with bent radius and ulna was noted, and an observation of bent fibula was noted in one fetus at that same dose level. These observations were not present in the control or other treated groups for this study, nor were they present in the historical control data from the performing laboratory (included with the study report).

Treatment-related variations included the following: 1) Increases in the fetal and/or litter incidences of skeletal variations occurred at the 50.0 mg/kg/day dose level in the vertebral arches (incompletely ossified), ribs (hypoplastic or wavy), sternebrae (incompletely ossified or unossified) and pelvis (incompletely ossified ischia or pubis). 2) A significant reduction in the mean numbers of caudal vertebral and metacarpal ossification sites was noted for both the 25.0 and 50.0 mg/kg/day dose groups. At 50.0 mg/kg/day, the ossification site averages were also significantly reduced for sternal centers, metatarsals, and hindpaw phalanges.

Developmental LOEL = 25.0 mg/kg/day
[based upon 1) decreased mean fetal weight and 2) retardation in skeletal development as evidenced by an increased number of litters with any variation and by decreased numbers of caudal vertebral and metacarpal ossification sites]
Developmental NOEL = 10.0 mg/kg/day

CORE CLASSIFICATION: **CORE-Guideline**; this study satisfies the guideline recomendations for a §83-3(a) developmental toxicity (teratology) study in rats.

A. Materials

1. Test compound:
   Name: F6285 technical
   Purity: 94.2%
   Reference No.: E7301-72
   Description: Solid
   CAS No.: 122836-35-5

2. Vehicle:
   Name: Mazola Corn Oil
   Purity: 100%
   Lot No.: WOA

3. Test animals:
   Species: Rat
   Strain: Crl:CD®BR
   Source: Charles River Laboratories, Portage, MI
   Age: Young adult
   Weight: 214-270 g at gestation day 0

17 X

4. Environment: Housing: Individual stainless steel cages
Lighting: 12 hours light/12 hours dark
Temperature: 64-72°F
Humidity: 45-83%
Food: Purina Rodent Chow 5002 ad libitum
Water: Municipal water, automatic watering system ad libitum

B. Methods - This study was conducted to evaluate the developmental toxicity of the test substance, F6285, when administered by gavage to pregnant female Sprague-Dawley rats during the period of major fetal organogenesis. A copy of the study methods is presented as Attachment 1.

1. Mating, Group Assignment, and Dosage Levels

Following a period of acclimation, the female rats were mated with young adult male rats of the same strain and source. Females were assigned to the following study groups on Day 0 of gestation according to a table of random numbers. No more than two females mated by the same male were assigned to any group.

Group No.	Dose[a] (mg/kg)	No. per Group
1 (Control)	0[b]	25
2 (Low)	1.0	25
3 (Low Mid)	10.0	25
4 (High Mid)	25.0	25
5 (High)	50.0	25

a Administered at constant volume of 5 ml/kg.
b Vehicle control (corn oil).

2. Rationale for Dosage Selection

In a range-finding study (MRID No. 429321-03), F6285 was administered orally to groups of 10 female rats each at dosages of 0, 10.0, 25.0, 100, and 500 mg/kg/day (w/v) in corn oil. The test solutions were administered at a constant volume of 5 ml/kg on days 6 through 15 of gestation, inclusive. Individual doses were adjusted daily to body weight. Cesarean sections were performed on day 20 of gestation. The uteri and ovaries were examined for the number and distribution of implantation sites, early and late resorptions, live and dead fetuses, and corpora lutea. Each fetus was weighed, sexed, and examined for external abnormalities.

id

Nine out of ten rats in the 500 mg/kg/day dose group died prior to gestation day 15; no other rats died prior to cesarean section. Clinical observations in the 500 mg/kg/day dose group included clonic convulsions, pale eyes and ears, walking on toes, dehydration, decreased feces, brownish-red feces, and hematuria; no clinical observations were noted in the other dose groups. Maternal body weight and body weight gain values were decreased from the onset of dosing (day 6) through termination at 100 mg/kg/day; however, food consumption was unaffected, as were body weight gains corrected for gravid uterine weight. Gravid uterine weight values were greatly reduced for 100 and 500 mg/kg/day rats, and absolute and relative (to brain weight) spleen weights were elevated. There were no viable fetuses in the 100 or 500 mg/kg/day dose groups; all implants resulted in resorptions. At 10.0 and 25.0 mg/kg/day, the litter information was similar to control, although fetal body weights were reported to be slightly depressed at 25.0 mg/kg/day. The only malformation noted was one fetus in the 10.0 mg/kg/day dose group with shortened phalanges. The maternal NOEL was 25.0 mg/kg/day, based on increased absolute and relative spleen weights at 100 mg/kg/day, and the developmental NOEL was 10.0 mg/kg/day, based upon reduced fetal body weights at the 25.0 mg/kg/day dose level.

Based upon the results of this range-finding study, 50.0 mg/kg/day was selected as a high dose for the subsequent full developmental toxicity study in rats. Low-, low mid-, and high mid-dose levels chosen were: 1.0, 10.0, and 25.0 mg/kg/day, respectively.

3. Test Material Formulation, Administration, and Analysis

Suspensions of F6582 in corn oil were formulated once prior to study start. The test substance was dissolved into a small quantity of acetone, then mixed with corn oil and stirred on a stirring plate. Nominal concentrations of 0, 1.0, 10.0, 25.0, and 50.0 mg/5 ml were prepared (based upon a constant dosage volume of 5 ml/kg) for administration to the respective test groups. Dosing suspensions were stored refrigerated and were mixed on a stirring plate for a minimum of 30 minutes prior to each use.

The test material was administered once daily to the study animals by gavage on Days 6-15 of gestation. Individual dosage volumes were adjusted daily, based upon the most recent body weight value. Control animals received the vehicle (corn oil) in the same manner.

Samples of the formulations from each dose level were analyzed prior to the treatment period. Concentration, homogeneity, and 5-week stability were determined by HPLC for all samples. Results indicated that the formulations were homogeneous, within adequate range of nominal concentrations, and were stable for at least the duration of the dosing period. The report did not indicate the storage conditions for the formulations that were analyzed for stability, i.e., whether they were stored at room temperature or under refrigeration.

4. Observations

The rats were observed for mortality twice daily. Clinical observations were recorded once daily, at approximately 4 hours posttreatment. Individual body weights were recorded on

gestation Days 0, 6-15 (inclusive), and 20. Food consumption was measured on gestation Days 0, 6, 15, and 20.

Surviving dams were sacrificed on Day 20 of gestation by CO_2 asphyxiation. A gross necropsy was performed on each rat. Spleen, brain, and gravid uterine weights were recorded. Corpora lutea of pregnancy were counted, and uterine contents were examined for pregnancy status, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses.

Histopathological evaluation was performed on the preserved, stained spleens of each dam. In addition to the standard hematoxylin and eosin stain, Prussian blue was used to identify iron positive pigment (hemosiderin).

Following removal from the uterus, each fetus was weighed, sacrificed by CO_2 asphyxiation, tagged with an identification number, and placed in a jar containing either methanol or Bouin's fixative (one half of each litter per solution). The fetuses were shipped to Argus Research Laboratories, Inc., where they were examined for gross external alterations. Fetuses preserved in Bouin's fixative were examined for visceral malformations and variations. Those fetuses preserved in methanol were eviscerated, examined for gender, stained with alizarin red S, and examined for skeletal malformations and variations. Malformations were defined as irreversible changes which occur at low incidence in this species and strain; variations were defined as common findings in this species and strain, reversible delays, or accelerations in development.

5. Statistical Analysis

The following methods of statistical analysis were performed, using SAS/STAT or proprietary programs (see report No. FMC A91-3410, pages 22-23; Attachment 1). Proportional data, i.e., the number of live and dead fetuses, number of total fetuses, resorption incidences, proportion of male fetuses, and fetal incidence data, were analyzed by the Variance Test for Homogeneity of the Binomial Distribution. Parametric data (maternal body weights, body weight changes, absolute and relative organ weights, fetal body weights, and mean fetal ossification or alteration data) were analyzed using Bartlett's Test of Homogeneity of Variances, the Analysis of Variance, and/or Dunnett's Test. Live fetal body weights, mean numbers of resorptions, implantations, corpora lutea minus implantations, and litter size were analyzed using the Kruskal-Wallis Test with Dunn's Method of Multiple Comparisons when statistical significance was achieved, or with Fisher's Exact Test. No test for linear trend was performed, and the unit of analysis was not indicated (i.e., the litter versus the fetus). The level of significance was $p \leq 0.05$.

Historical control data, from the performing laboratory, for reproductive indices; maternal necropsy observations; external, soft tissue, and skeletal alterations; and ossification site averages in the CD rat were presented in the report and are appended to this DER as Attachment 2.

6. Compliance

The following required signed and dated statements were provided:
- Statement of No Data Confidentiality Claims
- Good Laboratory Practice Certification
- Quality Assurance statement
- EPA Flagging Statement **(positive for criterium 5)**

C. Results

1. Maternal Mortality and Clinical Observations

During the course of the study, no rats died due to test substance administration. However, three rats, one each in the 0, 25.0, and 50.0 mg/kg/day dose groups, were sacrificed between days 9-12 of gestation due to intubation accidents.

At the 50.0 mg/kg/day dose level, fresh or dried blood was observed around the vagina of 12 of the 25 rats ($p \leq 0.01$) on one or more days beginning at gestation day 13 to 16. These observations were judged to be related to fetal loss and attributed to treatment. A similar observation was noted in one dam at the 25.0 mg/kg/day dose level but was not attributed to test substance administration.

2. Maternal Body Weight and Food Consumption Data

Summaries of mean maternal body weight change and food consumption values during gestation are presented in Tables 1 and 2, respectively.

Table 1. Mean Gestation Body Weight Change Data (g)

Days of Gestation	Dose level (mg/kg/day)				
	0	1.0	10.0	25.0	50.0
N	24	25	25	23	24
0-6	34	35	35	32	33
6-15	36	33	36	36	33
15-20	71	68	69	67	44**
0-20	141	136	141	135	109**
0-20 Corrected[a]	64	63	67	65	69

a Day 0-20 body weight change minus gravid uterine weight.
* Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A91-3410, page 39.

Significant decreases in mean body weight change values were noted for the 50.0 mg/kg/day group on days 15-20 and 0-20; however, when corrected for gravid uterine weight, the day 0-20 body weight gain value was not significantly different from control. The differences in body weight gain were attributed to treatment-related fetal loss at the 50.0 mg/kg/day dose level. This was supported by the gestation food consumption data which were statistically similar between control and treated groups.

Table 2. Mean Gestation Food Consumption Data (g)

Days of Gestation	Dose level (mg/kg/day)				
	0	1.0	10.0	25.0	50.0
N	24	25	25	23	24
0-6	128	134	131	130	126
6-15	172	173	174	173	165
15-20	127	129	131	132	131

Note: Data were extracted from report No. FMC A91-3410, page 40.

3. Maternal Gross Pathology, Organ Weight Data, and Histopathology

At necropsy, unilateral hydronephrosis associated with urinary calculi were reported for one rat at the 1.0 mg/kg/day dose level. This finding was not attributed to treatment, and no other abnormality was observed at any other dose level.

Mean maternal absolute and relative spleen weight values are summarized in Table 3. At the 50.0 mg/kg/day dose level, the absolute mean spleen weight value was similar to control; however, the mean spleen-to-brain weight ratio was significantly increased. This was determined to be a treatment-related effect.

Table 3. Mean Absolute and Relative[a] Spleen Weights (g)

Parameter	Dose Level (mg/kg/day)				
	0	1.0	10.0	25.0	50.0
N	24	25	25	23	24
Absolute spleen weight (g)	0.64	0.63	0.64	0.70	0.70
Relative spleen weight (%)	32.59	32.98	32.44	36.23	36.71*

a Mean spleen weight to brain weight ratio.
* Significantly different from control value, $p \leq 0.05$.
Note: Data were extracted from report No. FMC A91-3410, pages 41-42.

Microscopic evaluation of the spleens revealed a moderate increase in the degree of extramedullary hematopoiesis at the 50.0 mg/kg/day dose level; no similar effect was observed in the other treated or control groups. Average grading scores for extramedullary hematopoiesis were 2.08, 1.72, 1.72, 1.84, and 3.00 for the control through high-dose groups, respectively (Report No. FMC A91-3410, Histopathology Report, pages 163-174). These scores represent grades of differentiation between test and control spleens but are not reflective of severity. Hemosiderin pigment was not increased in the spleen of test animals as compared to control. The increased splenic extramedullary hematopoiesis was interpreted as being related to an increased physiological demand for erythrocyte production over and above that in the bone marrow and was considered associated to treatment.

4. Observations Noted at Cesarean Section

The results of the examination of uterine contents at cesarean section are presented in Table 4.

Table 4. Summary of Selected Cesarean Section Observations

Parameter	Dose level (mg/kg/day)				
	0	1.0	10.0	25.0	50.0
Number pregnant	25	25	25	24	24
Number of deaths[a]	1	0	0	1	1[b]
Number of litters totally resorbed	0	0	0	0	1
Number of viable litters	24	25	25	23	23
Number of implantations/dam	14.8	14.4	14.3	14.4	13.9
Mean implantation loss [c]	2.7	1.2	1.8	1.3	2.2
Number of fetuses/litter	14.5	13.4	13.6	14.0	7.9**
Percent live fetuses [d]	98.3	93.6	95.5	97.0	55.9**
Number dead fetuses (%[d])	0(0)	0(0)	0(0)	0(0)	3(0.9)**
Number resorptions/litter	0.3	0.9	0.6	0.4	6.0**
Total number (%[d])	6(1.7)	23(6.4)	16(4.5)	10(3.0)	144(43.2)**
Number early (%[d])	6(1.7)	23(6.4)	16(4.5)	7(2.1)	113(33.9)**
Number late (%[d])	0(0)	0(0)	0(0)	3(0.9)	31(9.3)**
Number (%) litters with resorptions	6(25.0)	13(52.0)	12(48.0)	8(34.8)	23(95.8)**
Mean fetal body weights (g)					
Total	3.35	3.47**	3.41*	3.12**	2.71**
Male	3.43	3.60**	3.52**	3.21**	2.83**
Female	3.27	3.33	3.30	3.03**	2.63**
Percent males	47.3	52.4	50.4	49.8	40.9

a Deaths were the result of gavage injury.
b Not pregnant.
c Number of corpora lutea minus implantations/dam
d Percent of total implantations.
* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A91-3410, pages 43-44.

The data demonstrated treatment-related decreases in mean litter size and in the percent of total fetuses and live fetuses at the 50.0 mg/kg/day dose level. In addition, a treatment-related increase was noted at that dose for the percent of dead fetuses; mean number of resorptions; percent of early, late, and total resorptions; and percent of rats with any resorption. Significant treatment-related decreases in the mean fetal weight values (total and by sex) were observed for the 25.0 and 50.0 mg/kg/day dose groups. Mean total and male fetal weight values were significantly increased for the 1.0 and 10.0 mg/kg/day dose groups as compared to control; however, this was not considered to be treatment-related.

5. Developmental Toxicity

Observations noted at external, visceral, and skeletal evaluation of fetuses are summarized in Table 5 and presented in further detail in Tables 6 (malformations) and 7 (skeletal variations). Table 8 summarizes selected mean number of ossification sites per litter.

Table 5. Summary of the Incidence of Fetal Alterations

Observation	Dose (mg/kg/day)				
	0	1.0	10.0	25.0	50.0
No. fetuses (litters) examined	349(24)	336(25)	341(25)	321(23)	186(23)
Litters with fetuses with any alteration observed N(%)	8(33.3)	11(44.0)	10(40.0)	12(52.2)	21(91.3)**
Fetuses with any alteration observed N(%)	14(4 0)	18(5.4)	14(4.1)	18(5.6)	48(25.8)**
% Fetuses with any alteration/ litter Mean±S.D.	3.94 ± 7.06	5.32 ± 7.00	3.93 ± 6.55	5.67 ± 7.70	30.24 ± 23.70**
Litters with fetuses with any malformation observed N(%)	3(12.5)	1(4.0)	3(12.0)	0*	7(30.4)**
Fetuses with any malformation observed N(%)	3(0.9)	1(0.3)	3(0.9)	0	9(4.8)**
% Fetuses with any malformation/ litter Mean±S.D.	0.80 ± 2.19	0.36 ± 1.82	0.92 ± 2.58	0.00 ± 0.00	6.63 ± 12.46**
Litters with fetuses with any variation observed N(%)	6(25.0)	11(44.0)	8(32.0)	12(52.2)*	20(87.0)**
Fetuses with any variation observed N(%)	11(3.2)	18(5.4)	12(3.5)	18(5.6)	43(23.1)**
% Fetuses with any variation/ litter Mean±S.D.	3.14 ± 6.33	5.32 ± 7.00	3.38 ± 6.56	5.67 ± 7.70	27.52 ± 22.49**

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

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

Note: Data were extracted from report No. FMC A91-3410, pages 90-91.

Table 6. Summary of Malformations

Observation		Dose (mg/kg/day) 0	1.0	10.0	25.0	50.0
EXTERNAL						
No. fetuses examined		349(24)	336(25)	341(25)	321(23)	186(23)
EYE - Bulge depressed	Fetus N(%)	0	1(0.3)	0	0	0
	Litter N(%)	0	1(4.0)	0	0	0
JAW - Micrognathia	Fetus N(%)	0	1(0.3)	0	0	1(0.5)
	Litter N(%)	0	1(4.0)	0	0	1(4.3)
PALATE - Cleft, medial	Fetus N(%)	0	1(0.3)	1(0.3)	0	0
	Litter N(%)	0	1(4.0)	1(4.0)	0	0
BODY - Umbilical hernia	Fetus N(%)	2(0.6)	0	0	0	0
	Litter N(%)	2(8.3)	0	0	0	0
BODY - Edema (anasarca)	Fetus N(%)	0	0	0	0	4(2.2)**
	Litter N(%)	0	0	0	0	4(17.4)**
HINDPAW - Digits short, left	Fetus N(%)	0	0	0	0	1(0.5)
	Litter N(%)	0	0	0	0	1(4.3)
TAIL - Absent	Fetus N(%)	0	0	1(0.3)	0	1(0.5)
	Litter N(%)	0	0	1(4.0)	0	1(4.3)
TAIL - Thread-like	Fetus N(%)	1(0.3)	0	0	0	0
	Litter N(%)	1(4.2)	0	0	0	0
VISCERAL						
No. fetuses examined		176(24)	167(25)	171(25)	163(23)	95(22)
HEART - Ventricular septal defect	Fetus N(%)	0	0	0	0	1(1.0)
	Litter N(%)	0	0	0	0	1(4.5)
HEART/GREAT VESSELS Situs inversus	Fetus N(%)	0	0	1(0.6)	0	0
	Litter N(%)	0	0	1(4.0)	0	0
STOMACH/INTESTINES Enlarged, filled	Fetus N(%)	0	0	0	0	1(1.0)
	Litter N(%)	0	0	0	0	1(4.5)
KIDNEYS - Fused	Fetus N(%)	1(0.6)	0	0	0	0
	Litter N(%)	1(4.2)	0	0	0	0

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

Note: Data were extracted from report No. FMC A91-3410, pages 92-98.

Table 6. Summary of Malformations - continued

Observation		Dose (mg/kg/day) 0	1.0	10.0	25.0	50.0
SKELETAL						
No. fetuses examined		173(24)	169(25)	170(25)	158(23)	91(21)
SKULL - Eye socket, small	Fetus N(%)	0	1(0.6)	0	0	0
	Litter N(%)	0	1(4.0)	0	0	0
SKULL - Mandibles, short and/or fused	Fetus N(%)	0	1(0.6)	0	0	1(1.1)
	Litter N(%)	0	1(4.0)	0	0	1(4.8)
SKULL - Tympanic rings, fused	Fetus N(%)	0	1(0.6)	0	0	0
	Litter N(%)	0	1(4.0)	0	0	0
RIBS - Short	Fetus N(%)	0	0	0	0	2(2.2)**
	Litter N(%)	0	0	0	0	1(4.8)
FORELIMBS - Radius and ulna, bent	Fetus N(%)	0	0	0	0	3(3.3)**
	Litter N(%)	0	0	0	0	2(9.5)
HINDLIMB - Fibula, bent	Fetus N(%)	0	0	0	0	1(1.1)
	Litter N(%)	0	0	0	0	1(4.8)

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

Note: Data were extracted from report No. FMC A91-3410, pages 92-98.

In the control and treated groups, fetuses with any alteration were noted in 33.3, 44.0, 40.0, 52.2, and 91.3 ($p \leq 0.01$) percent of the total litters (Table 5). At the high dose (50.0 mg/kg/day) 25.8% of the fetuses had any alteration, and the average percentage of fetuses with any alteration was 30.24 per litter; these values were both statistically significant ($p \leq 0.01$) as compared to control. The increased incidences of alterations at the high-dose were attributed to significant increases in the fetal and litter incidences of both malformations and variations at that dose. The percent of litters with fetuses with any malformation (30.4%) or variation (87.0%), the percent of fetuses with any malformation (4.8%) or variation (23.1%), and the mean percent of fetuses with any malformation (6.63%) or variation (27.52%) per litter were significantly increased ($p \leq 0.01$) as compared to control. In addition, at the 25.0 mg/kg/day level, a significant increase ($p \leq 0.05$) in the percentage of litters with any variation was noted. These findings were considered to be related to treatment with F6285.

External evaluation of the fetuses revealed a significant increase ($p \leq 0.01$) in the fetal and litter incidences of edema (anasarca) at the 50.0 mg/kg/day level (Table 6). Four fetuses (from four litters) were observed with anasarca at this dose, whereas no edematous fetuses were observed in the control or other treated groups. This malformation was judged to be treatment-related. Neither the incidence nor the distribution of any other external malformations noted suggested a response to treatment. These observations included sporadic incidences of depressed eye bulge, micrognathia, cleft palate, umbilical hernia,

short digits, and hypoplastic tail.

Likewise, visceral malformations noted (ventricular septal defect, situs inversus, enlarged stomach and intestines, and fused kidneys) occurred sporadically and without dose response, and therefore were not attributed to treatment (Table 6).

Skeletal malformations of the skull (including small eye socket, short and/or fused mandibles, and fused tympanic rings) were not attributed to treatment due to the nature of their incidence and distribution (Table 6). However, the fetal incidence of short ribs was significantly increased ($p \leq 0.01$) at the 50.0 mg/kg/day dose level. Since this malformation was believed to be related to significantly increased skeletal variations of the ribs (hypoplasia and/or wavy ribs, Table 7), it was judged to be attributed to treatment. In addition, a significant increase in the number of fetuses with bent radius and ulna was noted at the 50.0 mg/kg/day dose level, and an observation of bent fibula was noted in one fetus at that same dose level (Table 6). These observations were not present in the control or

Table 7. Summary of Selected Skeletal Variations

Observation		Dose (mg/kg/day)				
		0	1.0	10.0	25.0	50.0
No. fetuses examined		173(24)	169(25)	170(25)	158(23)	91(21)
VERTEBRAE - Lumbar arches, incompletely ossified	Fetus N(%)	0	0	1(0.6)	1(0.6)	4(4.4)**
	Litter N(%)	0	0	1(4.0)	1(4.3)	4(19.0)**
RIBS - Hypoplastic, incompletely ossified	Fetus N(%)	0	1(0.6)	1(0.6)	0	4(4.4)**
	Litter N(%)	0	1(4.0)	1(4.0)	0	3(14.3)
RIBS - Wavy	Fetus N(%)	1(0.6)	1(0.6)	1(0.6)	0	30(33.0)**
	Litter N(%)	1(4.2)	1(4.0)	1(4.0)	0	16(76.2)**
STERNEBRAE - Incompletely ossified or unossified	Fetus N(%)	4(2.3)	6(3.6)	4(2.4)	7(4.4)	13(14.3)**
	Litter N(%)	3(12.5)	4(16.0)	4(16.0)	4(17.4)	9(42.8)
STERNEBRAE - Incompletely ossified	Fetus N(%)	3(1.7)	5(3.0)	3(1.8)	5(3.2)	11(12.1)**
	Litter N(%)	3(12.5)	4(16.0)	3(12.0)	3(13.0)	8(38.1)
STERNEBRAE - Unossified	Fetus N(%)	1(0.6)	1(0.6)	1(0.6)	2(1.3)	2(2.2)
	Litter N(%)	1(4.2)	1(4.0)	1(4.0)	1(4.3)	2(9.5)
PELVIS - Incompletely ossified pubes and ischia	Fetus N(%)	6(3.5)	6(3.6)	7(4.1)	7(4.4)	16(17.6)**
	Litter N(%)	4(16.7)	5(20.0)	5(20.0)	3(13.0)	9(42.8)
PELVIS - Pubes, incompletely ossified	Fetus N(%)	6(3.5)	6(3.6)	7(4.1)	7(4.4)	16(17.6)**
	Litter N(%)	4(16.7)	5(20.0)	5(20.0)	3(13.0)	9(42 8)
PELVIS - Ischia, incompletely ossified	Fetus N(%)	1(0.6)	3(1.8)	1(0.6)	6(3.8)	10(11.0)**
	Litter N(%)	1(4.2)	3(12.0)	1(4.0)	2(8.7)	8(38.1)**

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

Note: Data were extracted from report No. FMC A91-3410, pages 92-98.

other treated groups for this study, nor were they observed historically by the performing laboratory in a two year period preceding the in-life portion of this study (Attachment 2). These limb malformations were considered to be treatment-related.

No external or visceral variations of concern were observed. All skeletal variations of note (Table 7) were judged to be reversible delays in ossification. Significant increases ($p \leq 0.01$) in the fetal and/or litter incidences occurred at the high-dose (50.0 mg/kg/day) for variations in the vertebral arches (incompletely ossified), ribs (hypoplastic or wavy), sternebrae (incompletely ossified or unossified) and pelvis (incompletely ossified ischia or pubis). These increases in skeletal variations were judged to be related to treatment.

As demonstrated in Table 8, a significant reduction in the mean numbers of caudal vertebral and metacarpal ossification sites was noted for both the 25.0 and 50.0 mg/kg/day dose groups. At 50.0 mg/kg/day, the ossification site averages were also significantly reduced for sternal centers, metatarsals, and hindpaw phalanges. All other ossification site averages (for the hyoid; cervical, thoracic, lumbar, and sacral vertebrae; ribs, manubrium and xiphoid sternebrae; carpals, digits, and phalanges of the forepaw; and tarsals and digits of the hindpaw) did not demonstrate any biologically or statistically significant difference between treated and control groups.

Table 8. Summary of Selected Fetal Ossification Sites

Ossification Sites per Litter (Mean ± S.D.)	Dose (mg/kg/day)				
	0	1.0	10.0	25.0	50.0
No. fetuses examined	173(24)	169(25)	170(25)	158(23)	91(21)
VERTEBRAE - Caudal	4.92 ± 0.49	4.85 ± 0.49	4.80 ± 0.45	4.56 ± 0.39*	4.45 ± 0.53**
STERNEBRAE - Sternal	3.64 ± 0.27	3.61 ± 0.33	3.62 ± 0.31	3.48 ± 0.26	3.39 ± 0.38*
FOREPAWS - Metacarpals	3.55 ± 0.36	3.51 ± 0.32	3.39 ± 0.31	3.24 ± 0.27**	3.08 ± 0.20**
HINDPAWS - Metatarsals	4.00 ± 0.00	4.00 ± 0.00	3.99 ± 0.04	3.99 ± 0.04	3.94 ± 0.14**
HINDPAWS - Phalanges	5.00 ± 0.00	5.00 ± 0.00	4.92 ± 0.24	4.92 ± 0.30	3.67 ± 1.75**

* Statistically different from control value, $p \leq 0.05$

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

Note: Data were extracted from report No. FMC A91-3410, page 99.

F. Discussion/Conclusions

1. Maternal toxicity

Following oral administration of the test substance, F6285, to pregnant rats on days 6-15 of gestation, evidence of treatment-related toxicity to the high-dose (50.0 mg/kg/day) dams consisted of significantly increased mean spleen-to-brain weight ratio and a moderate increase in splenic extramedullary hematopoiesis, which was interpreted as being related to an increased physiological demand for erythrocyte production over and above that in the bone marrow. Clinical observations (fresh or dried blood observed around the vagina) and significant decreases in mean maternal body weight change values on days 15-20 and 0-20 were considered to result from treatment-related fetal loss.

Maternal LOEL = 50.0 mg/kg/day
(based upon increased relative spleen weight and splenic extramedullary hematopoiesis)
Maternal NOEL = 25.0 mg/kg/day

2. Developmental toxicity

Fetal viability: At the 50.0 mg/kg/day dose level, treatment-related decreases in mean litter size and in the percent of total fetuses and live fetuses were noted. In addition, treatment-related increases were noted for the percent of dead fetuses; mean number of resorptions; percent of early, late, and total resorptions; and percent of rats with any resorption.

Fetal body weight: Treatment-related decreases in mean fetal weight values (total and by sex) were observed for the 25.0 and 50.0 mg/kg/day dose groups.

Fetal alterations: In the control and treated groups, fetuses with any alteration were noted in 33.3, 44.0, 40.0, 52.2, and 91.3 ($p \leq 0.01$) percent of the total litters for the control through high-dose groups, respectively. At the 50.0 mg/kg/day dose, 25.8% ($p \leq 0.01$) of the fetuses had any alteration, and the average percentage of fetuses with any alteration was 30.24% per litter ($p \leq 0.01$). The increased incidences of alterations at the high-dose were attributed to significant increases in the fetal and litter incidences of both malformations and variations at that dose. The percent of litters with fetuses with any malformation (30.4%) or variation (87.0%), the percent of fetuses with any malformation (4.8%) or variation (23.1%), and the mean percent of fetuses with any malformation (6.63%) or variation (27.52%) per litter were increased ($p \leq 0.01$). In addition, at the 25.0 mg/kg/day level, a significant increase ($p \leq 0.05$) in the percentage of litters with any variation was noted.

Malformations (treatment-related): At the 50.0 mg/kg/day dose, 1) the fetal and litter incidences of edema (anasarca) were increased ($p \leq 0.01$). Four fetuses (from four litters) were observed with anasarca at this dose, whereas no edematous fetuses were observed in the control or other treated groups. 2) The fetal incidence of short ribs was increased ($p \leq 0.01$). Since this malformation was believed to be related to significantly increased skeletal variations of the ribs (hypoplasia and/or wavy ribs), it was attributed

to treatment. 3) An increase ($p \leq 0.01$) in the number of fetuses with bent radius and ulna was noted, and an observation of bent fibula was noted in one fetus at that same dose level. These observations were not present in the control or other treated groups for this study, nor were they present in the historical control data from the performing laboratory (Attachment 2).

Variations (treatment-related): 1) Increases ($p \leq 0.01$) in the fetal and/or litter incidences of skeletal variations were reported at the 50.0 mg/kg/day dose level in the vertebral arches (incompletely ossified), ribs (hypoplastic or wavy), sternebrae (incompletely ossified or unossified) and pelvis (incompletely ossified ischia or pubis). 2) A significant reduction in the mean numbers of caudal vertebral and metacarpal ossification sites was noted for both the 25.0 and 50.0 mg/kg/day dose groups. At 50.0 mg/kg/day, the ossification site averages were also significantly reduced for sternal centers, metatarsals, and hindpaw phalanges.

Developmental LOEL = 25.0 mg/kg/day
(based upon 1) decreased mean fetal weight and 2) retardation in skeletal development as evidenced by an increased number of litters with any variation and by decreased numbers of caudal vertebral and metacarpal ossification sites)
Developmental NOEL = 10.0 mg/kg/day

3. CORE Classification: **CORE-Guideline**

Substantially Similar

Page _____ is not included in this copy.

Pages 31 through 52 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__✓__ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

(11176

Reviewed by: Susan L. Makris, M.S. Susan L. Makris 7/20/94
Section III, Toxicology Branch II (7509C)
Secondary reviewer: James N. Rowe, Ph.D James N. Rowe 7/20/94
Section III, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Dermal Developmental Toxicity Study in Rats (§ 83-3)

EPA NOS.:

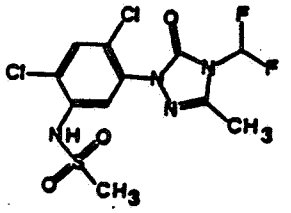
MRID NOS.: 430046-03 (Range-finding), 429321-05
PC CODE: 129081

DP BARCODE NOS.: D198408 and D198715
SUBMISSION NOS.: S456588 and S457101
CASE NOS.: 034954 and 285252
ID NOS.: 00279-EUP-RGR and 3G04272

TEST MATERIAL: F6285 Technical

SYNONYMS: 2-(2,4-dichloro-5-methylsulfonylamidophenyl)-4-difluoromethyl-2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one
FMC 97285
Sulfentrazone

CHEMICAL STRUCTURE:



STUDY NUMBER: FMC No.: A91-3428
Argus No.: 106-010

SPONSOR: FMC Corporation, Agricultural Chemical Group
1735 Market Street, Philadelphia, PA 19103

TESTING FACILITY: FMC Corporation Toxicology Laboratory (in-life)
Box 8, Princeton, NJ 08543

Argus Research Laboratories, Inc. (fetal exams)
905 Sheehy Drive, Horsham, PA 19044

TITLE OF REPORT: F6285 Technical, Teratology Study in Rats (Dermal)

AUTHOR: Christine Freeman

STUDY COMPLETION DATE: June 11, 1992

EXECUTIVE SUMMARY: The test substance, F6285, was administered by 6-hour dermal application to pregnant female Crl:CD®BR (Sprague-Dawley) rats on days 6-15 of gestation at dose levels of 5, 25, 50, 100, and 250 mg/kg/day. The rats were observed for signs of toxicity; body weight and food consumption values were recorded. On day 20 of gestation, the rats were sacrificed and necropsied; spleen and uterine weights were recorded. The uteri were examined, implantation sites were counted, and the numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external anomalies. They were then processed for visceral and skeletal evaluation.

There was no evidence of treatment-related maternal toxicity. All rats survived to cesarean section. Maternal body weight change, food consumption, gross pathological findings, and absolute and relative (to brain) spleen weight values were comparable between control and treated groups. Vaginal bleeding between gestation days 13 and 17 was observed in rats of all groups (including control) and was judged by the study author to be related to the extrusion of Reichert's membrane, which has been shown to occur during this stage of pregnancy and is frequently observed in dermal studies because the rats cannot groom themselves (Long and Evans, 1920). This finding, although attributed to treatment, was not considered a toxic effect, since the incidence of this finding in the control animals was high (14/24), and no correlation to fetal loss was observed in any group.

Maternal LOAEL = Not determined
Maternal NOAEL $\geq$ 250 mg/kg/day

Evidence of treatment-related developmental toxicity consisted of decreased fetal body weight and increased incidences of fetal alterations, comprised primarily of skeletal variations and reductions in mean numbers of ossification sites.

At the high-dose level (250 mg/kg/day), significant treatment-related decreases in mean fetal body weight (males, females, and combined) were observed. In addition, the percent of fetuses with any alteration observed (9.8%) was increased ($p \leq 0.01$) from the control incidence (3.2%). The percent of litters containing fetuses with any alteration (68.0%) was also significantly increased as compared to the control (37.5%) at the high dose, and was primarily attributable to increased incidences of skeletal variations.

Fetal malformations noted were sporadic and not attributed to treatment. No external or visceral variations of concern were observed. Significant treatment-related increases in the fetal and litter incidences of incompletely ossified lumbar vertebral arches, hypoplastic or wavy ribs, and incompletely ossified or nonossified ischia or pubes occurred at the high-dose (250 mg/kg/day). An additional significant increase in the high-dose fetal incidence of variations in the sternebrae (incompletely ossified or unossified) was not judged to be treatment-related. At 250 mg/kg/day, the mean numbers of thoracic vertebral and rib ossification sites were significantly decreased, a high-dose effect of treatment with F6285, consistent with the significant treatment-related hypoplasia observed in the skeletal evaluation of the ribs.

Developmental LOEL = 250 mg/kg/day
(based on decreased fetal body weight; increased incidences of fetal variations:

hypoplastic or wavy ribs, incompletely ossified lumbar vertebral arches, and incompletely ossified ischia or pubes; and reduced number of thoracic vertebral and rib ossification sites)

Developmental NOEL = 100 mg/kg/day

CORE CLASSIFICATION: **CORE-Guideline**; this study satisfies the guideline recommendations for a §83-3(a) developmental toxicity (teratology) study in rats.

A. Materials

1. Test compound:
   Name: F6285 technical
   Purity: 94.2%
   Reference No.: E7301-72
   Description: Solid

2. Vehicle:
   Name: Normal saline (Banco)
   Purity: 0.9%
   Lot No.: GO-30

3. Test animals:
   Species: Rat
   Strain: Crl:CD®BR VAF/Plus®
   Source: Charles River Laboratories, Portage, MI
   Age: Young adult
   Weight: 231-300 g at gestation day 0

4. Environment:
   Housing: Individual stainless steel cages
   Lighting: 12 hours light/12 hours dark
   Temperature: 61-74°F
   Humidity: 41-85%
   Food: Purina Rodent Chow 5002 ad libitum
   Water: Municipal water, automatic watering system ad libitum

B. Methods - This study was conducted to evaluate the developmental toxicity of the test substance, F6285, when administered by dermal application to pregnant female Sprague-Dawley rats during the period of major fetal organogenesis. A copy of the study methods is presented as Attachment 1.

1. Rationale for Dosage Selection

In a range-finding study (FMC study No. A91-3427, MRID No. 430046-03), F6285 was administered orally to groups of 10 female rats each at dosages of 0, 50, 100, 500, and 3000 mg/kg/day. The test material, moistened with saline, was applied on days 6 through 15 of gestation, inclusive. Exposure periods were 6 hours in duration, and individual doses were adjusted daily to body weight. Cesarean sections were performed on day 20 of gestation. The uteri and ovaries were examined for the number and distribution of implantation sites, early and late resorptions, live and dead fetuses, and corpora lutea.

Each fetus was weighed, sexed, and examined for external abnormalities.

All rats survived to study termination. No clinical signs of toxicity were noted in any treated group; vaginal bleeding in late pregnancy was attributed to the extrusion of Reichert's membrane due to the inability of the rats to groom while wrapped. Body weight and body weight gain values were similar to controls for the 50 and 100 mg/kg/day treatment groups. At 500 mg/kg/day, maternal body weight gains were reduced for gestation days 15-20 and 0-20; at 3000 mg/kg/day, maternal body weight gains were reduced for gestation days 6-15, 15-20, and 0-20. These decrements in body weight change were attributed to fetal loss, as confirmed by the finding that corrected maternal body weight gains and food consumption values were similar between control and treated groups, and that gravid uterine weights were severely decreased at 500 and 300 mg/kg/day. At necropsy, one enlarged placenta was observed at 50 mg/kg/day. At 3000 mg/kg/day, absolute and relative (to brain) spleen weights were elevated.

All litters at 3000 mg/kg/day were completely resorbed; reduced litter sizes were observed at 500 mg/kg/day with approximately 75% of the implants resorbed. Fetal body weights were slightly depressed at 500 mg/kg/day. Sex ratio was not affected.

External fetal abnormalities included one fetus at 100 mg/kg/day with a "small bubble" in the skin and three fetuses in one 500 mg/kg/day litter with malformations: 1) kinked tail, scaly skin, shortened right forelimb, and shortened hindlimbs; 2) scaly skin, shortened right forelimb, and shortened right hindlimb; and 3) scaly skin, shortened right forelimb, shortened neck, kinked tail, and both hindlimbs rotated inward.

The maternal NOEL was 500 mg/kg/day, based on increased absolute and relative spleen weights at 3000 mg/kg/day. The developmental NOEL was 100 mg/kg/day, based upon reduced fetal viability and decreased fetal body weights at the 500 mg/kg/day dose level.

Based upon the results of this range-finding study, 250 mg/kg/day was selected as a high dose for the subsequent full dermal developmental toxicity study in rats. The other treatment levels chosen were: 5, 25, 50, and 100 mg/kg/day.

2. Mating, Group Assignment, and Dosage Levels

At receipt, all rats were randomized into their cages using a table of random numbers. Following a period of acclimation, the female rats were mated with young adult male rats of the same strain and source. Females were assigned to the following study groups on Day 0 of gestation. No more than two females mated by the same male were assigned to any group.

Group No.	Dosea (mg/kg/day)	No. per Group
1 (Control)	0b	25
2	5	25
3	25	25
4	50	25
5	100	25
6	250	25

a Administered in approximately 2.0 ml of saline.
b Vehicle control (saline).

3. Test Material Formulation, Administration, and Analysis

The study animals were exposed to the control and test substances by dermal application on days 6-15 of gestation, inclusive. The dose for each rat was calculated daily, based upon individual body weight. The test substance was weighed onto a 2" x 2" gauze pad lined with weighing paper. The material was moistened with approximately 0.2 ml of saline and applied to the clipped back of each rat. The pad was held in place with hypoallergenic tape, covered with an elastic bandage, and occluded with plastic for a 6-hour exposure period. At the end of the exposure period, the bandages and pads were removed, and any residual test substance was wiped away with clean gauze. The test sites were then wiped with methanol, rinsed with water, and dried. Vehicle control animals were treated in the same manner, but only saline was applied to the patch.

The study report stated that the test substance was determined to be stable for the duration of the study (FMC A91-3428, page 18). Although no data were presented to verify this statement, other reports submitted to the Agency demonstrate the stability of F6285 (FMC Nos. A91-3410 and A92-3540). Since the test substance was applied without formulation in a vehicle, concentration and homogeneity data were not required.

4. Observations

The rats were observed for mortality twice daily. Clinical observations were recorded once daily, immediately after unwrapping. Individual body weights were recorded on gestation Days 0, 6-15 (inclusive), and 20. Food consumption was measured and calculated for gestation Days 0-6, 6-15, and 15-20.

Surviving dams were sacrificed on Day 20 of gestation by CO_2 asphyxiation. A gross necropsy was performed on each rat. Spleen, brain, and gravid uterine weights were recorded. Corpora lutea of pregnancy were counted, and uterine contents were examined

57

for pregnancy status, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses.

Following removal from the uterus, each fetus was weighed, sacrificed by CO_2 asphyxiation, tagged with an identification number, and placed in a jar containing either methanol or Bouin's fixative (one half of each litter per solution). The fetuses were shipped tc Argus Research Laboratories, Inc., where they were examined for gross external alterations. Fetuses preserved in Bouin's fixative were examined for gender and for visceral malformations and variations using Wilson's method of free-hand sectioning. Those fetuses preserved in methanol were eviscerated, examined for gender, stained with alizarin red S, and examined for skeletal malformations and variations. Malformations were defined as irreversible changes which occur at low incidence in this species and strain; variations were defined as common findings in this species and strain, reversible delays, or accelerations in development.

5. Statistical Analysis

The following methods of statistical analysis were performed, using SAS/STAT or proprietary programs (see report No. FMC A91-3428, pages 22-23; Attachment 1). Proportional data, i.e., the number of live and dead fetuses, number of total fetuses, resorption incidences, proportion of male fetuses, and fetal incidence data, were analyzed by the Variance Test for Homogeneity of the Binomial Distribution. Parametric data (maternal body weights, body weight changes, food consumption, absolute and relative organ weights, fetal body weights, and mean fetal ossification or alteration data) were analyzed using Bartlett's Test of Homogeneity of Variances, the Analysis of Variance, and/or Dunnett's Test. If the Analysis of Variance was not appropriate, the Kruskal-Wallis Test or Fisher's Exact Test was used. Live fetal body weights, mean numbers of resorptions, implantations, corpora lutea minus implantations, and litter size were analyzed using the Kruskal-Wallis Test with Dunn's Method of Multiple Comparisons when statistical significance was achieved, or with Fisher's Exact Test. No test for linear trend was performed, and the unit of analysis was not indicated (i.e., the litter versus the fetus). The level of significance was $p \leq 0.05$.

Historical control data for reproductive indices, maternal necropsy observations, and external, soft tissue, and skeletal alterations were presented in the report and appended as Attachment 2.

6. Compliance

The following required signed and dated statements were provided:

- Statement of No Data Confidentiality Claims
- Good Laboratory Practice Certification
- Quality Assurance statement
- EPA Flagging Statement (positive for criterium 5)

011176

C. Results

1. Maternal Mortality and Clinical Observations

All rats survived to cesarean section, and no signs of clinical toxicity were noted during the study. Incidental clinical findings included alopecia, a palpable lump, a scab, and unthriftiness. Vaginal bleeding between gestation days 13 and 17 was observed in rats of all groups (including control). The incidences of occurrence for the control, 5, 25, 50, 100, and 250 mg/kg/day dose groups, respectively, were 14, 15, 17, 19*, 21*, and 24* rats (* = significant, $p \leq 0.01$). This finding was judged by the study author to be related to the extrusion of Reichert's membrane, which has been shown to occur during this stage of pregnancy and is frequently observed in dermal studies because the rats cannot groom themselves [Long, J.A. and Evans, H.M. (1920) A characteristic sign of pregnancy in the rat detectable from the thirteenth to the sixteenth day. *Anat. Rec.* 18:249]. Although the incidence of this finding increased in a dose-related manner, with statistical significance achieved at the 50, 100, and 250 mg/kg/day dose levels, it was concluded that the bleeding was not a treatment-related adverse toxic effect.

2. Maternal Body Weight and Food Consumption Data

A summary of mean maternal body weight change values during gestation is presented in Table 2. Values were comparable between control and treated groups. Food consumption data were reported for days 0-6, 6-15, and 15-20 of gestation; the data did not demonstrate any significant differences between control and treated groups (data not presented in DER).

Table 2. Mean Gestation Body Weight Change Data (g)

Days of Gestation	Dose level (mg/kg/day)					
	0	5.0	25.0	50.0	100	250
N	24	23	24	22	25	25
0-6	37	39	38	39	41	42
6-15	44	44	45	41	43	41
15-20	84	82	76	82	81	79
0-20	165	165	158	162	165	162
0-20 Corrected a	78	79	79	81	82	84

a Corrected body weight = day 0-20 body weight change minus gravid uterine weight.

Note: Data were extracted from report No. FMC A91-3428, pages 39-40.

4. Maternal Gross Pathology and Spleen Weight Data

At necropsy, incidental observations (all observed in treated females) included splenic changes (discolorations, irregular shaping with adhesions, and firm areas), a cystic ovary, apparent unilateral uterine atrophy, and a subcutaneous mass. Neither the incidence nor the distribution of findings were suggestive of a relationship to treatment.

Mean maternal absolute and relative (to brain) spleen weight values are summarized in Table 3. No significant or biologically important differences were observed.

Table 3. Mean Absolute and Relative[a] Spleen Weights (g)

Parameter	Dose Level (mg/kg/day)					
	0	5.0	25.0	50.0	100	250
N	24	23	24	22	25	25
Absolute spleen weight (g)	0.79	0.80	0.81	0.95	0.80	0.80
Relative spleen weight (%)	40.28	42.65	41.81	49.18	40.96	41.39

a Mean spleen weight to brain weight ratio.
Note: Data were extracted from report No. FMC A91-3428, pages 43-44.

5. Observations Noted at Cesarean Section

The results of the examination of uterine contents at cesarean section are presented in Table 4. The data demonstrated significant treatment-related decreases in mean fetal body weight (males, females, and combined) at the high-dose (250 mg/kg/day). Mean fetal weight values were significantly increased for the 5.0 mg/kg/day dose group as compared to control; however, this was not considered to be treatment-related. Embryo/fetal survival was not affected by treatment of the dams.

6. Developmental Toxicity

Observations noted at external, visceral, and skeletal evaluation of fetuses are summarized in Tables 5 (the incidence fetuses and litters with any alteration), 6a (external malformations), 6b (skeletal malformations), and 7 (skeletal variations). Table 8 summarizes the mean number of ossification sites per litter.

Table 5 demonstrates that at the high-dose level (250 mg/kg/day), the percent of fetuses with any alteration observed (9.8%) was increased ($p \leq 0.01$) from the control incidence (3.2%). The percent of litters containing fetuses with any alteration (68.0%) was also significantly increased as compared to the control (37.5%) at the high dose. The study author did not consider these high-dose increases to be treatment-related. However, it is the opinion of the reviewer that the increased incidence of fetal alterations at the 250 mg/kg/day dose level is an effect of treatment. Although a breakdown of the alterations into malformations and variations was not provided in the report, it appears from the

individual incidence data (summarized in Tables 6 and 7) that the overall increase in alterations at the high-dose was primarily attributable to increased incidences of skeletal variations (Table 7). In addition, fetal body weights were significantly decreased at the same dose level (Table 4), a finding which is commonly associated with reductions in skeletal development. A significant increase in the percent of fetuses with any alteration at the 50.0 mg/kg/day level was not considered to be related to treatment, due to the lack of dose-response and the lack of significance in the litter incidence at that level.

External evaluation of the fetuses (Table 6a) revealed two malformed control fetuses, one with micrognathia and one with anasarca. Among the treated groups, three 50.0 mg/kg/day fetuses were observed with micrognathia and aglossia, one 100 mg/kg/day fetus had spina bifida and one was observed to have exencephaly with depressed eye bulges and open lids, and one 250 mg/kg/day fetus had micrognathia. No visceral malformations were reported. Observed skeletal malformations of the skull, vertebrae, and ribs (Table 6b) were related almost entirely to the malformations noted at external examination (micrognathia, exencephaly, and spina bifida). Neither the incidence nor the distribution of the external or skeletal malformations suggested a response to treatment.

No external or visceral variations of concern were observed. All skeletal variations of note (Table 7) were judged to be reversible delays in ossification. Significant treatment-related increases ($p \leq 0.01$) in the fetal and litter incidences of incompletely ossified lumbar vertebral arches, hypoplastic or wavy ribs, and incompletely ossified or nonossified ischia or pubes occurred at the high-dose (250 mg/kg/day). An additional significant increase in the high-dose fetal incidence of variations in the sternebrae (incompletely ossified or unossified) was not judged to be treatment-related because a dose response was not evident, the litter incidence was not significant, and the fetal and litter numerical incidences of the finding on this study were comparable to ranges observed historically (Attachment 2). The study author did not consider the incompletely ossified lumbar arches or the reduction in pelvic ossification at 250 mg/kg/day to be treatment-related; however, these findings, although seen primarily in the high-dose and not in a dose-related pattern, were outside of historical control ranges (Attachment 2). Such reductions in ossification are also consistent with treatment-related decreases in mean fetal body weight at the high-dose level (which was also observed as a high-dose response, i.e., without a dose-response). Other significant increases or decreases in the fetal and/or litter incidences of skeletal findings in the 5.0, 25.0, 50.0, and 100 mg/kg/day treated groups were not attributed to treatment.

The mean numbers of ossification sites (Table 8), were decreased ($p \leq 0.05$) at the 250 mg/kg/day dose level for thoracic vertebrae and ribs. The study author did not judge these findings to be treatment-related due to a lack of dose-response and the comparability with historical control values. This reviewer, however, considers this to be a high-dose effect of treatment with F6285, consistent with the significant treatment-related hypoplasia observed in the skeletal evaluation of the ribs (Table 7). Slight, nonsignificant decreases in ossification site averages were noted at the same dose level in the sacral and caudal vertebrae, manubrium, sternebrae, metacarpals, metatarsals, and fore- and hindlimb phalanges but were not attributed to treatment due to lack of significance and dose-response; most of these findings also fell within the historical control ranges presented in the study report (Attachment 2). All other ossification site averages (for the hyoid; cervical

Table 4. Summary of Selected Cesarean Section Observations

Parameter	Dose level (mg/kg/day)					
	0	5.0	25.0	50.0	100	250
Number pregnant	24	23	24	22	25	25
Number of deaths	0	0	0	0	0	0
Number of litters totally resorbed	0	0	0	0	0	0
Number of viable litters	24	23	24	22	25	25
Number of implantations/dam	16.6	15.8	15.3	15.9	15.6	15.6
Mean implantation loss a	1.3	1.9	2.7	2.7	2.5	2.5
Number of fetuses/litter	15.8	15.3	14.2	15.2	14.9	14.8
Percent live fetuses b	94.5	96.7	92.7	95.4	95.6	94.4
Number dead fetuses (%b)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.3)
Number resorptions/litter	0.9	0.5	1.1	0.7	0.7	0.8
Total number (%b)	21(5.3)	12(3.3)	27(7.3)	16(4.6)	17(4.4)	21(5.4)
Number early (%b)	21(5.3)	12(3.3)	26(7.1)	15(4.3)	16(4.1)	20(5.1)
Number late (%b)	0(0.0)	0(0.0)	1(0.3)	1(0.3)	1(0.3)	1(0.3)
Number (%) litters with resorptions	17(70.8)	10(43.5)	12(50.0)	11(50.0)	11(44.0)	15(60.0)
Mean fetal body weights (g)						
Total	3.56	3.70**	3.60	3.53	3.57	3.35**
Male	3.66	3.77*	3.69	3.65	3.69	3.41**
Female	3.44	3.61**	3.50	3.42	3.44	3.30**
Percent males	52.0	53.4	51.6	48.2	52.0	49.5

a Number of corpora lutea minus implantations/dam.
b Percent of total implantations.
** Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A91-3428, pages 45-48.

Table 5. Summary of the Incidence of Fetuses with Any Alteration

Observation		Dose (mg/kg/day)					
		0	5.0	25.0	50.0	100	250
No. fetuses (litters) examined		377(24)	352(23)	341(24)	334(22)	373(25)	368(25)
Litters with fetuses with any alteration observed	N(%)	9(37.5)	7(30.4)	6(25.0)	11(50.0)	10(40.0)	17(68.0)**
Fetuses with any alteration observed	N(%)	12(3.2)	9(2.6)	8(2.3)	29(8.7)**	13(3.5)	36(9.8)**
% Fetuses with any alteration/ litter	Mean±S.D.	3.30 ± 4.99	2.52 ± 4.60	2.17 ± 4.19	8.21 ± 11.06	3.67 ± 6.08	10.60 ± 12.56

** Statistically different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A91-3428, page 104.

Table 6a. Summary of External Malformations

Observation		Dose (mg/kg/day)					
		0	5.0	25.0	50.0	100	250
No. fetuses (litters) examined		377(24)	352(23)	341(24)	334(22)	373(25)	368(25)
HEAD - Exencephaly	Fetus N(%)	0	0	0	0	1(0.3)[a]	0
	Litter N(%)	0	0	0	0	1(4 0)	0
EYES - Bulge, depressed	Fetus N(%)	0	0	0	0	1(0.3)[a]	0
	Litter N(%)	0	0	0	0	1(4.0)	0
EYES - Lids, open	Fetus N(%)	0	0	0	0	1(0.3)[a]	0
	Litter N(%)	0	0	0	0	1(4.0)	0
TONGUE - Absent	Fetus N(%)	0	0	0	3(0.9)[b]	0	0
	Litter N(%)	0	0	0	1(4.5)	0	0
JAW - Micrognathia	Fetus N(%)	1(0.3)	0	0	3(0.9)[b]	0	1(0.3)
	Litter N(%)	1(4.2)	0	0	1(4.5)	0	1(4.0)
BODY - Edema (anasarca)	Fetus N(%)	1(0.3)	0	0	0	0	0
	Litter N(%)	1(4.2)	0	0	0	0	0
BODY - Spina bifida	Fetus N(%)	0	0	0	0	1(0.3)[a]	0
	Litter N(%)	0	0	0	0	1(4.0)	0

a,b Observed in the same fetus(es).
Note: Data were extracted from report No. FMC A91-3428, pages 105-106.

Table 6b. Summary of Skeletal Malformations

Observation		Dose (mg/kg/day)					
		0	5.0	25.0	50.0	100	250
No. fetuses (litters) examined		191(24)	178(23)	171(23)	169(22)	190(25)	185(25)
SKULL - Nasals, maxillae, pre-maxillae, incompletely ossified	Fetus N(%) Litter N(%)	0 0	0 0	0 0	0 0	0 0	1(0.5)[a] 1(4.0)
SKULL - Frontals, parietals, interparietals, supraoccipitals, not ossified	Fetus N(%) Litter N(%)	0 0	0 0	0 0	0 0	1(0.5)[b] 1(4.0)	0 0
SKULL - zygomatics, squamosals, incompletely ossified	Fetus N(%) Litter N(%)	0 0	0 0	0 0	0 0	0 0	1(0.5)[a] 1(4.0)
SKULL - Sphenoid, incompletely ossified	Fetus N(%) Litter N(%)	0 0	0 0	0 0	0 0	0 0	1(0.5)[a] 1(4.0)
SKULL - Sphenoid alae, not ossified	Fetus N(%) Litter N(%)	0 0	0 0	0 0	1(0.6)[a] 1(4.5)	0 0	0 0
SKULL - Sphenoid body, irregularly shaped	Fetus N(%) Litter N(%)	0 0	0 0	0 0	1(0.6)[a] 1(4.5)	0 0	0 0
SKULL - Tympanic rings, irregularly shaped and fused	Fetus N(%) Litter N(%)	0 0	0 0	0 0	1(0.6)[a] 1(4.5)	0 0	0 0
SKULL - Mandibles, short and fused	Fetus N(%) Litter N(%)	0 0	0 0	0 0	1(0.6)[a] 1(4.5)	0 0	1(0.5)[a] 1(4.0)
SKULL - Eye socket, unilateral, small	Fetus N(%) Litter N(%)	0 0	0 0	0 0	0 0	1(0.5)[b] 1(4.0)	0 0
VERTEBRAE - Cervical, 5 present	Fetus N(%) Litter N(%)	0 0	0 0	0 0	0 0	1(0.5)[c] 1(4.0)	0 0
VERTEBRAE - Cervical, fused	Fetus N(%) Litter N(%)	0 0	0 0	0 0	0 0	1(0.5)[c] 1(4.0)	0 0

a Observation associated with micrognathia.
b Observation associated with exencephaly, same fetus.
c Observation associated with spina bifida, same fetus.
Note: Data were extracted from report No. FMC A91-3428, pages 108-114.

Table 6b. Summary of Skeletal Malformations - continued

Observation		Dose (mg/kg/day)					
		0	5.0	25.0	50.0	100	250
No. fetuses (litters) examined		191(24)	178(23)	171(23)	169(22)	190(25)	185(25)
VERTEBRAE - Cervical, arches open	Fetus N(%)	0	0	0	0	1(0.5)c	0
	Litter N(%)	0	0	0	0	1(4.0)	0
VERTEBRAE - Thoracic, centra not ossified	Fetus N(%)	0	0	0	0	1(0.5)c	0
	Litter N(%)	0	0	0	0	1(4.0)	0
VERTEBRAE - Thoracic, arches fused	Fetus N(%)	0	0	0	0	1(0.5)c	0
	Litter N(%)	0	0	0	0	1(4.0)	0
VERTEBRAE - Thoracic, arches open	Fetus N(%)	0	0	0	0	1(0.5)c	0
	Litter N(%)	0	0	0	0	1(4.0)	0
VERTEBRAE - Thoracic, arches small	Fetus N(%)	0	0	0	0	1(0.5)c	0
	Litter N(%)	0	0	0	0	1(4.0)	0
VERTEBRAE - Lumbar, arches open	Fetus N(%)	0	0	0	0	1(0.5)c	0
	Litter N(%)	0	0	0	0	1(4.0)	0
RIBS - Fused	Fetus N(%)	0	0	0	0	1(0.5)c	0
	Litter N(%)	0	0	0	0	1(4.0)	0
SCAPULAE - Irregularly shaped	Fetus N(%)	0	0	0	0	0	1(0.5)
	Litter N(%)	0	0	0	0	0	1(4.0)
FORELIMBS - Humerus, radius, and ulna, bent	Fetus N(%)	0	0	0	0	0	1(0.5)
	Litter N(%)	0	0	0	0	0	1(4.0)

a Observation associated with micrognathia.
b Observation associated with exencephaly, same fetus.
c Observation associated with spina bifida, same fetus.
Note: Data were extracted from report No. FMC A91-3428, pages 108-114.

Table 7. Summary of Selected Skeletal Variations

Observation		Dose (mg/kg/day)					
		0	5.0	25.0	50.0	100	250
No. fetuses (litters) examined		191(24)	178(23)	171(23)	169(22)	190(25)	185(25)
VERTEBRAE - Lumbar arches, incompletely ossified	Fetus N(%)	0	0	2(1.2)**	0	0	4(2.2)**
	Litter N(%)	0	0	1(4.3)	0	0	4(16.0)**
RIBS - Hypoplastic, incompletely ossified	Fetus N(%)	0	0	0	3(1.8)	1(0.5)	9(4.9)**
	Litter N(%)	0	0	0	2(9.1)	1(4.0)	7(28.0)**
RIBS - Wavy	Fetus N(%)	0	0	1(0.6)	4(2.4)	3(1.6)	12(6.5)**
	Litter N(%)	0	0	1(4.3)	2(9.1)	3(12.0)*	7(28.0)**
STERNEBRAE - Incompletely ossified or unossified	Fetus N(%)	1(0.5)	5(2.8)	2(1.2)	10(5.9)**	5(2.6)	11(5.9)**
	Litter N(%)	1(4.2)	3(13.0)	2(8.7)	5(22.7)	4(16.0)	8(32.0)
STERNEBRAE - Incompletely ossified	Fetus N(%)	1(0.5)	5(2.8)*	1(0.6)	8(4.7)**	2(1.0)	9(4.9)**
	Litter N(%)	1(4.2)	3(13.0)	1(4.3)	5(22.7)	2(8.0)	7(28.0)
STERNEBRAE - Unossified	Fetus N(%)	0	0	1(0.6)	2(1.2)	3(1.6)	2(1.1)
	Litter N(%)	0	0	1(4.3)	1(4.5)	3(12.0)	2(8.0)
PELVIS - Incompletely ossified and unossified pubes and ischia	Fetus N(%)	6(3.1)	1(0.6)	2(1.2)	21(12.4)**	4(2.1)	19(10.3)**
	Litter N(%)	6(25.0)	1(4.3)*	2(8.7)	10(45.4)	3(12.0)	13(52.0)**
PELVIS - Pubes, incompletely ossified	Fetus N(%)	6(3.1)	0	2(1.2)	16(9.5)**	4(2.1)	14(7.6)*
	Litter N(%)	6(25.0)	0**	2(8.7)	7(31.8)	3(12.0)	12(48.0)**
PELVIS - Ischia, incompletely ossified	Fetus N(%)	2(1.0)	1(0.6)	1(0.6)	11(6.5)**	0	8(4.3)**
	Litter N(%)	2(8.3)	1(4.3)	1(4.3)	7(31.8)**	0	4(16.0)

* Statistically different from control value, $p \leq 0.05$.
** Statistically different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A91-3428, pages 108-114.

Table 8. Summary of Selected Fetal Ossification Sites

Ossification Sites per Litter (Mean ± S.D.)	Dose (mg/kg/day)					
	0	5.0	25.0	50.0	100	250
No. fetuses (litters) examined	191(24)	178(23)	171(23)	169(22)	190(25)	185(25)
VERTEBRAE - Thoracic	13.04 ± 0.09	13.05 ± 0.07	13.05 ± 0.12	13.03 ± 0.06	13.01 ± 0.04	13.00 ± 0.02*
VERTEBRAE - Sacral	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	2.93 ± 0.34
VERTEBRAE - Caudal	4.66 ± 0.39	4.87 ± 0.51	4.80 ± 0.40	4.74 ± 0.33	4.84 ± 0.45	4.49 ± 1.04
RIBS	13.03 ± 0.06	13.03 ± 0.06	13.03 ± 0.07	13.02 ± 0.05	13.01 ± 0.0[illegible]	13.00 ± 0.00*
STERNEBRAE - Manubrium	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.95 ± 0.20
STERNEBRAE - Sternal	3.68 ± 0.31	3.67 ± 0.25	3.70 ± 0.27	3.61 ± 0.37	3.49 ± 0.26	3.45 ± 0.79
FOREPAWS - Metacarpals	3.40 ± 0.30	3.50 ± 0.33	3.42 ± 0.33	3.34 ± 0.30	3.46 ± 0.28	3.18 ± 0.60
FOREPAWS - Phalanges	5.04 ± 0.11	5.10 ± 0.34	5.03 ± 0.10	5.01 ± 0.04	4.98 ± 0.12	4.81 ± 1.01
HINDPAWS - Metatarsals	3.99 ± 0.04	4.01 ± 0.04	4.00 ± 0.00	4.00 ± 0.02	3.98 ± 0.05	3.81 ± 0.74
HINDPAWS - Phalanges	4.91 ± 0.24	5.00 ± 0.00	5.00 ± 0.00	4.94 ± 0.21	4.92 ± 0.22	4.60 ± 1.17

* Statistically different from control value, $p \leq 0.05$

Note: Data were extracted from report No. FMC A91-3428, page 115.

and lumbar vertebrae; xiphoid sternebrae; carpals and digits of the forepaw; and tarsals and digits of the hindpaw) did not demonstrate any biologically or statistically significant difference between treated and control groups.

E. Discussion/Conclusions

1. Maternal toxicity

Following 6-hour daily dermal administration of the test substance, F6285, to pregnant rats during days 6-15 of gestation, there was no evidence of treatment-related toxicity to the dams.

All rats survived to cesarean section. Vaginal bleeding between gestation days 13 and 17 was observed in rats of all groups (including control). The incidences of occurrence for the control, 5, 25, 50, 100, and 250 mg/kg/day dose groups, respectively, were 14, 15, 17, 19*, 21*, and 24* rats (* = significant, $p \leq 0.01$). This finding was judged by the study author to be related to the extrusion of Reichert's membrane, which has been shown to occur during this stage of pregnancy and is frequently observed in dermal studies because the rats cannot groom themselves (Long and Evans, 1920). Although the incidence of this finding increased in a dose-related manner, with statistical significance achieved at the 50, 100, and 250 mg/kg/day dose levels, it was concluded that the bleeding, although attributed to treatment, was not an adverse toxic effect, since the incidence of this finding in the control animals was high (14/24), and no correlation to fetal loss was observed in any group. The number of resorptions seen in this study is comparable to the numbers seen historically (Attachment 2) and is indicative of the lack of a toxic response to treatment with F6285.

Maternal body weight change, food consumption, gross pathological findings, and absolute and relative (to brain) spleen weight values were comparable between control and treated groups.

Maternal LOAEL = Not determined
Maternal NOAEL $\geq$ 250 mg/kg/day

2. Developmental toxicity

Significant treatment-related decreases in mean fetal body weight (males, females, and combined) were observed at the high-dose (250 mg/kg/day). Mean fetal weight values were significantly increased for the 5.0 mg/kg/day dose group as compared to control; however, this was not considered to be treatment-related. Embryo/fetal survival was not affected by treatment of the dams.

At the high-dose level (250 mg/kg/day), the percent of fetuses with any alteration observed (9.8%) was increased ($p \leq 0.01$) from the control incidence (3.2%). The percent

of litters containing fetuses with any alteration (68.0%) was also significantly increased as compared to the control (37.5%) at the high dose. The study author did not consider these high-dose increases to be treatment-related. However, it is the opinion of the reviewer that the increased incidence of fetal alterations at the 250 mg/kg/day dose level is an effect of treatment. Although a breakdown of the alterations into malformations and variations was not provided in the report, it appears from the individual incidence data that the overall increase in alterations at the high-dose was primarily attributable to increased incidences of skeletal variations. In addition, fetal body weights were significantly decreased at the same dose level, a finding which is commonly associated with reductions in skeletal development. A significant increase in the number of fetuses with any alteration at the 50.0 mg/kg/day level was not considered to be related to treatment, due to the lack of dose-response and the lack of significance in the litter incidence at that level.

Neither the incidence nor the distribution of the external or skeletal malformations noted suggested a response to treatment. At external evaluation of the fetuses, two malformed control fetuses were observed, one with micrognathia and one with anasarca. Among the treated groups, micrognathia and aglossia were noted in three 50.0 mg/kg/day fetuses, exencephaly with depressed eye bulges and open lids was observed in one 100 mg/kg/day fetus and spina bifida was observed in another fetus at that dose level, and micrognathia was reported in one 250 mg/kg/day fetus. No visceral malformations were reported. Observed skeletal malformations of the skull, vertebrae, and ribs were related almost entirely to the micrognathia, exencephaly, and spina bifida noted at external examination.

No external or visceral variations of concern were observed. All skeletal variations of note were judged to be reversible delays in ossification. Sig[illegible] treatment-related increases in the fetal and litter incidences of incompletely ossified [illegible] vertebral arches, hypoplastic or wavy ribs, and incompletely ossified or nonossified ischia or pubes occurred at the high-dose (250 mg/kg/day). An additional significant increase in the high-dose fetal incidence of variations in the sternebrae (incompletely ossified or unossified) was not judged to be treatment-related because a dose response was not evident, the litter incidence was not significant, and the fetal and litter numerical incidences of the finding on this study were comparable to ranges observed historically. The study author did not consider the incompletely ossified lumbar arches or the reduction in pelvic ossification at 250 mg/kg/day to be treatment-related; however, these findings, although seen primarily in the high-dose and not in a dose-related pattern, were outside of historical control ranges (Attachment 2). Such reductions in ossification are also consistent with treatment-related decreases in mean fetal body weight at the high-dose level (which was also observed as a high-dose response, i.e., without a dose-response). Other significant increases or decreases in the fetal and/or litter incidences of skeletal findings in the 5.0, 25.0, 50.0, and 100 mg/kg/day treated groups were not attributed to treatment.

At 250 mg/kg/day, the mean numbers of thoracic vertebral and rib ossification sites were significantly decreased. The study author did not judge these findings to be treatment-related due to a lack of dose-response and the comparability with historical control values. This reviewer, however, considers this to be a high-dose effect of treatment with F6285,

70

consistent with the significant treatment-related hypoplasia observed in the skeletal evaluation of the ribs, relying preferably upon concurrent control values. Slight, nonsignificant decreases in ossification site averages were noted at the same dose level in the sacral and caudal vertebrae, manubrium, sternebrae, metacarpals, metatarsals, and fore- and hindlimb phalanges but were not attributed to treatment due to lack of significance and dose-response; most of these findings also fell within the historical control ranges presented in the study report (Attachment 2). All other ossification site averages (for the hyoid; cervical and lumbar vertebrae; xiphoid sternebrae; carpals and digits of the forepaw; and tarsals and digits of the hindpaw) did not demonstrate any biologically or statistically significant difference between treated and control groups.

Developmental LOEL = 250 mg/kg/day
(based on decreased fetal body weight; increased incidences of fetal variations: hypoplastic or wavy ribs, incompletely ossified lumbar vertebral arches, and incompletely ossified ischia or pubes; and reduced number of thoracic vertebral and rib ossification sites)
Developmental NOEL = 100 mg/kg/day

3. CORE Classification: **CORE-Guideline**

Subsequent Review

Page _____ is not included in this copy.

Pages 72 through 95 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

Reviewed by: Susan L. Makris, M.S. Susan L Makris 7/14/94
Section IV, Toxicology Branch II (7509C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 7/14/94
Section III, Toxicology Branch II (7509C)

C11176

DATA EVALUATION REPORT

STUDY TYPE: Oral Developmental Toxicity Study in Rabbits (§83-3)

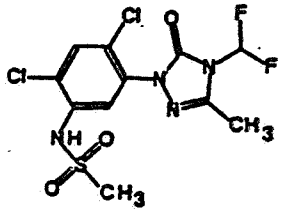
EPA NOS.: MRID NOS.: 429321-06
PC CODE: 129081

DP BARCODE NOS.: D198408 and D198715
SUBMISSION NOS.: S456588 and S457101
CASE NOS.: 034954 and 285252
ID NOS.: 00279-EUP-RGR and 3G04272

TEST MATERIAL: F6285 Technical

SYNONYMS: 2-(2,4-dichloro-5-methylsulfonylamidophenyl)-4-difluoromethyl-2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one
FMC 97285
Sulfentrazone
Methanesulfonam

CHEMICAL STRUCTURE:



STUDY NUMBER: FMC No.: A92-3540
Argus No.: 106-012

SPONSOR: FMC Corporation, Agricultural Chemical Group
1735 Market Street, Philadelphia, PA 19103

TESTING FACILITY: FMC Corporation Toxicology Laboratory (in-life)
Box 8, Princeton, NJ 08543

Argus Research Laboratories, Inc. (fetal exams)
905 Sheehy Drive, Horsham, PA 19044

TITLE OF REPORT: F6285 Technical, Teratology Study in Rabbits (Oral)

AUTHOR: Christine Freeman

STUDY COMPLETION DATE: June 22, 1993

EXECUTIVE SUMMARY: The test substance, F6285, was administered by gavage to pregnant female New Zealand White rabbits (20/group) on days 7-19 of gestation (with the day of mating defined as gestation Day 0) at dose levels of 100, 250, and 375 mg/kg/day. The rabbits were observed for signs of toxicity; body weight and food consumption values were recorded. Cesarean section was performed on Day 28 of gestation; the does were necropsied, uterine weights were recorded, and uterine contents were examined. Fetal specimens were evaluated for external, visceral, and skeletal abnormalities by standard methodologies.

In the does, treatment-related incidences of decreased feces and hematuria were noted at the 250 mg/kg/day or greater. In addition, at the 375 mg/kg/day dose level, five rabbits aborted. Significant reductions in mean body weight change were observed for the dosing period (GD 7-19) and for the study duration (GD 0-29, both before and after adjustment for gravid uterine weight) at the 250 and 375 mg/kg/day dose levels.

Maternal LOEL = 250 mg/kg/day, based upon increased abortions, clinical signs (hematuria and decreased feces), and reduced body weight gain
Maternal NOEL = 100 mg/kg/day

At the 250 and 375 mg/kg/day dose levels, significant decreases in the percent live fetuses per litter, significant increases in the percent early resorptions per litter, and significantly decreased fetal body weight (8 and 15% below control, respectively) were observed. These decrements in litter size, survival, and weight were also observed as a significantly decreased mean gravid uterine weight value in does at the 375 mg/kg/day dose level.

No external or visceral findings in fetuses suggested a response to treatment; however, skeletal evaluation revealed dose- and treatment-related findings at the 375 mg/kg/day dose level. These included significant increases in both the fetal and litter incidences of fused caudal vertebrae (a malformation) and of partially fused nasal bones (a variation). In addition, at 375 mg/kg/day, significant treatment-related reductions in ossification site averages were observed for metacarpals and both fore- and hindpaw phalanges.

Developmental LOEL = 250 mg/kg/day, based upon increased resorptions, decreased live fetuses per litter, and decreased fetal weight
Developmental NOEL = 100 mg/kg/day

CORE CLASSIFICATION: **CORE-Guideline**; this study satisfies the guideline recommendations for a §83-3(b) developmental toxicity (teratology) study in rabbits

A. Materials

1. Test compound: Name: F6285 technical
Purity: 94.2%
Reference No.: E7301-72
Description: Solid
CAS No.: 122836-35-5

2. Vehicle: Name: Mazola Corn Oil
Purity: Not provided
Lot No.: Not provided

3. Test animals: Species: Rabbit
Strain: New Zealand White
Source: Hazleton Research Products, Inc.
Denver, Pennsylvania
Age: Not specified
Weight: 2.60-3.45 kg at gestation day 0

4. Environment: Housing: Individual stainless steel cages
Lighting: 12 hours light/12 hours dark
Temperature: 65-77°F
Humidity: 33-81%
Food: Purina Rabbit Chow 5522, 150 g/day, ad libitum
Water: Municipal water, automatic watering system ad libitum

B. Methods - This study was conducted to evaluate the developmental toxicity of the test substance, F6285, when administered by gavage to pregnant female New Zealand White rabbits during the period of major fetal organogenesis. A copy of the study methods is presented as Attachment 1.

1. Rationale for Dosage Selection

It was reported that in a range-finding study (FMC study No. A92-3539; data not provided), F6285 was administered orally to groups of 7 presumed pregnant rabbits each at dosages of 0, 250, 500, and 1000 mg/kg/day (w/v) in corn oil. The test solutions were administered at a constant volume of 2 ml/kg on days 7 through 19 of gestation, inclusive. Individual doses were adjusted daily to body weight. Cesarean sections were performed on day 29 of gestation. The uteri and ovaries were examined for the number and distribution of implantation sites, early and late resorptions, live and dead fetuses, and corpora lutea. Each fetus was weighed, sacrificed, examined for external abnormalities, and sexed.

In the 1000 mg/kg/day dose group, only two rabbits survived to cesarean section, and of these, one had previously aborted. At 500 mg/kg/day, three animals survived to study termination; one was not pregnant and the other two had previously aborted. No treatment-related mortality occurred at 250 mg/kg/day. In rabbits receiving 500 mg/kg/day, treatment-related clinical signs consisted of vaginal bleeding, decreased locomotion, oral discharge, pallor, unthriftiness, and hematuria. Clinical observations in the 1000 mg/kg/day dose group included these observations (except for vaginal bleeding) with the addition of tremors and grinding teeth. No treatment-related clinical observations were noted at 250 mg/kg/day. Maternal body weight and gravid uterine weight values, litter size, number of implantations, and number of resorptions were similar between the control and 250 mg/kg/day dose groups; no gross external alterations were noted in the

98

fetuses. At 500 and 1000 mg/kg/day, there were no live fetuses; all were resorbed or aborted. The maternal and developmental NOEL was 250 mg/kg/day, and the maternal and fetal maximum tolerated dose was between 250 and 500 mg/kg/day.

Based upon the results of this range-finding study, 375 mg/kg/day was selected as a high dose for the subsequent definitive developmental toxicity study in rabbits. Low- and mid-dose levels chosen were: 100 and 250 mg/kg/day, respectively.

2. Mating, Group Assignment, and Dosage Levels

Following a period of acclimation, the female rabbits were mated with naive male rabbits of the same strain and source. Mating was confirmed by visual verification of copulation and microscopic evidence of sperm in a vaginal smear. Females were assigned to the following study groups on Day 0 of gestation (apparently not by random assignment).

Group No.	Dose[a] (mg/kg)	No. per Group
1 (Control)	0[b]	20
2 (Low)	100	20
3 (Mid)	250	20
4 (High)	375	20

a Administered at constant volume of 2 ml/kg.
b Vehicle control (corn oil).

3. Test Material Formulation, Administration, and Analysis

Suspensions of F6582 in corn oil were formulated once prior to study start. The test substance was diluted in corn oil and mixed on a stirring plate. Nominal concentrations of 0, 100, 250, and 375 mg/2 ml were prepared (based upon a constant dosage volume of 2 ml/kg) for administration to the respective test groups. Dosing suspensions were stored refrigerated and were mixed on a stirring plate prior to and during use.

The test material was administered once daily to the study animals by gavage on Days 7-19 of gestation. Individual dosage volumes were adjusted daily, based upon the most recent body weight value. Control animals received the vehicle (corn oil) in the same manner.

Samples of the formulations from each dose level and from each mixing flask were analyzed prior to the treatment period. Concentration and homogeneity were determined by HPLC for all samples. Results indicated that the formulations were homogeneous and were within adequate range (± 5%) of nominal concentrations (FMC study No. A92-3540,

page 90). The stability of the test substance in corn oil for the duration of the dosing period was reported to have been demonstrated on a previous study (FMC study No. A92-3539; data not provided), although the conditions of storage were not indicated. Results of a stability study of F6285 stored at room temperature for 24 months were provided (FMC study No. A92-3540, page 79) and indicated only a 0.2% difference between initial purity and that determined at 24 months.

4. Observations

Clinical observations were recorded once daily. Body weights were recorded once daily, on gestation Days 0, 7-19, 24, and 29. Food consumption was not measured because the rabbits were maintained on limited feed to prevent enteritis.

Surviving does were sacrificed on Day 29 of gestation by CO_2 inhalation. A gross necropsy was performed on each rabbit. Gravid uterine weights were recorded. Corpora lutea of pregnancy were counted, and uterine contents were examined for pregnancy status, number and placement of implantations, early and late resorptions, and live and dead fetuses. No indication was given in the study report if the uteri of apparently nongravid females were further examined (i.e., stained with ammonium sulfide) to confirm pregnancy status.

For females dying prior to study termination, a gross necropsy was performed, and the uterus was examined for pregnancy status. Gravid uterine weights were recorded; abnormalities and uterine contents were noted but not retained. Does that aborted were sacrificed and necropsied; the report does not address examination of the uterus or fetuses for these animals.

Following removal from the uterus, the fetuses were individually weighed, identified, sacrificed by CO_2 inhalation, and examined to identify gross external alterations. A gross necropsy (examination for visceral anomalies), including coronal sectioning of the head to identify internal brain abnormalities, was performed on each fetus. The study report did not indicate whether a detailed dissection of the heart was performed on each fetus to detect cardiac anomalies. The sex of each fetus was determined by examination of the internal reproductive organs. After visceral evaluation, all fetuses were fixed, macerated in an aqueous potassium hydroxide solution, stained with alizarin red S, cleared, and examined for skeletal alterations. A full description of the methods for processing the fetal specimens for skeletal examination was not included in the study report.

5. Statistical Analysis

The following methods of statistical analysis were performed, using proprietary programs (see report No. FMC A92-3540, pages 11-14; Attachment 1). Parametric data (maternal body weights, body weight changes, gravid uterine weights, and adjusted weight gains) were analyzed using the Welch Trend Test. The number of does with complete resorptions, fetal body weights, and sex ratios were analyzed using Exact Permutation Trend Test, Analysis of Covariance, and the Test for Binomial Proportions, respectively.

10C

All other cesarean section data were analyzed by Jonkheere's Trend Test. Fetal ossification sites and mean percent of fetuses with any malformation or variation per litter, were analyzed using Bartlett's Test of Homogeneity of Variance, Dunnett's Test, the Analysis of Variance, Kruskal-Wallis Test, and/or Dunn's Method of Multiple Comparisons, as appropriate. The litter was used as the base unit of analysis; the level of significance was $p \leq 0.05$.

Historical control data, from the performing laboratory, for skeletal alterations and ossification site averages in New Zealand White rabbits were presented in the report and appended to this DER as Attachment 2.

6. Compliance

The following required signed and dated statements were provided:
- Statement of No Data Confidentiality Claims
- Good Laboratory Practice Certification
- Quality Assurance statement
- EPA Flagging Statement (negative)

C. Results

1. Maternal Mortality

There were no treatment-related deaths during the study. However, five rabbits at the 375 mg/kg/day dose level were sacrificed following abortions on gestation days 21 (2 does), 22, 23, and 24. In addition, five rabbits (one at 375 mg/kg/day, two at 250 mg/kg/day, and two at 100 mg/kg/day) died or were sacrificed due to misdosing. All other animals survived to cesarean section.

2. Maternal Clinical Observations

Selected clinical observations noted during gestation are presented in Table 2. Treatment-related incidences of decreased feces and hematuria were noted at the 250 mg/kg/day dose level or greater. Other clinical observations noted during the study, including abdominogenital staining, diarrhea, mucoid anal discharge, vaginal distention, and unkempt appearance, occurred sporadically and were not considered treatment-related.

3. Maternal Body Weight and Gravid Uterine Weight Data

A summary of mean maternal body weight change and gravid uterine weight data is presented in Table 2. Significant reductions in mean body weight were observed for the 250 and 375 mg/kg/day rabbits on gestations days 19 and 29 (data not presented in DER). At these dose levels, body weight change was significantly reduced during the dosing period

Table 1. Selected Maternal Clinical Observations[a]

Observation	Dose Level (mg/kg/day)			
	0	100	250	375
Decreased feces	41(13)	28(13)	88(16)	156(18)
Hematuria	0	0	3(1)	107(16)

a The total number of times an observation occurred (number of animals with the finding).

N = 20

Note: Data were extracted from report No. FMC A92-3540, page 23.

(gestation days 7-19) and for the study duration (gestation days 0-29, before and after adjustment for gravid uterine weight, which was also significantly reduced at the 375 mg/kg/day dose level). No treatment-related effects on maternal body weight were observed at the 100 mg/kg/day level.

Table 2. Mean Maternal Body Weight Change and Gravid Uterine Weight (kg)

Days of Gestation	Dose Level (mg/kg/day)			
	0	100	250	375
N	16	18	18	13
0-7	0.19	0.21	0.19	0.21
7-19	0.17	0.14	0.05*	-0.11**
19-29	0.26	0.27	0.28	0.25
0-29	0.62	0.62	0.52*	0.35**
Gravid uterine weight	0.47	0.48	0.45	0.32**
Adjusted[a] 0-29	0.15	0.14	0.06*	0.03*

a Day 0-29 body weight change minus gravid uterine weight.

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

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

Note: Data were extracted from report No. FMC A92-3540, page 44.

4. Maternal Gross Pathology

No treatment-related observations were noted at necropsy of the does. The only gross lesions found were associated with intubation errors.

5. Observations Noted at Cesarean Section

The results of the examination of uterine contents at cesarean section are presented in Table 3. As noted previously in this DER, there was a treatment-related increase in the number of does aborting at the high-dose level (375 mg/kg/day).

Table 3. Summary of Selected Cesarean Section Observations

Parameter	Dose level (mg/kg/day)			
	0	100	250	375
Number inseminated	20	20	20	20
Number pregnant	16	20	20	20
Number not pregnant	0	0	0	1
Number of deaths[a]	0	2	2	1
Number of litters aborted	0	0	0	5
Number of litters totally resorbed	0	0	0	1
Number viable litters	16	18	18	12
Number of implantations/doe	8.13	8.61	9.22*	9.23*
Mean preimplantation loss[b]	1.1	1.2	0.4	1.2
Number of fetuses/litter	7.6	8.0	8.2	5.5
Mean percent live	94	94	89*	57**
Mean percent dead	0	0	0	0
Percent early resorptions/litter	1	3	8*	33**
Percent late resorptions/litter	5	3	2	10
Mean fetal body weights (g)	42.43	42.94	39.01*	35.97**
Mean sex ratio	0.60	0.58	0.57	0.50

a Deaths were the result of intubation errors; all rabbits that died were pregnant.
b Number of corpora lutea minus implantations/doe.
* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A92-3540, pages 49-53.

Significant increases in the number of implantation sites per doe were noted at the mid- and high-dose levels (250 and 375 mg/kg/day); these increases were not judged to be related to administration of F6285. Treatment- and dose-related observations at these levels included significant decreases in the percent live fetuses per litter and significant increases in the percent early resorptions per litter. An additional treatment- and dose-related finding, significantly decreased fetal body weight, was also noted at the mid- and high-dose

levels (8 and 15% decreased from control, respectively). No effects of treatment were observed at the low-dose (100 mg/kg/day) level.

7. Developmental Toxicity

Observations noted at external, visceral, and skeletal evaluation of fetuses are presented in Tables 4 (malformations) and 5 (variations). Table 6 summarizes the mean number of selected ossification sites observed per litter.

Both the fetal and litter incidences of the malformation described as fused caudal vertebrae were statistically significant for the 375 mg/kg/day dose group as compared to control; this was judged to be a treatment-related effect, since the incidence was above the historical control value for this strain of rabbit (Attachment 2). All other malformations noted were considered to be incidental and not related to test substance administration, since incidences of specific findings in treated groups were not statistically significant, no dose-related patterns were apparent, and the incidences fell within those observed historically by the performing laboratory.

The only external variation presented was a description of bulging eyes, which was judged by the Study Director to be related to the small size of the fetuses for which this finding was noted. No visceral variations were noted at any dose level. Reported skeletal variations included common alterations in the degree of ossification of the skull (especially the nasal and frontal bones), sternebrae, and pelvis. A significant increase in the fetal and litter incidences of partially fused nasal bones, which was also greater than historically observed, was considered to be dose- and treatment-related. This finding was the major contributing factor to the significant fetal increase of an irregular ossification of the nasals. Other sporadic statistically significant incidences noted in the degree of ossification in the skull and pelvis, as well as nonsignificant findings noted in the hyoid, ribs, sternebrae, and scapulae, were within the historical range and were not considered to be related to treatment.

As demonstrated in Table 6, a significant reduction in the mean numbers of caudal vertebral ossification sites was noted for the 250 mg/kg/day dose group; however, since a dose-related effect was not observed, this finding was not attributed to treatment. At 375 mg/kg/day significant treatment-related reductions in ossification site averages were also observed for metacarpals and both fore- and hindpaw phalanges. All other ossification site averages (for the hyoid; cervical, thoracic, lumbar, and sacral vertebrae; ribs; manubrium, sternal centers, and xiphoid; carpals and digits of the forepaw; and tarsals, metatarsals, and digits of the hindpaw) did not demonstrate any biologically or statistically significant difference between treated and control groups.

Table 4. Summary of Malformations

Observation		Dose (mg/kg/day) 0	100	250	375
No. fetuses (litters) examined		122(16)	144(18)	148(18)	71(12)
EXTERNAL					
No. fetuses (litters) affected		0	0	0	5(2)
FORELIMB(S) - abnormal flexure	Fetus N(%)	0	0	0	2(2.8)
	Litter N(%)	0	0	0	1(8.3)
TAIL - short	Fetus N(%)	0	0	0	1(1.4)
	Litter N(%)	0	0	0	1(8.3))
HEAD - exencephaly	Fetus N(%)	0	0	0	1(1.4)
	Litter N(%)	0	0	0	1(8.3)
HEAD - domed[a]	Fetus N(%)	0	0	0	1(1.4)
	Litter N(%)	0	0	0	1(8.3)
VISCERAL					
No. fetuses (litters) affected		1(1)	0	0	0
BRAIN - Dilated lateral ventricle[b]	Fetus N(%)	1(0.8)	0	0	0
	Litter N(%)	1(6.2)	0	0	0
SKELETAL					
No. fetuses (litters) affected		1(1)	1(1)	2(2)	4(4)
SKULL - incompletely ossified frontals, parietal, interparietal, supraoccipital[c]	Fetus N(%)	0	0	0	1(1.4)
	Litter N(%)	0	0	0	1(8.3)
VERTEBRAE - caudal, fused	Fetus N(%)	0	0	0	3(4.2)**
	Litter N(%)	0	0	0	3(25.0)**
VERTEBRAE - caudal, misaligned	Fetus N(%)	1(0.8)	0	2(1.4)	2(2.8)
	Litter N(%)	1(6.2)	0	2(11.1)	1(8.3)
VERTEBRAE - caudal, 11 present[d]	Fetus N(%)	0	0	0	1(1.4)
	Litter N(%)	0	0	0	1(8.3)
RIBS - split (R7/R8, unilateral)	Fetus N(%)	0	1(0.7)	0	0
	Litter N(%)	0	1(5.6)	0	0

a Internal evaluation of the brain revealed no abnormalities.
b Noted at internal evaluation of brain; not observed at external examination.
c Associated with exencephaly.
d Associated with short tail.
** Statistically different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A92-3540, pages 55 and 126-130.

Table 5. Summary of Selected Variations

Observation		Dose (mg/kg/day) 0	100	250	375
No. fetuses (litters) examined		122(16)	144(18)	148(18)	71(12)
EXTERNAL					
No. fetuses (litters) affected		0	0	5(2)	4(1)
EYES - bulging[a]	Fetus N(%)	0	0	5(7.0)	4(5.6)
	Litter N(%)	0	0	2(16.7)	1(8.3)
VISCERAL					
No. fetuses (litters) affected		0	0	0	0
SKELETAL					
SKULL - summary of all irregular ossifications	Fetus N(%)	30(24.6)	49(34.0)*	60(40.5)**	28(39.4)**
	Litter N(%)	14(87.5)	14(77.8)	16(88.9)	11(91.7)
NASALS - summary of all irregular ossifications[b]	Fetus N(%)	24(19.7)	30(20.8)	45(30.4)**	24(33.8)**
	Litter N(%)	12(75.0)	14(77.8)	15(83.3)	10(83.3)
NASALS - partially fused	Fetus N(%)	0	0	1(0.7)	5(7.0)**
	Litter N(%)	0	0	1(5.6)	3(25.0)**
NASALS/FRONTALS - irregular suture	Fetus N(%)	0	9(6.2)**	6(4.0)*	5(7.0)**
	Litter N(%)	0	6(33.3)	5(27.8)	4(33.3)
FRONTALS - summary of all irregular ossification[c]	Fetus N(%)	10(8.2)	25(17.4)	23(15.5)	10(14.1)
	Litter N(%)	7(43.8)	9(50.0)	13(72.2)	5(41.7)
SCAPULAE - ala, irregularly shaped or wavy	Fetus N(%)	0	2(1.4)	2(1.4)	2(2.8)
	Litter N(%)	0	2(11.1)	2(11.1)	1(8.3)
PELVIS - pubes, not ossified	Fetus N(%)	0	0	0	4(5.6)**
	Litter N(%)	0	0	0	1(8.3)

a Bulging eyes attributed to small size of fetuses.
b Includes internasals; intranasals; irregular suture; fused; midline suture displaced; nasals/frontals, irregular suture.
c Includes interfrontal; intrafrontal; irregular suture; incompletely ossified.
* Statistically different from control value, $p \leq 0.05$.
** Statistically different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. A92-3540, pages 55 and 126-130.

Table 6. Summary of Selected Fetal Ossification Sites

Ossification Sites per Litter (Mean ± S.D.)	Dose (mg/kg/day)			
	0	100	250	375
No. fetuses examined	122(16)	144(18)	148(18)	71(12)
VERTEBRAE - Caudal	16.78 ± 0.36	16.70 ± 0.27	17.14 ± 0.40**	16.74 ± 0.27
FORELIMB - Metacarpals	4.99 ± 0.03	5.00 ± 0.00	5.00 ± 0.00	4.92 ± 0.13**
FORELIMB - Phalanges	13.78 ± 0.31	13.74 ± 0.32	13.79 ± 0.31	13.39 ± 0.50**
HINDLIMB - Phalanges	12.00 ± 0.00	11.99 ± 0.02	12.00 ± 0.00	11.83 ± 0.35**

** Statistically different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A92-3540, page 132.

D. Discussion/Conclusions

1. Maternal Toxicity

Administration of the test substance, F6285, to female New Zealand White rabbits on Days 7-19 of presumed gestation by oral gavage at doses of 100, 250, and 375 mg/kg/day produced evidence of maternal (systemic) toxicity at the 250 mg/kg/day (mid-dose) and 375 mg/kg/day (high-dose) levels.

Treatment-related incidences of decreased feces and hematuria were noted at 250 mg/kg/day or greater. In addition, at the 375 mg/kg/day dose level, five rabbits aborted. Significant reductions in mean body weight change were observed for the dosing period (GD 7-19) and for the study duration (GD 0-29, both before and after adjustment for gravid uterine weight) at the 250 and 375 mg/kg/day dose levels.

Maternal LOEL = 250 mg/kg/day, based upon increased abortions, clinical signs (hematuria and decreased feces), and reduced body weight gain

Maternal NOEL = 100 mg/kg/day

2. Developmental Toxicity

At the 250 and 375 mg/kg/day dose levels, significant decreases in the percent live fetuses per litter, significant increases in the percent early resorptions per litter, and significantly decreased fetal body weight (8 and 15% of control, respectively) were observed. These decrements in litter size, survival, and weight were also observed as a significantly decreased mean gravid uterine weight value in does at the 375 mg/kg/day dose level.

No external or visceral findings in fetuses suggested a response to treatment; however, skeletal evaluation revealed dose- and treatment-related findings at the 375 mg/kg/day dose level. These included significant increases in both the fetal and litter incidences of fused caudal vertebrae (a malformation) and of partially fused nasal bones (a variation). In addition, at 375 mg/kg/day, significant treatment-related reductions in ossification site averages were observed for metacarpals and both fore- and hindpaw phalanges.

Developmental LOEL = 250 mg/kg/day, based upon increased resorptions, decreased live fetuses per litter, and decreased fetal weight

Developmental NOEL = 100 mg/kg/day

3. CORE Classification: **CORE-Guideline**

Confidential Information Deleted

Page _____ is not included in this copy.

Pages 119 through 119 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__✓__ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

Reviewed by: Susan L. Makris, M.S. Susan L Makris 8/8/94
Section IV, Toxicology Branch II (7509C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 8/8/94
Section III, Toxicology Branch II (7509C)

011176

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Subchronic Toxicity Study in Rats (§82-1)

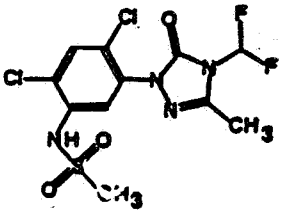
EPA NOS.: MRID NOS.: 430046-01
PC CODE: 129081

DP BARCODE NOS.: D198408 and D198715
SUBMISSION NOS.: S456588 and S457101
CASE NOS.: 034954 and 285252
ID NOS.: 00279-EUP-RGR and 3G04272

TEST MATERIAL: F6285 Technical

SYNONYMS: 2-(2,4-dichloro-5-methylsulfonylamidophenyl)-4-difluoromethyl-2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one
FMC 97285
Sulfentrazone

CHEMICAL STRUCTURE:



STUDY NUMBER: FMC No.: A89-2881

SPONSOR: FMC Corporation, Agricultural Chemical Group
1735 Market Street, Philadelphia, PA 19103

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

TITLE OF REPORT: F6285 Technical, Ninety-Day Feeding Study in Rats

AUTHOR: Donald E. Nye

STUDY COMPLETION DATE: November 6, 1990
(revised August 16, 1993 and October 28, 1993)

EXECUTIVE SUMMARY: The test substance, F6285, was administered to Fischer 344 rats (10/sex/group) for 90 days at dietary levels of 0, 50, 100, 300, 1000, 3000, and 7000 ppm. Ten

additional rats per sex per group at the control, 1000, and 3000 ppm levels were maintained an additional 4 weeks to assess recovery. The rats were observed for signs of toxicity; body weight and food consumption values were recorded weekly. Ophthalmoscopic examinations were conducted prior to treatment and at study termination. Blood samples were collected from all animals at termination, and hematology and clinical chemistry evaluations were performed. After 90-days of treatment, 10 rats/sex/group were sacrificed and necropsied. Recovery animals were sacrificed and necropsied after 4-weeks on control feed. Organ weight data were recorded, and tissues were processed for subsequent specified histopathological examination.

Administration of the test substance caused severe anemia complicated by inanition. The anemia was postulated to result from the interference of heme biosynthesis through the inhibition of protoporphyrin oxidase and the accumulation of protoporphyrin IX. As a result of the anemia and inanition, all high-dose (7000 ppm) rats died before Week 6 of study and one 3000 ppm female died during Week 2. Treatment-related clinical observations in all 7000 ppm animals and all 3000 ppm females included decreased or brownish-red feces, red abdominogenital staining, decreased locomotion, hypersensitivity to touch, dehydration, pale eyes and ears, shedding fur, unthriftiness, and walking on toes. At 3000 and 7000 ppm, mean body weight values for both sexes were significantly decreased through the periods of treatment and recovery. Decreased body weight gain was also noted for males at 1000 ppm during recovery. Food consumption was decreased for all animals that died on study and at 3000 ppm for some measured intervals.

During the treatment phase, increased white blood cell counts, decreased hemoglobin and hematocrit, decreased mean corpuscular volume and mean corpuscular hemoglobin measurements in 1000 and 3000 ppm rats were attributed to treatment; some of these effects remained through the recovery period. Treatment-related decreases in platelet counts were noted in 1000 ppm males after treatment, and in 1000 and 3000 ppm females at both bleeding intervals. The number of nucleated red blood cells was significantly higher in 3000 ppm rats after treatment, and in 1000 and 3000 ppm females after the recovery period; red blood cell counts were significantly elevated at 1000 and 3000 ppm after the recovery period. ALT levels were significantly decreased for 1000 and 3000 ppm males after treatment, and for 1000 ppm males following the recovery period.

Gross and microscopic pathological treatment-related findings in the spleen were attributed to the observed anemia. Enlarged spleen was observed in 1/10 males and 9/10 females at 3000 ppm, and in 8/10 males and 1/10 females at 7000 ppm. At 3000 ppm, absolute and relative spleen weights were increased for both sexes, with the effects more pronounced in the females After 4-weeks of recovery, enlarged spleen was noted for 1/10 females at 3000 ppm, and although spleen weights remained increased, some recovery was evident.

Significantly increased liver weight (relative to body weight) for males and females following treatment and significantly decreased liver weight (relative to brain weight) for females following the recovery period were noted at 3000 ppm. These changes may be related to treatment. Other significant organ weight findings, in the adrenals, heart, brain, kidneys, and testes of animals in the 3000 ppm treatment group and in the heart and testes of males at 1000 ppm were considered to be secondary toxic effects resulting from the treatment-related body weight depression and anemia.

Following the treatment period, histopathological findings were observed in both sexes at the 3000 and 7000 ppm levels. No treatment-related lesions were observed following the recovery period. For both the 7000 and 3000 ppm treatment groups, anemia was directly associated with microscopic alterations in the bone marrow and spleen and with related lesions in the heart, liver, lungs, and kidneys. Effects were more severe in females, resulting in earlier mortality at 7000 ppm (average time to mortality was 18 days for females, as compared to 32 days for males); in the males, the longer exposure time prior to death resulted in increased severity of erythroid hyperplasia in the bone marrow. The anemia resulted in a loss of mature erythrocytes and accumulations of large numbers of nucleated erythrocyte precursors (reticulocytes) in the bone marrow and spleen. Splenic effects also included increased extramedullary hematopoiesis. Other microscopic changes noted in both sexes at 3000 and 7000 ppm were associated with inanition.

Based upon findings following a 4-week recovery period, the effects of dietary administration of F6285 appear to be reversible.

NOEL = 300 ppm (19.9 mg/kg/day in males; 23.1 mg/kg/day in females)
LOEL = 1000 ppm (65.8 mg/kg/day in males; 78.1 mg/kg/day in females)
based on clinical anemia (reduced hematocrit, hemoglobin, mean cell volume, and mean cell hemoglobin values during treatment; increased red blood cell count during recovery)

CORE CLASSIFICATION: CORE-Guideline; this study satisfies the requirements for a §82-1 subchronic toxicity study in rats and is acceptable for regulatory purposes.

A. Materials

1. Test compound:
   Name: F6285 technical
   Purity: 90.7%
   Reference No.: E6529-31-1
   Description: Tan solid
   CAS No.: 122836-35-5

2. Test animals:
   Species: Rat
   Strain: Fisher 344 (CDF strain)
   Source: Charles River Laboratories, Kingston, NY
   Age at start of treatment (Day 1): 7.5 weeks
   Weight (Day 1): 104-154 g (males); 104-126 g (females)

4. Environment:
   Housing: Individual stainless steel cages
   Lighting: 12 hours light/12 hours dark
   Temperature: 68-76°F
   Humidity: 26-87%
   Food: Purina Rodent Chow 5001 ad libitum
   Water: Municipal water, automatic watering system ad libitum

B. Methods - This study was conducted to evaluate the subchronic toxicity of the test

122

substance, F6285, when administered in the diet to male and female Fisher 344 rats for approximately 90 days, followed by a 4-week recovery period.

1. Group assignment and dose level

Following quarantine, rats were assigned, via a computer-generated body weight dependent randomization procedure, to the following test and control groups:

Group	Dietary Concentration[a] (ppm)	No. Males		No. Females	
		90-Days	Recovery	90-Days	Recovery
1	0 (Control)	10	10	10	10
2	50	10	-	10	-
3	100	10	-	10	-
4	300	10	-	10	-
5	1000	10	10	10	10
6	3000	10	10	10	10
7	7000	10	-	10	-

a Not adjusted for purity.

2. Test material formulation, administration, and analysis

The test substance, F6285, was ground into a fine powder, then weighed and added to approximately 500 g of diet to form a pre-mix. After mixing by hand, the pre-mix was added to the appropriate quantity of diet for each treatment level, and mixed in a Patterson-Kelly 16 quart blender for approximately 10 minutes. The control diet was not blended.

The appropriate dietary mixes were administered fresh weekly to the test animals for a period of at least 90 days. The control group was maintained on basal diet throughout the study, and the animals in the recovery groups were provided basal diet from study day 91 through to termination.

The frequency of diet preparation was not indicated. Diet mixtures were stored in closed containers at room temperature until used.

Diet samples were analyzed by reversed phase HPLC using UV detection. Mixtures of F6285 in rodent chow (method of storage not specified) were found to be stable for a period of at least 58 days (FMC report No. A89-2881, page 664). Homogeneity of

a non-study dietary mix (550 ppm) was determined prior to study start and once during the in-life phase. Adequate homogeneity was demonstrated; however, the mixing times indicated for the homogeneity samples (15-20 minutes) exceeded the 10-minute mixing time reportedly used in diet formulation procedures. Samples of each dietary level were analyzed for concentration of F6285 throughout most of the study duration (FMC report No. A89-2881, pages 627-632). It is not possible to correlate the analysis dates with the dates or intervals of sample collection. A summary of concentration analyses was provided (page 618); however, the data presented appear to be derived from a different FMC study. Nevertheless, actual concentrations were generally determined to be within ±10% of nominal. More difficulty was encountered in achieving nominal concentrations for the lower dose levels (50-300 ppm); this may have been related to the mixing procedure (time in the blender). These issues, although of concern, were not judged to compromise the overall adequacy of the study.

3. Observations

a. Clinical observations - All rats were observed twice daily for mortality and once daily for clinical signs of toxicity.

b. Body weight and food consumption - Body weight data were recorded at study start (Day 1), weekly thereafter, and at termination. Food consumption was measured weekly, from study start until termination.

c. Ophthalmoscopic examination - All rats were examined by a veterinary ophthalmologist prior to the initiation of treatment and prior to study termination. Animals with pretreatment abnormalities were not placed on study.

d. Clinical pathology - On study day 95 or on day 32 of the recovery period, blood samples were collected, via puncture of the orbital sinus, from all surviving rats for hematology and clinical chemistry analyses. The rats were fasted overnight prior to bleeding. The following parameters were evaluated:

a) Hematology

- hematocrit
- hemoglobin
- erythrocyte count
- total leukocyte count
- differential leukocyte count
- platelet count
- mean corpuscular hemoglobin concentration (MCHC)
- mean corpuscular volume (MCV)
- mean corpuscular hemoglobin (MCH)

b) Clinical chemistry

- calcium
- phosphorus
- chloride
- sodium
- potassium
- glucose
- blood creatinine
- total bilirubin

albumin
total serum protein
albumin/globulin ratio (calculated)
alanine aminotransferase (ALT/SGPT)
aspartate aminotransferase (AST/SGOT)
blood urea nitrogen (BUN)

e. Sacrifice and necropsy - All rats that died prior to study termination were subjected to a complete necropsy. After 96 to 99-days of continuous dietary test substance administration, or after day 33 of the recovery period, all surviving rats were sacrificed by intraperitoneal injection of thiamylal sodium and exsanguination and were necropsied. Organ weight values were recorded for the brain, heart, liver, kidneys, gonads, adrenals, and spleen. Tissues collected and processed included:

Brain (medulla/pons, cerebral cortex, cerebellar cortex)
Spinal cord (cervical, thoracic, lumbar)
Pituitary
Thyroid/parathyroid
Thymus
Lungs/trachea
Heart
Sternum with bone marrow
Salivary glands
Liver
Spleen
Kidneys
Pancreas
Uterus
Vagina
Prostate
Epididymis
Esophagus
Skin
Exorbital/lachrymal glands
Small intestine (duodenum, jejunum, ileum)
Large intestine (cecum, colon, rectum)
Femur including articular surface
Urinary bladder
Lymph nodes (submandibular, mesenteric)
Sciatic nerve with muscle attached
Mammary gland
Adrenals
Ovaries
Testes
Aorta
Stomach
Eyes
Gross lesions

f. Histopathology - Tissues from the control and Group 6 (3000 ppm) rats that were sacrificed at the termination of the study, as well as tissues from rats that died prior to study termination, were examined microscopically. In addition, the lungs, liver, kidneys, spleen, and all organs with gross lesions in Groups 2, 3, 4, and 5 (50, 100, 300, and 1000 ppm, respectively) were examined microscopically.

4. Statistics

The following methods of statistical analyses were performed, using SAS/STAT or proprietary programs (FMC report No. A89-2881, pages 15-16). An analysis of variance (ANOVA) was performed on data that were determined to be normally distributed (by Proc Univariate). Homogeneity of variance was analyzed by Bartlett's test. For homogeneous data, treated group means were compared to control by Dunnett's test; for non-homogeneous data, a T-test was conducted. Data which were not normally distributed were log-transformed prior to analysis. If log-transformation did not achieve normal distribution, nonparametric methods (Kruskal-Wallis Non-Parametric ANOVA, and Dunn's Test for Multiple Comparisons) were used to determine if treatment groups were significantly different from control.

5. Compliance

The following signed and dated statements were included:
Statement of No Data Confidentiality
GLP Compliance Statement
Flagging statement for potential adverse effects (none noted)
Quality Assurance Documentation

C. Results

1. Mortality and clinical observations

All high-dose (7000 ppm) rats died before Week 6 of study. The average time to mortality for males was 32 days and for females 18 days. In addition, one 3000 ppm female died during Week 2 of treatment-related toxicity. All other rats survived to termination of the 90-days of treatment.

Treatment-related clinical observations were observed in all 7000 ppm animals and all 3000 ppm females. These observation included decreased or brownish-red feces, red abdominogenital staining, decreased locomotion, hypersensitivity to touch, dehydration, pale eyes and ears, shedding fur, unthriftiness, and walking on toes (data from FMC report No. A89-2881, pages 171-241). During the recovery phase, no treatment-related observations were noted.

2. Body weight

Mean body weight and body weight change data are presented in Table 1. At 3000 and 7000 ppm, mean body weight values for both sexes were significantly decreased as compared to control from Week 1 of study through the entire period of treatment (or until death), and throughout the 4-week recovery period. For 3000 ppm males, the mean body weight gain was 83.3% of control at the end of treatment, and 85.9% of control at the end of the recovery period. For females in that group, body weight gain was 71.6% of control at the end of treatment, and 79.5% of control at the end

of recovery. The mean body weight values of males at 1000 ppm were similar to control values throughout the treatment period; however, during the recovery period, significant decreases in mean body weight and body weight gain were noted. At the end of the recovery period, the body weight gain for 1000 ppm males was 90.1% of control. The body weight data for females at 1000 ppm were similar to control values, as were body weight data for all treatment groups less than 1000 ppm.

Table 1. Selected mean body weight and body weight change values (g)

Interval	Treatment level (ppm)						
	0	50	100	300	1000	3000	7000
Males							
Day 1	128	128	130	128	128	128	127
Wk 1	155	156	156	156	154	146	101**
Wk 5	228	223	224	236	222	205**	95**
Wk 8	258	252	255	267	249	232**	a
Wk 13	296	290	292	304	284	267**	—
Wks 0-13	168	161	163	176	156	140**	—
Recovery Wk 1	311	—	—	—	288*	276**	—
Recovery Wk 4	328	—	—	—	309*	300**	—
Day 1-Recovery Wk 4	191	—	—	—	172*	164**	—
Females							
Day 1	114	114	115	115	114	115	115
Wk 1	129	129	129	127	127	107**	95**
Wk 5	158	159	157	158	157	144**	101
Wk 8	168	169	166	167	166	153**	a
Wk 13	181	181	179	183	179	163**	—
Wks 0-13	67	68	64	68	65	48	—
Recovery Wk 1	188	—	—	—	190	169**	—
Recovery Wk 4	198	—	—	—	201	181**	—
Day 1-Recovery Wk 4	78	—	—	—	82	62**	—

a All high-dose (7000 ppm) animals died prior to Week 6.
* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A89-2881, pages 27-30.

3. Food consumption and test substance intake

A summary of selected food consumption data is presented in Table 2. Food consumption was decreased for all animals that died on study. In addition, at 3000 ppm, food consumption values were significantly reduced for males at weeks 2-4, 13, and recovery week 1 and for females at weeks 1-3 and 9-13.

Table 2. Selected mean food consumption values (g)

Interval	Treatment level (ppm)						
	0	50	100	300	1000	3000	7000
Males							
Wk 1	101	101	103	103	101	94	47**
Wk 5	103	101	105	110	103	96	37**
Wk 13	111	109	111	115	109	102*	a
Recovery Wk 1	116	—	—	—	109	108*	—
Recovery Wk 4	118	—	—	—	110*	112	—
Females							
Wk 1	89	85	91	90	91	61**	32**
Wk 5	86	87	84	85	86	86	59
Wk 13	86	89	83	87	86	76**	—
Recovery Wk 1	92	—	—	—	96	92	—
Recovery Wk 4	91	—	—	—	94	88	—

a All high-dose (7000 ppm) animals died prior to Week 6.
* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A89-2881, pages 31-34.

Compound intake values were calculated from individual body weight and food consumption data and are summarized in Table 3.

Table 3. Mean test substance intake (mg/kg/day)

Sex	Treatment (ppm)					
	50	100	300	1000	3000	7000
Males	3.3	6.7	19.9	65.8	199.3	534.9 a
Females	4.0	7.7	23.1	78.1	230.5	404.3 a

a All high-dose (7000 ppm) animals died prior to Week 6.
Note: Data were extracted from report No. FMC A89-2881, pages 35-38.

4. Ophthalmological examination

Evaluation of the study animals for ophthalmological findings did not identify any effects that were attributed to treatment (report No. FMC A89-2881, pages 672-673).

5. Clinical pathology

Selected hematology and serum chemistry findings are summarized in Tables 4a and 4b, respectively.

White blood cell counts were significantly elevated for rats at 3000 ppm during the treatment phase of the study. Significant decreases in hemoglobin and hematocrit counts were observed for rats at 1000 and 3000 ppm after 13 weeks of treatment; red blood cell counts were significantly elevated at these levels after the recovery period. Mean corpuscular volume and mean corpuscular hemoglobin measurements were significantly decreased for males at 300, 1000, and 3000 ppm after the treatment phase, and for 1000 and 3000 ppm males after recovery; for females, significant decreases in MCV and MCH were observed at 1000 and 3000 ppm both 13 weeks after treatment and after the 4-week recovery period. Platelet counts were significantly decreased in 300 and 1000 ppm males after treatment, and in 1000 and 3000 ppm females at both bleeding intervals. The number of nucleated red blood cells was significantly higher in 3000 ppm rats after treatment, and in 1000 and 3000 ppm females after the recovery period.

Mean sodium levels were significantly increased for males at 3000 ppm after treatment, and for 1000 ppm males and 3000 ppm males and females following recovery. These increases fell within published historical ranges and were therefore not considered by the study author to be treatment-related (Mitruka, B.M. and H.M. Rawnsley, Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals and Normal Humans, 2nd edition, Year Book Medical Publishers, Chicago, 1981). Alanine aminotransferase (ALT) levels were significantly decreased for 1000 and 3000 ppm males after treatment, and for 1000 ppm males following the recovery period. Although the recovery values fell within reference ranges, the posttreatment values did not and were considered to be potentially treatment-related.

Other sporadic statistically significant findings noted during clinical pathological evaluation were not considered to be treatment-related due to a lack of dose response and/or because the values fell within the published historical control reference ranges.

011176

Table 4a. Selected mean clinical pathology findings - Males

ppm	Hematology								Clinical chemistry	
	WBC	RBC	HGB	HCT	MCV	MCH	PLT	NRBC	NA	ALT
Treatment Phase										
0	7.5	8.18	16.3	49.4	60	19.9	740	0.3	140.5	57
50	7.4	8.33	16.3	49.8	60	19.6	715	0.7	140.7	57
100	7.5	8.39	16.4	50.1	60	19.5	729	0.8	141.0	57
300	7.4	8.38	16.0	49.4	59**	19.1**	657*	0.6	140.3	67
1000	7.3	8.44	15.5**	47.8**	57**	18.4**	611**	1.0	141.2	53
3000	10.1**	8.63	13.5**	41.7**	48**	15.7**	669	26.3**	141.8**	41**
Recovery Phase										
0	7.7	8.60	16.8	51.5	60	19.6	799	0.4	144.4	65
1000	7.8	8.88**	16.9	51.6	58*	19.0**	774	0.7	145.7**	56*
3000	7.6	9.35**	17.0	51.5	55**	18.1**	768	0.5	145.8**	54**

* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A89-2881, pages 39-43 and 49-51.

Table 4b. Selected mean clinical pathology findings - Females

ppm	Hematology								Clinical chemistry	
	WBC	RBC	HGB	HCT	MCV	MCH	PLT	NRBC	NA	ALT
Treatment Phase										
0	5.8	7.66	16.1	49.6	65	21.0	773	2.2	140.8	50
50	5.4	7.65	15.9	49.8	65	20.9	728	0.9	141.2	50
100	6.1	7.76	16.2	50.3	65	20.9	785	1.3	141.3	47
300	6.0	7.79	16.2	50.2	65	20.8	739	1.0	141.5	52
1000	6.4	7.86	14.9**	46.5**	59**	18.9**	657**	3.2	141.1	44
3000	10.1**	7.74	11.6**	36.8**	47**	14.9**	661**	86.4**	141.2	43
Recovery Phase										
0	5.0	7.88	16.6	50.7	64	21.0	803	2.4	144.5	64
1000	4.9	8.21*	16.4	50.3	61*	20.0**	660**	0.8*	145.9	87
3000	4.9	8.60**	17.1	51.4	60**	19.8**	647**	0.8*	146.6**	56

* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from report No. FMC A89-2881, pages 44-48 and 52-54.

6. Postmortem Studies

a. Gross pathology - Enlarged spleen was observed in 1/10 males and 9/10 females at 3000 ppm, and in 8/10 males and 1/10 females at 7000 ppm (FMC report A89-2881, page Nos. 490-505). After 4-weeks of recovery, this finding was also noted for 1/10 females at 3000 ppm (FMC report A89-2881, page Nos. 571-576). Other incidental gross pathology findings reported were considered to be unrelated to treatment.

b. Organ weight data - Absolute and relative (to body weight and brain weight) organ weight data are summarized in Tables 5a (males) and 5b (females). At 3000 ppm, absolute and relative spleen weights were increased for both sexes, with the effects more pronounced in the females. At the end of the 4-week recovery period, spleen weights remained enlarged, but some recovery was evident. These findings were considered to be related to treatment.

Significant organ weight findings noted at 3000 ppm included increased liver weight (relative to body weight) for males and females following treatment, and decreased relative (to brain weight) liver weights for females following the recovery period. These changes may be related to treatment.

Other significant organ weight findings, in the adrenals, heart, brain, kidneys, and testes of animals in the 3000 ppm treatment group and in the heart and testes of males at 1000 ppm were considered to be secondary toxic effects resulting from the treatment-related body weight depression and anemia.

c. Histopathology - Treatment-related histopathological findings were observed in both sexes at the 3000 and 7000 ppm levels following 13 weeks of dietary administration of F6285 (Pathology Report, page Nos. 462-475). Microscopic findings noted in the tissues of rats from the 50, 100, 300, and 1000 ppm groups following 13 weeks of dietary administration did not suggest a relationship to treatment. Following a 4-week recovery period for rats at 1000 and 3000 ppm, no treatment-related lesions were observed (Pathology Report, page Nos. 561-570).

Administration of the test substance caused severe anemia complicated by inanition, with resulting mortality, at 7000 ppm. Table 6 presents a summary of lesions associated with anemia at the 3000 and 7000 ppm treatment levels. For both treatment groups, the anemia was directly associated with microscopic alterations in the bone marrow and spleen and with related lesions in the heart, liver, lungs, and kidneys. Affects were more severe in females, resulting in earlier mortality at 7000 ppm (average time to mortality was 18 days for females, as compared to 32 days for males); in the males, the longer exposure time prior to death resulted in increased severity of erythroid hyperplasia in the bone marrow. The anemia resulted in a loss of mature erythrocytes and accumulations of large numbers of nucleated erythrocyte precursors (reticulocytes) in the bone

Table 5a. Mean absolute and relative organ weight data in males

Organ	Treatment (ppm)						Recovery (ppm)		
	0	50	100	300	1000	3000	0	1000	3000
Absolute organ weight (g)									
Adrenals	0.046	0.047	0.051	0.052	0.054	0.049	0.053	0.056	0.052
Brain	1.74	1.80	1.83	1.81	1.83	1.81	1.85	1.81	1.86
Heart	0.87	0.89	0.88	0.92	0.90	0.86	1.01	0.90**	0.90**
Kidneys	1.76	1.94	1.93	1.98*	1.84	1.73	2.20	2.09	1.99
Liver	8.71	9.01	9.39	9.79	9.17	8.85	10.51	9.82	9.72
Spleen	0.64	0.66	0.68	0.68	0.68	0.82**	0.64	0.59*	0.59*
Testes	2.81	2.93	2.85	2.98	2.80	2.78	3.03	3.08	3.01
Organ-to-body weight ratio (%)									
Adrenals	0.017	0.017	0.017	0.017	0.020	0.019	0.017	0.019	0.018
Brain	0.635	0.636	0.639	0.609	0.666	0.719**	0.586	0.614	0.644*
Heart	0.318	0.314	0.306	0.310	0.328	0.338	0.319	0.305	0.312
Kidneys	0.642	0.682	0.669	0.665*	0.670	0.681	0.697	0.708	0.689
Liver	3.17	3.18	3.25	3.27	3.34	3.49**	3.34	3.31	3.36
Spleen	0.232	0.231	0.235	0.226	0.246	0.323**	0.204	0.200	0.205
Testes	1.025	1.037	0.995	1.003	1.023	1.095	0.963	1.042*	1.045**
Organ-to-brain weight ratio (%)									
Adrenals	2.65	2.65	2.74	2.88	2.94	2.72	2.88	3.07	2.79
Heart	50.54	49.51	48.07	51.12	49.48	47.19	54.82	50.02	48.63*
Kidneys	101.90	107.58	105.05	109.76	100.65	95.26	119.79	115.75	107.42*
Liver	502.98	500.92	511.88	541.33	503.26	487.25	572.35	543.08	523.86
Spleen	36.94	36.40	36.93	37.40	36.95	45.19**	34.92	32.75	31.98
Testes	162.34	163.24	155.73	165.04	154.14	153.06	165.01	170.40	162.55

* Significantly different from control value, $p<0.05$.
** Significantly different from control value, $p<0.01$.
Note: Data were extracted from report No. FMC A89-2881, pages 59-70.

Table 5b. Mean absolute and relative organ weight data in females

Organ	Treatment (ppm)						Recovery (ppm)		
	0	50	100	300	1000	3000	0	1000	3000
Absolute organ weight (g)									
Adrenals	0.055	0.056	0.056	0.053	0.052	0.061*	0.059	0.065	0.061
Brain	1.69	1.76	1.70	1.72	1.67	1.69	1.73	1.69	1.75
Heart	0.63	0.69	0.64	0.65	0.66	0.69	0.66	0.69	0.68
Kidneys	1.26	1.39	1.26	1.35	1.21	1.37	1.37	1.37	1.31
Liver	6.16	6.42	6.13	6.28	6.28	6.82	6.48	6.57	6.10*
Spleen	0.63	0.66	0.64	0.67	0.70	1.45**	0.56	0.57	0.64**
Ovaries	0.08	0.08	0.08	0.08	0.07	0.08	0.07	0.08	0.08
Organ-to-body weight ratio (%)									
Adrenals	0.032	0.031	0.032	0.030	0.031	0.038*	0.031	0.034	0.035
Brain	0.978	0.980	0.970	0.967	0.989	1.058	0.917	0.875	1.010**
Heart	0.366	0.380	0.364	0.367	0.388	0.433*	0.350	0.356	0.392**
Kidneys	0.730	0.771	0.716	0.758	0.715	0.854	0.726	0.709	0.754
Liver	3.56	3.54	3.46	3.52	3.70	4.26**	3.43	3.40	3.51
Spleen	0.363	0.365	0.363	0.378	0.411	0.902**	0.298	0.293	0.366**
Ovaries	0.044	0.043	0.046	0.044	0.040	0.047	0.039	0.042	0.046
Organ-to-brain weight ratio (%)									
Adrenals	3.24	3.18	3.30	3.09	3.13	3.60	3.44	3.89	3.47
Heart	37.54	39.30	37.73	37.95	39.31	41.08	38.24	40.70	38.93
Kidneys	74.86	79.34	74.17	78.28	72.50	80.36	79.40	81.21	74.83
Liver	365.60	365.34	360.28	365.53	374.96	404.44	375.16	389.96	348.40*
Spleen	37.26	37.48	37.69	39.15	41.57	86.13**	32.65	33.51	36.33
Ovaries	4.59	4.37	4.76	4.52	4.04	4.49	4.22	4.87	4.55

* Significantly different from control value, $p<0.05$.
** Significantly different from control value, $p<0.01$.
Note: Data were extracted from report No. FMC A89-2881, pages 59-70.

marrow and spleen. Enlargement of the spleen due to extramedullary hematopoiesis was also observed. Anemia-related hypoxemia led to associated histopathological cardiac and pulmonary lesions, including: atrial thrombosis and hypertrophy, perivascular pulmonary edema, increased numbers of alveolar macrophages, medial and endothelial cell hypertrophy of pulmonary arteries, pulmonary arterial thrombosis, and emphysema. Renal alterations associated with anemia included eosinophilic granular/proteinaceous casts, dilated cortical tubules with flattened or hypertrophied epithelial cells, and scattered individual epithelial cell necrosis.

Other microscopic changes noted in both sexes at 3000 and 7000 ppm were associated with inanition and included varying degrees of hepatocyte fatty change, central lobular hepatocyte necrosis with or without irregular areas of coagulation necrosis, reduced numbers of mineralized concretions in the kidneys, reduced parabronchial lymphoid cuffs, lymphoid hypoplasia of the spleen and lymph nodes, involution of the thymus, generalized hypoplasia of the bone marrow, reduction of thyroid follicular colloid, atrophy of the mammary gland, atrophy of skeletal muscle, acinar cell degranulation of the pancreas, azospermia and germinal epithelial atrophy or degeneration of the testes, prostatic atrophy, and anestrus. Hypertrophy of the adrenal cortex in rats at 7000 ppm was considered to be related to stress and a contributing factor in the lymphoid hypoplasia and thymic involution observed at that treatment level.

D. Discussion/Conclusion

Administration of the test substance caused severe anemia complicated by inanition. The anemia was postulated to result from the interference of heme biosynthesis through the inhibition of protoporphyrin oxidase and the accumulation of protoporphyrin IX.

As a result of the anemia and inanition, all high-dose (7000 ppm) rats died before Week 6 of study. Due to a greater severity of toxicity in females, the average time to mortality for females was 18 days and for males 32 days. One 3000 ppm female also died during Week 2 of treatment-related toxicity. All other rats survived to termination of the 90-days of treatment.

Treatment-related clinical observations in all 7000 ppm animals and all 3000 ppm females included decreased or brownish-red feces, red abdominogenital staining, decreased locomotion, hypersensitivity to touch, dehydration, pale eyes and ears, shedding fur, unthriftiness, and walking on toes. During the recovery phase, no treatment-related observations were noted.

Evaluation of the study animals for ophthalmological findings did not identify any effects that were attributed to treatment.

At 3000 and 7000 ppm, mean body weight values for both sexes were significantly decreased as compared to control from Week 1 of study through the entire period of

Table 6. Selected incidences of microscopic lesions related to anemia

Tissue and finding		Males			Females		
		0 ppm	3000 ppm	7000[a] ppm	0 ppm	3000 ppm	7000[a] ppm
HEART							
Atrial thrombosis		0	1	6	0	0	1
Atrial hypertrophy		0	0	8	0	0	2
LIVER							
Central lobular hepatocyte necrosis		0	0	9	0	1	3
Coagulation necrosis (irregular regions)		0	0	4	0	1	4
SPLEEN							
Extramedullary hematopoiesis	- moderate	0	4	1	9	10	1
	- marked	0	0	8	0	0	1
Hemosiderin pigment	- moderate	0	9	5	8	8	1
	- marked	0	1	1	0	0	0
Reticulocytes in vessels		0	10	10	0	10	6
Increased reticulocytes, sinusoids		0	10	0	0	10	0
BONE MARROW/STERNUM							
Erythroid hyperplasia	- moderate	0	3	0	0	10	2
	- marked	0	7	9	0	0	1
Granulocytic hypoplasia	- mild	0	10	0	0	3	2
	- moderate	0	0	1	0	5	2
	- marked	0	0	8	0	3	0
BONE MARROW/FEMUR							
Erythroid hyperplasia	- mild	0	9	1	0	7	2
	- moderate	0	0	8	0	3	2
Granulocytic hypoplasia	- moderate	0	0	8	0	1	2
LUNGS							
Foamy alveolar macrophages in alveoli		1	5	8	0	7	4
Thickening alveolar septal walls		0	0	9	0	1	4
Chronic passive congestion		0	0	9	0	3	9
Perivascular edema		0	0	9	0	2	3
Alveolar macrophage hyperplasia		0	6	9	0	9	3
Medial hypertrophy, arterial walls		0	0	9	0	1	6
Fibrin thrombi, arteries		0	0	4	0	0	1
Intimal cell hypertrophy, arteries		0	0	9	0	1	5
Emphysema		0	0	10	0	0	9
KIDNEYS							
Eosinophilic granular casts		0	1	0	0	5	1
Dilated cortical tubules with flattened and/or hypertrophied epithelial cells		0	0	0	0	4	1
Tubular epithelial necrosis		0	0	0	0	3	1

N = 10

a All high-dose (7000 ppm) animals died prior to Week 6.

Note: Data were extracted from report No. FMC A89-2881, pages 476-486.

treatment (or until death), and throughout the 4-week recovery period. For 3000 ppm males, the mean body weight gain was 83.3% of control at the end of treatment, and 85.9% of control at the end of the recovery period. For females in that group, body weight gain was 71.6% of control at the end of treatment, and 79.5% of control at the end of recovery. The mean body weight values of males at 1000 ppm were similar to control values throughout the treatment period; however, during the recovery period, significant decreases in mean body weight and body weight gain were noted. At the end of the recovery period, the body weight gain for 1000 ppm males was 90.1% of control. The body weight data for females at 1000 ppm were similar to control values, as were body weight data for all treatment groups less than 1000 ppm.

Food consumption was decreased for all animals that died on study. At 3000 ppm, food consumption values were significantly reduced for males at weeks 2-4, 13, and recovery week 1 and for females at weeks 1-3 and 9-13.

White blood cell counts were significantly elevated for rats at 3000 ppm during the treatment phase of the study. Significant decreases in hemoglobin and hematocrit counts were observed for rats at 1000 and 3000 ppm after 13 weeks of treatment; red blood cell counts were significantly elevated at these levels after the recovery period. Mean corpuscular volume and mean corpuscular hemoglobin measurements were significantly decreased for males at 300, 1000, and 3000 ppm after the treatment phase, and for 1000 and 3000 ppm males after recovery; for females, significant decreases in MCV and MCH were observed at 1000 and 3000 ppm both 13 weeks after treatment and after the 4-week recovery period. Platelet counts were significantly decreased in 300 and 1000 ppm males after treatment, and in 1000 and 3000 ppm females at both bleeding intervals. The number of nucleated red blood cells was significantly higher in 3000 ppm rats after treatment, and in 1000 and 3000 ppm females after the recovery period. Although significant depressions were noted in MCV, MCH, and platelet values for males at 300 ppm, these changes were considered not to be biologically significant since the changes were not observed in both sexes and did not always occur in a dose-response pattern. The study author did not consider the hematological effects at 1000 ppm to be biologically significant since they were not supported by histopathological evidence and were within published reference ranges; however, since the effects were observed in both sexes, were dose-related, and elicited a posttreatment recovery response (increased red blood cell production), it is hypothesized by this reviewer that these changes represent a true response to treatment at or near the actual lowest effect level and should not be minimized or ignored.

Mean sodium levels were significantly increased for males at 3000 ppm after treatment, and for 1000 ppm males and 3000 ppm males and females following recovery. These increases fell within published historical ranges and were therefore not considered by the study author to be treatment-related. Alanine aminotransferase (ALT) levels were significantly decreased for 1000 and 3000 ppm males after treatment, and for 1000 ppm males following the recovery period. Although the recovery values fell within reference ranges, the posttreatment values did not and were considered to be potentially treatment-related.

Gross and microscopic pathological treatment-related findings in the spleen were attributed

to the observed anemia. Enlarged spleen was observed in 1/10 males and 9/10 females at 3000 ppm, and in 8/10 males and 1/10 females at 7000 ppm. At 3000 ppm, absolute and relative spleen weights were increased for both sexes, with the effects more pronounced in the females. After 4-weeks of recovery, enlarged spleen was noted for 1/10 females at 3000 ppm, and although spleen weights remained increased, some recovery was evident.

Significantly increased liver weight (relative to body weight) for males and females following treatment and significantly decreased liver weight (relative to brain weight) for females following the recovery period were noted at 3000 ppm. These changes may be related to treatment. Other significant organ weight findings, in the adrenals, heart, brain, kidneys, and testes of animals in the 3000 ppm treatment group and in the heart and testes of males at 1000 ppm were considered to be secondary toxic effects resulting from the treatment-related body weight depression and anemia.

Treatment-related histopathological findings were observed in both sexes at the 3000 and 7000 ppm levels. Microscopic findings noted in the tissues of rats from the 50, 100, 300, and 1000 ppm groups following 13 weeks of dietary administration did not suggest a relationship to treatment. Following the 4-week recovery period for rats at 1000 and 7000 ppm, no treatment-related lesions were observed.

For both the 7000 and 3000 ppm treatment groups, observed anemia was directly associated with microscopic alterations in the bone marrow and spleen and with related lesions in the heart, liver, lungs, and kidneys. Affects were more severe in females, resulting in earlier mortality at 7000 ppm (average time to mortality was 18 days for females, as compared to 32 days for males); in the males, the longer exposure time prior to death resulted in increased severity of erythroid hyperplasia in the bone marrow. The anemia resulted in a loss of mature erythrocytes and accumulations of large numbers of nucleated erythrocyte precursors (reticulocytes) in the bone marrow and spleen. Enlargement of the spleen due to extramedullary hematopoiesis was also observed. Anemia-related hypoxemia, resulting in tachycardia, increased pulse volume and pressure, pulmonary congestion, and dyspnea, led to associated pathological lesions, including: atrial thrombosis and hypertrophy, perivascular pulmonary edema, increased numbers of alveolar macrophages, medial and endothelial cell hypertrophy of pulmonary arteries, pulmonary arterial thrombosis, and emphysema. Renal alterations associated with anemia included eosinophilic granular/proteinaceous casts, dilated cortical tubules with flattened or hypertrophied epithelial cells, and scattered individual epithelial cell necrosis.

Other microscopic changes noted in both sexes at 3000 and 7000 ppm were associated with inanition and included varying degrees of hepatocyte fatty change, central lobular hepatocyte necrosis with or without irregular areas of coagulation necrosis, reduced numbers of mineralized concretions in the kidneys, reduced parabronchial lymphoid cuffs, lymphoid hypoplasia of the spleen and lymph nodes, involution of the thymus, generalized hypoplasia of the bone marrow, reduction of thyroid follicular colloid, atrophy of the mammary gland, atrophy of skeletal muscle, acinar cell degranulation of the pancreas, azospermia and germinal epithelial atrophy or degeneration of the testes, prostatic atrophy, and anestrus. Hypertrophy of the adrenal cortex in rats at 7000 ppm was considered to be

related to stress and a contributing factor in the lymphoid hypoplasia and thymic involution observed at that treatment level.

Based upon findings following a 4-week recovery period, the effects of dietary administration of F6285 appear to be reversible.

NOEL = 300 ppm (19.9 mg/kg/day in males; 23.1 mg/kg/day in females)
LOEL = 1000 ppm (65.8 mg/kg/day in males; 78.1 mg/kg/day in females)
based on clinical anemia (reduced hematocrit, hemoglobin, mean cell volume, and mean cell hemoglobin values during treatment; increased red blood cell count during recovery)

CORE Classification: CORE-Guideline; this study satisfies the requirements for a §82-1 subchronic toxicity study in rats and is acceptable for regulatory purposes.

011176

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy Aug. 9, 1994
Section IV, Tox. Branch II (7509C)

Secondary reviewer: Susan L. Makris, M.S. Susan L Makris 8/9/94 011176
Section IV, Tox. Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic (90-day) Study - Mice (§82-1A)

TEST MATERIAL: F6285; Methanesulfonamide; Sulfentrazone

MRID No.: 430046-02 **PC Code:** 129081

STUDY NUMBER: A89-2882

SPONSOR: FMC Corporation, Philadelphia, PA

TESTING FACILITY: FMC Corporation, Toxicology Laboratory
Princeton, NJ

TITLE OF REPORT: F6285 Technical, Ninety-Day Feeding Study in Mice

AUTHOR: Donald E. Nye

REPORT ISSUED: Original Date - December 10, 1990
Revised Date - August 10, 1993
Second Revised Date: October 28, 1993

EXECUTIVE SUMMARY:

In a subchronic toxicity study, F6285 was administered by dietary admix to Charles River B6C3F1 mice (10/sex/group) at doses of 0, 50, 100, 300, 550, 1,000 and 3,000 ppm (mg/kg/day: males = 0, 10.3, 17.8, 60.0, 108.4, 194.4 and all dead by day 9; females = 0, 13.9, 29.0, 79.8, 143.6, 257.0 and all dead by day 9). There was a 4-week recovery period (10/sex/group) for 0, 550 and 1,000 ppm animals of both sexes. The following parameters were examined: mortality, clinical signs, body weights, food consumption, hematology, macroscopic pathology, organ weights and microscopic pathology.

All 3,000 ppm mice died by study day 9; histopathological evaluation revealed erythroid hypoplasia of the bone marrow and evidence of inanition. The following test article effects were observed at 550 and 1,000 ppm: decreases in body weights and/or gains; decreased erythrocytes, hemoglobin and hematocrit values; and splenic microscopic pathology (increased incidence and severity of extramedullary hematopoiesis). A 4-week recovery period reversed all of the test-article effects with the exception of the splenic hematopoietic findings in females only; however, the post-recovery splenic alterations in 1,000 ppm females were reduced in severity. **The NOEL is 300 ppm (60.0 mg/kg/day for males; 79.8 mg/kg/day for females) and the LOEL is 550 ppm (108.4 mg/kg/day for males; 143.6 mg/kg/day for females).**

Core classification is **Minimum.** This study satisfies the data requirement (§82-1A) for a 13-week subchronic toxicity study in mice.

126

I. MATERIALS, METHODS AND RESULTS

A. Statistical Analyses (Report pages 14 and 15)

Data considered normal by the use of Proc Univariate were analyzed by an Analysis of Variance (ANOVA) test. If there were differences between treatment groups and there was no unusual variability between groups (Bartlett's Test), Dunnett's test was used to compare treated group means with the control group mean. If the variance was not homogeneous (Bartlett's Test), the differences between treated and control groups were subjected to the T-test. Log transformed data were used to analyze data considered to be not normally distributed (Proc Univariate). Non-parametric methods were used if the data were still not normal (log transformed). Significant differences among groups were determined by the Kruskal-Wallis Non-Parametric ANOVA. Identification of groups that were different from the control was done by Dunn's Test for Multiple Rank Comparison.

B. Regulatory Compliance

A Good Laboratory Practice Compliance statement, Quality Assurance statement and a list of Quality Assurance inspections were included in the Report.

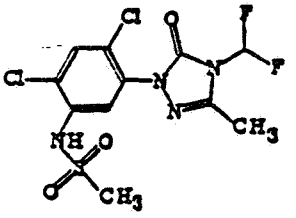
A flagging statement for potential adverse effects, 40 CFR 158.34, was included and the study neither met nor exceeded any of the applicable criteria.

A signed statement of no confidentiality claim was provided.

C. Test Article

Name: F6285 (FMC97285); methanesulfonamide; Sulfentrazone; 2-(2,4-dichloro-5-methylsulfonylamidophenyl)-4-difluoromethyl-2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one

Formula:



Purity: 99.8%
Stability: in the diet for a minimum of 58 days
Physical Description: tan solid
Reference No.: E6529-31-1

D. Dose Selection

There was no mention in the Report as to how the doses for this 90-day study were chosen. The number of ppm examined in this study were 0 (control), 50, 100, 300, 550, 1,000 and 3,000. There were 10 mice/sex/group dosed with the control or test article concentrations for at least 90 days. There were an additional 10/sex/group in the 0, 550 and 1,000 ppm groups which were placed on basal diet (recovery mice) for 4 weeks following the 90-day treatment period.

E. Test Article Stability, Concentration and Homogeneity

Table 1

DIETARY ADMIX STABILITY ANALYTICAL DATA FOR A 90-DAY MOUSE STUDY WITH F6285

Sample	Concentration (ppm)			Percent Differences	
	4/5/89	5/15/89	6/2/89	4/5-5/15	4/5-6/2
A	549	485	472	11.7	14.0
B	554	511	541	7.8	2.4
C	561	512	549	8.7	2.1

Data extracted from Report Appendix E, page 531.

Table 2

A SUMMARY OF CONCENTRATION ANALYTICAL DATA FOR A 90-DAY MOUSE STUDY WITH F6285

Week	Target Concentration (ppm)					
	50	100	300	550	1000	3000
1	41(82)	111(111)	286(95)	538(98)	1001(100)	2937
3	51(102)	103(103)	304(101)	579(105)	1115(112)	+
5	49(98)	108(108)	301(100)	579(105)	1115(112)	+
7	51(102)	103(103)	304(101)	512(93)	1012(101)	+
9	47(94)	98(98)	276(92)	630(115)	1142(114)	+
11	43(86)	86(86)	309(103)	422(77)	913(91)	+
13	44(88)	94(94)	314(105)	517(94)	960(96)	+
Mean	47(94)	101(101)	300(100)	541(98)	1025(103)	-

NOTE: Admixes for each concentration were analyzed every week (this table presents data for odd-numbered weeks only).
() = % of target concentration
+ = All mice dead before week 3.
Data extracted from Report Appendix E, page 486.

Table 3

SELECTED HOMOGENEITY ANALYTICAL DATA FOR A 90-DAY MOUSE STUDY WITH A THEORETICAL CONCENTRATION OF 550 PPM OF F6285

Date	Bottom (ppm) (20 minute)	Right (ppm) (15 minute)	Left (ppm) (15 minute)
4/6/89	549(605)a	554(611)	561(619)
5/15/89	481(530)	504(556)	506(558)
6/2/89	455(502)	521(574)	529(583)

a = The ppm are the "actual" which are based on the assay of the F6285 as active ingredient being 0.907 (active ingredient ÷ 0.907 = "actual"; i.e. 549 ÷ 0.907 = 605).
Data extracted from Report Appendix E, pages 495-499.

Stability, concentration and homogeneity were considered to be within acceptable limits.

F. Dietary Admixes

A dietary pre-mix was prepared by adding a weighed amount of finely powdered test article to about 500 g of basal diet. This hand-mixed pre-mix was then added to an appropriate amount of basal diet and mixed for about 10 minutes in a Patterson-Kelly blender. [The Reviewer presumed that each dietary concentration was prepared separately (rather than by diluting a higher concentration), although Report page 11 did not state this.] The concentration of test article for each batch was analyzed before being given to the mice. Diets were stored at room temperature until used. The Report did not describe the frequency of diet formulation.

G. Animals

Male and female, approximately 28-day old, B6C3F1 mice were received from Charles River Laboratories, Kingston, NY. There was a 23 day acclimation period prior to the start of the study. The animals were individually housed in stainless steel cages in a room with a temperature range of 66-74°F, humidity of 31-92% and a 12-hour light/dark cycle. Food and water were available ad libitum.

The mice were placed in treatment groups by a computer generated randomization program (taking into consideration weight variation).

H. Mortality, Moribundity and Clinical Signs

Observations were made once each day for clinical signs and twice each day for mortality.

Mortality was as follows:

0, 50, 100, 300 and 550 ppm = none
1,000 ppm = males, none; females, 1/20 on study day 39
3,000 ppm = males, 10/10; females, 10/10 - all by study day 9

In addition, the following four died during the terminal bleeding: 8650F, 0 ppm; 8689F, 300 ppm; 8603M, 550 ppm; and 8725F, 1,000 ppm. Also, 8633M, 1,000 ppm, died on recovery day 6 due to "mechanical injury."

Clinical signs were reported only for mice given a concentration of 3,000 ppm and included: tremors, red abdominal staining, decreased feces, ataxia, hematuria and hypersensitivity to touch. These observations were made primarily during dosing days 6-8 (all 3,000 ppm mice died by day 9).

I. Body Weights

Animals were weighed at study initiation and weekly thereafter: 13 weeks (test-article administration) and 17 weeks (test-article administration + 4 weeks for recovery).

From treatment weeks 6 through 11, there were statistically significant lower group mean body weights in males administered 550 ($p<0.05$) or 1,000 (5/6 intervals = $p<0.01$, 1/6 = $p<0.05$) ppm. Body weight gains for interval weeks 0-5, 5-9 and 9-13 for treated males showed slightly lower values compared with controls. For weeks 0-13, the control male group mean gain was 8.8 g; whereas, for the 50, 100, 300, 550 and 1,000 ppm groups, the values were 7.3, 7.[illegible], 7.3, 6.8 ($p<0.05$) and 6.7 ($p<0.05$), respectively. Recovery males (0, 550 and 1,000 ppm) showed lower ($p<0.05$) week 4 weights and lower ($p<0.01$) weeks 0-4 (start of dosing through recovery) gains at the 550 ppm dose, but not for the 1,000 ppm mice.

There were no statistically significant differences between female treated and control group mean body weights at any interval during the 13-week dosing period. Group mean body weight gains for treated groups were greater than the control means for the 13-week dosing period. During the 4-week recovery, group mean female values for body weight gains (550 and 1,000 ppm) were essentially equal to or greater than the control mean.

011176

Table 4

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 90-DAY DIETARY ADMIX MOUSE STUDY WITH F6285

Week	Males (ppm)						Females (ppm)					
	0	50	100	300	550	1000	0	50	100	300	550	1000
DOSE												
0a	24.0	24.1	24.0	24.1	24.2	24.0	19.8	19.7	19.5	19.8	19.6	19.6
1	25.1	25.2	25.2	24.8	24.8	24.8	21.3	21.2	21.3	21.7	20.8	20.9
3	26.8	26.9	27.0	26.1	26.1	25.9	22.7	22.8	23.4	23.0	22.6	22.7
5	28.4	28.2	28.2	27.9	27.5	27.2	24.3	24.5	24.6	24.4	24.2	24.5
7	29.6	29.3	29.0	28.4	28.2	28.0	25.4	25.5	26.3	25.4	25.4	25.4
9	30.9	30.0	30.1	29.6	29.3	28.8	25.7	26.8	26.4	26.1	25.5	26.0
11	32.2	30.4	31.2	30.3	30.0	29.8	25.9	26.7	26.6	25.9	26.4	26.7
13	32.8	31.4	31.4	31.4	31.0	30.6	26.0	27.3	27.8	26.9	28.2	27.4
GAIN												
0-5	4.4	4.1	4.2	3.8	3.3	3.2	4.5	4.8	5.1	4.6	4.6	4.9
5-9	2.5	1.8	1.9	1.7	1.8	1.6	1.4	2.3	1.8	1.7	1.3	1.5
9-13	1.9	1.4	1.3	1.8	1.7	1.8	0.3	0.5	1.4	0.8	2.7	[illegible]
0-13	8.8	7.3	7.3	7.3	6.8	6.7	6.2	7.6	8.2	7.1	8.5	8.
REC												
1	33.9	-	-	-	32.0	32.8	27.7	-	-	-	28.5	29.2
4	37.2	-	-	-	34.3	36.3	30.5	-	-	-	30.7	32.5
GAIN												
13-4	4.4	-	-	-	3.3	5.7	4.5	-	-	-	2.5	5.1
0-4	12.5	-	-	-	9.5	11.7	10.1	-	-	-	10.3	12.2

DOSE = dosing period
REC = recovery period
- = no recovery mice
NOTES: Body weights for 3,000 ppm males and females are not included in this Table as all mice died by dosing day 9.
Number of mice (ppm):
13 weeks of dosing: males - 0, 550 and 1,000 = 20
50, 100 and 300 = 10
females - 0, 550 and 1,000 = 20
except for 1,000 where 20 through week 5 and 19 for weeks 6-13
50, 100 and 300 = 10

4 weeks of recovery: males - 0 and 550 = 10; 1,000 = 9
females - 0 and 550 = 10; 1,000 = 9
Statistical Significance: underline of decimal point = $p<0.05$;
underline of entire number = $p<0.01$
a = "week 0" is day 1 of dosing
13-4 = the difference between dosing week 13 value and the recovery week 4 value
Body weight gains were calculated by the Reviewer except for the day 1 to recovery week 4 values which were in the Report.
Data extracted from Report Tables 1 and 2, pages 25-28.

44

J. Food Consumption

Food consumption was measured for individual animals weekly for 13 weeks (dosing only) or 17 weeks (dosing plus recovery) and was presented as grams/mouse/week.

The group mean consumption for the entire 13-week dosing period was as follows for concentrations 0, 50, 100, 300, 550 and 1,000 ppm (g/mouse/week): males = 36.9, 41.6, 36.2, 39.7, 39.4 and 37.8; females = 47.1, 49.5, 51.8, 46.2, 46.0 and 45.4. Recovery group means for the four weeks were as follows (0, 550 and 1,000 ppm): males = 37.3, 38.8 and 43.3; females = 49.8, 48.8 and 58.0. [The mean of 58.0 g/mouse/week for the 1,000 ppm females may have been partially due to spillage as the control and 550 ppm means were at least 8 g lower.]

K. Compound Consumption

The group mean compound consumptions (mg/kg body weight/day) for the 13-week dosing period for the 50, 100, 300, 550 and 1,000 ppm groups were as follows: males = 10.3, 17.8, 60.0, 108.4 and 194.4; females = 13.9, 29.0, 79.8, 143.6 and 257.0.

L. Clinical Pathology

After an overnight fast, orbital sinus blood was taken from surviving mice on dosing day 96 or recovery day 33 for the following hematology determinations: hematocrit, hemoglobin, erythrocyte count, leukocyte count, leukocyte differential, platelet count, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration.

During dosing of males, there were statistically significant ($p<0.01$) lower group mean erythrocyte, hemoglobin and hematocrit values at 550 and 1,000 ppm (also hematocrit at 300 ppm). Mean Corpuscular Volume was less than control ($p<0.05$ or 0.01) at 550 and 1,000 ppm with Mean Corpuscular Hemoglobin being less than control at 1,000 ppm ($p<0.01$). Erythrocyte group means were above the control value at 550 and 1,000 ppm ($p<0.05$ or 0.01) during the 4-week recovery period; whereas, Mean Corpuscular Hemoglobin was below ($p<0.01$) the control in both dose groups.

For females during the 13-week dosing period, there were statistically significant lower hemoglobin, hematocrit and Mean Corpuscular Volume for the 550 ($p<0.05$) and 1,000 ($p<0.01$) ppm animals. Also, at 1,000 ppm, the Mean Corpuscular Hemoglobin was below ($p<0.01$) the control. During recovery, both doses of treated females had lower Mean Corpuscular Volumes ($p<0.01$) than did the control group.

The 1,000 ppm group mean platelet count for males of 1107 ± 107.0 (S.D.) compared with a control value of 933 ± 60.7 was not statistically different, nor was this considered to be of biological significance. In recovery females, group mean platelet counts of 976 ± 216.3 at 550 ppm and 952 ± 126.4 at 1,000 ppm were greater ($p<0.05$) than the control value of 818 ± 41.8, but, due to the animal-to-animal variability and the lower post-recovery control value compared with the post-treat-treatment control value (901 ± 73.1), this was not considered to have been of toxicologicical significance.

Table 5

HEMATOLOGY PARAMETERS WHICH MAY HAVE BEEN EFFECTED BY THE DIETARY ADMIX ADMINISTRATION OF F6285 IN MICE DURING A 13-WEEK STUDY WITH A 4-WEEK RECOVERY PERIOD

PARAMETER	13-Week Dosing (ppm)						4-Week Recovery (ppm)		
	0	50	100	300	550	1000	0	550	1000
MALES									
RBC	7.8	7.3	7.6	7.4	7.2**	7.2**	7.7	8.0*	8.1**
HGB	15.1	14.2*	14.7	14.4	13.6**	12.4**	15.3	15.2	15.1
HCT	47.2	44.5	46.0	44.8**	42.4**	38.6**	47.6	47.6	47.2
MCV	61	61	61	61	59*	54**	62	60	59
MCH	19.5	19.3	19.3	19.4	19.0	17.3**	19.7	19.0**	18.8**
MCHC	32.1	31.8	32.0	32.1	32.1	32.0	32.1	31.9	32.0
FEMALES									
RBC	7.5	7.7	7.5	7.7	7.4	7.3	7.5	7.5	7.6
HGB	14.8	15.3	14.8	14.9	14.2*	13.8**	14.9	14.7	14.7
HCT	46.8	47.6	46.9	47.1	45.0*	43.3**	46.8	45.9	46.4
MCV	63	62	62	62	61*	59**	63	61**	61**
MCH	19.8	19.9	19.7	19.5	19.3	18.8**	19.8	19.6	19.5
MCHC	31.7	32.2	31.5	31.7	31.6	31.8	31.8	32.0	31.7

RBC = erythrocyte count ($x10^6/mm^3$)
HGB = hemoglobin (g/dl)
HCT = hematocrit (%)
MCV = Mean Corpuscular Volume (μm^3)
MCH = Mean Corpuscular Hemoglobin ($\mu\mu g$)
MCHC = Mean Corpuscular Hemoglobin Concentration (%)
Statistical Significance: * = $p<0.05$; ** = $p<0.01$
Data extracted from Report Tables 7 and 8, pages 37-46.

M. Sacrifice and Pathology

Surviving animals were weighed, injected intraperitoneally with Thiamylal Sodium anesthetic, exsanguinated (femoral artery) and necropsied on dosing days 97-99 or recovery days 34-35. Found dead mice were also necropsied. The following

organs were weighed and organ-to-body weight as well as organ-to-brain weight were calculated (scheduled sacrifice mice only): brain, heart, liver, kidneys, gonads, adrenals and spleen.

The following tissues were preserved and slides were prepared:

DIGESTIVE	RESPIRATORY	UROGENITAL
Salivary glands*	Trachea*	Kidneys*
Esophagus*	Lung*	Urinary bladder*
Stomach*		Testes*
Duodenum*	CARDIOV/HEMAT	Epididymides
Jejunum*	Aorta*	Prostate
Ileum*	Heart*	Ovaries
Cecum*	Bone marrow*	Uterus*
Colon*	Lymph nodes*	Cervix
Rectum*	Spleen*	Vagina
Liver*	Thymus*	
Pancreas*		
NEUROLOGIC	GLANDULAR	OTHER
Brain*	Adrenals*	Bone*
Peripheral nerve*	Lacrimal gland*	Skeletal muscle*
Spinal cord (3 levels)*	Mammary gland*	Skin
Pituitary*	Parathyroid*	
Eyes*	Thyroid*	

* = EPA Guideline Requirements [neither gallbladder nor gross lesions and masses were mentioned in Report]

Microscopic tissue examination was performed as follows:

all tissues - all mice at 0, 550 and 1,000 ppm as well as all found dead

lungs, liver, kidneys, spleen and gross lesions - all mice at 50, 100 and 300 ppm

MACROSCOPIC

There were no gross necropsy findings which were considered to be test article related.

ORGAN WEIGHTS

Report page 19 (G. Organ Weight Data) states, "Females receiving 1000 ppm had significantly increased adrenal weights and adrenal to body weight ratio and adrenal to brain weight ratios. Adrenal weights were increased, although not significantly for this group following the recovery period."

Report Table 12, page 53, presented the female group mean absolute adrenal weights (g) where the mean ± S.D. for the 0, 50, 100, 300, 550 and 1,000 ppm values (9 or 10 mice/group) were: 0.0126 ± 0.00181, 0.0139 ± 0.00249, 0.0132 ± 0.00164, 0.0141 ± 0.00240, 0.0138 ± 0.00259 and **0.0258 ± 0.03005** ($p<0.01$). Individual absolute organ weight data for 1,000 ppm females (Report Appendix A, page 180) had the adrenal weights for mouse 8721 as **0.1059** g. The individual necropsy sheet (Report page 296) for this mouse had adrenals weighing **0.0159** g. Therefore, the group mean should have been **0.0158** g.

Recovery female adrenal weights may have been heavier (not statistically significant) in the 1,000 ppm group compared with the control, although one mouse in this treated group had the organ weight considerably higher than the next lowest weight. [Group mean and individual absolute as well as relative adrenal weights were reviewed.]

This Reviewer concludes that there was no definitive effect of F6285 on any organ weights in this study.

NOTE: The Registrant needs to make appropriate changes in the tables and text of this Report, regarding female adrenal weights.

MICROSCOPIC

Histopathological examination of the 3,000 ppm males and females which died between study days 6 and 9, indicated "erythroid hypoplasia of the bone marrow and histomorphological evidence of inanition in all animals." The livers of all mice were reported to have evidence of proliferation of a cytoplasmic ultrastructural organelle. It was "suspected" that death was related to "increased erythrocyte fragility and hypoxemia secondary to increased circulating levels of protoporphyrin." [Report page 19]

The only test article effect reported was splenic extramedullary hematopoiesis.

As noted in Table 6, the number of, as well as severity in, males of splenic extramedullary hematopoiesis in 1,000 ppm mice during 13 weeks of dosing, was greater than in the control group. Most 550 ppm males also had this finding. During recovery, there was only 1 male (550 ppm) in the 0, 550 or 1,000 ppm groups with this observation (severity of 1, "minimal").

For females, 8/10 control mice had a severity of 1 or 2 ("minimal" or "mild") as compared with the 1,000 ppm group in which 6/10 had "mild" and 4/10 had "moderate" (severity of 3). Lower doses had more mice with a severity of mild than did the controls. Recovery females at 550 (10 mice) and 1,000 (9 mice)

ppm had all 19 animals with a severity of 1 or 2 compared with 6/10 controls with "normal" and 4/10 with "minimal".

Table 6

INCIDENCE AND SEVERITY OF SPLENIC INCREASED EXTRAMEDULLARY HEMATOPOIESIS IN A 13-WEEK DIETARY ADMIX MOUSE STUDY WITH F6285

ppm	Males (individual severity)										Females (individual severity)									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
DOSING																				
0	-	-	-	-	-	-	1	1	-	1	-	1	1	1	-	1	2	2	2	2
50	1	-	1	-	-	-	-	-	1	1	1	1	2	-	2	1	1	2	2	3
100	1	1	-	-	1	-	-	1	2	-	1	1	2	2	1	2	2	2	2	2
300	-	-	-	-	1	1	-	-	1	-	2	1	2	1	1	2	2	2	2	2
550	1	1	1	1	-	1	1	3	2	1	2	2	2	1	1	2	2	2	3	2
1000	1	2	-	3	1	3	3	2	3	3	2	2	2	2	3	2	3	3	3	2
3000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RECOV																				
0	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	1	-	1	-
550	-	-	-	-	-	1	-	-	-	-	1	1	1	2	2	2	2	1	1	2
1000	-	-	-	-	-	-	-	-	-	-	1	1	1	1	1	2	1	2	2	X

Numbers 1-10 = individual animal
X = only 9 tissues in this group
- = indicated change not present
Severity: 1 = minimal
2 = mild
3 = moderate
NOTE: All 3,000 ppm mice died by study day 9.
Data extracted from Report Appendix C, pages 385-472.

II. DISCUSSION

Analytical data for test article stability, concentration and homogeneity were considered to be within acceptable limits.

There was no mortality in the 0, 50, 100, 300 or 550 ppm groups. One female (from a total of 20) at 1,000 ppm died on dosing day 39. At 3,000 ppm, 10/10 males and 10/10 females died by dosing day 9. One control female, one 300 ppm female, one 550 ppm male and one 1,000 ppm female died during the terminal bleeding. A 1,000 ppm male died on recovery day 6 due to "mechanical injury."

The only clinical signs were in the 3,000 ppm mice and included: tremors, red abdominal staining, decreased feces, ataxia, hematuria and hypersensitivity to touch. These were noted primarily during dosing days 6-8.

Statistically significant ($p<0.05$ or 0.01) lower group mean body weights were observed in 550 and 1,000 ppm males from about dosing week 6 until termination. Slight decreases in weight gains in males for these doses were reported for weeks 0-5, 5-9 or 9-13; however, the gains for weeks 0-13 were below control ($p<0.05$). During the 4 weeks of recovery, there were lower ($p<0.05$) group mean weights only for the 550 ppm males. Body weight gain for 550 ppm males during recovery was less ($p<0.01$) than control.

For females, all dosed group mean body weights were similar to controls during the 13 weeks of dosing, with increases ($p<0.05$ or 0.01) in weeks 0-13 weight gain for the 550 and 1,000 ppm mice. During recovery, group mean body weights or weight gains were similar to or greater than the control values.

Food consumption for all treated groups (g/mouse/week) was equal to or greater than respective control values during both dosing and recovery.

Group mean test article consumption (mg/kg body weight/day) for the 13-week dosing period was as follows (50, 100, 300, 550 and 1,000 ppm): males = 10.3, 17.8, 60.0, 108.4 and 194.4; females = 13.9, 29.0, 79.8, 143.6 and 257.0).

There were lower (primarily $p<0.01$) group mean hemoglobin, hematocrit, MCV and MCH values in the 550 and 1,000 ppm mice compared with the respective control values (female 550 ppm MCH, similar to control). In addition, for males at 550 and 1,000 ppm, mean erythrocyte values were increased ($p<0.01$). For males during recovery, both dose group values were similar to or greater than controls with the exception of MCH which was lower ($p<0.01$). For females during recovery, only MCV values at both doses were below ($p<0.01$) control.

There were no definitive macroscopic pathology or organ weight differences between treated and control mice which appeared to be related to test article administration.

In 3,000 ppm mice that died prior to study day 9, treatment-related microscopic findings included erythroid hypoplasia of the bone marrow and histopathological evidence of inanition.

For mice that survived to 13 weeks, the only microscopic pathology findings that were interpreted as being test article related concerned the increased incidence and severity of splenic extra medullary hematopoiesis. Three of 10 control males had a severity of "minimal"; whereas, 9/10 550 ppm males had "minimal", "mild" or "moderate" and 9/10 at 1,000 ppm had the observation with 7 being "mild" or "moderate". Only one 550 ppm male had "minimal" after the recovery period. For control females, 4/10 showed "minimal", 4/10 "mild" and 2/10 "no change". At 550 ppm, 7/10 had "mild" (2 had "minimal" and 1/10 "moderate") and at 1,000 ppm, 6/10 "mild" and 4/10 "moderate". Recovery control females showed 6/10 "no change" and 4/10 "minimal" compared with 10/10 550 ppm and 9/10 1,000 ppm females

showing "minimal" or "mild". Splenic changes noted in females during recovery were judged to be a lingering effect of treatment and were considered to be reversible with additional recovery time.

The Report stated that F6285 inhibits the enzyme protoporphyrinogen oxidase which results in protoporphyrin IX accumulation. In this 90-day mouse study, hematopoiesis was affected as evidenced by changes in hematology parameters and spleen microscopic pathology.

III. CONCLUSIONS

In a subchronic toxicity study, F6285 was administered by dietary admix to Charles River B6C3F1 mice (10/sex/group) at doses of 0, 50, 100, 300, 550, 1,000 and 3,000 ppm (mg/kg/day: males = 0, 10.3, 17.8, 60.0, 108.4, 194.4 and all dead by day 9; females = 0, 13.9, 29.0, 79.8, 143.6, 257.0 and all dead by day 9). There was a 4-week recovery period (10/sex/group) for 0, 550 and 1,000 ppm animals of both sexes. The following parameters were examined: mortality, clinical signs, body weights, food consumption, hematology, macroscopic pathology, organ weights and microscopic pathology.

All 3,000 ppm mice died by study day 9; histopathological evaluation revealed erythroid hypoplasia of the bone marrow and evidence of inanition. The following test article effects were observed at 550 and 1,000 ppm: decreases in body weights and/or gains; decreased erythrocytes, hemoglobin and hematocrit values; and splenic microscopic pathology (increased incidence and severity of extramedullary hematopoiesis). A 4-week recovery period reversed all of the test-article effects with the exception of the splenic hematopoietic findings in females only; however, the post-recovery splenic alterations in 1,000 ppm females were reduced in severity. **The NOEL is 300 ppm (60.0 mg/kg/day for males; 79.8 mg/kg/day for females) and the LOEL is 550 ppm (108.4 mg/kg/day for males; 143.6 mg/kg/day for females).**

Core classification is **Minimum.** This study satisfies the data requirement (§82-1A) for a 13-week subchronic toxicity study in mice.

011176

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy Aug. 9, 1994
Section IV, Tox. Branch II (7509C)

Secondary reviewer: Susan L. Makris, M.S. Susan L Makris 8/9/94
Section IV, Tox. Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Feeding Study - Dogs (§82-1)

TEST MATERIAL: F6285 Technical; methanesulfonamide; Sulfentrazone

MRID No.: 429321-02 **PC Code:** 129081

STUDY NUMBER: Project No.: 91-3657 FMC No.: A 91-3415

SPONSOR: FMC Corporation, Agricultural Chemical Group
Philadelphia, PA

TESTING FACILITY: Bio/dynamics, Inc., East Millstone, NJ

TITLE OF REPORT: A Subchronic (3-Month) Oral Toxicity Study of F6285 (FMC 97285) in the Dog via Dietary Administration

AUTHOR: Carol S. Auletta

REPORT ISSUED: July 21, 1992 and revised August 12, 1992

EXECUTIVE SUMMARY:

In a subchronic toxicity study, F6285 was administered by dietary admix to Marshall Farms beagle dogs (4/sex/group) at doses of 0, 300, 800 and 2,000 ppm (mg/kg body weight/day: males = 0, 10, 28 and 57; females = 0, 10, 28 and 73) for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, macroscopic pathology, organ weights and microscopic pathology.

The highest dose tested (2,000 ppm) caused: lower body weights (7-10%) and weight gains in males and females mostly during the first 5 weeks of the study; decreases in hemoglobin and hematocrit (as well as MCV, MCH and MCHC); elevated alkaline phosphatase levels; increased liver weights; and microscopic liver as well as splenic changes. **The NOEL is 800 ppm (28 mg/kg/day both sexes) and the LOEL is 2,000 ppm (57 and 73 mg/kg/day, males and females).**

Core classification is **Guideline.** This study satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

I. MATERIALS, METHODS AND RESULTS

A. Statistical Analyses (Report Appendix A, pages 38-41)

MULTIPLE GROUP ANALYSIS

One-way analysis of variance, followed by a multiple comparison procedure, was used to evaluate equality of means. To determine if the groups had equal variance, Bartlett's test was performed. Parametric procedures were used if the variances were equal; if they were not equal, nonparametric were used. Parametric were one-way ANOVA and used the F distribution for significance. If there were significant differences among the means, Dunnett's test was utilized to ascertain which means were significantly different from the control. If nonparametric was needed, the Kruskal-Wallis test was employed; and if differences were indicated, the Dunn summed rank test was used.

To test for trend, standard regression techniques with a test for trend and lack of fit were used for parametric (equal variance). Jonckheere's test for monotonic trend was utilized in nonparametric.

Bartlett's test for equal variance was conducted at the 1%, 2-sided risk level. All other statistical analyses were conducted at 5 and 1%, 2-sided.

TWO GROUP ANALYSIS

The F test was used to test the variances of two groups for equality. If variances were equal, a standard t-test was used. Welch's t-test was used if variances differed at the 1% level. The t-tests were conducted at the 5 and 1%, 2-sided risk level.

B. Regulatory Compliance

A Good Laboratory Practice Compliance statement, Quality Assurance statement and a list of Quality Assurance inspections were included in the Report.

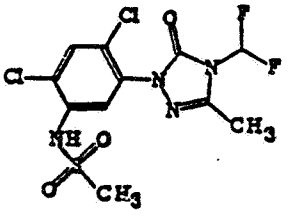
A flagging statement for potential adverse effects, 40 CFR 158.34, was included and the study neither met nor exceeded any of the applicable criteria.

A signed statement of no confidentiality claim was provided.

C. Test Article

Name: F6285 Technical (FMC 97285); methanesulfonamide; Sulfentrazone; 2-(2,4-dichloro-5-methylsulfonyl-amidophenyl)-4-difluoromethyl-2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one

Formula:



Purity: 94.2%
Lot Number: E7301-72
Description: white powder

D. Dose Selection

No criteria for doses chosen were found in this Report (i.e. acute or <90-day data).

The doses selected for this 90-day study were 0, 300, 800 and 2,000 ppm.

E. Study Design

There were 4 dogs/sex/group. The dietary concentrations were 0 (control), 300, 800 and 2,000 ppm. Each dog was presented 400 g of the appropriate diet daily for 4.5 hours.

Because of weight loss and concerns for the survival of dog 4761F (2,000 ppm), it received 400 g of canned dog food in addition to 400 g of treated diet from test day 51 to the end of the 90-day study (total of 800 g/day), resulting in a dietary concentration of 1,000 ppm instead of 2,000 ppm.

F. Test Article Stability, Concentration and Homogeneity

Report Appendix N, Tables I-IV, pages 398-407.

Table 1

TEST ARTICLE STABILITY CONDUCTED PRIOR TO A 90-DAY DIETARY ADMIX TOXICITY STUDY IN DOGS WITH F6285

Dietary Concentration	Day 4 mean % Recoveries	Week 6 mean % Recoveries	% Difference
500 ppm	97.9	94.4	-3.50
3,000 ppm	97.8	105	+7.20

Table 2

TEST ARTICLE CONCENTRATION IN A 90-DAY DIETARY ADMIX TOXICITY STUDY IN DOGS WITH F6285

Dietary Concentration	Mean % Nominal ± S.D.
300 ppm	95.2 ± 3.00
800 ppm	99.8 ± 3.90
2,000 ppm	102 ± 1.93

NOTE: All values are the mean of 4 intervals (weeks 1-5, 6-8, 9-13 and 14) with duplicate samples at each interval.

Table 3

TEST ARTICLE HOMOGENEITY CONDUCTED PRIOR TO A 90-DAY DIETARY ADMIX TOXICITY STUDY IN DOGS WITH F6285

Dietary Concentration (ppm)	Percent Recovery		
	Top	Middle	Bottom
500	99,98,100	99,96,98	99,99,98
3,000	101,100,101	101,104,96	102,101,103
300	97,97,94	99,97,97	101,97,97

Analytical data for stability, concentration and homogeneity were considered to be within acceptable limits.

G. Dietary Admixes

Report page 15 states that, "Appropriate amounts of the test substance were mixed with standard laboratory diet to achieve the desired concentrations." Control dogs received basal diet. Fresh diets were prepared once every 3-5 weeks.

H. Animals

Male and female, about 4-month old, beagle dogs were received from Marshall Farms, U.S.A., Inc., North Rose, NY. There was an acclimation period of about 4 weeks. The animals were individually housed in a room with actual temperature and humidity ranges of 66-75°F and 31-95%, respectively. There was a 12 hour light/dark cycle. Water was available ad libitum.

Based on physical examinations, body weights and clinical laboratory data, dogs were "randomly" assigned to groups.

I. Mortality, Moribundity and Clinical Signs

Observations for mortality and clinical signs were made at least twice daily. Detailed physical examinations were performed prior to the start of the study and weekly during the study.

There was no mortality.

No test article-related clinical signs were reported during the first 4 study weeks. Starting at the 5th week, 2,000 ppm male No. 4261 and female No. 4761 were observed to be thin. The female had "pale gums" during weeks 7 and 8. During the first 7 weeks of treatment, there was "poor food consumption" and body weight losses of 1.9 and 2.0 kg for the male and female, respectively. The female was given an additional 400 g of non-treated commercial canned dog food (beginning on test day 51 and continuing until study termination). Therefore, this resulted in a dietary concentration of 1,000 ppm instead of 2,000 ppm. A "thin appearance" was described through week 12 for the male and through week 10 for the female. By the time of scheduled sacrifice, both animals were considered to be "normal".

No other clinical signs considered to be test article related were reported.

J. Body Weights (Tables 4 and 5)

Animals were weighed prior to the start of dosing, weekly during treatment and at sacrifice (after fasting).

Group mean body weights and weight gains for the 300 and 800 ppm males were similar to or greater than controls throughout the study. For 2,000 ppm males, there was a group mean body weight loss for the first 5 weeks of the study, after which, the animals gained weight, and, by study week 13, the group mean weight equaled the control. Individual body weight gains (kg) during the 13 weeks of dosing were as follows: 0 ppm = 2.8, 1.7, -0.4, 1.8; 300 ppm = 2.7, 3.8, 2.8, 0.9; 800 ppm = 1.7, 1.6, 2.7, 2.4; 2,000 ppm = 1.7, 0.0, 3.0, 1.1.

The week 13 female group mean body weights at 800 and 2,000 ppm were 8.4 kg compared with a control mean of 9.1 kg, and the 13 week group mean body weight gains for these two treated groups were 1.1 kg compared with 1.8 kg for the control. Individual body weight gains during the 13 weeks of dosing were as follows: 0 ppm = 0.6, 3.2, 2.8, 0.7; 300 ppm = 1.0, 0.8, 1.8, 2.3; 800 ppm = 1.3, 0.4, 1.6, 1.1; 2,000 ppm = 1.1, 1.1, 0.5, 1.8.

Table 4

GROUP MEAN BODY WEIGHTS (kg) AND WEIGHT GAINS (kg) IN A 90-DAY DIETARY ADMIX TOXICITY STUDY IN DOGS WITH F6285

Week	Males (ppm)				Females (ppm)			
	0	300	800	2000	0	300	800	2000
B.W.								
0	8.9	8.9	8.9	8.9	7.3	7.3	7.3	7.3
1	9.1	9.1	9.1	8.9	7.6	7.3	7.5	7.1
2	9.0	9.4	9.2	8.7	7.5	7.5	7.7	7.1
3	9.1	9.6	9.4	8.5	7.5	7.6	7.9	7.1
4	9.4	10.0	9.7	8.5	7.8	7.7	7.8	7.3
7	9.7	10.1	10.1	8.8	8.3	8.1	8.0	7.3
10	10.1	10.7	10.4	9.5	8.8	8.6	8.1	7.7
13	10.4	11.5	11.0	10.4	9.1	8.8	8.4	8.4
GAIN								
0-4	0.5	1.1	0.8	-0.4	0.5	0.4	0.5	0.0
4-7	0.3	0.1	0.4	0.3	0.5	0.4	0.2	0.0
7-10	0.4	0.6	0.3	0.7	0.5	0.5	0.1	0.4
10-13	0.3	0.8	0.6	0.9	0.3	0.2	0.3	0.7
0-13	1.5	2.6	2.1	1.5	1.8	1.5	1.1	1.1

Number of dogs = 4/sex/group

Body weight gains: weeks 0-4 and 0-13 from Report; 4-7, 7-10 and 10-13 calculated by the Reviewer

Data extracted from Report Appendix E, pages 53-60.

157

Table 5

INDIVIDUAL BODY WEIGHTS FOR CONTROL AND 2,000 ppm ANIMALS IN A 90-DAY DIETARY ADMIX TOXICITY STUDY IN DOGS WITH F6285

INDIVIDUAL BODY WEIGHT VALUES - kilograms
MALES

ANIMAL NO.	SEX	WEEK -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP I			0 PPM													
1260	M	9.0	9.6	9.9	10.0	10.1	10.1	10.3	10.4	10.5	11.0	11.1	11.5	11.4	12.0	12.[illegible]
1261	M	8.8	9.1	9.5	9.5	9.7	10.7	10.2	10.3	10.5	10.7	10.6	10.7	10.7	10.6	10.[illegible]
1262	M	8.3	9.0	9.0	8.1	8.3	8.3	8.5	8.7	8.5	8.0	7.9	8.0	8.5	8.6	8.[illegible]
1263	M	8.1	8.0	8.0	8.3	8.4	8.6	8.5	8.8	9.3	9.5	9.8	10.0	10.1	9.7	9.[illegible]
MEAN		8.6	8.9	9.1	9.0	9.1	9.4	9.4	9.6	9.7	9.8	9.9	10.1	10.2	10.2	10.[illegible]
S.D.		0.4	0.7	0.8	0.9	0.9	1.2	1.0	0.9	1.0	1.4	1.4	1.5	1.2	1.6	1.[illegible]
N		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
GROUP IV			2000 PPM													
4260	M	9.0	9.3	9.2	8.5	8.5	8.6	9.4	9.4	9.5	9.7	10.1	10.0	10.3	10.8	11.[illegible]
4261	M	8.5	9.0	9.0	8.4	8.0	8.1	7.7	7.8	7.1	8.0	8.1	8.0	8.3	8.7	9.[illegible]
4262	M	8.[illegible]	8.0	8.1	8.5	8.4	8.0	8.4	8.5	8.7	9.1	9.6	9.8	10.0	10.2	11.[illegible]
4263	M	8.8	9.4	9.4	9.3	9.1	9.3	9.4	9.6	9.7	9.7	10.1	10.0	10.0	10.3	10.[illegible]
MEAN		8.7	8.9	8.9	8.7	8.5	8.5	8.7	8.8	8.8	9.1	9.5	9.5	9.7	10.0	10.[illegible]
S.D.		0.3	0.6	0.6	0.4	0.5	0.6	0.8	0.8	1.2	0.8	0.9	1.0	0.9	0.9	0.[illegible]
N		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

INDIVIDUAL BODY WEIGHT VALUES - kilograms
FEMALES

ANIMAL NO.	SEX	WEEK -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP I			0 PPM													
1760	F	6.5	6.8	7.3	7.0	7.1	7.2	7.1	7.2	6.8	7.1	7.4	7.3	7.4	7.4	7.[illegible]
1761	F	7.0	7.7	8.0	7.9	7.7	8.1	8.[illegible]	9.2	9.5	10.2	9.9	10.2	10.3	10.7	10.[illegible]
1762	F	7.1	7.8	8.1	8.3	8.4	8.7	[illegible]	9.3	9.3	9.8	9.8	10.2	10.3	10.4	10.[illegible]
1763	F	6.3	6.9	6.9	6.9	6.7	7.1		7.0	7.4	7.5	7.5	7.4	7.5	7.8	7.[illegible]
MEAN		6.7	7.3	7.6	7.5	7.5	7.8	[illegible]	8.2	8.3	8.7	8.7	8.8	8.9	9.1	9.[illegible]
S.D.		0.4	0.5	0.6	0.7	0.7	0.8	1.0	1.2	1.4	1.6	1.4	1.6	1.6	1.7	1.[illegible]
N		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
GROUP IV			2000 PPM													
4760	F	7.1	7.9	7.6	7.4	7.5	7.6	7.8	8.0	8.2	8.2	8.4	8.3	8.4	8.2	9.0
4761	F	6.4	6.7	6.5	6.1	6.2	5.6	5.1	5.2	4.7	5.5	5.8	5.9	6.4	7.5	7.[illegible]
4762	F	7.0	7.5	7.5	7.5	7.6	8.0	7.9	8.1	8.1	8.4	8.2	8.2	8.1	7.9	8.0
4763	F	6.6	7.1	7.1	7.3	7.2	7.8	7.7	7.6	8.2	8.2	8.3	8.4	8.5	8.7	8.9
MEAN		6.8	7.3	7.1	7.1	7.1	7.3	7.1	7.2	7.3	7.6	7.7	7.7	7.9	8.1	8.4
S.D.		0.3	0.5	0.6	0.7	0.6	1.1	1.4	1.4	1.7	1.4	1.3	1.2	1.0	0.5	0.6
N		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Data reproduced from Report Appendix E, pages 61-68.

K. Food Consumption (Report Appendix F, pages 85-105)

This parameter was measured prior to the start of the study and daily during the study.

Each dog was given 400 g of dietary admix for 4.5 hours every day. The remaining food was weighed and a visual estimate of spillage was made. Food consumption was expressed as g/kg body weight/day and was calculated as follows:

$$\text{g/kg/day} = \frac{\text{g/interval}}{\text{average body weight (kg)}} \div 7 \text{ days}$$

$$\text{average body weight (BW)} = \frac{\text{previous BW} + \text{current BW}}{2}$$

For males, the control, 300 and 800 ppm dogs ate an average of 32-38 g/kg/day. The 2,000 ppm males consumed an average of 22-25 g/kg/day during weeks 0-5 ($p<0.01$), 26-28 g/kg/day for weeks 6 and 7 and 31-36 g/kg/day during weeks 8-14.

Females in all 4 groups ate an average of 27-41 g/kg/day. Smaller amounts (group mean) were eaten by the 2,000 ppm group essentially only during weeks 1 and 2. Consumption values for the 2,000 ppm group for weeks 9-14 were for 3 dogs as the 4th received 400 g of treated food plus 400 g of non-treated canned food.

L. Test Article Intake

Test article intake was calculated from food consumption and was based on nominal concentrations.

Table 6

TEST ARTICLE INTAKE (mg/kg body weight/day) IN A 90-DAY DIETARY ADMIX STUDY IN DOGS WITH F6285

Nominal Concentration (ppm)	Actual Test Article Intake		Study Average Test Article Intake	
	Males	Females	Males	Females
300	9-11	8-12	10	10
800	26-30	23-31	28	28
2,000	44-71	60-84	57	73

Data reproduced from table on Report page 23.

M. Ophthalmology

Ophthalmoscopic examinations were performed on all dogs prior to test article administration and at study termination by Lionel F. Rubin, V.M.D., DACVO.

There was no indication of dose or test article related abnormality.

N. Clinical Pathology

After an overnight fast, blood was taken from the jugular vein of unanesthetized dogs prior to the start of dosing as well as at one month, two months and at study termination.

HEMATOLOGY

The following parameters were examined:

Hemoglobin*	Mean Corpuscular Volume
Hematocrit*	Mean Corpuscular Hemoglobin
Erythrocyte count*	Mean Corpuscular Hemoglobin Conc.
Reticulocyte count*	Activated partial thromboplastin time*
Platelet count*	Total leukocyte count*
Prothrombin time*	Differential leukocyte count*
Erythrocyte morphology	Bone marrow differential count (terminal)

* = EPA Guideline Requirement

NOTE: Report page 24 stated that the toxicity of F6285 was related to its mode of action. The material acts through disruption of hemoglobin biosynthesis, specifically by inhibiting protoporphyrinogen oxidase.

The 2,000 ppm concentration caused statistically significant ($p<0.05$ or 0.01) decreases in hemoglobin, hematocrit, Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) in males and females at all three intervals (months 1 and 2 as well as at termination). This concentration in females only showed lower ($p<0.01$) Mean Corpuscular Hemoglobin Concentration (MCHC) at month 2 and termination. Report page 24 indicated that these hematological changes were consistent with those reported in a preliminary study (Bio/dynamics Study No. 91-3656; not reviewed by the Agency).

Activated partial thromboplastin time (APTT) group mean values were below ($p<0.05$) controls for males and females only at month 1 with an apparent non-statistically

significant trend at month 2 and termination (not shown in Table).

No hematology effects appeared to be caused by 300 or 800 ppm nor were there any bone marrow changes reported in any treatment group.

Table 7

SELECTED GROUP MEAN HEMATOLOGY VALUES IN A 90-DAY DIETARY ADMIX TOXICITY STUDY IN DOGS WITH F6285

Parameter	Month	Males (ppm)				Females (ppm)			
		0	300	800	2000	0	300	800	2000
Hemog.	0	14.5	14.5	14.0	14.6	14.3	13.9	14.3	14.2
	1	15.5	14.9	14.5	12.7*	15.2	15.8	15.0	12.6*
	2	15.3	15.1	14.9	10.0**	15.4	15.6	15.8	11.4**
	3	15.6	15.2	15.0	10.9*	15.6	16.1	15.8	11.8**
Hemat.	0	43.0	43.2	41.3	42.9	42.2	41.1	42.3	42.7
	1	44.7	42.7	42.3	36.3*	43.5	46.1	43.4	37.0
	2	46.6	46.2	45.7	32.0*	47.5	47.5	48.6	37.6**
	3	47.6	45.6	45.1	36.4**	47.8	49.0	47.8	38.4*
Eryth.	0	6.58	6.55	6.38	6.56	6.26	5.99	6.34	6.49
	1	6.81	6.58	6.49	6.00	6.51	6.70	6.68	6.07
	2	6.77	6.78	6.81	5.65	6.70	6.67	7.21	6.36
	3	6.92	6.76	6.79	6.96	6.80	6.94	7.20	6.99
MCV	0	65.4	66.1	64.7	65.4	67.6	68.7	66.8	65.9
	1	65.7	65.0	65.2	60.5**	67.2	68.7	65.0	60.8**
	2	68.8	68.1	67.1	56.5**	70.8	71.3	67.4	59.7**
	3	68.8	67.4	66.5	52.5**	70.5	70.6	66.3	55.1**
MCH	0	22.0	22.1	22.0	22.2	23.0	23.1	22.6	22.0
	1	22.7	22.6	22.3	21.0**	23.3	23.6	22.4	20.7**
	2	22.5	22.2	21.9	17.7**	23.0	23.3	21.9	18.0**
	3	22.6	22.4	22.1	15.6*	23.0	23.2	22.0	16.9**
MCHC	0	33.7	33.5	34.0	34.0	33.9	33.7	33.8	33.3
	1	34.6	34.8	34.3	34.8	34.8	34.3	34.4	34.1
	2	32.8	32.6	32.7	31.4	32.4	32.7	32.5	30.1**
	3	32.8	33.2	33.3	29.8	32.5	32.9	33.2	30.6**

Hemog. = Hemoglobin, g/dl
Hemat. = Hematocrit, %
Eryth. = Erythrocyte count, $10^6/\mu l$
MCV = Mean Corpuscular Volume, fl
MCH = Mean Corpuscular Hemoglobin, pg
MCHC = Mean Corpuscular Hemoglobin Concentration, g/dl
Statistical Significance: * = $p<0.05$; ** = $p<0.01$
Data extracted from Report Appendix H, pages 127-135.

161

011176

CLINICAL CHEMISTRY

The following parameters were examined:

Aspartate aminotransferase*
Alanine aminotransferase*
Alkaline phosphatase
Blood urea nitrogen*
Creatinine*
Glucose*
Total protein*
Albumin*
Globulin (calculated)
A/G ratio (calculated)
Total bilirubin*
Sodium*
Potassium*
Chloride*
Calcium*
Inorganic phosphorus*

* = EPA Guideline Requirement

Table 8

SELECTED GROUP MEAN CLINICAL CHEMISTRY VALUES IN A 90-DAY DIETARY ADMIX STUDY IN DOGS WITH F6285

Parameter		Males (ppm)				Females (ppm)			
Month		0	300	800	2000	0	300	800	2000
SGPT	0	19	19	15	19	18	20	19	
	1	19	21	17	97a	22	25	20	
	2	23	26	21	106*e	26	31	23	
	3	21	24	20	37	26	28	19	
Alk Ph	0	106	102	96	132	103	115	129	
	1	97	96	121	395*b	93	104	122	4*d
	2	87	97	134	495**f	88	96	109	401*h
	3	76	91	130	358**i	88	87	99	330*j
Tot Pro	0	5.2	5.6	5.7	5.5	5.6	5.6	5.4	5.5
	1	6.3	6.3	6.3	5.3*	6.0	5.9	5.8	5.3
	2	6.3	6.4	6.3	5.4*	6.3	6.3	5.9	5.6
	3	6.0	6.0	6.1	5.1*	5.9	5.9	5.5	5.4
Albumin	0	2.8	3.0	2.9	3.1	3.1	3.1	3.0	3.1
	1	3.2	3.3	3.2	2.8**	3.5	3.5	3.3	2.9
	2	3.3	3.3	3.2	2.3**	3.5	3.5	3.4	2.8
	3	3.2	3.2	3.2	2.4**	3.3	3.3	3.2	2.8*

SGPT (Alanine Aminotransferase) = IU/L
Alk Ph (Alkaline Phosphatase) = IU/L
Tot Pro (Total Protein) = g/dl
Albumin = g/dl
INDIVIDUAL VALUES OF:

a = 36, 133, 199 and 19
b = 239, 260, 724 and 357
c = 16, 250, 15 and 13 (250, dog No. 4761F)
d = 548, 392, 185 and 209
e = 191, 147, 40 and 44
f = 460, 426, 600 and 493
g = 19, 111, 15 and 19
h = 403, 577, 330 and 292
i = 330, 404, 309 and 388
j = 287, 226, 522 and 283

Statistical Significance: * = $p<0.05$; ** = $p<0.01$
Data extracted from Report Appendix K, pages 189-269.

Serum alanine aminotransferase (SGPT) group mean values in 2,000 ppm males were elevated at months 1 and 2 primarily due to 2 dogs. In females at this dose at months 1 and 2, elevation over control values was due to one dog.

Alkaline phosphatase group mean values at 2,000 ppm in males and females were statistically ($p<0.05$ or 0.01) greater than controls at all 3 treatment intervals (elevation in all dogs at all intervals).

Total protein for the 2,000 ppm males was below control values ($p<0.05$) at all treatment intervals. For females, there were non-statistically significant lower group mean values at the treatment intervals.

Albumin group mean values for 2,000 ppm males were below controls ($p<0.01$) at all treatment intervals. At 2,000 ppm, female values were also below controls, but significance ($p<0.05$) appeared only at the terminal interval.

There were no definitive test article effects on any other clinical chemistry parameters.

O. Sacrifice and Pathology

Fasted dogs were anesthetized with sodium pentobarbital, exsanguinated and necropsied. The following organs were weighed and organ-to-body weight as well as organ-to-brain weight ratios were calculated: brain, kidneys, liver, ovaries, spleen, testes with epididymides and thyroid/parathyroid.

All of the tissues listed below were preserved and examined histopathologically for all animals with the following <u>not</u> being examined: eyes, gallbladder, mammary gland, skeletal muscle, skin, spinal cord and trachea.

DIGESTIVE	RESPIRATORY	UROGENITAL
Salivary glands*	Trachea*	Kidneys*
Esophagus*	Lungs*	Urinary bladder*
Stomach*		Testes*
Duodenum*	CARDIOV/HEMAT	Epididymides
Jejunum*	Aorta*	Ovaries
Ileum*	Heart*	Uterus*
Cecum*	Bone marrow*	Cervix
Colon*	Lymph node (mesenteric & submandibular)*	
Rectum*		
Liver*	Spleen*	
Pancreas*	Thymus*	
Gallbladder*		

011176

NEUROLOGIC	GLANDULAR	OTHER
Brain*	Adrenals*	Bone (sternum/femur)*
Peripheral nerve*	Mammary gland*	Skeletal muscle*
Spinal cord (3 levels)*	Parathyroid*	Skin
Pituitary*	Thyroid*	All gross lesions and masses*
Eyes with optic n.*		

* = EPA Guideline Requirement

MACROSCOPIC

There were no gross necropsy findings which were considered to be test article related.

ORGAN WEIGHTS

The liver was the only organ for which weight (absolute and relative) was affected by test article administration.

Table 9

GROUP MEAN ABSOLUTE AND RELATIVE LIVER WEIGHTS IN A 90-DAY DIETARY ADMIX STUDY IN DOGS WITH F6285

	Males (ppm)				Females (ppm)			
	0	300	800	2000	0	300	800	2000
Final body weight	10.3	11.1	10.8	10.3	9.0	8.6	8.2	8.1
Liver weight (g)	287	319	352*	360*	249	239	232	308*
Organ-to-body wt	2.81	2.89	3.28	3.51*	2.79	2.80	2.85	3.85*
Organ-to-brain wt	3.84	4.05	4.38	4.79	3.58	3.36	3.32	4.02

Body weights = kg
Statistical Significance: * = $p<0.05$; ** = $p<0.01$
Data extracted from Report Appendix L, pages 270-300.

Absolute and organ-to-body weight group mean liver values for males and females administered 2,000 ppm were higher ($p<0.05$ or 0.01) than control values. The 800 ppm absolute male value was also greater than control ($p<0.05$). Organ-to-brain weights at the highest dose tested were also greater than controls for both sexes.

164

Testicular/epididymis group mean absolute weight fo the 2,000 ppm dose was 12.1 g compared with a control mea of 18.1. This was due primarily to one dog which had lost 1.9 kg of body weight and had eaten less food (absolute organ weight was 5.9 g compared with 11.5, 13.5 and 17.4 g for the others in the same group). [The Report indicated that microscopic examination of the small testes confirmed them to be prepubertal."]

MICROSCOPIC

Test article related microscopic changes were reported for the liver.

Table 10

MICROSCOPIC LIVER CHANGES IN A 90-DAY DIETARY ADMIX STUDY IN DOGS WITH F6285

Observation	Males (ppm)				Females (ppm)			
	0	300	800	2000	0	300	800	2000
Sinusoidal macrophages: brown pigment	--11	--1-	----	2411	----	----	-1--	12--
Centrilobular hepatocytes: swelling	----	----	22--	2-22	----	----	----	2222
Hepatocytes: brown pigment	----	----	----	-31-	----	----	----	-1--
Periportal hepatocytes: swelling	----	----	----	-2--	----	----	----	----
Bile duct: hyperplasia	----	----	----	----	----	----	----	1---
Portal lymphoid cell infiltrate	----	----	----	----	----	----	----	1---

NOTE: the "-" or digits (1,2,3,4) represent the "finding" for an individual dog
Severity: - = finding not present
1 = minimal or very slight degree or amount present
2 = slight degree or amount present
3 = moderate degree or amount present
4 = moderately severe degree or amount present
Data extracted from Report Appendix M, pages 354-393.

The primary liver changes were: hepatocytes with brown pigment in 2/4 males and 1/4 females at 2,000 ppm compared with no other dogs in the study with this observation; centrilobular hepatocyte swelling, with a severity of 2 ("slight degree or amount present"), was reported only in 2/4 males at 800 ppm, 3/4 males at 2,000 ppm and 4/4 females at 2,000 ppm; and brown pigment in sinusoidal macrophages in 4/4 2,000 ppm males (also

increased severity) compared with 2/4 controls and 2/4 2,000 ppm females with none in controls.

There may have been the suggestion of an effect of test article administration on the presence and severity of splenic brown pigment in reticuloendothelial cells (there were statistically significant decreases in hemoglobin and hematocrit in 2,000 ppm males and females). [The study pathologist did not consider this change to be biologically significant.]

Table 11

MICROSCOPIC SPLEEN CHANGES IN A 90-DAY DIETARY ADMIX STUDY IN DOGS WITH F6285

Observation	Males (ppm)				Females (ppm)			
	0	300	800	2000	0	300	800	2000
Congestion	2142	1412	2321	1411	1421	1312	1422	1214
Siderotic nodule	----	----	----	----	----	--P-	----	----
Reticuloendothelial cells: brown pigment	1--1	111-	--11	2222	11-1	--11	11--	2211
Extramedullary hematopoiesis ..	----	-11-	--1-	[illegible]	1---	1111	1---	11--

NOTE: the "-" or digits (1,2,3,4) represent the "finding" for an individual dog
P = finding present (where grading inappropriate)
Severity: - = finding not present
1 = minimal or very slight degree or amount present
2 = slight degree or amount present
3 = moderate degree or amount present
4 = moderately severe degree or amount present
Data extracted from Report Appendix M, pages 354-393.

Considered, by the study Pathologist, to be of "questionable significance", was urothelial hyperplasia with lymphoid cell infiltrates or subacute inflammation of the urinary bladders of one control male, one 800 ppm female and two 2,000 ppm females.

II. DISCUSSION

Analytical data for test article stability, concentration and homogeneity were considered to be within acceptable limits.

There was no mortality.

The only reported clinical signs concerned one male and one female in the 2,000 ppm group. Both dogs appeared to be thin at about study week 5. During weeks 7 and 8, the female had "pale gums". There were decreased food consumption and body weight losses (1.9 and 2.0 kg for the male and female, respectively) for both dogs during the first 7 weeks of the study. In addition to the 400 g/day of treated food, the female was given 400 g of non-treated canned dog food (Treatment day 51 through study termination - 90 + days). The "thin appearance" was reported for the male through week 12 and for the female, through week 10. Both dogs were described as "normal" at study termination.

For males, there was a group mean body weight loss at 2,000 ppm during the first 5 weeks of the study, with sufficient gain during weeks 5-13 so that at sacrifice, the group mean was equal to the control. One control lost 0.4 kg during the study (others gained 2.8, 1.7 and 1.8 kg), with one 2,000 ppm male not gaining weight and the other three gaining 1.7, 3.0 and 1.1 kg.

Group mean female body weight was essentially below control values for the 2,000 ppm group throughout the study, with the 13-week weight gain being 1.1 kg compared to a control gain of 1.8 kg. At 800 ppm, after dosing week 7, there appeared to be lower group mean weights, so that by week 13, this group of dogs had gained an average of 1.1 kg.

The lower body weights for 2,000 ppm males for weeks 1-5 appeared to be a reflection of decreased food consumption during this time. For the 2,000 ppm females, group mean amounts of food consumed during the first 2 weeks of the study were lower than the control values.

Study average test article intake (mg/kg body weight/day) was as follows (300, 800, 2,000 ppm): males = 10, 28 and 57; females = 10, 28 and 73).

There were no opththalmic findings associated with test article administration.

Changes in hematology parameters were observed in the 2,000 ppm dogs of both sexes at the 1, 2 and 3 month intervals (mostly $p<0.05$ or 0.01). Hemoglobin, hematocrit, Mean Corpuscular Volume and Mean Corpuscular Hemoglobin group means were below controls. Erythrocyte group means were similar to control with Mean Corpuscular Hemoglobin Concentration being below controls ($p<0.01$) for 2,000 ppm females at the 2 and 3 month intervals. It was stated in the Report that F6285 has as its method of action, the inhibition of protoporphyrinogen oxidase, thus disrupting hemoglobin biosynthesis.

The only clinical chemistry parameter which was definitively effected by the test article was an increase in group mean (3-5 fold) and individual alkaline phosphatase levels at all three intervals at 2,000 ppm (both sexes). Albumin levels were below control (not significant, <0.05 or 0.01) at all intervals at the same dose level.

Group mean total protein values for 2,000 ppm dogs were below (not significant or $p<0.05$) controls at all intervals (both sexes).

The liver was the target organ in this study. In addition to the elevated alkaline phosphatase levels, male and female organ weights as well as organ-to-body weights were above control ($p<0.05$ or 0.01) with organ-to-brain weights also being above (not significant). Histopathologically, the incidence and severity of liver lesions (brown pigmented sinusoidal macrophages, swelling of centrilobular hepatocytes and the suggestion of brown pigmented hepatocytes) was greater in 2,000 ppm males and females than in respective controls.

The number of dogs and/or severity of splenic brown pigmented reticuloendothelial cells were increased slightly in both sexes of the 2,000 ppm group compared with respective controls. There was no firm evidence of an increase in the number of treated dogs with splenic extramedullary hematopoiesis.

III. CONCLUSIONS

In a subchronic toxicity study, F6285 was administered by dietary admix to Marshall Farms beagle dogs (4/sex/group) at doses of 0, 300, 800 and 2,000 ppm (mg/kg body weight/day: males = 0, 10, 28 and 57; females = 0, 10, 28 and 73) for 13 weeks. The following parameters were examined: mortality, clinical signs, body weights, food consumption, ophthalmology, hematology, clinical chemistry, macroscopic pathology, organ weights and microscopic pathology.

The highest dose tested (2,000 ppm) caused: lower body weights (7-10%) and weight gains in males and females mostly during the first 5 weeks of the study ; decreases in hemoglobin and hematocrit (as well as MCV, MCH and MCHC); elevated alkaline phosphatase levels; increased liver weights; and microscopic liver as well as splenic changes. **The NOEL is 800 ppm (28 mg/kg/day both sexes) and the LOEL is 2,000 ppm (57 and 73 mg/kg/day, males and females).**

Core classification is **Guideline.** This study satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

011176

FINAL

DATA EVALUATION REPORT

F6285

Study Type: Mutagenicity: Gene Mutation in Cultured Mammalian Cells (Mouse Lymphoma Cells)

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S Date 5/12/94

Independent Reviewer Marisa B. Brett, B.A. Date 5/12/94

QA/QC Manager William McLellan, Ph.D. Date 5/12/94

Contract Number: 68D10075
Work Assignment Number: 3-57
Clement Number: 239
Project Officer: Caroline Gordon

169

F6285

011176

GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATIONS

EPA Reviewer: Susan L. Makris, M.S.
Review Section III, Toxicology
Branch (II)/HED (7509C)

Signature: Susan L. Makris
Date: 5/17/94

EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D.
Review Section II, Toxicology
Branch (II)/HED (7509C)

Signature: Byron T. Backus
Date: 5/17/94

DATA EVALUATION REPORT

CHEMICAL: F6285

TOX. CHEM. NUMBER: Not available

PC Code: 129081

STUDY TYPE: Mutagenicity: Gene mutation in cultured mammalian cells (mouse lymphoma cells)

MRID Number: 430046-04

SYNONYMS: None provided

SPONSOR: FMC Corporation, Princeton, NY

TESTING FACILITY: Microbiological Associates, Inc., Rockville, MD

TITLE OF REPORT: L5178Y $TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay

AUTHORS: Bigger, C.A.H. and Clarke, J.J.

STUDY NUMBER: TA 136.701020; FMC Project No. A91-3434

REPORT ISSUED: March 20, 1992

EXECUTIVE SUMMARY: In two independently performed mouse lymphoma L5178Y $TK^{+/-}$ forward mutation assays, F6285 nonactivated doses of 424, 522, 620, 718, 817, 915, 1013, 1112, 1210, and 1308 µg/mL (Trial 1) and 1308, 1407, 1505, 1603, 1702, 1800, 2000, 2400, 2700, and 3000 µg/mL (Trial 2) were evaluated. In the S9-activated phase of testing, F6285 doses of 424, 620, 817, 1013, 1112, 1210, 1308, and 1407 µg/mL (Trial 1) and 915, 1013, 1112, 1210, 1308, 1407, 1505, 1603, 1702, and 1800 µg/mL (Trial 2) were assayed. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1242/1254 (2:1), and F6285 was delivered to the test system in dimethyl sulfoxide.

In the presence of S9 activation, the test material was cytotoxic (≈1800 µg/mL) but did not induce a mutagenic response. Results from the first nonactivated trial also indicated that F6285 at levels up to 1308 µg/mL was

170

not mutagenic. However, dose-related increases in the mutation frequencies (MFs) occurred at precipitating levels (≥2400 µg/mL) in the absence of S9 activation in the second trial. Although we conclude that the results are not sufficient to classify F2685 as mutagenic, we do consider the findings to be equivocal (see Section D, Reviewers' Discussion and Interpretation of Results).

The study is classified as **Acceptable** and satisfies the guideline requirements for an in vitro mammalian cell mutation assay (84-2).

A. MATERIALS:

1. Test Material: F6285

Description: Tan powder
Identification No.: Lot no. E 7301-72
Purity: 94.2%
Receipt date: September 24, 1991
Stability: Not provided
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO).
Other provided information: The test material was stored at room temperature, protected from light. Dosing solutions prepared for the initial and confirmatory assays were analyzed for actual concentrations.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMSO to yield final concentrations of 0.25 and 0.50 µL/mL.

Activation (concentrations, solvent): 7,12-Dimethylbenz(a)anthracene (DMBA) was prepared in DMSO to yield final concentrations of 2.5 and 5.0 µg/mL.

3. Activation: S9 derived from adult male Sprague-Dawley

x Aroclor 1242/1254 (2:1 mixture)	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 liver homogenate was prepared by the performing laboratory. Prior to use, the S9 fraction was characterized for its ability to convert 2-aminoanthracene and DMBA to mutagenic forms.

S9 mix composition:

Component	Concentration/mL of S9 mix
NADP	6.0 mg
Isocitric acid	11.25 mg
S9 homogenate	0.25 mL
Culture medium	0.75 mL

4. Test Cells: Mammalian cells in culture

__x__ mouse lymphoma L5178Y cells
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

__x__ thymidine kinase (TK)
selection agent:
(give concentration)
_____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
3 µg/mL trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent:
(give concentration)
_____ 8-azaguanine (8-AG)
_____ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
Selection agent:
(give concentration)
_____ ouabain

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Cytotoxicity assay: Nine doses (0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, and 5000 µg/mL) were evaluated in the presence and absence of S9 activation.

(b) Mutation assay:

(1) Nonactivated conditions:

Initial assay: 227 to 2000 µg/mL, cultures exposed to 424, 522, 620, 718, 817, 915, 1013, 1112, 1210, or 1308 µg/mL were cloned.

Confirmatory assay: 718 to 3000 µg/mL, cultures exposed 1308, 1407, 1505, 1603, 1702, 1800, 2000, 2400, 2700, or 3000 µg/mL were cloned.

(2) S9-activated conditions:

Initial assay: 424 to 3000 μg/mL; cultures exposed to 424, 620, 817, 1013, 1112, 1210, 1308, or 1407 μg/mL were cloned.

Confirmatory assay: 325 to 1800 μg/mL; cultures exposed to 915, 1013, 1112, 1210, 1308, 1407, 1505, 1603, 1702, or 1800 μg/mL were cloned.

B. TEST PERFORMANCE:

1. Cell Treatment:

(a) Cells exposed to test compound, solvent, or positive control for: 4 hours (nonactivated) 4 hours (activated)

(b) After washing, cells were cultured for 2 days (expression period) before cell selection

(c) After expression, cells were cultured for 10 to 12 days in selection medium to determine numbers of mutants and for 10 to 12 days without selection medium to determine cloning efficiency. (CE)

2. Statistical Methods: The data were not evaluated statistically.

3. Evaluation Criteria:

(a) Assay validity: For the assay to be considered valid, the following criteria must be satisfied: (1) the CE of the solvent control must exceed 50%; (2) the mutation frequency (MF) of the solvent control must be between 0.2 and 1.0 mutant colonies/10^4 survivors; and (3) the MF of the positive controls must be ≥2-fold higher than the corresponding solvent control value.

b. Positive response: The test material was considered positive if it induced a reproducible dose-related increase in the MF that exceeded 2 times the MF of the solvent control at one or more doses with ≥10% total survival.

C. REPORTED RESULTS:

1. Cytotoxicity Assays: Doses evaluated in the preliminary cytotoxicity assay ranged from 0.5 to 5000 μg/mL +/-S9. There was no indication in the report that the test material was insoluble at any level. No cells survived treatment with 5000 μg/mL +/- S9. For the remaining treatment groups, survival was generally dose-related and ranged from 32% at 1000 μg/mL to 99% at 100 μg/mL under nonactivated conditions and from 67% at 1000 μg/mL to 102% at 100 μg/mL in the presence of S9.

Based on these findings, doses selected for the initial mutation assay were 227 to 2000 μg/mL -S9 and 424 to 3000 μg/mL +S9.

2. Mutation Assays:

(a) Nonactivated conditions: Representative data from the initial and confirmatory assays conducted with F6285 are presented in Table 1. In the initial trial, a marked reduction in cell survival (i.e. ≤9% of the solvent control) was reported at 1407 and 1603 μg/mL. These cultures were not plated for mutant selection. Presumably, levels >1603 μg/mL were severely cytotoxic since no data were reported. At 1308 μg/mL, relative survival posttreatment was 23%. There was, however, no evidence of a mutagenic effect at any concentration. The first confirmatory assay was aborted due to the poor CEs for the solvent control groups; results presented in Table 1 are, therefore, from the successfully completed confirmatory trial conducted with a dose range of 1308 to 3000 μg/mL. No explanation was provided for increasing the dose levels or for using the highest cloned dose (1308 μg/mL) in the initial trial as the low dose for the confirmatory assay. Posttreatment survival data for the confirmatory trial were not in good agreement with the findings from Trial 1. As shown, a much higher level (2700 μg/mL) was required in the confirmatory trial to induce a cytotoxic effect comparable (i.e., ~25% relative suspension growth) to that observed in Trial 1 at 1308 μg/mL. Similarly, doses ≥1407 μg/mL in Trial 1 were too cytotoxic to be cloned. Findings from the confirmatory trial also showed dose-related increases in total mutant colonies and ≥2-fold increases in the MF at 2400 to 3000 μg/mL. Colony size distribution analysis indicated an increase in the frequency of small colonies (i.e., ≤0.6 mm) with no appreciable rise in large colony mutants. This finding suggests that the effect at the TK locus may have occurred primarily through a clastogenic mechanism.

Based on the overall findings, the study authors concluded that the results were equivocal for the following reasons. The increased MFs were seen at precipitating doses of the test article. The MF for the solvent control group was low. The MFs for the 2400- and 2700-μg/mL treatment groups fell within the historical range for the solvent control.

(b) S9-Activated assays: Cultures exposed to doses ranging from 424 to 1603 μg/mL were cloned in the initial trial and cells treated with 915 to 1800 μg/mL were cloned in the successfully completed confirmatory trial. Representative findings are presented in Table 2. Although posttreatment survival data were not in full agreement between the two trials, the difference was not as dramatic as noted for the nonactivated assays. We, therefore, attributed this discrepancy to the narrow dose ranges that were investigated and the likelihood that minor dosing errors

TABLE 1. Representative Results of the Nonactivated Mouse Lymphoma Forward Mutation Assays with F6285

Substance	Dose	Percent Relative Suspension Growth	Mutant Colonies[a] ±S.D.	Viable Colonies[a] ±S.D.	Percent Relative Cloning Efficiency	Percent Relative Total Growth	Mutation Frequency per 10^6 survivors[b]	Fold Increase
Solvent Control								
Dimethyl sulfoxide	1%	100[d]	39	219	100	100	35.6	--
(Test Material)	1%	100[e]	27	216	100	100	25.0	--
Positive Control[f]								
Ethylmethane sulfonate	0.25 µL/mL	79[d]	311±23	175±3	83	66	355.4	10.0
	0.25 µL/mL	78[e]	272±30	135±8	72	56	403.0	16.1
Test Material								
F6285	1308 µg/mL[g,h]	23[d]	35±5	186±3	85	20	37.6	1.1
	1603 µg/mL[g]	60[e]	24±4	155±11	72	43	31.0	1.2
	1702 µg/mL	58	33±4	195±7	90	52	33.8	1.3
	1800 µg/mL[i,j]	58	33±5	189±8	88	51	34.9	1.4
	2400 µg/mL[j]	37	50±9	178±10	83	31	56.2	2.2
	2700 µg/mL[j]	26	55±7	185±5	86	22	59.5	2.4
	3000 µg/mL[j]	15	86±8	168±9	78	12	102.4	4.1

[a]Means and standard deviations from the counts of triplicate plates from single cultures. Values without standard deviations (solvent control) were from at least duplicate cultures (3 plates/culture) that were reported separately by the study authors. Presented average values were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 200$

[c]Fold Increase = $\frac{\text{MF (treatment group)}}{\text{MF (solvent control group)}}$

[d]Results from the initial trial
[e]Results from the confirmatory trial
[f]Two levels of the positive control were assayed; results from the lower dose were selected as representative. Independent solvent control cultures were also processed for the positive control group; since there MF were comparable to the solvent control cultures for the test material, they were not selected as representative data.
[g]Findings for lower doses (424, 522, 620, 718, 817, 915, 1013, 1112, or 1210 µg/mL--initial trial or 1308, 1407, or 1505 µg/mL--confirmatory trial) did not suggest a mutagenic response.
[h]Higher levels (1407 and 1603 µg/mL) in the initial trial were too cytotoxic to be cloned.
[i]Cells exposed to 2000 µg/mL were reported to be lost; no further explanation was provided.
[j]Reported to be precipitating levels of the test material.

Note: Data were extracted from the study report, pp. 18, 19, 22, and 23.

TABLE 2. Representative Results of the S9-activated Mouse Lymphoma Forward Mutation Assays with F6285

Substance	Dose	Percent Relative Suspension Growth	Mutant Colonies[a] ±S.D.	Viable Colonies[a] ±S.D.	Percent Relative Cloning Efficiency	Percent Relative Total Growth	Mutation Frequency Per 10^6 Survivors[b]
Solvent Control							
Dimethyl sulfoxide	1%	100[c]	45	204	100	100	44.1
	1%	100[d]	28	175	100	100	32.0
Positive Control[e]							
7,12-Dimethylbenz(a) anthracene	2.5 µg/mL	72[c]	209±7	199±4	96	84	210.1
	2.5 µg/mL	74[d]	151±1	170±6	97	74	177.6
Test Material							
F6285	1407 µg/mL	18[c,f,g]	49±4	197±16	97	17	49.7
	1800 µg/mL	13[d,f]	50±2	214±8	122	16	46.7

[a]Means and standard deviations from the counts of triplicate plates from single cultures. Values without standard deviations (solvent controls) were from at least duplicate cultures (3 plates/culture) that were reported separately by the study authors. Presented average values were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 200$

[c]Results from the initial trial
[d]Results from the confirmatory trial
[e]Two levels of the positive control were assayed; results from the lower dose were selected as representative. Independent solvent control cultures were also processed for the positive control group; since there MF were comparable to the solvent control cultures for the test material, they were not selected as representative data.
[f]Findings for lower doses (424, 817, 1013, 1112, 1210, or 1308 µg/mL -- initial trial or 915, 1013, 1112, 1210, 1308, 1407, 1505, 1603, or 1702 µg/mL--confirmatory trial) did not suggest a mutagenic effect. Note: The cultures treated with 620 µg/mL -- initial trial were lost; no further explanation was provided.
[g]Higher levels (1505 or 1603 µg/mL) in the initial trial were too cytotoxic to be cloned.

Note: Data were extracted from the study report, pp. 20, 21, 24, and 25.

occurred. Overall, the results indicate that S9-activated F6285 was assayed to levels that induced cytotoxicity with no evidence of a mutagenic response.

3. Analytical Determinations: Dosing solutions prepared for the initial and confirmatory nonactivated and S9-activated trials were verified analytically. Results indicated that with a single exception (82-mg/ml solution prepared for the first nonactivated trial), all dosing solutions were within ±10% of the intended concentration.

From the overall results, the study authors concluded:

"Under the conditions of the assays described in this report, F6285, Lot# E 7301-72 was found to be negative in the presence of exogenous metabolic activation and equivocal in the absence of exogenous metabolic activation based on marginal increases in mutant frequency against low background at precipitating doses."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the mutation assays were properly conducted, and we agree with the study authors' conclusions. In the presence of S9 activation, F6285 was evaluated to cytotoxic levels but failed to cause an increase in mutation at the $TK^{+/-}$ locus in mouse lymphoma cells. In the nonactivated confirmatory trial, dose-related increases in mutant colonies and the MF were observed at levels that were not excessively cytotoxic (≥2400 µg/mL). However, several factors argue against the finding being indicative of a genotoxic effect. The elevated MFs (56.2 to 102.4 mutants/10^6 survivors) fell within the generally accepted spontaneous MF range for mouse lymphoma cells (15-110 mutants/10^6 survivors)[1]. The effect was limited to precipitating levels and was not seen in the S9 activated phase of testing, although the highest S9-activated dose that was tested (1800 µg/mL) was below 2400 µg/mL, the level at which precipitation was first seen without S9 activation. Similarly, the increased incidence of small colony mutants points to an adverse effect on chromosomes rather than the induction of gene mutations[2]. Although findings from the micronucleus assay in mice conducted with the same lot number of F6285 were negative (see Data Evaluation Record 3-57/240; MRID No. 430046-05), this presumably involved exposure to nonprecipitating dose levels.

Based on the above considerations, we conclude that the data are insufficient to classify nonactivated F6285 as genotoxic in this test system. We, nevertheless, agree that the findings should be considered equivocal because elevations in the MF occurred at doses that were not evaluated in the initial nonactivated trial, or in the presence of S9 activation.

---

[1]Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. Environ. Mol. Mutagen. 12:19-36.

[2]Moore, M.M. and Clive, D. (1982). The quantitation of $TK^{-/-}$ and $HGPRT^{-}$ mutants of L5178/$TK^{-/-}$ mouse lymphoma cells at varying times posttreatment. Environ. Mutagen. 4:499-519.

E. Quality Assurance Measures: Was the test performed under GLPs? Yes. (A quality assurance statement from the performing laboratory was signed and dated March 23, 1992).

F. Appendix: No.

0111

FINAL

DATA EVALUATION REPORT

F6285

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jennifer E. Alexander Date 5/16/94
Jennifer E. Alexander, B.S.

Independent Reviewer Nancy E. McCarroll Date 5/16/94
Nancy E. McCarroll, B.S.

QA/QC Manager William L. McLellan Date 5/16/94
William L. McLellan, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-57
Clement Number: 240
Project Officer: Caroline Gordon

F6285

GUIDELINE SERIES 84: MUTAGENICITY
MICRONUCLEUS

EPA Reviewer: Susan L. Makris, M.S. Signature: Susan L Makris
Review Section IV, Date: 5/17/94
Toxicology Branch II/HED (7509C)
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Review Section III, Date: 5/17/94
Toxicology Branch II/HED (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

TOX. CHEM. NUMBER: Not provided

PC CODE: 129081

MRID NUMBER: 430046-05

TEST MATERIAL: F6285

SYNONYM(S): None listed

SPONSOR: FMC Corporation, Princeton, NJ

STUDY NUMBER(S): A91-3433 (FMC); TA136.122019 (MA)

TESTING FACILITY: Microbiological Associates, Inc., Bethesda, MD

TITLE OF REPORT: Micronucleus Cytogenetic Assay in Mice

AUTHOR(S): Donald L. Putman and Robert R. Young

REPORT ISSUED: Final Report: March 23, 1992; Amended Report: October 27, 1993

EXECUTIVE SUMMARY: In an in vivo mouse micronucleus assay, groups of five male and five female ICR mice were administered single intraperitoneal (IP) injections of 85, 170, or 340 mg/kg F6285. The test material was delivered to the animals in corn oil, and bone marrow cells were harvested 24, 48, and 72 hours posttreatment.

Based on preliminary testing, 340 mg/kg was estimated to be approximately 80% of the $LD_{50/7}$. Lethargy was noted in the high-dose animals; however, no evidence of a cytotoxic effect on the target organ was seen. Similarly, no significant increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow cells harvested for either sex at any dose or sacrifice time occurred.

This study is classified as Acceptable and satisfies the guideline requirement for an in vivo mouse micronucleus assay (84-2).

181

F6285 MICRONUCLEUS

A. MATERIALS:

1. Test Material: F6285

Description: Tan powder
Identification no.: Lot Number E7301-72
Purity: 94.2%
Receipt date: September 24, 1991
Stability: Not provided
Contaminants: None listed
Solvent used: Corn oil
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions were prepared at the time of use; samples were retained for determination of actual concentration used in the study.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Corn oil (dosing volume of 20 mL/kg) was administered by intraperitoneal (IP) injection.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was dissolved in deionized water and administered by IP injection at a final dose of 30 mg/kg.

3. Test Compound:

Route of administration: IP

Dose levels used:

- Pilot study: 10, 100, 500, 1000, or 5000 mg/kg (male mice); 5000 mg/kg (female mice)
- Toxicity study: 133, 200, 300, or 450 mg/kg (males and females)
- Repeat toxicity study: 334, 374, or 412 mg/kg (males and females)
- Micronucleus assay: 85, 170, or 340 mg/kg (males and females)

4. Test Animals:

(a) Species: mouse Strain: ICR Age: 6-8 weeks
Weight range:

- Pilot Study: 22.8-26.9 g (males), 19.4-24.3 g (females)
- Toxicity Study: 32.7-37.7 g (males), 21.9-25.2 g (females)

- Repeat Toxicity Study: 26.8-33.1 g (males), 21.5-25.3 g (females,
- Micronucleus Assay: 26.4-34.9 g (males), 22.0-27.6 g (females)

Source: Harlan Sprague Dawley, Inc., Frederick, MD

(b) Number of animals used per test dose:

- Pilot study: 5 males; 5 females at 5000 mg/kg; 2 males per group at 10, 100, 500, and 1000 mg/kg
- Toxicity study: 5 males; 5 females per group
- Repeat toxicity study: 5 males; 5 females per group
- Micronucleus assay: 15 males; 15 females per vehicle, low-, mid-, and high-dose groups (an additional group of 5 animals/sex received the high dose and were used as replacement animals in the event of unscheduled deaths in the primary group)

  5 males; 5 females per positive control groups

Note: Dosing was based on individual body weights.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr (low-, mid-, and high-dose groups)

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr x 48 hr
x 72 hr

(c) Positive control:
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): __x__ 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

__x__ bone marrow _____ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal:
1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Not specified; however, the reviewers assume that NCEs were tallied while counting the 1000 PCEs

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the test material or the vehicle, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Sacrifice time for the positive control group was 24 hours. Bone marrow cells were flushed from both femurs with fetal calf serum and centrifuged. Supernatants were discarded; pellets were resuspended in residual supernatant and spread onto slides. Prepared slides were fixed in methanol, stained with May-Grunwald and Giemsa solutions, coverslipped, coded and scored.

4. Statistical Methods: The results were evaluated for statistical significance using the Kastenbaum-Bowman tables which are based on the binomial distribution.

5. Evaluation Criteria: The test material was considered positive for micronuclei induction if a significant increase ($p \leq 0.05$) in micronucleated polychromatic erythrocytes (MPEs) compared to the vehicle control was seen. The response must be either dose- or time-dependent.

C. REPORTED RESULTS:

1. Pilot Study: This study was performed to determine the appropriate doses for the toxicity study. Groups of two male mice received single IP injections of 10, 100, 500, or 1000 mg/kg F6285, and groups of five male and five female mice received a single IP injection of 5000 mg/kg F6285. Animals were observed daily for mortality and clinical signs for 7 days; body weights were recorded immediately prior to dosing and 1 and 3 days after dosing. Deaths occurred in all animals at doses of 500, 1000, and 5000 mg/kg. Signs of toxicity seen in these animals included prostration, convulsions, and irregular breathing. No clinical signs or deaths were reported in the 10- or 100- mg/kg groups. Based on these findings, 450 mg/kg was chosen as the high dose for the toxicity study.

2. Toxicity Study: Groups of five males and five females received single IP injections of 133, 200, 300, or 450 mg/kg F6285 in order to determine the $LD_{50/7}$ for this compound. Animal observations, mortality, and body weights were recorded as described for the pilot study. Mortality was limited to the high-dose group: 5/5 males and 4/5 females died. Only two females in the high-dose group exhibited signs of toxicity, which included prostration, hunched posture, ataxia, crusty eyes, and irregular breathing. However, an $LD_{50/7}$ value could not be determined; therefore, a repeat study was initiated using a narrower range of doses.

3. Repeat Toxicity Study: The initial toxicity study protocol was used, with doses of 334, 374, and 412 mg/kg F6285. Mortality occurred in 1/5 males and 1/5 females in the high-dose group. Signs of toxicity, including prostration (mid- and high-dose), convulsions (high-dose), ataxia (high-dose), tremors (low-and high-dose), lethargy (low-dose), and irregular breathing (low-dose) were seen. Based on the combined results of the initial and repeat toxicity studies, an $LD_{50/7}$ value of 424 mg/kg was estimated. Approximately 80% of the $LD_{50/7}$ (340 mg/kg) was chosen as the starting concentration for the micronucleus assay.

4. Micronucleus Assay:

   a. Analytical determinations: Results from the analytical determinations indicated that low-, mid-, and high-dose solutions differed from the nominal concentrations by 6.8%, 1%, and 1.2%, respectively.

   b. Animal observations: Groups of 15 male and 15 female ICR mice were given a single IP injection of 85, 170, or 340 mg/kg F6285. No deaths were reported at any dose or sacrifice period. The only sign of toxicity, lethargy, was seen in 3/20 males and 3/20 females from the high-dose group.

   c. Micronucleus assay: Representative findings from the micronucleus assay are shown in Table 1. F6285 was neither cytotoxic to the target organ nor caused a significant increase in the frequency of MPEs in bone marrow cells harvested from male or female mice 24, 48, or 72 hours postexposure to 85, 170, or 340 mg/kg. By contrast, the positive control (30 mg/kg CP) induced a significant ($p \leq 0.05$) genotoxic effect in both sexes.

   From the overall results, the study author concluded that F6285 was not genotoxic in this *in vivo* assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess, in agreement with the study author, that F6285 did not increase the frequency of micronuclei in the PCEs harvested from animals treated with the test material. Acceptable doses were used based on the clear evidence of compound toxicity in the preliminary studies as well as the minimal toxic effects recorded for the high dose in the micronucleus assay. Additionally, the sensitivity of the

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with F6285

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean MPEs/1000 PCEs ±S.D.	Mean PCE/NCE Ratio
Vehicle Control								
Corn oil	20 mL	24	M	5	5000	4	0.8±0.8	0.45
			F	5	5000	2 (6)[b]	0.4±0.6 (0.6)	0.64
		48	M	5	5000	3	0.6±0.9	0.40
			F	5	5000	3 (6)	0.6±0.9 (0.6)	0.56
		72	M	5	5000	0	0.0±0.0	0.62
			F	5	5000	1 (1)	0.2±0.5 (0.1)	0.63
Positive Control								
Cyclophosphamide	30 mg	24	M	5	5000	66*	13.2±3.3	0.59
			F	5	5000	76* (142)	15.2±2.3 (14.2)	0.68
Test Material								
F6285	340 mg[c]	24	M	5	5000	2	0.4±0.6	0.52
			F	5	5000	5 (7)	1.0±0.7 (0.7)	0.70
		48	M	5	5000	1	0.2±0.5	0.47
			F	5	5000	7 (8)	1.4±1.1 (0.8)	0.65
		72	M	5	5000	2	0.4±0.9	0.61
			F	5	5000	2 (4)	0.4±0.6 (0.4)	0.55

[a]Time after compound administration by intraperitoneal injection
[b]Values in () are the combined results for both sexes, calculated by the reviewers.
[c]Results for the low- and mid-dose groups sacrificed 24, 48, or 72 hours postexposure to 85 or 170 mg/kg, respectively, did not suggest a genotoxic effect.

*Significantly higher ($p \leq 0.05$) than the corresponding vehicle control by the Kastenbaum-Bowman tables.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes

Note: Data were extracted from from the study report, pp. 16-19.

test system to detect a genotoxic response in male and female mouse bone marrow cells was shown by the significant ($p \leq 0.05$) results obtained with the positive control (30 mg/kg CP). We conclude, therefore, that F6285 was adequately tested and found to be not genotoxic in this in vivo micronucleus assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated March 23, 1992).

F. APPENDIX?: No.

FINAL 011176

DATA EVALUATION REPORT

F6285

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jennifer E. Alexander Date 5/16/94
Jennifer E. Alexander, B.S.

Independent Reviewer Nancy E. McCarroll Date 5/16/94
Nancy E. McCarroll, B.S.

QA/QC Manager William L. McLellan Date 5/16/94
William L. McLellan, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-57
Clement Number: 240
Project Officer: Caroline Gordon

19

F6285

GUIDELINE SERIES 84: MUTAGENICITY
MICRONUCLEUS

EPA Reviewer: Susan L. Makris, M.S. Signature: Susan L Makris
Review Section IV, Date: 5/17/94
Toxicology Branch II/HED (7509C)
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Review Section III, Date: 5/17/94
Toxicology Branch II/HED (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

TOX. CHEM. NUMBER: Not provided

PC CODE: 129081

MRID NUMBER: 430046-05

TEST MATERIAL: F6285

SYNONYM(S): None listed

SPONSOR: FMC Corporation, Princeton, NJ

STUDY NUMBER(S): A91-3433 (FMC); TA136.122019 (MA)

TESTING FACILITY: Microbiological Associates, Inc., Bethesda, MD

TITLE OF REPORT: Micronucleus Cytogenetic Assay in Mice

AUTHOR(S): Donald L. Putman and Robert R. Young

REPORT ISSUED: Final Report: March 23, 1992; Amended Report: October 27, 1993

EXECUTIVE SUMMARY: In an in vivo mouse micronucleus assay, groups of five male and five female ICR mice were administered single intraperitoneal (IP) injections of 85, 170, or 340 mg/kg F6285. The test material was delivered to the animals in corn oil, a bone marrow cells were harvested 24, 48, and 72 hours posttreatment.

Based on preliminary testing, 340 mg/kg was estimated to be approximately 80% of the $LD_{50/7}$. Lethargy was noted in the high-dose animals; however, no evidence of a cytotoxic effect on the target organ was seen. **Similarly, no significant increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow cells harvested for either sex at any dose or sacrifice time occurred.**

This study is classified as **Acceptable** and satisfies the guideline requirement for an in vivo mouse micronucleus assay (84-2).

A. MATERIALS:

1. Test Material: F6285

Description: Tan powder
Identification no.: Lot Number E7301-72
Purity: 94.2%
Receipt date: September 24, 1991
Stability: Not provided
Contaminants: None listed
Solvent used: Corn oil
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions were prepared at the time of use; samples were retained for determination of actual concentration used in the study.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Corn oil (dosing volume of 20 mL/kg) was administered by intraperitoneal (IP) injection.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was dissolved in deionized water and administered by IP injection at a final dose of 30 mg/kg.

3. Test Compound:

Route of administration: IP

Dose levels used:

- Pilot study: 10, 100, 500, 1000, or 5000 mg/kg (male mice); 5000 mg/kg (female mice)
- Toxicity study: 133, 200, 300, or 450 mg/kg (males and females)
- Repeat toxicity study: 334, 374, or 412 mg/kg (males and females)
- Micronucleus assay: 85, 170, or 340 mg/kg (males and females)

4. Test Animals:

(a) Species: mouse Strain: ICR Age: 6-8 weeks
Weight range:

- Pilot Study: 22.8-26.9 g (males), 19.4-24.3 g (females)
- Toxicity Study: 32.7-37.7 g (males), 21.9-25.2 g (females)

- Repeat Toxicity Study: 26.8-33.1 g (males), 21.5-25.3 g (females)
- Micronucleus Assay: 26.4-34.9 g (males), 22.0-27.6 g (females)

Source: Harlan Sprague Dawley, Inc., Frederick, MD

(b) Number of animals used per test dose:

- Pilot study: 5 males; 5 females at 5000 mg/kg; 2 males per group at 10, 100, 500, and 1000 mg/kg
- Toxicity study: 5 males; 5 females per group
- Repeat toxicity study: 5 males; 5 females per group
- Micronucleus assay: 15 males; 15 females per vehicle, low-, mid-, and high-dose groups (an additional group of 5 animals/sex received the high dose and were used as replacement animals in the event of unscheduled deaths in the primary group)

  5 males; 5 females per positive control groups

Note: Dosing was based on individual body weights.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr (low-, mid-, and high-dose groups)

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr x 48 hr
x 72 hr

(c) Positive control:
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): __x__ 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

__x__ bone marrow _____ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Not specified; however, the reviewers assume that NCEs were tallied while counting the 1000 PCEs

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the test material or the vehicle, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Sacrifice time for the positive control group was 24 hours. Bone marrow cells were flushed from both femurs with fetal calf serum and centrifuged. Supernatants were discarded; pellets were resuspended in residual supernatant and spread onto slides. Prepared slides were fixed in methanol, stained with May-Grunwald and Giemsa solutions, coverslipped, coded and scored.

4. Statistical Methods: The results were evaluated for statistical significance using the Kastenbaum-Bowman tables which are based on the binomial distribution.

5. Evaluation Criteria: The test material was considered positive for micronuclei induction if a significant increase ($p \leq 0.05$) in micronucleated polychromatic erythrocytes (MPEs) compared to the vehicle control was seen. The response must be either dose- or time-dependent.

C. REPORTED RESULTS:

1. Pilot Study: This study was performed to determine the appropriate doses for the toxicity study. Groups of two male mice received single IP injections of 10, 100, 500, or 1000 mg/kg F6285, and groups of five male and five female mice received a single IP injection of 5000 mg/kg F6285. Animals were observed daily for mortality and clinical signs for 7 days; body weights were recorded immediately prior to dosing and 1 and 3 days after dosing. Deaths occurred in all animals at doses of 500, 1000, and 5000 mg/kg. Signs of toxicity seen in these animals included prostration, convulsions, and irregular breathing. No clinical signs or deaths were reported in the 10- or 100- mg/kg groups. Based on these findings, 450 mg/kg was chosen as the high dose for the toxicity study.

2. Toxicity Study: Groups of five males and five females received single IP injections of 133, 200, 300, or 450 mg/kg F6285 in order to determine the $LD_{50/7}$ for this compound. Animal observations, mortality, and body weights were recorded as described for the pilot study. Mortality was limited to the high-dose group: 5/5 males and 4/5 females died. Only two females in the high-dose group exhibited signs of toxicity, which included prostration, hunched posture, ataxia, crusty eyes, and irregular breathing. However, an $LD_{50/7}$ value could not be determined; therefore, a repeat study was initiated using a narrower range of doses.

3. Repeat Toxicity Study: The initial toxicity study protocol was used, with doses of 334, 374, and 412 mg/kg F6285. Mortality occurred in 1/5 males and 1/5 females in the high-dose group. Signs of toxicity, including prostration (mid- and high-dose), convulsions (high-dose), ataxia (high-dose), tremors (low-and high-dose), lethargy (low-dose), and irregular breathing (low-dose) were seen. Based on the combined results of the initial and repeat toxicity studies, an $LD_{50/7}$ value of 424 mg/kg was estimated. Approximately 80% of the $LD_{50/7}$ (340 mg/kg) was chosen as the starting concentration for the micronucleus assay.

4. Micronucleus Assay:

   a. Analytical determinations: Results from the analytical determinations indicated that low-, mid-, and high-dose solutions differed from the nominal concentrations by 6.8%, 1%, and 1.2%, respectively.

   b. Animal observations: Groups of 15 male and 15 female ICR mice were given a single IP injection of 85, 170, or 340 mg/kg F6285. No deaths were reported at any dose or sacrifice period. The only sign of toxicity, lethargy, was seen in 3/20 males and 3/20 females from the high-dose group.

   c. Micronucleus assay: Representative findings from the micronucleus assay are shown in Table 1. F6285 was neither cytotoxic to the target organ nor caused a significant increase in the frequency of MPEs in bone marrow cells harvested from male or female mice 24, 48, or 72 hours postexposure to 85, 170, or 340 mg/kg. By contrast, the positive control (30 mg/kg CP) induced a significant ($p \leq 0.05$) genotoxic effect in both sexes.

   From the overall results, the study author concluded that F6285 was not genotoxic in this in vivo assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess, in agreement with the study author, that F6285 did not increase the frequency of micronuclei in the PCEs harvested from animals treated with the test material. Acceptable doses were used based on the clear evidence of compound toxicity in the preliminary studies as well as the minimal toxic effects recorded for the high dose in the micronucleus assay. Additionally, the sensitivity of the

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with F6285

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean MPEs/1000 PCEs ±S.D.	Mean PCE/NCE Ratio
Vehicle Control								
Corn oil	20 mL	24	M	5	5000	4	0.8±0.8	0.45
			F	5	5000	2 (6)[b]	0.4±0.6 (0.6)	0.64
		48	M	5	5000	3	0.6±0.9	0.40
			F	5	5000	3 (6)	0.6±0.9 (0.6)	0.56
		72	M	5	5000	0	0.0±0.0	0.62
			F	5	5000	1 (1)	0.2±0.5 (0.1)	0.63
Positive Control								
Cyclophosphamide	30 mg	24	M	5	5000	66*	13.2±3.3	0.59
			F	5	5000	76* (142)	15.2±2.3 (14.2)	0.68
Test Material								
F6285	340 mg[c]	24	M	5	5000	2	0.4±0.6	0.52
			F	5	5000	5 (7)	1.0±0.7 (0.7)	0.70
		48	M	5	5000	1	0.2±0.5	0.47
			F	5	5000	7 (8)	1.4±1.1 (0.8)	0.65
		72	M	5	5000	2	0.4±0.9	0.61
			F	5	5000	2 (4)	0.4±0.6 (0.4)	0.55

[a]Time after compound administration by intraperitoneal injection
[b]Values in () are the combined results for both sexes, calculated by the reviewers.
[c]Results for the low- and mid-dose groups sacrificed 24, 48, or 72 hours postexposure to 85 or 170 mg/kg, respectively, did not suggest a genotoxic effect.

*Significantly higher ($p \leq 0.05$) than the corresponding vehicle control by the Kastenbaum-Bowman tables.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes

Note: Data were extracted from from the study report, pp. 16-19.

test system to detect a genotoxic response in male and female mouse bone marrow cells was shown by the significant ($p \leq 0.05$) results obtained with the positive control (30 mg/kg CP). We conclude, therefore, that F6285 was adequately tested and found to be not genotoxic in this in vivo micronucleus assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated March 23, 1992).

F. APPENDIX?: No.