UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: RfD/Peer Review Report of Sulfentrazone; 2-(2,4-dichloro-5-methylsulfonylamidophenyl)-4-difluoromethyl-2,4-dihydro-5-methyl-3H-1,2,4-triazol-3-one.

CASRN: 122836-35-5
EPA Chem. Code: 129081
Caswell No.: 951

FROM: George Z. Ghali, Ph.D. G. Ghali
Manager, RfD/QA Peer Review Committee
Health Effects Division (7509C)

THRU: William Burnam
Chairman, RfD/QA Peer Review Committee
Health Effects Division (7509C)

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

The Health Effects Division-RfD/Peer Review Committee met on February 15, 1996 to discuss and evaluate the toxicology data submitted in support of Sulfentrazone registration. The Committee reconvened again on April 4, 1996 to follow-up on recommendations made in the earlier meeting and to assess the Reference Dose (RfD) for this chemical.

Material available for review consisted of data evaluation records (DERs) for a chronic toxicity/carcinogenicity study in rats (83-5 or 83-1a and -2a), a chronic feeding study in dogs (83-1b), a carcinogenicity study in mice (83-2b), a multi-generation reproductive toxicity study in rats (83-4), developmental toxicity studies in rats (83-1a) and rabbits (83-1b), subchronic feeding toxicity studies in rats, mice (82-1a) and dogs (82-1b), acute and subacute neurotoxicity studies in rats (81-7 and 82-6), and a battery of mutagenicity studies (84-2).

A. Chronic and Subchronic Toxicity:

The Committee considered the chronic toxicity phase of the rat study (83-1a, MRID No. 43345409) to be acceptable, and the data evaluation record for this study (HED Doc. No. 011834) to be adequate.

In females of the 1000 and 2000 ppm dose ranges, statistically significant, dose-related decreases in body weights (up to 11 and 19%, respectively), body weight gain (13 and 26%), and food consumption (up to 13 and 16%) were observed. Hemoglobin concentrations decreased up to 13-18%, respectively, in males receiving 2000 and 3000 ppm of the test material, and up to 15-25%, respectively, in females receiving 1000 and 2000 ppm. Corresponding statistically significant decreases in hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) occurred at the same doses in both sexes. Statistically significant increases occurred in the nucleated red blood cell (NRBC) count in peripheral blood and in the reticulocyte count in bone marrow for females receiving 2000 ppm and in the red blood cell (RBC) count in males and females receiving the 2000 and 3000 ppm diets. The DER stated that the clinical pathology findings were consistent with the reported mechanism of action of sulfentrazone, i.e. inhibition of protoporphyrinogen oxidase and disruption of heme synthesis. A significant increase in the incidence of cataracts was noted among females of the 2000 ppm group. Cataracts were also observed in males of the 3000 ppm group, highest dose tested. On this basis, the NOEL/LOEL were considered to be 1000 ppm (40 and 36.4 mg/kg/day in males and females, respectively) and 2000 ppm (82.8 and 67.0 mg/kg/day for males and females, respectively).

The Committee considered the chronic toxicity study in dogs (83-1b, MRID No. 43345406) to be acceptable and the data evaluation record (HED Doc No. 011834) to be adequate.

Treatment with the test material induced a compensated RBC normochromatic microcytosis in both males and females receiving 1800 ppm, the highest dose tested. Serum alkaline phosphatase activity of males of the 1800 ppm group and females of the 800 and 1800 ppm groups was increased. However, because of the variability of findings at 800 ppm and lack of supportive evidence for toxicity at that dose, the increased alkaline phosphatase activity was not considered to be adverse. The only microscopic treatment-related effects at necropsy were found in the liver and gallbladder and consisted primarily of cytoplasmic pigmentation (minimal at 800 ppm, but with increased severity at 1800 ppm). The biological significance of pigmentation was questioned by the Committee. The Committee first considered the pigmentation to be a marker of other toxic effects but not a toxic effect by itself. However, the Committee determined that further investigation might be necessary.

The induction of normochromatic microcytosis in animals of the 1800 ppm group, although compensated by increased red cell production, reflects an adverse treatment-related effect. The DER stated that microcytosis may have arisen from the inhibition of heme synthesis as indicated by the presence of brown to yellow/brown pigmentation in hepatocytes and reticuloendothelial cells of the liver. On the basis of these findings the NOEL/LOEL were considered to be 24.9 and 61.2 mg/kg/day, respectively, in males and 29.6 and 61.9 mg/kg/day, respectively, in females.

The Committee did not discuss the subchronic toxicity studies in rats (MRID No. 43004601, HED Doc. No. 011176), mice (MRID No. 43004602, HED Doc. No. 011176) and dogs (MRID No. 42932102, HED Doc. No. 011176). However, these studies were considered by the scientific reviewer to be acceptable.

The Committee agreed with the reviewer's evaluation and interpretation of the chronic toxicity data and recommended no revisions to the data evaluation records as presented.

The Committee recommended that a subchronic (21-day) dermal toxicity study be submitted by the registrant.

B. Carcinogenicity:

The Committee considered the carcinogenicity phases of the combined chronic toxicity/carcinogenicity studies in rats (83-2a, MRID No. 43345409) and mice (83-2b, MRID No. 43345407) to be acceptable and the data evaluation records (HED Doc. No. 011834) were considered adequate. The highest dose levels tested in both the rat (3,000 ppm) and mouse (2,000 ppm) studies were considered to be a adequate for carcinogenicity testing in these strains of rats and mice.

In both rat and mouse studies, there were no treatment-related increases in tumors of any kind observed at any dose level. The Committee concluded that the treatment did not alter the spontaneous tumor profile in these strains of rat and mouse.

The Committee recommended that the chemical be classified as "Group E", evidence of none carcinogenicity for humans; i.e. the chemical is not likely to be carcinogenic to humans via relevant routes of exposure. This weight of the evidence judgment is largely based on the absence of significant tumor increases in two adequate rodent carcinogenicity studies. It should be noted, however, that the designation of an agent as being in Group E is based on the available evidence and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

C. Reproductive and Developmental Toxicity:

The Committee considered the reproductive toxicity study in rats (83-4, MRID No. 43345408) to be acceptable and the data evaluation record (HED Doc. No. 011834) to be adequate.

The reproductive/developmental toxicity NOEL/LOEL were considered to be 200 ppm (14 and 16 mg/kg/day for males and females, respectively) and 500 ppm (33 and 40 mg/kg/day for males and females, respectively). At 500 and 700 ppm (46 and 56 mg/kg/day, respectively), treatment-related effects included decreased body weight gain during gestation in both generations, and reduced premating body weight gains in the second generation (F1) males. Gestation body weight gain decrements were the result of litter loss. Reductions in F1 male premating body weight gain may have been the result of developmental toxicity in these animals. For both generations, the following were noted: increased duration of gestation, reduced prenatal viability (fetal and litter), reduced litter size, increased number of stillborn pups, reduced pup and litter postnatal survival, and decreased pup body weights throughout lactation. In the F1 generation males at 500 and 700 ppm, reduced male fertility and degeneration and/or atrophy of the germinal epithelium of the testes and oligospermia and intratubular degeneration of seminal product in the epididymides were observed.

The Committee reviewed the oral developmental toxicity study in rats (83-3a, MRID No. 42932103, 42932104) and supplemental data submitted to further clarify the study findings (MRID No. 42932104) and considered these studies to be acceptable and the data evaluation records (HED Doc. No 011176 and 011834, respectively) to be adequate. The maternal NOEL was determined to be 25 mg/kg/day. The maternal LOEL, 50 mg/kg/day, was based upon increased spleen-to-brain weight ratios and increased splenic extramedullary hematopoiesis. Observations of significantly decreased gestation body weight change and vaginal bleeding in the dams were attributed to fetal loss during gestation. It was noted that the developmental NOEL (10 mg/kg/day) was lower than the maternal NOEL. The developmental LOEL of 25 mg/kg/day was based upon decreased fetal weight and retardation in skeletal development (increased number of litters with any variation and decreased numbers of caudal vertebral and metacarpal ossification sites). Also at the highest dose tested (50 mg/kg/day), increased early and late resorptions, increased percent dead fetuses, decreased fetuses/litter, and overall increased incidences of fetal variations and malformations, including increased numbers of fetuses with anasarca, short ribs, and bent radius and ulna, were observed. The supplemental study demonstrated that administration of Sulfentrazone to the dams during gestation did not result in fetal cardiac anomalies.

A dermal prenatal developmental toxicity study in rats was

reviewed by the Committee (83-3a, MRID No. 43004603, 42932105); the study was considered to be acceptable and the data evaluation record was judged to be adequate (HED Doc. No. 011176). A maternal NOAEL of 250 mg/kg/day was identified, with a LOAEL >250 mg/kg/day. Treatment-related vaginal bleeding observed at 250 mg/kg/day was not correlated to fetal loss and not considered a toxic effect. The developmental NOEL, 100 mg/kg/day, was noted to be lower than the maternal NOEL. The developmental LOEL (250 mg/kg/day) was based upon decreased fetal body weight, and increased fetal variations (hypoplastic or wavy ribs, incompletely ossified lumbar vertebral arches, incompletely ossified ischia or pubes, and reduced number of thoracic vertebral and rib ossification sites).

An oral developmental toxicity study in rabbits (83-3b, MRID No. 42932106) was considered acceptable by the Committee, and the data evaluation record (HED Doc. No. 011176) was deemed adequate. The maternal NOEL/LOEL were identified at 100 mg/kg/day and 250 mg/kg/day, respectively. The LOEL was based upon increased abortions, clinical signs (decreased feces and hematuria), and reduced body weight gain during gestation. The developmental NOEL was determined to be 100 mg/kg/day. The developmental LOEL of 250 mg/kg/day was based upon increased resorptions, decreased live fetuses per litter, and decreased fetal weight; also at 375 mg/kg/day, there were significant increases in the fetal and litter incidences of fused caudal vertebrae (a malformation) and partially fused nasal bones (a variation), and reductions in phalanges ossification.

It was the conclusion of the Committee that, under the conditions of the studies reviewed, Sulfentrazone caused developmental and reproductive toxicity. The results of these studies elicited a high level of concern by the Committee, since the developmental toxicity studies demonstrated embryo/fetal toxicity at treatment levels that were not maternally toxic, and significant toxic effects were observed primarily in the second generation animals of the reproduction study. Because these animals had been exposed to Sulfentrazone in utero, the possibility that the observed reproductive toxicity resulted from a developmental and/or genotoxic mechanism was suggested.

D. Acute and Subchronic Neurotoxicity:

The Committee considered the acute neurotoxicity study (81-7, MRID No. 43345405) and subchronic neurotoxicity study (82-6, MRID No. 43651001) to be acceptable and the data evaluation records (HED Doc. No. 0011834) to be adequate. There was no evidence of a primary neurotoxic effect resulting from treatment with Sulfentrazone.

E. Mutagenicity:

The Committee considered the following mutagenicity studies to

be acceptable:

1) Reverse gene mutation assay in Salmonella typhimurium (MRID No. 41911601, Doc. No. 009263): The test is negative in all strains up to the highest dose level tested of 10,000 µg/plate in the presence and absence of metabolic activation system (+/-S9).

2) Mouse lymphoma L5178Y $TK^{+/-}$ forward gene mutation assay (MRID No. 43004604, Doc. No. 011176): Equivocal but dose-related positive results were recorded at precipitating levels (2400, 2700 and 3000 µg/ml in the absence of S9). Increased mutation frequencies (to 4-fold over control) were accompanied by an increase in total mutant colonies and an increased frequency of small colony mutants; suggestive of clastogenic activity. The response was neither confirmed nor seen in the S9-activated phase of testing at doses up to 1800 µg/mL. It is not known if the test material would have induced a positive effect in the presence of S9 activation had higher precipitating levels been assayed.

3) Mouse micronucleus assay (MRID No. 43004605, Doc. No. 011176): The test is negative in ICR mice up to 340 mg/kg (estimated to be ≈ 80% of the $LD_{50/7}$) administered once by intraperitoneal injection. Minimal toxicity was reported, but no bone marrow cytotoxicity was observed.

When considered in isolation, the equivocal results from the mouse lymphoma assay provide weak evidence of possible mutagenic activity. In light of the evidence from the 2-generation reproduction study and developmental toxicity studies, discussed above, which suggest a potential concern for germ cell genotoxicity, the in vitro data, suggesting a potential for clastogenicity, assume greater importance. In the 2-generation reproduction study, major indicators of germ cell genotoxicity were apparent at the highest dose tested, 700 ppm (i.e., gonadal pathology--F1 generation, reduced pregnancies--F1 generation; and reduced litter sizes--both generations). Similarly, post-implantation loss was markedly increased in the P generation and to a lesser extent in the F1 generation at the highest dose tested. Increased postimplantation loss was also observed in the P generation at the mid-dose of 500 ppm. The Committee indicated that the increased incidence of postimplantation loss in the F1 generation is of particular concern since the possibility exists that genetic defects could have been inherited by viable progeny. Crude estimates of the dominant lethality index (DLI) derived from dead implants per total implants indicate such a possibility. The estimated DLI for the parental high-dose generation was 0.54. This value is ≈7-fold higher than the expected spontaneous rate of dominant lethal in rats (0.08) and indicative of a powerful effect. The ≈2.4-fold increase in DLI for the high-dose F1 generation would also be considered a positive response.

In the two developmental oral gavage studies conducted with

rats, reproducible and significant decreases in total implants and live fetuses and significant increases in early resorptions were seen at the highest dose level of 50 mg/kg/day. Dose-related decreases in live fetuses and increases in early resorptions also occurred at the mid- and high dose (250 and 375 mg/kg/day) in the rabbit developmental study.

Given all of the above considerations, the Committee concluded that the weight-of-evidence supports a justifiable concern for a potential mutagenic risk to germinal cells. Since the overall findings suggest that highly proliferating tissue is a target for sulfentrazone, the Committee recommended that a rat dominant lethal assay should be conducted to determine if the effects noted in the reproduction and developmental studies are associated with genetic damage to male germinal cells. The assay should be performed using a 5-day dosing regime with doses and route of administration selected to insure optimal conditions to detect test material/target cell interaction.

F. Structural-Activity Relationship (SAR):

Sulfentrazone is structurally similar to four other pesticide products. These pesticides are Flumioxazin (PC Code 129034), Oxadiazon (PC Code 109001), LS 82-556 (FEBS Letters 245: 35-38, 1989), and M&B 39279 (FEBS Letters 245: 35-38, 1989). All these pesticides also produce protoporphyrin IX following inhibition of protoporphyrinogen oxidase.

Three of these chemicals (flumioxazin, oxadiazon, and M&B 39279) possess, like sulfentrazone, a phenyl ring attached to a heterocyclic ring. However, because of the diversity of functional groups in these molecules it is not possible to determine whether properties other than protoporphyrinogen oxidase inhibition will be common to these compounds.

Oxadiazone, on the other hand, was associated with liver tumors in rats and mice and has been classified by the HED-CPRC as a "Group C(q)" carcinogen, and as a "Group C" carcinogen by the Agency Science Advisory Panel (SAP).

G. Reference Dose (RfD):

In the meeting of February 15, the Committee recommended that an RfD for this chemical not to be established at that time since critical data deemed necessary for the overall assessment of the toxicological profile and/or hazard characterization of this chemical were lacking.

Subsequently, a meeting was held between Agency representatives and the registrant, FMC Corporation. In this meeting the registrant agreed to provide the necessary data.

In the meeting of April 15, the Committee recommended that a provisional RfD be established based upon the reproductive toxicity study in rats with a reproductive/developmental toxicity NOEL of 200 ppm (14 mg/kg/day in males and 16 mg/kg/day in females). At the next higher dose level of 500 ppm (33 mg/kg/day in males and 44 mg/kg/day in females) the following effects were observed: decreased maternal body weight and/or body weight gain during gestation in both generations (P and F1), reduced premating body weight gains in the second generation (F1 adults), increased duration of gestation in both F1 and F2 dams, reduced parental viability (fetal and litter), reduced litters size, increased number of stillborn pups, reduced pup and litter postnatal survival, and decreased pup body weights throughout gestation. Male fertility was reduced in the F1 generation at 500 and 700 ppm, with degeneration and/or atrophy of the germinal epithelium of the testes and oligospermia and intratubular degeneration of seminal product in the epididymides.

An uncertainty factor (UF) of 100 was applied to account for inter-species extrapolation and intra-species variability and an additional modifying factor (MF) of 3 was applied to account for the nature and severity of effects noted in the study and the high level of concern regarding potential mutagenic concern and other reproductive and developmental toxicity effects observed. On this basis, the RfD was estimated to be 0.05 mg/kg/day.

It should be noted that this chemical has not yet been reviewed by the FAO/WHO Joint Meeting on Pesticide Residue (JMPR) and an acceptable daily intake (ADI) has not been established.

H. Individuals in Attendance:

Peer Review Committee members and associates present were William Burnam (Chief, SAB; Chairman, RfD/Peer Review Committee), George Ghali (Manager, RfD/Peer Review Committee), Karl Baetcke (Chief, TB I), Mike Ioannou (Acting Chief, TB II), Stephen Dapson, Roger Gardner, Guruva Reddy, Henry Spencer and Rick Whiting. In attendance also was Kit Farwell, Steve Robbins, Yung Yang of HED as observers.

Scientific reviewers (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report)

Susan Makris _____

James Rowe James N. Rowe

Respective Branch Chief (Committee member; signature indicates concurrence with the peer review unless otherwise stated)

for Mike Ioannou James N. Rowe

CC: Stephanie Irene
Debra Edwards
Albin Kocialski
Mike Ioannou
Susan Makris
James Rowe
Beth Doyle
Karen Whitby
Amal Mahfouz (OW)
RfD File
Caswell File

I. Material Reviewed:

1. Emmerling, D. C. (1994). A chronic oral toxicity and oncogenicity study of F6285 technical in the rat. MRID No. 43345409. HED Doc. No. 011384. Classification: Acceptable. This study satisfies data requirement 83-5 (83-1a and 83-2a) of Subpart F of the Pesticide Assessment Guideline for chronic toxicity/carcinogenicity testing in rats.

3. Auletta, C. S. (1994). A chronic (12 month) oral toxicity study of F6285 Technical (FMC 97285) in the dog via dietary administration. MRID No. 43345406. HED Doc. No. 011384 Classification: Acceptable. This study satisfies data requirement 83-1b of Subpart F of the Pesticide Assessment Guideline for chronic toxicity testing in dogs.

4. Ponnock, K. S. (1994). A two-generation reproduction study in rats with F6285 technical. MRID No. 43345408. HED Doc. No. 011384. Classification: Acceptable. This study satisfies data requirement 83-4 of Subpart F of the Pesticide Assessment Guideline for reproductive toxicity testing in rats.

5. Freeman, C. (1992). F6285 Technical; Teratology study in rats (Oral). MRID No. 42932103. HED Doc. No. 011176. Classification: Core guideline data. This study satisfies data requirement 83-3a of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

6. Freeman, C. (1994). F6285 Technical; Modified oral teratology study in rats (cardiac). MRID No, 42932104. HED Doc. No. 011384. Classification: Supplementary data. This study does not satisfy data requirement 83-3a of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

7. Freeman, C. (1995). F6285 Technical modified oral teratology study in rats (cardiac). MRID No. 43651003. HED Doc. No. 011384. Classification: Supplementary data. This study does not satisfy data requirement 83-3a of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

8. Freeman, C. (1992). F6285 Technical; Teratology study in rats (dermal). MRID No. 43004603, 42932105. HED Doc. No. 011176. Classification: Core guideline data. This study satisfies data requirement 83-3a of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in

rats.

9. Freeman, C. (1993). F6285 Technical; Teratology study in rabbits (oral). MRID No. 42932106. HED Doc. No. 011176. Classification: Core guideline data. This study satisfies data requirement 83-3b of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rabbits.

10. Nye, D. E. (1990). F6285 Technical; Ninety-day feeding study in rats. MRID No. 43004601. HED Doc. No. 011176. Classification: Core guideline data. This study satisfies data requirement 82-1a of Subpart F of the Pesticide Assessment Guideline for subchronic toxicity testing in rats.

11. Auletta, C. S. (1992). A subchronic (3-Month) oral toxicity study of F6285 (FMC 97285) in the dog via dietary administration. MRID No. 42932102. HED Doc. No. 011176. Classification: Guideline data. This study satisfies data requirement 82-1b of Subpart F of the Pesticide Assessment Guideline for subchronic toxicity testing in dogs.

12. Nye, D. E. (1990). F6285 Technical; Ninety-day feeding study in Mice. MRID No. 43004602. HED Doc. 011176. Classification: Core minimum data. This study satisfies data requirement 82-1a of Subpart F of the Pesticide Assessment Guideline for subchronic toxicity testing in mice.

13. Freeman, C. (1995). F6285 Technical; Subchronic neurotoxicity screen in rats. MRID No. 43651002. HED Doc. No. 011384. Classification: Acceptable. This study satisfies data requirement 82-6 of Subpart F of the Pesticide Assessment Guideline for subchronic neurotoxicity testing in rats.

14. Freeman, C. (1994). F6285 Technical; Twenty-eight day neurotoxicity range-finding study in rats. MRID No. 43651001. HED No. 011384. Classification: Acceptable. This study satisfies data requirement 82-6 of Subpart F of the Pesticide Assessment Guideline for subchronic neurotoxicity testing in rats.

15. Freeman, C. (1994). F6285 technical; Acute neurotoxicity screen in rats. MRID No. 43345405. HED Doc. No. 011384. Classification: Acceptable. This study satisfies data requirement 81-7 of Subpart F of the Pesticide Assessment Guideline for acute neurotoxicity testing in rats.

16. Wojciechowski, J. and Cascieri, T. (1986). Salmonella/ Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test). MRID No. 41911611. HED Doc. NO. 009283. Classification: Acceptable. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing in rats.

17. Bigger, C.A.H. and Clarke, J. J. (1992). L1578Y TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay. MRID No. 43004604. HED Doc. No. 011176. Classification: Acceptable. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing in rats.

18. Putnam, D. L. and Young, R. R. (1992). Micronucleus Cytogenetic Assay in Mice. MRID No. 43004605. HED Doc. No. 011176. Classification: Acceptable. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for mutagenicity testing in rats.