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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: RfD/Peer Review Report of Isoxaflutole [4-(2-methylsulphonyl-4-trifluotopmethyl-benzoyl)-5-cyclopropyl isoxazole].

CASRN: 14112-29-0
EPA Chem. Code: 123000
Caswell No.:

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: Philip Errico, PM 25
Fungicide-Herbicide Branch
Registration Division (7505C)

The Health Effects Division-RfD/Peer Review Committee met on April 24, 1997 to discuss and evaluate the existing and/or recently submitted toxicology data in support of Isoxaflutole registration and to assess the Reference Dose (RfD) for this chemical.

Isoxaflutole is a new chemical proposed for use as a herbicide for the control of grasses and broadleaf weeds in field corn.

Material available for review consisted of data evaluation records (DERs) for a combined chronic toxicity/carcinogenicity study in rats **(83-5)**, a carcinogenicity study in mice **(83-2b)**, a chronic toxicity study in dogs **(83-1b)**, a reproductive toxicity study in rats **(83-4)**, developmental toxicity studies in rats and rabbits **(83-3a and -3b)**, acute and subchronic neurotoxicity studies in rats **(81-8 and 82-7)** and a battery of mutagenicity studies **(84-2)**.

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A. Chronic and Subchronic Toxicity:

The Committee considered the chronic toxicity phase (83-1a) of the combined chronic toxicity/carcinogenicity study in rats (83-5, 1995, MRID No. 43904806) to be acceptable and the data evaluation record (HED Doc. No. 012255) to be adequate.

In this study, Isoxaflutole (93-99.2% a.i.) was administered to Sprague-Dawley rats at dietary levels of 0.5, 2, 20 or 500 mg/kg/day for 104 weeks. The NOEL/LOEL were established at 2 and 20 mg/kg/day, respectively, based on liver, thyroid, ocular, and nervous system toxicity in males and liver toxicity in females.

The Committee considered the chronic toxicity study in dogs (83-1b, 1995, MRID No. 43573218) to be acceptable and the data evaluation record (HED Doc. No. 012255) to be adequate.

In this study, Isoxaflutole (98.7% a.i.) was administered to beagle dogs at dietary levels of 240, 1,200, 12,000 or 30,000 ppm (8.56, 44.41, 453, mg/kg/day for males and 8.41, 45.33, 498, or 1,254 mg/kg/day for females) for 52 weeks; all males in the high dose group of 30,000 ppm were sacrificed after 26 weeks due to severe chronic reaction to the test substance. The NOEL/LOEL were established at 1,200 and 12,000 ppm, respectively, based on reduced weight gains and intravascular hemolysis with associated clinical chemistry and hematological changes.

B. Carcinogenicity:

The Committee did not discuss the carcinogenicity phase (81-2a) of the combined chronic toxicity/carcinogenicity study in rats (83-5, 1995, MRID No. 43904806) or the carcinogenicity phase of the carcinogenicity study in mice (83-2b, 1995, MRID No. 43904807). The carcinogenicity issue had already been referred by the respective toxicology branch to the HED-Carcinogenicity Peer Review Committee (CPRC) for a weight-of-the-evidence evaluation.

C. Reproductive and Developmental Toxicity:

I. Reproductive Toxicity:

The Committee considered the reproductive toxicity study in rats (83-4, 1995, MRID 43904809) to be acceptable and the data evaluation record (HED Doc. No. 012255) to be adequate.

In this study, Isoxaflutole (98.7%) was administered to Sprague-Dawley rats in the diet at nominal doses of 0.5, 2, 20, or 500 mg/kg/day; actual doses were approximately 0.45, 1.76, 17.4, or 414 mg/kg/day for males and 0.46, 1.79, 17.7 or 437 mg/kg/day for females. The parental systemic NOEL was 1.76 mg/kg/day and the parental LOEL was 17.4 mg/kg/day, based on increased liver weights and hypertrophy in both sexes and

generations. At 500 mg/kg/day additional findings included decreased body weight, body weight gain, and/or food consumption during premating and gestation and increased incidence of subacute inflammation of the cornea of the eye in P generation adults as well as keratitis in F1 adults. Reproductive performance was not affected by treatment. The developmental/systemic NOEL for the offspring was 1.76 mg/kg/day, and the developmental systemic LOEL for the offspring was 17.4 mg/kg/day, based upon reduced litter survival in both generations (F1 and F2 pups). Litter survival was also reduced at 500 mg/kg/day; also at 500 mg/kg/day, F1 and F2 pup body weights were decreased throughout the entire lactation period and there was an increased incidence of chronic keratitis and low incidence of inflammation of the iris in both generations; retinal and vitreous bleeding were also observed in the F2 pups and weanlings. An increased number of pups with no milk in the stomach and underdeveloped renal papillae were noted in 500 mg/kg/day F1 and F2 pups at necropsy on postnatal day 4.

II. Developmental Toxicity:

The Committee considered the developmental toxicity study in rats (83-3a, 1995, MRID 43904808) to be acceptable and the data evaluation record (HED Doc. No. 011675) to be adequate.

In this study, Isoxaflutole (98.2%) was administered to Sprague-Dawley rats (25/group) by gavage at doses of 10, 100, or 500 mg/kg/day on gestation days 6-15 in 5% aqueous methylcellulose at a dose volume of 10 ml/kg. The maternal NOEL was 100 mg/kg/day, and the maternal LOEL was 500 mg/kg/day, based on an increased incidence of salivation within one and one-half hours after dosing, and on decreased body weight, body weight gain, and food consumption during the treatment period. The developmental NOEL was 10 mg/kg/day. The developmental LOEL, 100 mg/kg/day, was based on growth retardation (decreased fetal body weight and increased incidence of delayed ossification of sternebrae, metacarpals and metatarsals). Additionally, at 500 mg/kg/day, increased incidences of vertebral and rib anomalies and of subcutaneous edema were observed.

The Committee considered the developmental toxicity study in rabbits (83-3b, 1995, MRID 43904808) to be acceptable and the data evaluation record (HED Doc. No. 012255) to be adequate.

In this study, Isoxaflutole (99.6%) was administered to Zealand White rabbits (25/group) by gavage at doses of 5, 20, or 100 mg/kg/day on gestation days 6-19. The test substance was delivered in 1% aqueous methylcellulose at a dose volume of 5 ml/kg. The maternal NOEL was 20 mg/kg/day. The maternal LOEL, 100 mg/kg/day, was based on an increased incidence of clinical signs (anorexia and few feces) and on decreased body weight gain and food consumption during the dosing period. Developmental

toxicity was noted at all dose levels. At 5 mg/kg/day, the study developmental LOEL, an increased incidence of 27 presacral vertebrae was observed; the fetal incidence of this finding was distributed in a dose-dependant manner and exceeded concurrent and historical control values. At 20 and 100 mg/kg/day, additional findings included increased implantation loss and late resorptions, growth retardation (generalized reduction in skeletal ossification), and increased incidence of 13th ribs. At 100 mg/kg/day, the incidence of fetuses with incisors not erupted was also increased. The developmental NOEL was <5 mg/kg/day. Although a developmental NOEL was not identified, the Committee considered this study adequate for use in the risk assessment since the developmental LOEL of 5 mg/kg/day was believed to be at or near the developmental NOEL. The Committee recommended that benchmark dose calculation be performed to test this hypothesis.

D. FQPA Considerations:

The toxicology data base for Isoxaflutole included an acceptable two-generation reproduction study in rats and an acceptable prenatal developmental toxicity study in rats. Additionally, a prenatal developmental toxicity study in rabbits was submitted but did not establish a developmental NOEL in that species. Although there are no data gaps for the assessment of pre- and postnatal toxicity in rats, the assessment of prenatal toxicity in a non-rodent species is not complete.

The data provided no indication of increased sensitivity of rats following postnatal exposure to isoxaflutole. In the two-generation reproduction study in rats, the parental systemic NOEL (1.76 mg/kg/day), based upon increased liver weights and hypertrophy in both sexes and generations at the LOEL of 17.4 mg/kg/day, was equivalent to the developmental/systemic NOEL for the offspring. At 17.4 mg/kg/day (the developmental/systemic LOEL) decreased litter survival was observed in both generations (F1 and F2 pups).

The data from both the prenatal developmental toxicity studies in rats and rabbits suggested the potential for increased sensitivity of the fetus to *in utero* exposure to isoxaflutole. For both of these studies, the developmental NOEL was less than the maternal NOEL. In the rat study, maternal toxicity (increased incidence of salivation and decreased body weight, body weight gain, and food consumption during the treatment period) was observed at the LOEL of 500 mg/kg/day; the maternal NOEL was 100 mg/kg/day. The developmental NOEL in the rat study (10 mg/kg/day) was based on growth retardation (decreased fetal body weight and increased incidence of delayed ossification of sternebrae, metacarpals and metatarsals) at 100 mg/kg/day. In the prenatal developmental study in rabbits, no developmental NOEL was established, since the developmental LOEL (5 mg/kg/day, based upon an increased incidence of 27th presacral vertebrae)

occurred at the lowest dose tested. The maternal NOEL in this study was 20 mg/kg/day, and the maternal LOEL was 100 mg/kg/day, based on an increased incidence of clinical signs (anorexia and few feces) and on decreased body weight gain and food consumption during the dosing period.

E. Neurotoxicity:

The Committee considered the subchronic neurotoxicity study in rats (82-7, 1995, MRID No. 43904805) to be acceptable and the data evaluation record (HED Doc. No. 012255) to be adequate. The NOEL/LOEL were 250 and 750 mg/kg/day, respectively, in males, and 750 and 7750 mg/kg/day, respectively, in females based on decreases in body weight gain.

The Committee considered the neurotoxicity study in rats (81-8, 1995, MRID No. 43904804) to be acceptable and the data evaluation record (HED Doc. No. 012255) to be adequate. The NOEL/LOEL for neurobehavioral effects were 500 and 2000 mg/kg/day, respectively, in males based on impairment of neuromuscular junction. The NOEL in females was 2000 mg/kg/day, the highest dose tested.

F. Mutagenicity:

Four acceptable studies with Isoxaflutole (RPA 201772), an acceptable study with the major metabolite (RPA 202248) and an acceptable study with a minor metabolite (RPA 203328) were available for review. The following are Summaries of the acceptable studies and the Committee's conclusions.

I. Gene Mutations:

a. Isoxaflutole (RPA 201772):

1) Salmonella typhimurium reverse gene mutation assay (MRID No. 43588002, HED Doc. No. 011675): Independently performed tests were negative in S.typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 up to insoluble and non-cytotoxic doses (≥500 µg/plate +/- S9).

2) Mouse lymphoma L5178Y forward gene mutation assay (MRID No. 43573222, HED Doc. No. 011675): Independently performed tests were negative up to insoluble (≥150 µg/mL +/-S9) or soluble (≤75 µg/mL +/-S9) doses.

b. Major Metabolite (RPA 202248):

1) Salmonella typhimurium reverse gene mutation assay (MRID No. 43904811, HED Doc. No. 011675): Independently performed plate incorporation or preincubation modification to the standard plate incorporation tests were negative in S. typhimurium strains

TA1535, TA1537, TA98, TA100 and TA102 up to the highest dose assayed (5000 μg/plate +/- S9).

c. Minor Metabolite (RPA 203328)

1) Salmonella typhimurium reverse gene mutation assay (MRID No. MRID No. 43904814, HED Doc. No. 011675): Independently performed plate incorporation tests were negative in S. typhimurium strains TA1535, TA1537, TA98, and TA100 up to cytotoxic doses (≥2500 μg/plate +/- S9).

II. Chromosomal Aberrations:

a. Isoxaflutole (RPA 201772)

1) In vitro cytogenetic assay in cultured human lymphocytes (MRID NO. 43573221, HED Doc. No. 011675). The test was negative up to insoluble and non-cytotoxic concentrations (≥300 μg/mL -S9; 600 μg/mL +S9).

4) Mouse micronucleus assay (MRID No. 43573223, HED Doc. No. 011675): The test was negative in male or female CD-1 mice up to the highest administered oral gavage dose (5000 mg/kg). No evidence of an overt toxic response in the treated animals or a cytotoxic effect on the target cells was observed.

IV. Conclusions: The acceptable studies satisfy the new mutagenicity initial testing battery guidelines. Based on the findings of the acceptable studies, there is no concern for mutagenicity at this time.

G. Reference Dose (RfD):

The Committee recommended that an RfD for this chemical be established based on the chronic rat study with a NOEL of 1.74 mg/kg/day. At the next higher dose level of 17.6 mg/kg/day, liver, thyroid, ocular, and nervous system toxicity were observed in males and liver toxicity was observed in females.

The Committee considered the reproductive toxicity study in rats to be co-critical. In this study, the parental systemic NOEL was 2 mg/kg/day and the parental LOEL was 20 mg/kg/day, based on increased liver weights and hypertrophy in both sexes of the two generations.

An Uncertainty Factor (UF) of 100 was applied to account for both the interspecies extrapolation and intraspecies variability. The Committee determined that an additional UF (3X) should be applied to the risk calculations, based upon the lack of a NOEL in the developmental rabbit study and the potential for increased sensitivity to fetuses following *in utero* exposure.

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), Clark Swentzel (for Mike Ioannou, Acting Chief, TB II), Marion Copley, Byron Backus (for Nancy McCarroll), Stephen Dapson (for Susan Makris), Kit Farwell, Albin Kocialski, Henry Spencer, and Rick Whiting. In attendance also were Kathleen Raffaele and Barbara Madden of HED as observers.

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

Sanjivani Diawan Sanjivani Diwan

Jess Rowland Jess Rowland

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

Mike Ioannou M. Ioannou

CC: Stephanie Irene
Debra Edwards
Mike Ioannou
Jess Rowland
Sanjivani Diawan
Amal Mahfouz (OW)
RfD File
Caswell File

H. Material Reviewed:

1. Chase, K. R. (1995). Combined Oncogenicity and Toxicity Study by Dietary Administration to CD rats for 104 weeks. MRID No. 43904806. HED Doc. No. 012255. Classification: Acceptable.

2. Chase, K. R. (1995). Oncogenicity study by dietary administration to CD-1 mice for 78 weeks. MRID No. 43904807. HED Doc. No. 012255. Classification: Acceptable.

3. Brooker, A. J. (1994). Toxicity to Dogs by Repeated Dietary Administration for 52 Weeks. MRID No. 43573218. HED Doc. No. 012255. Classification: Acceptable.

4. Henwood, S. M. (1995). Two Generation Reproduction Study with RPA 201772 in Rats. MRID No. 43904809. HED Doc. No. 012255. Classification: Acceptable.

5. Reader, S. C. J. (1995). RPA 201772 (active ingredient): Teratology study in the rat. MRID No. 4357320. HED Doc. No. 011675. Classification: Acceptable.

6. Reader, S. C. J. (1995). RPA 201772 (Active Ingredient). Study of Embryo-Foetal Toxicity in the Rabbit by Oral Gavage) Administration. MRID No. 43904808. HED Doc. No. 012255. Classification: Acceptable.

7. Mandella, R.C. (1955). An acute neurotoxicity study of RPA 201772 in the rat via oral gavage administration. MRID No. 43904804. HED Doc. No. 012255. Classification: Acceptable.

8. Mandella, R. C. (1955). A subchronic (3-month) neurotoxicity study of RPA 201772 in the rat via dietary administration. MRID No. 43904805. HED Doc. No. 012255. Classification: Acceptable.

9. Dance, C. A. (1993). In vitro assessment of the clastogenic activity of RPA 201772 in cultured human lymphocytes. MRID No. 43573221. HED Doc. No. 011675. Classification: Acceptable.

10. Strang, P. (1993). RPA 201772: Investigation of mutagenic activity in the $RK^{+/-}$ mouse lymphoma cell system. MRID No. 43573222. HED Doc. No. 011675. Classification: Acceptable.

11. Edwards, C. N. (1993). RPA 201772: Mouse Micronucleus Test to comply with O.E.C.D. MRID No. 43573223. HED Doc.

No. 011675. Classification: Acceptable.

12. Percy, A. (1993). RPA 201772: Salmonella typhimurium Reverse Mutation Assay (Ames Test). MRID No. 43588002. HED Doc. No. 011675. Classification: Acceptable.

13. Percy, A. (1995). RPA 202248: Salmonella typhimurium reverse mutation assay (Ames Test). MRID No. 43904811, HED Doc. No. 012255. Classification: Acceptable.

14. Percy, A. (1994). RPA 202248: Salmonella typhimurium reverse mutation assay (Ames Test). MRID No. 43904814, HED Doc. No. 012255. Classification: Acceptable.