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

FEB 27 1995
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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Fipronil (REGENT® 1.5G): Request for an EUP for use on Field Corn (EPA Registration No. 264-EUP-OC; EPA PP No. 5G4263)

P.C. Code: 129121
D211468 Submission: S480904
D211202 Submission: S480292
D212067 Submission: S481736

FROM: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

Y M Ioannou 2/23/95

TO: Marion Johnson / PM 10
Registration Division (7505C)

THRU: Marcia Van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C)

M. VanGemert 2/24/95

Registrant: Rhone Poulenc Ag Company

Action Requested: Determine if the additional data provided by the registrant are adequate to support an EUP for use of REGENT® 1.5G insecticide on field corn.

Recommendation: Based on the additional data provided by the registrant, Toxicology Branch II has determined that the Toxicology data base for Fipronil is now adequate to support the Experimental Use Permit for use of REGENT® 1.5G insecticide on field corn with a proposed temporary tolerance (for Fipronil and/or its metabolites), in or on corn grain of 0.02 ppm.

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Background:

Rhone-Poulenc AG Company has previously (August, 1993) submitted a request for an Experimental Use Permit/Temporary Tolerance (EUP/TT) for use of Fipronil (REGENT® 1.5G soil insecticide) on field corn. As the toxicology data base was not complete at that time, Toxicology Branch II recommended against the issuance of the EUP/TT. Subsequently, the registrant submitted to the Agency new studies and/or additional data to fulfill the data requirement for an EUP/TT. Recently (January 31, 1995), the registrant again petitioned the Agency for an EUP/TT for use on field corn for the 1995 season. In this revised EUP program, the registrant is proposing to use REGENT® 1.5G soil insecticide on 242 acres of field corn (compared to 1000 acres originally) in 8 states, using a total active ingredient of Fipronil of 31.46 lbs. This pesticide will be incorporated in to the soil at the rate of ~0.13 lb. a.i./acre (single application) to control northern and western corn rootworm and/or wireworms. The requested temporary tolerance on field corn grain is 0.02 ppm. Temporary tolerances for corn forage and corn fodder are not proposed since label restrictions will prohibit the grazing of treated fields and the feeding of corn silage and fodder to livestock.

Review of Additional Data:

Acute Inhalation LC_{50} - Rat (MRID # 435444-01)

Male and female (5/sex) Sprague-Dawley rats were exposed to Fipronil technical (nose-only) at concentrations of 0.33, 0.52, and 0.72 mg/l for four hours.
The acute inhalation LC_{50} of Fipronil technical = 0.36 (0.23-0.44) mg/l in male rats and 0.42 (0.35-0.44) mg/l for female rats. The combined acute inhalation LC_{50} = 0.39 (0.35-0.44) mg/l.

Toxicity Category II; the study is acceptable.

Mouse Micronucleus Assay (MRID # 429186-50 [original]; **MRID # 435017-03** - [company response]).

This study, "Assessment of Clastogenic Action on Bone Marrow Erythrocytes in the Micronucleus Test," was reviewed earlier (5/16/94; document # 011086) and found to be unacceptable based on the fact that no toxicity to the target organ was elicited at the highest dose level tested (25 mg/kg). The scoring of slides from the 50 mg/kg dose group of the range-finding study, similarly, did not indicate any appreciable increase in the MPE frequency and only two animals per sex were used.

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The registrant, in their initial response, indicated that the high dose tested was adequate for a valid test and that Fipronil is not genotoxic in this or any other in vivo or in vitro assays.

The Agency (Dr. B. Backus, Mutagenicity expert in Toxicology Branch II), maintains that this micronucleus assay was not conducted properly to test the genotoxicity of Firponil. This study remains classified as unacceptable and the registrant is required to repeat this assay using higher dose levels (60-70 mg/kg). Although an acceptable study will be required for a Section 3 registration of Fipronil, for the purpose of the EUP this requirement is waived based on the fact that all other mutagenicity studies with Fipronil indicate that this is not a genotoxic agent and the expected exposure, based on the proposed revised EUP program, will be minimal.

Submission of Additional Data (not pertaining to the EUP)

The registrant submitted additional data for the following studies:

1) M&B 46030: Toxicity Study by Dietary Admnistration to CD rats for 13 weeks (MRID # 429186-43 [original]; MRID # 435017-01 [company response]).

This study was reviewed earlier (3/10/94) and classified as core-supplementary data based on the fact that the registrant did not submit data from the neurological examinations conducted in this study. In the present submission, the registrant provided the Agency with the requested data. These data indicate that there were no neurological effects in the treated groups that could be attributed to Fipronil. All reported values were "zero" in the controls and treated groups.

These additional data do not change any of the original conclusions in this study.

This study is upgraded to core minimum classification and satisifies the guideline requirement for a subchronic toxicity study (§82-1) in rats.

2) M&B 46030: Oncogenicity Study by Dietary Administration to CD-1 mice for 78 Weeks (MRID # 429186-49 [original]; MRID # 435017-02 [company response]).

The registrant submitted additional historical control data (on hepatocellular adenoma and carcinoma for male and female CD-1 mice). These data satisfy the deficiency reported in the data evaluation record of this study (reviewed on 5/17/94). These data do not change any of the conclusions or the core-classification of the study.

Review of Proposed Label

The proposed EUP label does not reflect the acute toxicity of Fipronil. Based on the fact that Fipronil meets the criteria for Toxicity Category II, the signal word "Warning" should appear on the label and not the word "Caution."

Conclusions/Recommendations

Based on the review of the recently submitted toxicology data and the revision of the original EUP program, Toxicology Branch II has determined that the data base for Fipronil is now adequate to support the EUP/TT for use of REGENT® 1.5G insecticide on field corn with the proposed temporary tolerance (for Fipronil and/or its metabolites) in or on corn grain of 0.02 ppm.

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Reviewed by: Timothy F. McMahon, Ph.D. T.F.M. 2/23/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.J 2/23/95
Section I, Toxicology Branch II (H7509C)

Data Evaluation Record

Study type: Acute inhalation-rats (81-3) P.C. Code.: 129121

MRID number: 435444-01

Test material: Fipronil technical

Study number: 94N1501

Testing Facility: Bushy Run Research Center, Union Carbide Corporation
Export, PA

Sponsor: Rhone-Poulenc Ag Company

Title of report: Fipronil: Acute Nose-Only Dust Inhalation Toxicity Study in Rats

Author(s): D.J. Nachreiner

Study Completed: February 9, 1995

Executive Summary:
In an acute inhalation toxicity study (MRID # 435444-01), male and female Sprague-Dawley albino rats (5/sex) were exposed nose-only to Fipronil technical at concentrations of 0.33, 0.52, and 0.72 mg/l for four hours. At the 0.72 and 0.52 mg/l exposure concentrations, 100% mortality was observed. Clinical signs at all dose levels consisted of perinasal, periocular, and perioral red encrustation; urogenital, body, and periocular wetness; unkempt fur; hyper- and hypoactivity; fine whole body tremors (day 0 only); and incoordination. **Under the conditions of this study, the acute inhalation LC_{50} of Fipronil technical (with 95% C.I.) was 0.36 (0.23-0.44) mg/l in male rats and 0.42 (0.35-0.44) mg/l for female rats. For the sexes combined, the acute inhalation LC_{50} was 0.39 (0.35-0.44) mg/l.**

This study is classified as **acceptable** (Toxicity Category II) and satisfies the guideline requirements (§81-3) for an acute inhalation toxicity study in rats.

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I. MATERIALS

A. Test Material: Fipronil Technical; purity: stated as 96.72%. batch no: 10MTD20. description: fine white powder. storage: ambient temperature

B. Test Animals: Male and Female Sprague-Dawley albino rats. Source: Harlan Sprague-Dawley, Indianapolis, IN. Age upon arrival: 42 days. Weight (day of exposure): males; 215-251g; females, 182-216g.

II. METHODS

A. Animal Husbandry

For this study, groups of 5 male and 5 female rats per dose group were used. The specific number of rats obtained was not stated, although enough were obtained for the dose groups used in this study. These animals were not subject to a pre-test health screen, but five other rats housed in the same isolation room used for acute testing were subjected to quality control evaluation once every 3 months. These rats were found to be in good health and suitable for use. During non-exposure periods, rats were housed 5 per cage in suspended, wire bottom, stainless steel cages, with food (AGWAY PROLAB animal diet rat, mouse, hamster 3000) and tap water *ad libitum*, except during exposure. Temperature was maintained at 66-77 ^{o}F, and humidity at 40-70%. Rats were acclimated for at least 5 days prior to exposure.

B. Chamber Apparatus

During exposure, rats were housed in individual animal holding tubes constructed of clear plastic. Tubes were sealed such that outside air could not leak past the rats and into the chamber. The apparatus was a nose-only modular vertical system constructed of polyvinyl chloride and contained animal holding tubes constructed of clear plastic. The apparatus was designed so that after presentation of test atmosphere to the animals, the exhaled gases from each animal were not entrained into the breathing zone of other animals. The apparatus was configured for two levels of exposure, each level having ports for 8 animals. A diagram of the apparatus as well as a flow diagram illustrating the flow of air through the apparatus was provided and is attached to this review.

Air supply was of a push/pull design. Dry air was supplied from a compressed air line. Air was metered to the auger-type dust feed inserted into the top of a glass chromatography tank, where the dust was mixed prior to being pulled through the nose-only apparatus at an airflow rate of 8 liters/minute. Excess air from the chromatography tank was exhausted by way of a vacuum line. Airflow rate through the animal ports was maintained at approximately 500 ml/min/port with a

slightly negative pressure in the chamber. Airflow rate was determined prior to loading the animals and was monitored with a flowmeter and controlled with valves on the compressed air and vacuum lines. The theoretically-derived time required for the chromatography tank and central cylinder of the nose-only apparatus to reach 99% of the target concentration was calculated to be 4.0 minutes. Exhaust air was scrubbed through an 8-liter glass bottle containing approximately 4 liters of water and through an empty 4 liter glass bottle. Additionally, a dust cartridge was used to filter air from the nose-only apparatus which was being monitored with a flowmeter.

C. Atmosphere Generation

Dust atmosphere generation was accomplished by means of a stainless steel auger-type dust feed. The dust was placed into a rectangular hopper where it was constantly agitated. The dust fell through a hole in the base of the hopper where it was transported by an auger to the end of a tube. The rotation rate of the auger was controlled by a variable speed motor. As the dust fell from the end of the tube, a compressed air stream entrained the dust and carried it into the chromatography tank mentioned above. The chromatography tank, which served as a mixing chamber, was also used to remove large non-respirable particles.

Concentration of test material was determined gravimetrically, with 4-6 samples obtained during each 4 hour exposure. A glass fiber filter (Type A/E, 25mm, Gelman Sciences, Inc.) used to collect the dust was connected to a dry gas meter, a critical orifice, and a vacuum pump. Following collection of dust from the chamber atmosphere, filters were weighed without drying. Nominal concentration of test material was determined by dividing the weight of the test substance delivered by the volume of air which passed through the chromatography tank during the exposure period.

Particle size distribution was measured using a TSI Aerodynamic Particle Sizer Model APS 3000. Particle size determinations were made 2 times for each exposure. Particle size determinations were obtained approximately 16 to 23 minutes prior to animal exposure and within 2 minutes following animal exposure, since size determinations could not be obtained during exposure without affecting the dust concentration in the nose-only chamber ports. Data collected were used to obtain the mass median aerodynamic diameter and geometric standard deviation. Chamber temperature was recorded during each exposure using a Type K thermocouple attached to a Fluke 51 K/J temperature indicator. Relative humidity could not be monitored during exposure using nose-only apparatus. Instead, the relative humidity of the dry compressed air was determined in the chromatography tank during each exposure using an Airguide humidity indicator. Oxygen content in the nose-only apparatus was monitored using a Model 245R Oxygen Indicator.

D. Exposure

The report provided the following information on target concentration selection: "The target concentration for the first exposure was 0.70 mg/l. The target concentration for subsequent exposures were selected by the Sponsor based on the incidence of mortality following previous fipronil exposures. The target concentration for the 0.33 and 0.52 mg/l exposures were 0.35 and 0.50 mg/l, respectively."

E. Animal Observations

Prior to exposure, physical condition of the animals was monitored for at least 5 days prior to exposure, and body weights were obtained prior to placement into the exposure group. Cage side observations for clinical toxicity and mortality were conducted at least once daily in the morning. Animals considered unacceptable for the study, based on clinical signs or body weights, were rejected.

During exposure, all animals were individually observed for toxic signs. Observations were recorded approximately every 30 minutes. Detailed individual examinations were performed just prior to and shortly following exposure, at approximately 1 and 2 hours following exposure, and once each day during the post-exposure period. Mortality checks were also made once daily.

Body weight data were collected on the morning prior to exposure, at 7 days following exposure, and at 14 days following exposure immediately preceding sacrifice. Complete necropsy was performed in all animals. The respiratory tract was saved from all animals at necropsy. In addition, tissues were saved at necropsy from all animals with gross lesions.

F. Statistical Analysis

The 4-hour LC_{50} value was determined by the moving average method of Thompson for males, females, and the combined sexes using the 0.33 and 0.52 mg/l dose groups. The 0.72 mg/l group could not be used for calculation of the LC_{50} value, since the moving average method requires the ratio between exposure groups to be constant. In addition, the 0.72 mg/l dose group could not be used to permit determination of the LC_{50} value by a probit analysis method, due to similar mortality observed at both the 0.72 and 0.52 mg/l dose levels.

III. RESULTS

A. Atmosphere Generation

Uniformity of test material in the chamber atmosphere was presented in Table 2 of Appendix 1, page 36 of the report. These data showed a uniform dust distribution as indicated by a low coefficient of variation of 6.0%. In addition, results obtained from the normal analytical sampling position were in close agreement with the other data presented in the Table. Data from Tables 3 and 5 of the report are summarized below:

Table 1

Atmosphere Characterization in the Acute Inhalation Toxicity of Fipronil Technical[a]

gravimetric particle conc. (mg/l)	Sample no.	nominal conc. (mg/l)	MMAD ± GSD (microns)
0.72	1	14.5	1.73±1.37
0.72	2		1.69±1.36
0.52	1	5.4	1.56±1.30
0.52	2		1.74±1.37
0.33	1	5.0	1.60±1.32
0.33	2		1.65±1.34

[a]data from pages 37 and 40 of the report.

As shown by the above data, the MMAD was less than 2 microns at all exposure concentrations. Thus, it can be expected that deposition within all regions of the rat respiratory tract would have occurred in this study. In addition, the report provided a graph of the log probability of particle mass. In this graph (page 41 of the report), it is seen that the cumulative percent of particles with a diameter of 3 microns or less encompassed 97% of the total mass of particles. Approximately 5% of the dust was ≤ 1 micron.

B. Animal Observations

At the 0.72 and 0.52 mg/l exposures, 100% mortality was observed. The majority of deaths occurred within 2 days post-exposure. At the 0.33 mg/l exposure, 2 male rats died, one on day 1 post-exposure, and 1 on day 6 post-exposure.

At the 0.72 mg/l exposure concentration, the following were noted in all rats on the day of exposure: urogenital, body, and periocular wetness; unkempt fur; fine whole body tremors. In one male at this dose, incoordination was observed on post-exposure days 4 through 7, hyperactivity on post-exposure day 4, and hypoactivity and swollen penis on post-exposure day 7. This rat died on day 8.

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In one female rat at this dose, perioral, perinasal, and periocular red encrustation was observed on post-exposure days 1 through 11, hyperactivity on post-exposure days 2 through 4, hypoactivity on post-exposure days 7 through 10, and incoordination on post-exposure days 6 through 11. This rat died on day 12.

At the 0.52 mg/l exposure concentration, the same signs as mentioned for all rats at the 0.72 mg/l exposure concentration were observed without any additional signs.

At the 0.33 mg/l exposure concentration, similar signs were again recorded, with the following additions in males: perioral, perinasal, and periocular red encrustation up to day 5 post-exposure; incoordination in 4 of 5 male rats up to post-exposure day 3; hypoactivity in 4 of 5 male rats up to post-exposure day 2. In female rats at this exposure concentration, similar signs were observed as for males. The incoordination did not appear until post-exposure day 3 in 4 of 5 female rats and did not last beyond this point.

Body weight data (pages 19-24 of the report) showed weight gain in those animals surviving the 14 day post-exposure period at the 0.33 mg/l exposure concentration. At the 0.52 and 0.72 mg/l exposure concentrations, most rats had died prior to 7 days. In those rats surviving to post-exposure day 7 (one male and one female each at the 0.72 mg/l dose), significant body weight loss was recorded (-97 grams for the male; -99 grams for the female).

Gross necropsy of the rats in this study showed an increased incidence of the following at the 0.52 and/or 0.72 mg/l dose levels: meningeal hemorrhage and color change of the stomach (includes red or black foci in 6 of 10 rats, possible ulcerated area in 1 of 10 rats, and thickened white surface in 2 of 10 rats). Meningeal hemorrhage was considered incidental in animals dying an agonal death, while the stomach lesions were considered treatment-related, as no such lesions were observed from the control animals used in similar studies at the performing laboratory.

IV. CONCLUSIONS

In an acute inhalation toxicity study, male and female Sprague-Dawley albino rats (5/sex) were exposed nose-only to Fipronil technical at concentrations of 0.33, 0.52, and 0.72 mg/l for four hours. At the 0.72 and 0.52 mg/l exposure concentrations, 100% mortality was observed. Clinical signs at all dose levels consisted of perinasal, periocular, and perioral red encrustation; urogenital, body, and periocular wetness; unkempt fur; hyper- and hypoactivity; fine whole body tremors (day 0 only); and incoordination. **Under the conditions of this study, the acute inhalation LC_{50} of Fipronil technical (with 95% C.I.) was 0.36 (0.23-0.44) mg/l in male rats and 0.42 (0.35-0.44) mg/l for female rats. For the sexes combined, the acute inhalation LC_{50} was 0.39 (0.35-0.44) mg/l.**

This study is classified as **acceptable** (Toxicity Category II) and satisfies the guideline requirements (§81-3) for an acute inhalation toxicity study in rats.