Apv. 4/19/94

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

013923

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Fipronil - Review of Data to Support EUP for Domestic Animal Product (0.25% fipronil)

PC Code: 129121
DP Barcode: D199677
Case: 040837
Submission: S458528

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer Virginia A Dobozy 4/7/94
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Robert Brennis/Daphne Waldo/PM 10
Registration Division (7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head Y.M. Ioannou 4/8/94
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 4/19/94
Toxicology Branch II
Health Effects Division (7509C)

Registrant: Rhone Merieux, Inc.

Action Requested: Review domestic animals safety data submitted to support an EUP for a 0.25% fipronil formulation

Recommendation: Toxicology Branch II has completed its review of the above referenced studies. It is recommended that an EUP be granted for this 0.25% formulation for use in testing on adult dogs only. There is insufficient data to demonstrate that the product can be safely used in kittens, puppies and cats, even in this limited testing program.

DATA REVIEW

Domestic Animal Safety Study (86-1) - MRID # 431211-10

Groups of three male and three female beagle dogs were topically administered six treatments of 0.25% fipronil at dosages of either 0, 3, 9 or 15 ml/kg (0, 7.5, 22.5 and 37.5 mg/kg, respectively) at 28-day intervals. (The proposed recommended dose for the product is 3-6 ml/kg.)

There was a statistically significant increase in the number of animals judged to have abnormal eye examinations in the 9 ml and 15 ml groups after the second treatment and in the 9 ml group after the sixth treatment. The increases were not dose-responsive and were not considered to be treatment-related. Two treated dogs, one in the 3 ml group and another in the 9 ml group, were noted to have skin lesions at 21 days after the last treatment. Additional treatment did not exacerbate the lesions. There was no other evidence of treatment-related toxicity.

Classification: Unacceptable because: 1) the highest dosage level was not 5X the highest recommended dose; and 2) the study was not conducted in compliance with the GLP regulations.

Domestic Animal Safety Study (86-1) - MRID # 431211-11

A total of 27 male and 21 female nursing puppies (9 litters of either beagle, spaniel or griffon breeds) was topically administered two treatments of the 0.25% formulation at a dosage of 6 ml/kg at 28-day intervals. (The proposed recommended dosage is 6 ml/kg.) The dams of the litters were treated at the same dosage. Twenty-six (26) male and 15 females served as untreated controls.

Six treated puppies, four from the same spaniel litter, died before weaning and another died after weaning. Parvovirus was isolated from the spaniel litter. One puppy in the control group died. There was no statistical difference in mortality between the groups. Growth rates were either comparable or exceeded the applicable control group for the beagle and griffon breeds, however were lower for the spaniel breed. This decrease may have been the result of the parvovirus infection.

Classification: Unacceptable because: 1) the study was conducted with nursing rather than weaned puppies; 2) the highest dosage level was not at 5X the highest recommended dose; 3) the study was not conducted in compliance with GLP regulations.

Domestic Animal Safety Study (86-1) - MRID # 431211-12

Groups of three male cats were topically administered six treatments of the 0.25% fipronil formulation at dosages of either 0, 3, 9 or 15 ml/kg (0, 7.5, 22.5 and 37.5 mg/kg, respectively) at 28-day intervals. (The proposed recommended dose for cats is 3-6 ml/kg.)

Food consumption was significantly decreased in the 15 ml/kg group as compared to the control group at weeks 2 and 4 after the first treatment. All other time points were comparable. The incidences of vomiting and soft/liquid feces were higher in the treated animals as compared to the controls.

Classification: Unacceptable because: 1) the highest dosage level was not at 5X the highest recommended dose; 2) only three male cats per dosage group were included in the study; and 3) the study was not conducted in compliance with the GLP regulations.

Additional Toxicity Data

The registrant also submitted six acute toxicity studies with the 0.25% formulation. They are being reviewed in the Registration Support Branch of the Registration Division. The following summarizes the conclusions of the studies with no evaluation of their conduct or acceptability.

Acute Oral Toxicity/Rats (MRID # 431211-04) - acute oral LD_{50} was greater than 5000 mg/kg - Toxicity Category IV

Acute Dermal Toxicity/Rats (MRID # 431211-05) - acute dermal LD_{50} was greater than 2000 mg/kg - Toxicity Category III

Acute Inhalation Toxicity/Rats (MRID # 431211-06) - acute inhalation LC_{50} was greater than 5.06 mg/l (12.7 µg/l active ingredient) - Toxicity Category IV

Primary Eye Irritation/Rabbits (MRID # 431211-07) - corneal involvement or irritation which cleared within 6 days - Toxicity Category III

Primary Dermal Irritation/Rabbits (MRID # 431211-08) - very slight irritation - Toxicity Category IV

Dermal Sensitization/Guinea Pigs (MRID # 431211-09) - not dermal sensitizer

Proposed Testing Program

In a document titled "Proposed Testing Program", the registrant submitted a protocol for a field study designed to test product efficacy against fleas. The protocol proposes testing

in six veterinary hospitals in four states, Georgia, South Carolina, Texas and Illinois. The maximum number of animals (any age, sex or breed of dogs and cats) to be included in the study is 180. The EUP request is for May to November 1994.

The protocol indicates that the study will be blinded with both a treated and vehicle control group. At the initial visit to the veterinarian, the animal will have a physical examination, a comb count to assess and remove the flea and tick population and a bath with a non-insecticidal shampoo. Then, a dose of 3-6 ml/kg of either the product or the vehicle control will be applied to the animal. (The range is based on the amount of product needed to obtain a damp to thoroughly wet hair coat.) At the one-month visit, the code will be broken and all animals with fleas or ticks will be treated. A comb count will assess and remove fleas and ticks if they are present. If fleas are present, the animal will be bathed with a non-insecticidal shampoo and the product applied at 3-6 ml/kg. If not, the animal will receive no treatment. The procedure is repeated at the two-month visit. At the three-month visit, all animals will be combed but only animals which were initially in the placebo group will be treated. An evaluation of that treatment will be done at the four-month visit. The animals will be observed for 1-2 hours after each treatment for clinical signs of toxicity. Any serious signs of adverse effects will be reported to the registrant within 10 days. If a pet is entered into the study, all the pets in the household must be included. The animals will receive no other insecticides/acaricides nor will the premise be treated during the study.

Discussion

The registrant acknowledges that the domestic animal safety studies discussed above are unacceptable and is presently conducting other studies in the United States. Protocols for these studies were reviewed by Toxicology Branch II (DP Barcode: D194332; September 3, 1993 memo from Virginia Dobozy to Joseph Tavano/Richard Mountfort/PM 10). Toxicology studies with the technical material, including oral studies in the dog, are presently under review for an EUP for agricultural use.

The concern in granting an EUP for use of the 0.25% formulation for the efficacy testing program is whether there is enough data to conclude that the margin of safety with the product is adequate to allow this limited use. The six acute toxicity studies show that the formulation is relatively non-toxic in laboratory animals by all routes tested. To determine the maximum amount of active ingredient which would be applied to an animal, the registrant was consulted. See attached Telefax dated April 6, 1994. For a single application, the maximum dermal exposure would be 15 mg/kg for a 4.5 kg animal. Allowing that an animal could have three treatments, the maximum for the overall study would be 45 mg/kg. In comparison,

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the dermal LD_{50} in rats was greater than 2,000 mg/kg; a margin of 44X. In addition, the French domestic animal safety study with dogs (MRID # 431211-10) demonstrated that there were no treatment-related effects on a wide variety of clinical and pathological parameters at 2.5X the highest recommended dose.

However, there is some concern about the findings in the French domestic animal safety study in cats (MRID # 431211-12). Only three male cats were used in each dose group so the validity of the results is questionable. In addition, the highest dose tested was only 2.5X the highest recommended dose. But, there were some indications that the treated animals may have been adversely affected. Food consumption was significantly decreased as compared to the controls in the 15 ml/kg group cats at weeks 2 and 4 after the first treatment. The incidences of vomiting and soft/liquid feces were higher in the treated animals as compared to the controls.

Cats are deficient in glucuronyl transferase activity which contributes to the poor detoxification of many xenobiotics.[1] The incidence of adverse reactions in cats submitted through the Incident Data System confirms that this susceptibility includes insecticidal pet products.

Recommendations

Toxicology Branch II recommends that an EUP be granted for this 0.25% formulation for use in testing on adult dogs only. There is insufficient data to demonstrate that the product can be safely used in kittens, puppies and cats, even in this limited testing.

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[1] Aronson AL, Riviere JE: Adverse drug reactions. p. 169. In Kirk (ed.): Current Veterinary Therapy. vol. IX. WB Saunders, Philadelphia, 1986

April 6, 1994

VIA TELEFAX

C10923

Dr. Virginia Dobozy
Insecticide and Rodenticide Branch
Registration Division (H7505C)
Office of Pesticide Programs
U.S. Environmental Protection Agency
Document Processing Desk (EUP)
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, VA 22202

SUBJECT: TELEPHONE CONVERSATION ON 6 APRIL 1994

FIPRONIL 0.25% TOPICAL SPRAY
EPA COMPANY NO. 65331

Dear Dr. Dobozy:

This is to assist you in your review regarding the testing program protocol and the maximum amount of active ingredient an animal could possibly be exposed to.

Example on Page 23 of 30 - Appendix 7.3:

weight of animal = 4.5 kg animal
maximum application possible to 4.5 kg animal = 81 ml (6 ml x 4.5 kg x 3 applications)
1 ml product (.25% formulation) = 2.5 mg a.i.
81 ml product (202.5 mg a.i.) applied to 4.5 kg animal (45 mg/kg)

If we compare it to the dermal in rat results which showed no signs of toxicity at 2000 mg/kg, the difference is 44X.

Should you have additional questions, please do not hesitate to contact me.

Sincerely,

Kandy Walker

Kandy Walker, Ph.D.
Manager, Regulatory Affairs
Pharmaceuticals and Pesticides

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Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 4/6/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I. 4/7/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Domestic Animal Safety Study/Cats (86-1)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 431211-12
TEST MATERIAL:	RM 1601C Synonym: Fipronil
STUDY NUMBER:	CLI 137
TESTING FACILITY:	Rhone Merieux Toulouse Cedex, France
SPONSOR:	Rhone Merieux, Inc. Athens, Georgia
TITLE OF REPORT:	Assessment of the Tolerance of a 0.25% RM 1601 Spray Formulation in Cats at 3, 9, and 15 Ml/kg When Applied 6 Times to the Haircoat at 28 Day Intervals
AUTHOR(S):	J.P. Arnaud and P.J. Consalvi
REPORT ISSUED:	December 27, 1993

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 431211-12), groups of three male cats were topically administered six treatments of RM 1601C (0.25% fipronil) at dosages of either 0, 3, 9 or 15 ml/kg (0, 7.5, 22.5 and 37.5 mg/kg, respectively) at 28-day intervals. (The proposed recommended dose for cats is 3-6 ml/kg.) A variety of parameters were evaluated after each treatment, including physical examination, body weight, food consumption, clinical observations and clinical pathology (hematology and clinical chemistry). Thyroid function tests were conducted before and 22 days after the treatment period. Skin biopsies were done on all cats in the high dose group and one control cat.

Food consumption was significantly decreased in the 15 ml/kg group as compared to the control group at weeks 2 and 4 after the first treatment. All other time points were comparable. The incidences of vomiting and soft/liquid feces were higher in the treated animals as compared to the controls.

The study is unacceptable because 1) the dosage levels were not at at 5X the recommended dose; 2) only three male cats per dosage group were included in the study; and 3) the study was not conducted in compliance with GLP regulations.

I. MATERIALS

A. Test Material

Name: RM 1601C
Synonym: RM 1601 (Fipronil)
Chemical Name: 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole
Purity: RM 1601C is 0.25% RM 1601
Batch Number: G 04 12
Description: Not provided
Storage Conditions: Not provided

The test material was supplied as a spray in a 100 ml bottle fitted with a manual trigger pump that delivered approximately 0.5 ml.

B. Administration: topical

C. Test Animals

Species: Male European cats
Source: IFFA CREDO, France
Age: Mean age of 8 months at start of study
Weight: Mean - 4.7 kg at start of study
Housing: Individually in cages
Environmental Conditions: Not provided
Food and Water: pelleted dry food (Alimet C32) and water *ad libitum*
Acclimation Period: Not provided

II. METHODS

A. Dosage and Administration

Twelve cats of each sex were randomly assigned to four groups as follows:

Group	Number	Dose of RMI 1601	Volume of RM 1601C/35	Number of Treatments
Control	3	0	0	0
3 ml	3	7.5 mg/kg	3 ml/kg	6
9 ml	3	22.5 mg/kg	9 ml/kg	6
15 ml	3	37.5 mg/kg	15 ml/kg	6

The treatments were administered at 28 day intervals. The cats were weighed immediately prior to treatment. The density of the spray solution (0.85) was used to establish the volume of test material to be applied since the trigger did not deliver exactly 0.5 ml. The weights of the product bottles were recorded before, during and after each application. The cats were anesthetized with ketamine IM before each treatment. The spray was applied against the lay of the hair over the entire animal avoiding the eyes. To avoid run-off of the product at the higher doses, the treatment procedure was repeated after drying the coat, two or three times within 30 minutes except for the first treatment of the 15 ml/kg group which was performed within 4 hours.

B. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies.

physical examinations - before dosing and at Days 1, 4, 7, 14 and 21 after each treatment (treatment day was designated Day 0)
observations for digestive disorders- cages were examined daily for presence of vomiting and consistency of feces
body weights - at weekly intervals during the study
food consumption - at weekly intervals during the study
skin examinations - at same time as physical examinations[1]
coat examinations - at same time as physical examinations[2]
hematology and clinical chemistry - before and 14 days after each monthly treatment
thyroid evaluations - 4 days before and 22 days after the treatment period
skin biopsies - at 23 days after the treatment period on all cats in the 15 ml group and one control cat

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[1] The skin was examined at four locations. Lesions were scored on a scale of 0 to 4. Skin fold thickness at the point of the shoulders was also measured at each examination.

[2] Three criteria were analyzed: 1) score for loss of hair (scale of 0-4); 2) appearance of coat; 4) score of scurfs (scale of 0-4).

C. Pathological Parameters

Hematology

The CHECKED (X) hematology parameters were examined.

X Hematocrit (HCT)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc. (MCHC)
X Mean corpuscular volume (MCV)
X Hemoglobin (HGB)
__Leukocyte count (WBC)
X Erythrocyte count (RBC)
X Platelet count

Clinical Chemistry

The CHECKED (X) clinical chemistry evaluations were done.

X Urea
X Uric acid
X Proteins
X AST
X Alkaline Phosphatases (ALP)
X Creatinine
X Glucose
X Cholesterol
X ALT

Thyroid Function

Assays of circulating thyroxine (T_4) were measured.

Skin Biopsies

At 23 days after the last treatment, the cats were anesthetized with ketamine and biopsies were taken from four sites: two areas with hair (root of tail and dorsal line at the level of the ileum) and two hairless areas (both groins).

D. Statistical Analyses

A description of the statistical methods from the study report is attached to the DER.

E. Compliance

The registrant submitted a signed statement indicating that the study was not conducted in compliance with the GLP regulations; the areas of non-compliance are not identified. A signed Statement of Data Confidentiality Claims indicates no confidentiality is claimed by the registrant.

III. RESULTS

A. Comparisons of Groups Before Treatment

The control and treated groups were comparable before treatment for

the following parameters: body weight, food consumption, physical examination, skin examination, hematology, clinical chemistry and thyroid function. On physical examination, the following were noted: pale conjunctiva in 1 cat (9 ml/kg); altered heart rate in 1 cat (control); cryptorchidism in 1 cat (3 ml/kg); and aggressiveness in 1 cat (9 ml/kg). On hematology evaluation, it was noted that one cat in the 9 ml/kg group had high levels of urea and creatinine pre-treatment.

B. Product Volume Administered

The product volumes administered were as follows:

Doses	Mean Volume (ml/kg)	Range (ml/kg)
3 ml/kg	3.20	2.92 to 3.47
9 ml/kg	9.42	8.54 to 10.09
15 ml/kg	15.98	15.05 to 17.00

C. Body Weights

Both the treated and control animals maintained their body weight during the course of the study.

D. Food Consumption

Food consumption was significantly decreased at weeks 2 and 4 after the first treatment for the 15 ml/kg group. All other time points were comparable between the treated and control groups.

E. Gastrointestinal Monitoring

There were no incidences of vomiting and soft/liquid feces in the control groups. The 3ml/kg and 15 ml/kg groups each had one animal which vomited after the fifth and sixth treatments. The incidences of soft/liquid feces was higher in the treated animals with sometimes 2-3 animals being affected in each of the treatment groups after the first, fifth and sixth treatments.

F. Physical Examinations

The number of "abnormal" cats for eye examination, heart and lung auscultations, legs and abdominal palpations, genitalia examination, behavior examination, reflexes examination were not

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different between groups for the twelve periods.[3] The incidence of nervousness or aggressiveness was increased in the cats receiving 9 and 15 ml/kg of the spray, however one of the cats in the 9 ml/kg group was aggressive pre-treatment.

G. Skin and Coat Examinations

There were no statistically significant differences in the number of animals with skin lesions or in skin thicknesses between the treated and control groups.

Coat examinations were comparable between the treated and control groups.

G. Clinical Pathology

Mean values for hematology and clinical chemistry are presented only for Day 0 and Day 154 (14 days after the last treatment). There were no significant differences between the treated and control groups and little variation in the pre- and post-treatment values for each group. There were no differences in T_4 levels between the treated and control groups at the end of the treatment period.

H. Skin Biopsies

The study report states that there were no histological lesions in skin biopsies taken from the three 15 ml/kg cats and the one control cat, however no individual animal data have been submitted.

IV. STUDY DEFICIENCIES

1. The product is identified as RM 1601 C/35 in Table 1 on page 12 of the study report, whereas RM 1601C is used throughout the rest of the study. The registrant should explain the difference.

2. The study report should state how the study does not comply with the GLP Guidelines.

3. All the individual animal data should be submitted.

V. CONCLUSIONS

In a domestic animal safety study (MRID # 431211-12), groups of three male cats were topically administered six treatments of RM

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[3] Twelve periods were considered for physical examinations: the first week after each monthly treatment (6 periods) and then the second, third and fourth weeks after each treatment were considered together (6 periods). A cat was considered "abnormal" if at least one single abnormal clinical parameter was recorded.

1601C (0.25% fipronil) at dosages of either 0, 3, 9 or 15 ml/kg (0, 7.5, 22.5 and 37.5 mg/kg, respectively) at 28-day intervals. (The proposed recommended dose for cats is 3-6 ml/kg.) A variety of parameters were evaluated after each treatment, including physical examination, body weight, food consumption, clinical observations and clinical pathology (hematology and clinical chemistry). Thyroid function tests were conducted before and 22 days after the treatment period. Skin biopsies were done on all cats in the high dose group and one control cat.

Food consumption was significantly decreased in the 15 ml/kg group as compared to the control group at weeks 2 and 4 after the first treatment. All other time points were comparable. The incidences of vomiting and soft/liquid feces were higher in the treated animals as compared to the controls.

The study is unacceptable for the following reasons:

1) Although there are no guidelines for the conduct of domestic animal safety studies, Toxicology Branch II has routinely required that the studies be conducted with 5X the recommended dose. The study report states that the minimal recommended dose is 3 ml/kg, however the protocol for the proposed testing program under the EUP indicated that the dose for cats is 3-6 ml/kg. Therefore, the highest dose in the study, 15 ml/kg, was only 2.5X the highest dose in that range.

2) There were only three cats in each dosage group. Toxicology Branch II recommended the use of six animals per sex per group for the U.S. studies. (September 3, 1993 memo from Virginia Dobozy to Joseph Tavano/Richard Mountfort/PM 10).

3) The study was not conducted in compliance with GLP guidelines.

Page 14 is not included in this copy.

Pages _____ through _____ are not included.

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: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 3/7/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. YMI 4/7/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Domestic Animal Safety Study/Dogs (86-1)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 431211-10
TEST MATERIAL:	RM 1601C Synonym: Fipronil
STUDY NUMBER:	CLI138
TESTING FACILITY:	Rhone Merieux Toulouse Cedex, France
SPONSOR:	Rhone Merieux, Inc. Athens, Georgia
TITLE OF REPORT:	Assessment of Tolerance of a 0.25% RM 1601 Spray Formulation in Dogs at 3, 9, and 15 ml/kg When Applied 6 Times to the Haircoat at 28 Day Intervals
AUTHOR(S):	J.P. Arnaud and P.J. Consalvi
REPORT ISSUED:	December 23, 1993

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 431211-10), groups of three male and three female beagle dogs were topically administered six treatments of RM 1601C (0.25% fipronil) at dosages of either 0, 3, 9 or 15 ml/kg (0, 7.5, 22.5 and 37.5 mg/kg, respectively) at 28-day intervals. (The proposed recommended dose for the product is 3-6 ml/kg.) A variety of parameters were evaluated after each treatment, including physical examination, body weight, food consumption, clinical observations and clinical pathology (hematology and clinical chemistry). Thyroid stimulation tests were conducted before and 22 days after the treatment period. Skin biopsies were done on all dogs in the high dose group, two from the control group and two with skin lesions.

There was a statistically significant increase in the number of animals judged to have abnormal eye examinations in the 9 ml and 15 ml groups after the second treatment and in the 9 ml group after the sixth treatment. The increases were not dose-responsive and were not considered to be treatment-related. Two treated dogs, one in the 3 ml group and another in the 9 ml group, were noted to have skin lesions at 21 days after the last treatment. Additional treatment with the product did not exacerbate the lesions. There was no other evidence of treatment-related toxicity.

The study is unacceptable because: 1) the dose levels were not 5X the recommended dose; and 2) the study was not conducted in compliance with the GLP regulations.

I. MATERIALS

A. Test Material

Name: RM 1601C
Synonym: RM 1601 (Fipronil)
Chemical Name: 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole
Purity: RM 1601C is 0.25% RM 1601
Batch Number: G 04 12
Description: Not provided
Storage Conditions: Not provided

The test material was supplied as a spray in a 500 ml bottle fitted with a manual trigger pump that delivered approximately 1 ml.

B. Administration: dermal

C. Test Animals

Species: Beagle dogs
Source: Dambriere Kennel and Interfauna, France
Age: Mean age of 9.3 months at start of study
Weight: Males - mean 11.9 kg; Females - mean 11.0 kg at start of study
Housing: Both individually and in groups[1]
Environmental Conditions: Not provided
Food and Water: 400 to 800 g daily of a pelleted food (Aliment MC) in the cages; groups of three animals were fed 3 kg daily in the pens; water was provided *ad libitum*
Acclimation Period: Not provided

II. METHODS

A. Dosage and Administration

Twelve dogs of each sex were randomly assigned to four groups as follows:

[1] For two days before and seven days after each monthly treatment, the dogs were housed individually in stainless steel cages at the testing facility. From the 7th day after the 1st, 2nd, 3rd, 4th, and 5th treatment until the 2 days before the following treatment, and between the 7th day after the 6th treatment until the 18th day after this treatment, dogs of the same sex were housed together in pens for animal welfare considerations.

Group	Number Males	Number Females	Dose of RMI 1601	Volume of RM 1601C/35	Number of Treatments
Control	3	3	0	0	0
3 ml	3	3	7.5 mg/kg	3 ml/kg	6
9 ml	3	3	22.5 mg/kg	9 ml/kg	6
15 ml	3	3	37.5 mg/kg	15 ml/kg	6

The treatments were administered at 28 day intervals. The dogs were weighed immediately prior to treatment. The density of the spray solution (0.851) was used to establish the volume of test material to be applied since the trigger did not deliver exactly 1 ml. The weightsof the product bottles were recorded before, during and after each application. The spray was applied against the lay of the hair over the entire animal avoiding the eyes. To avoid run-off of the product at the higher doses, the treatment procedure was repeated after drying the coat, two or three times within three hours.

An additional treatment was given to two dogs, one from the 3 ml group and another from the 9 ml group, which were noted to have skin lesions at 21 days after the last treatment. The animals were anesthetized and half of the lesion was sprayed with a volume of 3.2 ml for the 100 cm^2 skin surface which corresponded to the 15 ml/kg dose. The other half of the lesion remained as an untreated control.

B. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies.

physical examinations - before dosing and at Days 1, 2, 4, 7, 14 and 21 after each treatment (treatment day was designated Day 0)
observations for digestive disorders- during first seven days after each treatment, cages were examined for presence of vomiting and consistency of feces
body weights - at weekly intervals during the study
food consumption - measured for individual animals during the first seven days after each monthly treatment and as a group on a weekly basis for the remainder of the month
skin examinations - at same time as physical examinations[2]
coat examinations - at same time as physical examinations[3]

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[2] The skin was examined at four locations. Lesions were scored on a scale of 0 to 4. Skin fold thickness at the region 5 cm behind the umbilicus was also measured at each examination.

[3] Five criteria were analyzed: 1) score of diffuse loss of hair (scale of 0-4); 2) localized loss of hair; 3) appearance of coat; 4) score of fine scurfs (scale of 0-4); and 5) score of flaky scurfs (score of 0-4).

hematology and clinical chemistry - before and 14 days after each monthly treatment
thyroid evaluations - 10 days before and 22 days after the treatment period
skin biopsies - at 23 days after the treatment period on all dogs in the 15 ml group, two control dogs and 2 dogs with skin lesions

C. Pathological Parameters

Hematology

The CHECKED (X) hematology parameters were examined.

X Hematocrit (HCT)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc. (MCHC)
X Mean corpuscular volume (MCV)
X Hemoglobin (HGB)
__ Leukocyte count (WBC)
X Erythrocyte count (RBC)
X Platelet count

Clinical Chemistry

The CHECKED (X) clinical chemistry evaluations were done.

X Urea
X Uric acid
X Proteins
X AST
X Alkaline Phosphatases (ALP)
X Creatinine
X Glucose
X Cholesterol
X ALT

Thyroid Function

Thyroxine (T_4) was measured before and at 90 and 180 minutes after the IM administration of thyrotropin releasing hormone (TRH).

Skin Biopsies

The dogs were anesthetized with ketamine and biopsies were taken from four sites: two areas with hair (root of tail and dorsal line at the level of the ileum) and two hairless areas (both groins).

D. Statistical Analyses

A description of the statistical methods from the study report is attached to the DER.

E. Compliance

The registrant submitted a signed statement indicating that the study was not conducted in compliance with the GLP regulations; the areas of non-compliance are not identified. A signed Statement of Data Confidentiality Claims indicates no confidentiality is claimed by the registrant.

III. RESULTS

A. Comparisons of Groups Before Treatment

The control and treated groups were comparable before treatment for the following parameters: body weight, food consumption, physical examination, skin examination, hematology, clinical chemistry and thyroid function.

B. Actual Dosage Administered

The actual dosages administered were as follows:

Doses	Mean Volume (ml/kg)	Range (ml/kg)
3 ml/kg	3.01	2.84 to 3.30
9 ml/kg	8.94	8.63 to 9.50
15 ml/kg	14.90	14.22 to 16.08

C. Body Weights

Mean weekly body weight gain between the treated and control groups was comparable.

D. Food Consumption

Comparisons of individual daily food consumption data during the first week after each monthly treatment did not show any difference between the treated and control groups.

E. Gastrointestinal Monitoring

The incidences of vomiting and soft/liquid feces were comparable between the treated and control groups.

F. Physical Examinations

The incidence of findings on physical examination was comparable between the treated and control groups, except for eye examinations. There was a statistically significant increase in the number of animals judged to have abnormal eye examinations[4] in the 9 ml and 15 ml groups after the second treatment and in the 9 ml group after the sixth treatment. The increase in the incidence was not dose-responsive at either period. The specific ocular findings at either of the treatment periods were not stated.

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[4] A dog was considered abnormal if at least one single abnormal parameter was recorded. The eyes were examined for lacrimation, discharge, appearance of scleral blood vessels and color of conjunctivae.

G. Skin and Coat Examinations

There were no statistically significant differences in the number of animals with skin lesions or in skin thicknesses between the treated and control groups.

At 21 days after the last treatment, one dog in the 3 ml group had areas of hair loss on both sides of the flank, thorax and stifle and erythema on the ventral surface. Another dog in the 9 ml group had erythema on the neck, chest and entire ventral surface. These dogs were given an additional local treatment as described previously. There was no increase in edema or erythema after this treatment.

Coat examinations were comparable between the treated and control groups, except for the mean overall flaky scurf score which was increased in the 3 ml group.

G. Clinical Pathology

There were no significant differences between the treated and control groups, except for an increase in the mean ALP level in the 9 ml group (the sexes were not separated for reporting). There were no differences in T_4 levels between the treated and control groups at the end of the treatment period.

H. Skin Biopsies

There were no histological lesions in the six dogs in the 15 ml group or in the two control animals. The dog in the 3 ml group which had macroscopic skin lesions was found to have hyperkeratosis, acanthosis and parakeratosis on histology. No lesions were seen in the affected dog from the 9 ml group.

IV. STUDY DEFICIENCIES

1. The product is identified as RM 1601 C/35 in Table 1 on page 13 of the study report, whereas RM 1601C is used throughout the rest of the study. The registrant should explain the difference.

2. The study report should state how the study does not comply with the GLP Guidelines.

V. CONCLUSIONS

Groups of three male and three female beagle dogs were topically administered six treatments of RM 1601C (0.25% fipronil) at dosages of either 0, 3, 9 or 15 ml/kg (0, 7.5, 22.5 and 37.5 mg/kg, respectively) at 28-day intervals. (The proposed recommended dose for the product is 3-6 ml/kg.) A variety of parameters were evaluated after each treatment, including physical examination,

body weight, food consumption, clinical observations and clinical pathology (hematology and clinical chemistry). Thyroid stimulation tests were conducted before and 22 days after the treatment period. Skin biopsies were done on all dogs in the high dose group, two from the control group and two with skin lesions.

There was a statistically significant increase in the number of animals judged to have abnormal eye examinations in the 9 ml and 15 ml groups after the second treatment and in the 9 ml group after the sixth treatment. The increases were not dose-responsive and were not considered to be treatment-related. Two treated dogs, one in the 3 ml group and another in the 9 ml group, were noted to have skin lesions at 21 days after the last treatment. Additional treatment with the product did not exacerbate the lesions. There was no other evidence of treatment-related toxicity.

The study is unacceptable for the following reasons:

1) Although there are no guidelines for the conduct of domestic animal safety studies, Toxicology Branch II has routinely required that the studies be conducted with 5X the recommended dose. This study reached that level only for the lower dose of the dosage range.

2) The study was not conducted in compliance with the GLP regulations.

Page_____ is not included in this copy.

Pages 13 through 24 are not included.

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) _____.

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

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 4/6/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y M I 4/7/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Domestic Animal Safety Study/Puppies (86-1)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 431211-11
TEST MATERIAL:	RM 1601C Synonym: Fipronil
STUDY NUMBER:	CLI 180
TESTING FACILITY:	Rhone Merieux Toulouse Cedex, France
SPONSOR:	Rhone Merieux, Inc. Athens, Georgia
TITLE OF REPORT:	Assessment of the Tolerance of a 0.25% RM 1601 Spray in Nursing Puppies Administered Twice at a 28 Day Interval at a Dose Rate of 6 Ml/Kg
AUTHOR(S):	J.P. Arnaud and P.J. Consalvi
REPORT ISSUED:	February 8, 1994

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 431211-11), a total of 27 male and 21 female nursing puppies (9 litters of either beagle, spaniel or griffon breeds) was topically administered two treatments of RM 1601C (0.25% fipronil) at a dosage of 6 ml/kg at 28-day intervals. (The proposed recommended dosage is 3-6 ml/kg.) The dams of the litters were treated at the same dosage. Twenty-six (26) male and 15 female puppies served as untreated controls. The following parameters were evaluated: mortality, clinical examinations, body weights and skin/coat examinations.

Six treated puppies, four from the same spaniel litter, died before weaning and another died after weaning. Parvovirus was isolated from the spaniel litter. One puppy in the control died. There was no statistical difference in mortality between the groups. Growth rates were either comparable or exceeded the applicable control group for the beagle and griffon breeds, however was lower for the spaniel breed. This decrease may have been the result of the parvovirus infection.

The study is unacceptable because: 1) it was conducted with nursing rather than weaned puppies; 2) the dosage levels were not at 5X the recommended dose; 3) the study was not conducted in compliance with GLP regulations.

I. MATERIALS

A. Test Material

Name: RM 1601C
Synonym: RM 1601 (Fipronil)
Chemical Name: 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole
Purity: RM 1601C is 0.25% RM 1601
Batch Number: G 09 01
Description: Not provided
Storage Conditions: Not provided

The test material was supplied as a spray in two bottles, a 500 ml bottle fitted with a manual trigger pump that delivered approximately 1.0 ml for the treatment of the bitches and a 100 ml bottle with a trigger that delivered approximately 0.5 ml for the treatment of the puppies. A dose of 6 ml/kg of RM 1601C is equivalent to 15 mg/kg of RM 1601.

B. Administration: topical

C. Test Animals

Species: Beagle, Spaniel and Griffon puppies
Source: Not provided
Age: Mean age of 16.4 days (range: 2 to 38 days)
Weight: Mean - 740 g (range: 200 to 2250 g)
Housing: In farrowing rooms with dam
Environmental Conditions: Not provided
Food and Water: Fed by dam with mixed meat supplements
Acclimation Period: Not provided

II. METHODS

A. Dosage and Administration

According to the study report, the recommended dosage for the product will be 3 to 6 ml per kg of body weight. The duration of residual control is between one and three months.

A total of 53 male and 36 female puppies from three breeds (beagle, spaniel and griffon) were randomly assigned to the following groups.

Date of Inclusion	Group	Number of Litters	Number of Males	Number of Females
1/27/93	Control	3	11	4
	Treated	5	13	13
2/24/93	Control	1	5	0
3/24/93	Control	4	10	11
	Treated	4	14	8

Two treatments of 6 ml/kg (recommended dosage) were administered at 28 day intervals. The puppies were weighed immediately prior to treatment. The density of the spray solution (0.85) was used to establish the volume of test material to be applied since the trigger did not deliver exactly 0.5 ml. The weights of the product bottles were recorded before, during and after each application. The spray was applied against the lay of the hair over the entire animal avoiding the eyes. The dams of the litters were treated with the test spray on the same day as the puppies at a dosage of 6 ml/kg.

B. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies.

physical examinations* - before dosing and at Days 1, 2, 7, 14, 21, 28, 29, 30, 35, 42 and 49 after the first treatment (treatment day was designated Day 0)
body weights - at weekly intervals during the study; slope of growth curve for 49 days of the study was calculated for each puppy
skin examinations - at same time as physical examinations[1]
coat examinations - at same time as physical examinations[2]

* The following parameters were evaluated:

- examination of eyes for discharge and color of conjunctiva
- presence of nasal discharge
- presence of diarrhea
- examination of behavior

C. Statistical Analyses

A description of the statistical methods from the study report is attached to the DER.

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[1] The skin was examined and lesions were scored on a scale of 0 to 4.

[2] The presence of scurfs or losses of hair (diffuse and/or localized) were recorded.

D. Compliance

The registrant submitted a signed statement indicating that the study was not conducted in compliance with the GLP regulations; the areas of non-compliance are not identified. A signed Statement of Data Confidentiality Claims indicates no confidentiality is claimed by the registrant.

III. RESULTS

A. Comparisons of Groups Before Treatment

The study report states that the mean number of puppies per litter, mean ages of the litters and mean body weights of the puppies at Day 0 were not significantly different. Table II from the study report comparing the treated and control groups is attached to the DER.
The study report also indicates that the treated and control groups were comparable regarding clinical examinations at Day 0. However, according to Table III (page 17), the number of puppies in the treated group with abnormal eyes was increased [11/29 examined (38%) in the treated group vs. 2/22 (9%) in the control group].

B. Volume Administered

The volumes administered were as follows:

	First Treatment	Second Treatment
Mean volume of RM 1601C	7.22	6.81
Minimal volume	5.46	4.74
Maximal volume	8.40	7.82

C. Mortality

Six treated puppies died before weaning; another died after weaning. The age of the puppies at death ranged from 23 to 60 days. Two of the puppies died 5 and 21 days after the first treatment; five died 12, 13, 15, 17 and 18 days after the second treatment. Four were from the same litter and died over a period of 6 days. Parvovirus was found in the ingesta from one of the puppies. One puppy from the control group died at 18 days old, 14 days after inclusion in the study. There was no statistical difference in mortality between the groups.

D. Body Weights

To evaluate the effect of the test spray on growth, the slopes of the regression lines of growth of the treated and control groups were compared. There was no difference for beagle puppies and the treated griffon puppies showed significantly higher growth rates than the controls of the same breed. The spaniel puppies had a

significantly lower growth rate than the control spaniel puppies. However, these findings in spaniels were based on only one litter of puppies which was the litter infected with parvovirus and which had increased mortality.

E. Physical Examinations

There were no significant differences in the incidences of diarrhea, nasal discharge and abnormal behavior between the treated and control groups.

G. Skin and Coat Examinations

There were several time points at which there were statistically significant differences in the number of animals with either a scurf condition or hair loss, however many times the incidence was lower in the treated puppies.

IV. STUDY DEFICIENCIES

1. It is noted on page 11 of the study report that one control group was included in the study at a separate time from the treated groups. Since this was an untreated control, it is assumed that the group was used as a monitor of the background health of the puppies in the study. However, if there was no treated group included at the time, the purpose of this control group is unclear.

2. The study report should state how the study does not comply with the GLP Guidelines.

3. All the individual animal data should be submitted.

V. CONCLUSIONS

A total of 27 male and 21 female nursing puppies (9 litters of either beagle, spaniel or griffon breeds) was topically administered two treatments of RM 1601C (0.25% fipronil) at a dosage of 6 ml/kg at 28-day intervals. (The proposed recommended dosage is 3-6 ml/kg.) The dams of the litters were treated at the same dosage. Twenty-six (26) female and 15 male puppies served as untreated controls. The following parameters were evaluated: mortality, clinical examinations, body weights and skin/coat examinations.

Six treated puppies, four from the same spaniel litter, died before weaning and another died after weaning. Parvovirus was isolated from the spaniel litter. One puppy in the control died. There was no statistical difference in mortality between the groups. Growth rates were either comparable or exceeded the applicable control group for the beagle and griffon breeds, however was lower for the spaniel breed. This decrease may have been the result of the parvovirus infection.

This study is unacceptable for the following reasons:

1) Although there are no guidelines for the conduct of domestic animal safety studies, Toxicology Branch II recommends that such studies be conducted with weaned rather than nursing puppies. Additionally, this study was complicated by the treatment of the dams and therefore, the possible ingestion of the test chemical by the puppies.

2) Toxicology Branch II has routinely required that a test chemical be administered at 5X the recommended dose in domestic animal safety studies. This study was done at only 1X the highest dose of the recommended dose range.

3) The study was not conducted in compliance with the GLP regulations.

Page____ is not included in this copy.

Pages 31 through 33 are not included.

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