



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

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REVIEWER

JAN. 1/24/96

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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Fipronil Pet Formulations - EUP for Spot-On Product (10% a.i.) and Response to Review of Domestic Animal Safety Study in Puppies

DP Barcode: D215134; D219826
PC Code: 129121
Case: 007022; 014261
Submission: S483920; S494457

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer *Virginia A. Dobozy 12/5/95*
Registration and Special Review Section
Occupational and Residential Exposure Branch
Health Effects Division (7509C)

TO: Rick Keigwin/Ann Sibold, PM Team 10
Insecticide-Rodenticide Branch
Registration Division (7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head *J.M. Ioannou 1/17/96*
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Stephanie R. Irene, Ph.D., Acting Branch Chief
Toxicology Branch II
Health Effects Division (7509C) *Stephanie R. Irene 1/23/96*

Action Requested: 1) Review data submitted to support an EUP application for use of a 9.7% fipronil end use product for flea and tick control for use on dogs and cats; review registrant's response to Toxicology Branch II's review of the domestic animal safety study in puppies (MRID #43444905).

Recommendation: Toxicology Branch II recommends that an EUP for the 10% formulation be granted. The domestic animal safety study in puppies should be upgraded to Acceptable. See Status of Fipronil Pet Product Registrations on page seven for future actions with these products.

1. EUP for Spot-on Formulation

Summaries of Studies/ Documents

A. Discussion in Support of Bridging Domestic Animal Safety Data on Frontline® Spray Treatment to Frontline® Spot Treatment Data Package - MRID# 43577711

The document presents an argument on why there is justification for linking the new product, Frontline® Spot Treatment (10% w/v fipronil), to the existing product's (Frontline® Spray Treatment, 0.25% w/v fipronil) domestic animal data base. The following points are included:

- No adverse toxicity to dogs and cats were observed in the efficacy studies.
- The acute toxicity studies for both formulations have been classified Category III. (Toxicology Branch II did not review these studies for the 10% formulation.)
- In the domestic animal safety studies, the 0.25% formulation was administered to adult and juvenile animals at five times the recommended dose (30 ml/kg). A comparison of the actual active ingredient applied to the animal at maximum rates illustrates that less active ingredient was applied with the 10% formulation, except for cats weighing less than 2 kg. A comparison table shows the following comparisons.

Dog Bodyweight (kg)	Spot Treatment Total Dose (mg)	Spray Treatment Total Dose (mg)
5	67	75
10	134	150
20	268	300
Cat Bodyweight (kg)	Spot Treatment Total Dose (mg)	Spray Treatment Total Dose (mg)
2	50	30
4	50	60
8	50	120

Extracted from text table on page 5.

- The inerts in the two formulations differ. However, those found in the 10% formulation are listed on the Pesticide Product Inert Ingredients List or the GRAS list. The vehicle control for this formulation was tested in an acute toxicity battery and showed the same level of toxicity as the formulation.

B. Fipronil: Spot-On Position Paper - MRID# 43577714

This paper is a summary of pharmacokinetics studies conducted after the administration of various spot-on 10% fipronil formulations. Most of the studies referenced in the paper have not been submitted to Toxicology Branch II for review. The paper addresses blood and hair levels of the chemical found in these studies on both dogs and cats and an assessment of the minimum effective concentration of the chemical on hairs.

1. Blood Levels

Plasma levels of fipronil and its major sulfone metabolite (RM1602) were observed in the dog after application of various 10% fipronil spot formulations.¹ There was no time relationship between plasma levels and activity of fipronil and efficacy against fleas and ticks.

In the cat, no or very low fipronil concentrations could be detected after the spot-on application of various formulations.²

2. Hair Levels

Various studies demonstrated that fipronil can translocate quickly from the spot of application on the dog. Hair concentrations lasted more than two months. Similar findings were observed in the cat.

3. Minimum Effective Concentration (MEC)

The MEC to achieve 95% flea and tick control was 0.7 µg/g and 11.0 µg/g, respectively. In dogs, these levels were found on hair at the spotted area and distal to the spot showing a good translocation of the chemical. The spreading can be accomplished mechanically by scratching and rubbing along cage walls and physiologically by passive diffusion in sebum present on the skin and around the hairs. Similar findings were observed in the cat.

C. Special Study - Modified Dermal Absorption/Dogs - See attached DER

Percutaneous Absorption and Hair, Skin and Fat Distribution of Radioactivity After Spot-On Application of ¹⁴C-RM1601 in Beagle Dogs

¹ The bibliography identifies studies with the following formulations in the dog: RM1601E/16; RM1601 E/32; RM1601E/46; RM1601E/48; RM1601E/50; RM1601E/51; RM1601E/62. The Confidential Statement of Formula identifies the product as RM1601E/62.

² The bibliography identifies two formulations used in studies on cats, RM1601 E/46 and RM1601E/62. The Confidential Statement of Formula identifies the product as RM1601E/62.

- MRID# 43577715

Material Tested: ^{14}C -RM1601 (98.5% a.i.)

A single dermal 10 mg/kg dose of ^{14}C -RM1601 (fipronil) was administered to three male beagle dogs at a single location between the shoulder blades. Plasma levels of the radioactive chemical were determined at various time points. The animals were sacrificed at either day 7, 15 or 56, and hair, skin and fat samples from the application site and an untreated area were analyzed for radioactivity.

The study demonstrated that fipronil is absorbed systemically. Plasma levels of radioactivity were detected from day 2 to day 30 post-treatment; maximum concentration varied between days 4-7 in the three dogs. The highest levels of radioactivity were detected in the hair at the application and untreated sites, followed by the skin and then the fat. Levels in the subcutaneous fat at both the control area and application site were comparable to that from the perirenal area. At day 56 of the study, detectable radioactivity was still found in hair, skin and fat from the application site and from skin and fat from the untreated site.

Classification: Unacceptable. The study does not satisfy the guideline requirements for a dermal absorption study. The total dose of radioactive chemical was not accounted for in urine, feces and carcass. Therefore, a percentage of the dermal dose absorbed systemically could not be calculated.

D. Special Study - Modified Dermal Absorption/Cats - See attached DER

Percutaneous Absorption and Distribution of Radioactivity in Hair, Skin, and Fat After Spot-On Application of ^{14}C -RM1601 in Cats - MRID # 43577716

Material Tested: ^{14}C -RM1601 (96.6%)

A single dermal 50 mg dose of ^{14}C -RM1601 (fipronil) was administered to three male European cats at a single location between the shoulder blades. Plasma levels of the radioactive chemical were determined at various time points. The animals were sacrificed at either day 7, 15 or 30, and hair, skin and fat samples from the application site and an untreated area were analyzed for radioactivity.

The study demonstrated that systemic absorption of fipronil is insignificant in cats. The highest concentration of the chemical was found in hair at Day 30 post-treatment. Skin radioactivity was only detected at the application site and was highest 15 days post-treatment. There was a good distribution of the chemical in the

hair, with a gradient of treatment between the application site and the untreated area. The only radioactivity found in fat was at the application area at 30 days post-treatment.

Classification: Unacceptable. The study does not satisfy the guideline requirements for a dermal absorption study. The total dose of radioactive chemical was not accounted for in urine, feces, and carcass. Therefore, a percentage of the dermal dose absorbed systemically could not be calculated.

CONCLUSIONS

The registrant makes a convincing argument as to why the toxicology data base for the 0.25% formulation should be used to justify granting an EUP for the 10% formulation.

2. Response to Toxicology Branch II's Review of the Domestic Animal Safety Study in Puppies (MRID # 43444905)

Registrant's Response

In a September 25, 1995 letter, the registrant responded to the deficiencies in the study titled "Domestic Animal Safety Study of RM1601C Topical Spray in Juvenile Dogs". (See September 15, 1995 memo from Virginia A. Dobozy to Rick Keigwin and the accompanying review.) The response follow the deficiencies item by item below.

Deficiency No. 1:

The Toxicology Branch II review questioned the concentration of the product used in the study. The registrant replied that the concentration of active ingredient was originally expressed as 0.25% weight/volume (w/v). However, EPA requires the label of products of this type to express the active ingredient in weight/weight (w/w) concentrations. Therefore, 0.25% w/v was calculated to be 0.29% w/w.

Deficiency No. 2:

The Toxicology Branch II review requested clarification about how the recommended dosage on the label equates to the 1X dosage used in the study. The registrant provided the following calculations:

- The small bottle will contain a standard finger pump which delivers approximately 0.5 ml/pump. The large bottle will deliver approximately 1.5 ml/pump.
- Using a small bottle, the label recommendation of 3-6 pumps would deliver 1.4 - 2.7 ml per pound (3-6 ml/kg). Using the large bottle, the label recommendation of 1-2 pumps would deliver the same dose.
- The conversion factor to clarify the application volumes in

parentheses is one ounce equals 30 ml. Approximately one half ounce (15 ml) to one ounce (30 ml) is needed to treat an average size cat (5 kg) at 3-6 ml/kg. Approximately one ounce (30 ml) to two ounces (60 ml) is needed to treat a 25 pound dog at the same dosage.

The 6ml/kg dosage used in the study represents the highest dosage used in the acceptable ranges of doses (3-6 ml/kg or 1.4-2.7 ml/lb) for the product.

Toxicology Branch II Response: Using the calculations below, the dose used in the study was actually slightly less than the highest recommended dose.

Study Dose - 6 ml/kg

Label Recommendation - 3-6 pumps/lb

1.5 - 3.0 ml/lb (1 pump = 0.5 ml)

3.3 - 6.6 ml/kg (1 kg = 2.2 lb)

The 1X study dose was 9% less than the highest recommended dose.

Deficiency No. 3:

The Toxicology Branch II review requested clarification about the treatment of the replacement animals in the study.

Three dogs died during the study. All three showed symptoms of canine parvovirus infection, but the diagnosis was confirmed at necropsy for only two. The registrant states that two of the dogs that died early in the study (Days 16 and 22) were replaced. The data from these dogs were excluded from the data base. Analysis of the pre- and post-treatment data indicates that it was generally comparable to the main body of the data and therefore could be excluded. The third dog died late in the study; the data from this animal were included in the data base.

The replacement animals started and completed the entire study approximately one month after the other subjects. They received all applications of the test material and all required parameters were measured. Analysis of the data from these animals indicated that the chronological delay did not have an effect, and therefore, their data were combined with that of the other dogs.

Deficiency No. 4:

The Toxicology Branch II review stated that individual necropsy reports should have been provided on the animals which died during the study. The registrant has submitted those reports, along with laboratory data on those animals. The pathology reports give the following findings for individual animals:

- Dog 3M (control, died Day 62) - dark red in color or dark red

streaks in portions of gastrointestinal tract and lungs

- Dog 4M (control, died Day 22) - red mottled lungs; fluid in thoracic cavity

- Dog 27 (5x group, died day 16) - mottled, red and black lungs filled with reddish, mucous-like fluid; fluid in thoracic cavity; reddened mucosa of stomach, colon and rectum; moderately to severely reddened small intestine containing greenish mucoid substance

A review of the laboratory results showed that animal 4M had hematology parameters indicative of an infectious process with a very high white blood cell count and a differential with a shift to the left.

Conclusions

The registrant has satisfactorily addressed all of the deficiencies in the Toxicology Branch II review. Although the 1X dose was slightly less than the highest recommended dose, there was no evidence of toxicity at that level or 5X the recommended dose. Therefore, the dose is judged to be adequate.

Classification: Upgraded to Acceptable

Status of Fipronil Pet Product Registrations

The following is a summary of the status of the fipronil domestic animal products (spray and spot-on formulations).

- There are acceptable studies in puppies (MRID # 43444905) and kittens (MRID # 43444904).

- The studies in adult dogs (MRID # 43121110) and cats (MRID # 43121111) were judged to be unacceptable because they were not conducted in compliance with GLP regulations and the highest dose level was not 5X the highest recommended dose.

- The registrant has submitted a bridging argument that the data generated with the spray formulation should be used to satisfy the regulatory requirements of the spot-on product. Toxicology Branch II has considered this argument valid. (DP Barcode: D215134)

- The registrant previously submitted protocols for domestic animal safety studies with the spot-on formulation. In a telephone conversation on December 1, 1995, Kandy Walker of RMI said that these studies have been completed, but were not going to be submitted because of the bridging argument that was made. It was agreed that these studies would be submitted. The spray formulation is registered in French, and there are use data with the spray formulation. In addition, the registrant will summarize any adverse

reactions which have occurred during the field trials which they are presently doing with the spray formulation. Information for both of these uses will be submitted.

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. *Virginia A. Dobozy 1/17/96*
Registration & Special Review Section, OREB
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *J.M. Ioannou 1/17/96*
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Special Study - Modified Dermal Absorption Study/Cats

EPA I.D. NUMBERS: P. C. CODE: 129121
MRID NUMBER: 43577716

TEST MATERIAL: RM1601C
Synonym: Fipronil

STUDY NUMBER: CD-93/3748F

TESTING FACILITY: Centro de Investigacion y Desarrollo Aplicado, S.A.L.
Barcelona, Spain

SPONSOR: Rhone Merieux, Inc.
Athens, Georgia

TITLE OF REPORT: Percutaneous Absorption and Distribution of Radioactivity in Hair, Skin, and Fat After Spot-on Application of ¹⁴C-RM1601 in Cats

AUTHOR(S): J. Villageliu, S. Lopez, M. Germa, D. Moya, M. Canovas

REPORT ISSUED: October 25, 1994

EXECUTIVE SUMMARY: In a dermal absorption study (MRID # 43577716), a single dermal 50 mg dose of ¹⁴C-RM1601 (fipronil) was administered to three male European cats at a single location between the shoulder blades. Plasma levels of the radioactive chemical were determined at various time points. The animals were sacrificed at either day 7, 15 or 30, and hair, skin and fat samples from the application site and an untreated area were analyzed for radioactivity.

The study demonstrated that systemic absorption of fipronil is insignificant in cats. The highest concentration of the chemical was found in hair at Day 30 post-treatment. Skin radioactivity was only detected at the application site and was highest 15 days post-treatment. There was a good distribution of the chemical in the hair, with a gradient of treatment between the application site and the untreated area. The only radioactivity found in fat was at the application area at 30 days post-treatment.

Classification: Supplementary. The study does not satisfy the guideline requirements for a dermal absorption study. The total dose of radioactive chemical was not accounted for in urine, feces, and carcass. Therefore, a percentage of the dermal dose absorbed systemically could not be calculated.

I. MATERIALS

A. Test Materials

RM1601, batch no. 18BDM91

¹⁴C-RM1601, batch no. GHS 822A

Specific radioactivity: 43.71 μ Ci/mg (19.135 mCi/mmol)

Synonym: Fipronil

Chemical Name: 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole

Purity: 96.6%

Description: White powder

Storage Conditions: ¹⁴C-RM1601 was stored in the freezer between -20 and -25°C. RM1601 was stored at room temperature.

B. Administration: dermal

C. Test Animals

Species: Male European cats

Source: BIOCENTRE, S.A., Barcelona, Spain

Age: Approximately 2 years at start of study

Weight: 4.8-5.3 kg at start of study

Housing: In metabolism cages

Targeted environmental Conditions: Temperature: 22 $\pm$ 2°C

Relative humidity: 55 $\pm$ 10%

Food and Water: Gi'll Guy Mix and water were provided *ad libitum*

Acclimation Period: Twelve days

Routine hematology, clinical chemistry and urinalysis tests were conducted prior to the commencement of the study to assure that the animals were healthy.

D. Dose Preparation

Dose

RM1601: 50 mg of RM1601 (10% fipronil) per animal

¹⁴C-RM1601: 50 μ Ci per animal

Volume: 0.5 ml per animal

Vehicle: Solution of ethanol 10%, PVP K17 5%, Tween 80 5% and transcutol qs 100%

Preparation

RM1601 and ¹⁴C-RM1601 were dissolved in the vehicle using a magnetic shaker. The radioactivity in the dosing solution, measured in triplicate, was 87.81 μ Ci/g formulation. The activity of ¹⁴C-RM1601 was 2099.9 dpm/ μ g. The radiochemical purity of the final formulation was 99.68%.

II. METHODS

A. Dosage and Administration

A single spot-on dose of 50 mg/kg (0.5 ml/animal) was applied to three male cats directly to the skin after parting the hairs between the shoulder blades.

B. Experimental Design

Blood sample collection:

First cat (No. 607): T0-T10h, D1, D2, D3, D4, D5, D6 and D7

Second cat (No. 605): T0-T10h, D1, D2, D3, D4, D5, D6, D7 and D15

Third cat (No. 593): T0-T10h, D1, D2, D3, D4, D5, D6, D7, D15 and, D30

The blood samples were centrifuged and radioactivity in the plasma was measured without delay.

Skin, hair and fat distribution of radioactivity:

The cats were sacrificed on either day 7, 15 or 30 of the study.

Hair - A large area around the application site was shaved. The area was washed with a piece of gauze moistened with ethanol. The hair and gauze were analyzed to evaluate the amount of chemical on the haircoat of the cat.

Skin and Subcutaneous Fat - Skin samples and subcutaneous fat were taken separately from the application area to determine the total radioactivity present in each.

Untreated Area - Hair, skin and subcutaneous fat were also sampled from an untreated area in the lumbar region.

Deep Fat - Perirenal fat was taken to evaluate the deep storage of the chemical.

The samples were digested in NaOH 1N at 50°C for approximately 24 hours before radioactivity was determined. Duplicate samples were counted using a Beckman LS6000 liquid scintillation counter.

C. Compliance

The registrant submitted a signed statement indicating that the study was conducted in compliance with the Good Laboratory Practice regulations published by the OECD. Signed Quality Assurance and No Data Confidentiality Claims statements were also submitted.

III. RESULTS

Plasma Radioactivity

Radioactivity was not detected at any time points post-treatment.

Hair Distribution of ^{14}C -RM1601

The following total radioactivity levels (total ngEq) in hair were found at the indicated time periods (from Table 2, page 22 of the study report):

	Day 7 Cat No. 607	Day 15 Cat No. 605	Day 30 Cat No. 593
Hair, Control Area	63386	370809	97617
Hair, Application Zone	14303874	16910357	770314

Skin Distribution of ^{14}C -RM1601

The following total radioactivity levels (total ngEq) in skin were found at the indicated time periods (from Table 2, page 22 of the study report):

	Day 7 Cat No. 607	Day 15 Cat No. 605	Day 30 Cat No. 593
Skin, Control Area	-	-	-
Skin, Application Zone	749924	1407727	129787

Fat Distribution of ^{14}C -RM1601

The following total radioactivity levels (total ngEq) in fat were found at the indicated time periods (from Table 2, page 22 of the study report):

	Day 7 Cat No. 607	Day 15 Cat No. 605	Day 30 Cat No. 593
S.C. Fat, Control Area	-	-	-
Fat, Application Zone	-	-	40918
Fat, Perirenal Area	-	-	-

Radioactivity in Gauze

The amount of radioactivity (total ngEq) recovered from the gauze appears below (from Table 4, page 23 of the study report):

	Day 7 Cat No. 607	Day 15 Cat No. 605	Day 30 Cat No. 593
Skin, Control Area	1477	2548	266
Skin, Application Zone	235274	985875	22395

IV. STUDY DEFICIENCIES

1. The product tested in this study is identified as RM1601 E/59 (page 14), whereas the product in the Confidential Statement of Formula (CSF) is labeled RM1601 E/62. It appears that the difference is the addition of [REDACTED] to the CSF. The effect of these additional inert ingredients on the dermal absorption pattern of the chemical is unknown.

A position paper (MRID # 43577714) on the spot-on product indicates that multiple studies have been done to measure the plasma levels of fipronil and its major sulfone metabolite. Various formulations were tested, but none was conducted with radiolabeled chemical. The plasma levels obtained in these studies, especially the one using RM1601 E/62, should be compared to the results of the present study.

2. The study report indicates that 50 mg of RM1601/animal was approximately 12.5 mg/kg (page 14). However, based on the weights of the animals (4.8-5.3 kg), the dosage would range from 9.4 to 10.4 mg/kg.

V. CONCLUSIONS FROM STUDY REPORT

The study report makes the following conclusions:

- Only a minimal amount of the chemical was absorbed as evidenced by the lack of radioactivity in plasma and perirenal fat. A slight diffusion to the skin of the treatment zone and then to the subcutaneous fat at this area was observed.
- The highest concentration of the chemical was found in the hair at Day 30 post-treatment.
- There was a good distribution of the chemical in the hair, with a good gradient of treatment between the application area and the lumbar zone.
- Skin radioactivity was only detected at the application area and was highest 15 days post-treatment.
- These results show that systemic passage of the chemical is very low after a spot-on application.

VI. CONCLUSIONS

In a dermal absorption study (MRID # 43577715), a single dermal 50 mg dose of ^{14}C -RM1601 (fipronil) was administered to three male European cats at a single location between the shoulder blades. Plasma levels of the radioactive chemical were determined at various time points. The animals were sacrificed at either day 7, 15 or 30, and hair, skin and fat samples from the application site and an untreated area were analyzed for radioactivity.

The study demonstrated that systemic absorption of fipronil is insignificant in cats. The highest concentration of the chemical was found in hair at Day 30 post-treatment. Skin radioactivity was only detected at the application site and was highest 15 days post-treatment. There was a good distribution of the chemical in the hair, with a gradient of treatment between the application site and the untreated area. The only radioactivity found in fat was at the application area at 30 days post-treatment.

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. *Virginia A. Dobozy 12/5/93*
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Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Special Study - Modified Dermal Absorption Study/Dogs

EPA I.D. NUMBERS: P. C. CODE: 129121
MRID NUMBER: 43577715

TEST MATERIAL: RM1601C
Synonym: Fipronil

STUDY NUMBER: CD-93/3405F

TESTING FACILITY: Centro de Investigacion y Desarrollo Aplicado,
S.A.L.
Barcelona, Spain

SPONSOR: Rhone Merieux, Inc.
Athens, Georgia

TITLE OF REPORT: Percutaneous Absorption and Hair, Skin, and Fat Distribution of Radioactivity After Spot-on Application of ¹⁴C-RM1601 in Beagle Dogs

AUTHOR(S): M. Canovas, J. Villageliu, S. Lopez, M. Germa, D. Moya, P. Birckel

REPORT ISSUED: May 9, 1994

EXECUTIVE SUMMARY: In a modified dermal absorption study (MRID # 43577715), a single dermal 10 mg/kg dose of ¹⁴C-RM1601 (fipronil) was administered to three male beagle dogs at a single location between the shoulder blades. Plasma levels of the radioactive chemical were determined at various time points. The animals were sacrificed at either day 7, 15 or 56, and hair, skin and fat samples from the application site and an untreated area were analyzed for radioactivity.

The study demonstrated that fipronil is absorbed systemically. Plasma levels of radioactivity were detected from day 2 to day 30 post-treatment; maximum concentration varied between days 4-7 in the three dogs. The highest levels of radioactivity were detected in the hair at the application and untreated sites, followed by the skin and then the fat. Levels in the subcutaneous fat at both the control area and application site were comparable to that from the perirenal area. At day 56 of the study, detectable radioactivity was still found in hair, skin and fat from the application site and from skin and fat from the untreated site.

Classification: Supplementary. The study does not satisfy the guideline requirements for a dermal absorption study. The total dose of radioactive chemical was not accounted for in urine, feces and carcass. Therefore, a percentage of the dermal dose absorbed systemically could not be calculated.

I. MATERIALS

A. Test Materials

RM1601, batch no. 01BBP92

¹⁴C-RM1601, batch no. GHS707A

Specific radioactivity: 43.86 μ Ci/mg (19.2 mCi/mmol)

Synonym: Fipronil

Chemical Name: 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole

Purity: 98.5%

Description: Fine white powder

Storage Conditions: ¹⁴C-RM1601 was stored in the freezer between -20 and -25°C. RM1601 was stored at room temperature, and the vehicle of the formulation was kept at 4°C.

B. Administration: dermal

C. Test Animals

Species: Male beagle dogs

Source: BIOCENTRE, S.A., Barcelona, Spain

Age: 10-11 months at start of study

Weight: 10.0-10.8 kg at start of study

Housing: In kennel during acclimation period and then in metabolism cages, except for one animal which was housed in a kennel from day 30 onward

Targeted environmental Conditions: Temperature: 18 $\pm$ 3°C

Relative humidity: 40-70%

Food and Water: Food (UAR 125C) and water were provided *ad libitum*

Acclimation Period: Two weeks

Routine hematology, clinical chemistry and urinalysis tests were conducted prior to the commencement of the study to assure that the animals were healthy.

D. Dose Preparation

Dose

RM1601: 10 mg/kg

¹⁴C-RM1601: 10 μ Ci/kg

Volume: 0.1 ml/kg

Vehicle: Solution of ethanol 10%, PVP K17 10% and transcutol qs 100% (RM1601 E/46)

Preparation

RM1601 and ¹⁴C-RM1601 were dissolved in the vehicle using a

magnetic shaker. The radioactivity in the dosing solution, measured in triplicate, was 92.6 $\mu\text{Ci/g}$ formulation. The activity of ^{14}C -RM1601 was 2002.8 dpm/ μg . The radiochemical purity of ^{14}C -RM1601 and the final formulation was 99.34% and 99.60%, respectively.

II. METHODS

A. Dosage and Administration

A single spot-on dose of 10 mg/kg (0.1 ml/kg) was applied to three male beagle dogs directly to the skin after parting the hairs between the shoulder blades.

B. Experimental Design

Blood sample collection:

First dog (No. 6523): T0-T10h, D1, D2, D3, D4, D5, D6 and D7
Second dog (No. 6553): T0-T10h, D1, D2, D3, D4, D5, D6, D7 and D15
Third dog (No. 6529): T0-T10h, D1, D2, D3, D4, D5, D6, D7, D15, D30 and D56

The blood samples were centrifuged and radioactivity in the plasma was measured without delay.

Skin, hair and fat distribution of radioactivity:

The dogs were sacrificed on either day 7, 15 or 56 of the study.

Hair - An area 15 x 10 cm around the application site was shaved. The area was washed with a piece of gauze moistened with ethanol. The hair and gauze were analyzed to evaluate the amount of chemical on the haircoat of the dog.

Skin and Subcutaneous Fat - Skin samples and subcutaneous fat were taken separately from the application area to determine the total radioactivity present in each.

Untreated Area - Hair, skin and subcutaneous fat were also sampled from an untreated area in the lumbar region.

Deep Fat - Perirenal fat was taken to evaluate the deep storage of the chemical.

The samples were digested in NaOH 1N at 50°C for approximately 24 hours before radioactivity was determined. Duplicate samples were counted using a Beckman LS6000 liquid scintillation counter.

C. Compliance

The registrant submitted a signed statement indicating that the study was conducted in compliance with the Good Laboratory Practice regulations published by the OECD. Signed Quality Assurance and No Data Confidentiality Claims statements were also submitted.

III. RESULTS

Plasma Radioactivity

Non-quantitative levels of radioactivity were found in all three dogs at the 10h and Day 1 time periods. The maximum concentration (C_{max}) occurred between days 4 to 7 and ranged from 262.28 to 410.23 ngEq/ml. Attached Table 2 from the study presents the data from each time point.

Hair Distribution of ^{14}C -RM1601

The following total radioactivity levels (total ngEq) in hair were found at the indicated time periods (from Table 3, page 24 of the study report):

	Day 7 Dog No. 6523	Day 15 Dog No. 6553	Day 56 Dog No. 6529
Hair, Control Area	144015	15033	nq
Hair, Application Zone	633170	98170	17770

Skin Distribution of ^{14}C -RM1601

The following total radioactivity levels (total ngEq) in skin were found at the indicated time periods (from Table 3, page 24 of the study report):

	Day 7 Dog No. 6523	Day 15 Dog No. 6553	Day 56 Dog No. 6529
Skin, Control Area	3621	1329	nq
Skin, Application Zone	27132	5404	1414

Fat Distribution of ^{14}C -RM1601

The following total radioactivity levels (total ngEq) in fat were found at the indicated time periods (from Table 3, page 24 of the study report):

	Day 7 Dog No. 6523	Day 15 Dog No. 6553	Day 56 Dog No. 6529
S.C. Fat, Control Area	7853	3224	535
Fat, Application Zone	8798	2620	514
Fat, Perirenal Area	10059	2600	ng

Radioactivity in Gauze

The amount of radioactivity (total ngEq) recovered from the gauze appears below (from Table 4, page 25 of the study report):

	Day 7 Dog No. 6523	Day 15 Dog No. 6553	Day 56 Dog No. 6529
Skin, Control Area	24246	313	86
Skin, Application Zone	126668	138114	7853

IV. STUDY DEFICIENCIES

The product tested in this study is identified as RM1601 E/46 (page 14), whereas the product in the Confidential Statement of Formula (CSF) is labeled RM1601 E/62. It appears that the difference is the addition of [REDACTED]

[REDACTED] to the CSF. The effect of these additional inert ingredients on the dermal absorption pattern of the chemical is unknown.

A position paper (MRID # 43577714) on the spot-on product indicates that multiple studies have been done to measure the plasma levels of fipronil and its major sulfone metabolite. Various formulations were tested, but none was conducted with radiolabeled chemical. The plasma levels obtained in these studies, especially the one using RM1601 E/62, should be compared to the results of the present study.

V. CONCLUSIONS FROM STUDY REPORT

The study report makes the following conclusions:

- The chemical is absorbed by subcutaneous passage as shown by the presence of radioactivity in plasma and fat.
- There is a good distribution of the chemical in the hair, presenting a good gradient of concentration between the application zone and the untreated area.
- The persistence of the chemical at day 56 in the skin indicates that the skin has a storing effect.
- The results are in favor of a systemic passage of the chemical in

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

subcutaneous fat rather than a direct passage of the drug through the skin.

VI. CONCLUSIONS

In a modified dermal absorption study (MRID # 43577715), a single dermal 10 mg/kg dose of ^{14}C -RM1601 (fipronil) was administered to three male beagle dogs at a single location between the shoulder blades. Plasma levels of the radioactive chemical were determined at various time points. The animals were sacrificed at either day 7, 15 or 56, and hair, skin and fat samples from the application site and an untreated area were analyzed for radioactivity.

The study demonstrated that fipronil is absorbed systemically. Plasma levels of radioactivity were detected from day 2 to day 30 post-treatment; maximum concentration varied between days 4-7 in the three dogs. The highest levels of radioactivity were detected in the hair at the application and untreated sites, followed by the skin and then the fat. Levels in the subcutaneous fat at both the control area and application site were comparable to that from the perirenal area. At day 56 of the study, detectable radioactivity was still found in hair, skin and fat from the application site and from skin and fat from the untreated site.