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## Data Evaluation Record

Study type: Metabolism (85-1)

EPA identification numbers:
EPA MRID numbers: 429186-55
Submission: S454829
DP Barcode: D197450
P.C. Code: 129121

Laboratory Project ID: HUK Report No. 7040-68/117

Test materials: (5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl phenyl)-4-trifluoromethyl sulphinyl-pyrazole); (5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl [U- $^{14}$C]phenyl)-4-trifluoromethyl sulphinyl-pyrazole).

Other names: ($^{14}$C)-M&B 46,030; Fipronil

Testing Facilities: Hazleton UK, North Yorkshire, England.

Sponsor: Rhone Poulenc Agriculture, Ongar, Essex CM5 OHW.

Title of report: (14-C)-M&B 46,030: Absorption, Distribution, Metabolism, and Excretion in the Rat (2 Volumes)

Author(s): P Powles, C Biol, M I Biol

Report issued: June 26, 1992

Executive Summary:

In a rat metabolism study (MRID # 429186-55), $^{14}$C-Fipronil was administered orally in carboxymethylcellulose to groups (5 sex/dose) of male and female Sprague-Dawley rats at a low oral dose (4 mg/kg) repeated low oral dose (4 mg/kg x 14 days), and a single high dose (150 mg/kg).

The rate and extent of absorption appeared similar among all dose groups, but may have been decreased at the high dose. Distribution data showed significant amounts of residual radioactivity in carcass, g.i. tract, liver, adrenals, and abdominal fat at 168 hours post-dose for all rats in all dose groups. Repeated low oral dosing or a single high oral dose resulted in an overall decrease in the amount of residual radioactivity found, but an increase in the amount in abdominal fat, carcass, and adrenals.

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Feces appeared to be the major route of excretion for Fipronil derived radioactivity, where between 45-75% of an administered dose was excreted. Excretion in urine was between 5-25%. Increases in the percentages excreted in urine and feces were observed with repeated low oral dosing or a single high dose, while the percentage found in all tissues combined decreased. There were no significant sex-related differences in excretion.

Several metabolites were identified in urine and feces of Fipronil dosed rats. Major metabolites in urine included two ring-opened products of the metabolite M&B 45,897, two oxidation products (M&B 46,136 and RPA200766), and parent chemical (M&B 46,030). In feces, parent M&B 46,030 was detected as a significant fraction of the sample radioactivity as well as the oxidation products M&B 46,136 and M&B 45,950.

Pharmacokinetic investigations showed that at the single low oral dose, whole blood half-life ranged from 149.4-200.2 hr in male and female rats, with 0-168 hr AUCs approximately equal between sexes. At the single high oral dose, whole blood half-life was noticeably decreased to 54.4 hr in male rats and 51.2 hr in female rats. Blood AUCs at this dose were approximately proportional to the increase in dose.

Core Classification: minimum

This study satisfies the data requirements for a metabolism study in rats under Subdivision F guideline §85-1.

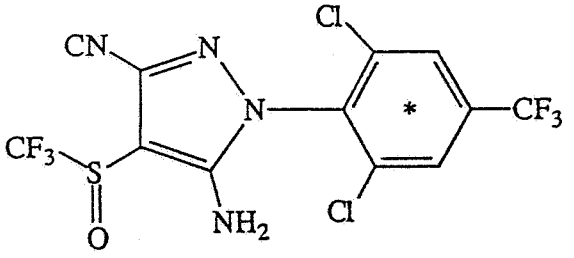
MATERIALS

A. Test Materials

[1]: [U-$^{14}$C] -M&B 46,030
Lot nos: 1 and 2
Batch No. IHR 1465
Radiochemical Purity: > 97.0%
Specific Activity: 19.62 mCi/mmol; 44.81 µCi/mg

[2]: Unlabelled M&B 46,030
Lot no: 1
Batch No. AJK 232
Chemical purity: >99.3%

Structure: (* indicates position of label for radiolabelled test substance)




B. Vehicles: aqueous methylcellulose (0.5% w/v) containing Tween 80 (0.01% w/v)

C. Test Animals: Species: rat
Strain: Crl:CD(SD) BR
Source: Charles River (UK) Ltd., Margate, Kent
Age: approximately 5-10 weeks on arrival
Weights (mean and range):

Dose groups (Definitive Study)

| | males | females |
|---|---|---|
| Low Dose (Group A) | 193.8g (183-204g) | 187.4 (180-193g) |
| Mult. Low Dose (Group B) | 296.2 (280-306g) | 208.0 (192-221g) |
| High Dose (Group C) | 208.4 (199-225g) | 180.2 (175-185g) |
| Low Dose PK study (Group D) | 211.8 (209-226g) | 175.2 (160-191g) |
| High Dose PK Study (Group E) | 202.4 (187-212g) | 171.4 (167-177g) |

II. METHODS

A. Study Design

A definitive rat metabolism study was conducted according to the Office of Pesticide Programs Subdivision F guidelines. In addition, preliminary tests were conducted to determine a proper high dose level for the definitive study as well as to determine the relevant routes of excretion for Fipronil in rats. For the toxicity study, one male and one female rat were dosed at 50, 100, and 150 mg/kg as a single oral dose, and observed for 192 hours post-dose for signs of toxicity. For the study of the routes of excretion for Fipronil, one male and one female rat were used at dose levels of 4 mg/kg and 150 mg/kg. Following a single oral dose, excreta and expired air were collected for up to 120 hours post-dose. Radioactivity was monitored in excreta. The results of these studies showed that the 150 mg/kg dose was appropriate for a high dose, and that excretion of Fipronil derived radioactivity through expired air was negligible (< 0.5% of the administered dose; Tables 7.11 and 7.13, pages 68 and 70 of the report).

The dose groups for the definitive study included Group A, B, and C as listed above, and in addition, two other groups (Groups D and E), which were used for determination of Fipronil derived radioactivity in whole blood. Groups D and E consisted of 5 rats/sex which received oral doses of 4 mg/kg and 150 mg/kg, respectively. Whole blood samples were obtained from the lateral tail vein at 0, 0.5, 1, 2, 4, 6, 24, 48, 72, 96, 120, 144, and 168 hours post-dose. Blood was collected into heparinized microhematocrit tubes, and the concentration of radioactivity in whole blood determined.

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2) Metabolite Characterization and Identification Studies

Metabolites of Fipronil were analyzed in urine, feces, fat, liver, kidney, muscle, and uterus from dose groups A to C.Identification was made using both HPLC and mass spectrometry.

Urine

Urine samples with > 50,000 dpm/ml were pooled by sex and time point. Aliquots were either filtered and injected onto HPLC, or subjected to enzymatic hydrolysis using *helix pomatia* juice followed by HPLC analysis. For mass spectrometry analysis, selected samples were pooled, buffered to pH 5 with 0.5M acetate buffer and then incubated overnight with *helix pomatia* juice. A portion of the incubate was then extracted with hexane and then ethyl acetate and the combined solvents passed through phase separating paper. The filtrate was rotary evaporated and then a portion of the concentrate mixed with 0.075M phosphate buffer, ß-glucuronidase from *E. coli*.(approx. 1mg; 1, 560,000 units/g), and toluene. Following overnight incubation at 37 degrees Celsius and addition of 1M sodium hydroxide, the material was extracted first with hexane and then ethyl acetate. Each extract was taken to dryness and the residue reconstituted in acetonitrile. Very little radioactivity was found in the hexane extract, and thus only the ethyl acetate extract was submitted for mass spectroscopic analysis.

Feces

For feces, approximately 3g samples were extracted with dichloromethane and then centrifuged. Following centrifugation, the dichloromethane was removed and the feces residues were soxhlet extracted with methanol overnight. Dichloromethane and methanol extracts were combined and then taken to dryness using rotary evaporation, and the residue reconstituted in methanol. Reconstituted samples were filtered through glass fiber plugs and then analyzed by HPLC. For mass spectroscopic analysis, selected extracts of pooled feces derived from those submitted for HPLC analysis were extracted with hexane by vortex mixing. Following centrifugation, the hexane was removed and taken to dryness using nitrogen convection. The residue was reconstituted in hexane for analysis by mass spectroscopy.

Tissues

Tissues were extracted with either acetonitrile (fat, muscle, and uterus) or acetonitrile and hexane. For fat, muscle, and uterus, the acetonitrile extract was mixed with hexane followed by shaking for 1 minute. The mixture was then allowed to settle and the acetonitrile layer decanted off. This was evaporated to dryness and reconstituted in acetonitrile for HPLC analysis.

For liver and kidney, the acetonitrile extract was evaporated to dryness and reconstituted in methanol. Water was mixed in and the extract loaded on to a C18 Sep-Pak cartridge. Following washings with water (10ml) and then methanol water (1:1, 5ml), radioactivity was eluted with

methanol. The washings were put through a second Sep-Pak cartridge and washed and eluted as before. The combined methanol extract was evaporated to dryness and reconstituted in acetonitrile for analysis by HPLC. For mass spectroscopic analysis, tissue samples were pooled and water added as appropriate. Samples were then extracted with acetonitrile for one hour and the supernatant passed through phase separating paper. The filtrate was partitioned with hexane and the hexane layer separated. There was negligible radioactivity within the hexane layer. The remaining acetonitrile layer was filtered again and then rotary evaporated to near dryness. After addition of water (2-10ml), the concentrated extract was passed through a primed C18 solid phase extraction column which was subsequently washed with water. Analytes were eluted with acetonitrile. The eluate was taken to dryness using nitrogen convection and the residue reconstituted in acetonitrile. A portion of this was used for spectroscopic analysis.

C. Experimental

a. Animal Husbandry

Rats were acclimated for 1 week prior to use, during which health status was monitored. Rats were housed in wire floor polypropylene cages (up to 5/sex) suspended over polypropylene dirt trays containing soft white wood sawdust. Rooms provided a minimum of 10 air changes/hour, and temperature was maintained at 19-23 $^{o}$C with a relative humidity of 40 to 70% with a 12 hour light/dark cycle.Food (SDS rat and mouse maintenance diet No.1, expanded) and tap water were provided *ad libitum*, except for the evening before dosing of radiolabel until approximately 4 hours following dosing.

b. Dosing

Dosing information was provided in the report for each dose group used in this study. Pertinent dosing information is summarized below:

| Group | Mean Dose Rate (mg/kg) | | % Nominal Dose | |
|---|---|---|---|---|
| | males | females | males | females |
| A | 4.02 | 4.15 | 100 | 103 |
| B[1] | 3.62 | 3.59 | 91 | 90 |
| C | 138.2 | 124.6 | 92 | 83 |
| D | 4.22 | 4.21 | 105 | 105 |
| E | 148.8 | 158.9 | 99 | 105 |

data taken from Tables 7.2-7.6, pages 59-63 of the report. [1]mean dose over the 14 day period was stated as 3.76 mg/kg for both sexes combined.

c. Sample Collection and Analysis

Rats in groups A to C were placed in individual all-glass metabolism cages for collection of urine and feces following dosing. Containers for urine and feces collection were surrounded by solid carbon dioxide. Collection times for urine were stated as: 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 post-dose. For feces, collection times were similar except for the first 24 hours, where only a 0-24 sample was collected. After each collection interval, cage debris was removed and the cages rinsed with water.
At the end of the last collection period, rats were exsanguinated under halothane anesthesia and the following tissues removed or sampled and assayed for radioactivity:

| | | |
|---|---|---|
| adrenals | blood | bone (femur) |
| bone marrow | brain | fat (abdominal) |
| g.i. tract | gonads | heart |
| kidney | liver | lung |
| muscle | pancreas | spleen |
| skin | stomach | thyroid |
| uterus | gross lesions | residual carcass |

d. Radioassay Preparation:

Levels of radioactivity in urine and cage washings were assayed directly by addition of aliquots to liquid scintillation fluid and counting. Feces, liver, g.i. tract, stomach, and pooled cage debris were homogenized in deionized water.The remaining tissues (except adrenals, ovaries, bone marrow and thyroids, which were analyzed as whole tissues) were mascerated with scissors and subject to analysis by combustion. Feces homogenates from the pilot study, blood, and cage debris were solubilized using Soluene-350 solubilizing agent, followed by incubation and addition of liquid scintillant prior to counting.

e. Metabolite Characterization and Identification

a. Sample Analysis:

Preparation of samples for metabolite analysis has been described above. To aid identification of metabolites, reference standards were supplied by the sponsor and are as follows:

| Standard Name | Batch No. |
|---|---|
| M&B 46,136 | AJK 165/1 |
| M&B 45,950 | JJW 2120/C1 |
| RPA200766 | WAB 414B |
| M&B 46,513 | 5A JHY65 |
| M&B 45,897 | JJW 2036 |

Metabolite data were collected using either selective ion monitoring and/or scan (100 to 600 amu) modes. The limit of detection for the analysis of each sample was taken as once (HPLC fractions) or twice the background disintegration rate obtained from the measurement of blank samples of the same type.

D. Compliance

A signed statement of No Data Confidentiality claims was provided.

A signed statement of GLP compliance was provided.This study was conducted in compliance with both 40 CFR 160.35 and the UK Principles of Good Laboratory Practice, The UK Compliance Program.

A signed statement of quality assurance was provided.

III. RESULTS

1. Absorption

As there were no intravenous data available for Fipronil (due to the lack of an intravenous dose group), the extent of absorption is inferred from available urinary excretion data. Pertinent data were presented in Tables 7.20-7.21 (pages 77-78 of the report), Tables 7.32-7.33 (pages 89-90 of the report), and Tables 7.44-7.45, pages 101-102 of the report and are summarized below for 0-24 hour urinary excretion of Fipronil derived radioactivity:

| Dose Group | 24 Hour Urine (% Dose) | |
|---|---|---|
| | males | females |
| A | 0.878 | 1.46 |
| B | 4.48 | 3.15 |
| C | 1.44 | 1.20 |

As shown, the extent of 24 hour urinary excretion was similar among male and female rats within a given dose group, but differed according to dosing regimen. At the single low and single high dose, less than 2% of the

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administered dose was excreted in urine in the first 24 hours, whereas repeated low dose administration (Group B) resulted in between 3.15-4.48% excreted in urine in the first 24 hours post-dose. Fecal excretion for the first 24 hours post-dose did not appear to differ significantly between male and female rats in dose groups A,B, and C (~ 20% of the administered dose). However, total urinary and fecal excretion over the 168 post-dose period increased in both sexes in going from group A to group C, while total tissue concentration decreased. The report stated that absorption was possibly affected at the high dose based on the dissolution rate of Fipronil. At the low dose, it was suggested that rapid distribution of phase I metabolites of Fipronil in to tissues accounted for the low percentage excreted in urine, as these metabolites were lipophilic. The lack of an intravenous dose group, with or without a biliary excretion study, makes interpretation of the available data difficult. The fact that the rate of urinary excretion was altered at the repeated low oral dose (wherein there was an apparent delay, with the peak percentage appearing at 24 and 48 hours, whereas no peak was observed at the single low dose) suggests that absorption at the least was slow even at the low dose, based perhaps upon the chemical nature of the parent chemical. This could be reasonably concluded, but it might also be inferred that induction was operative, although this was not suggested in the report.

2. Distribution

Distribution data were found in Tables 7.26-7.29, pages 83-86 of the report for the low dose, Tables 7.38-7.41, pages 95-98 of the report for the repeated low oral dose, and Tables 7.50-7.53, pages 107-110 for the single high dose. These data were presented in terms of both percent administered dose as well as µg equivalents per gram tissue. Relevant findings are summarized below in both formats:

**Table 1a**

Distribution of $^{14}$C-Labeled Fipronil Derived Radioactivity in Male and Female Rats (Percent Administered Dose)

| | LDM | LDF | RDM | RDF | HDM | HDF |
|---|---|---|---|---|---|---|
| carcass | 36.81± 8.09 | 36.74± 4.11 | 18.57± 3.51 | 15.17± 1.89 | 2.22± 1.39 | 3.75± 2.68 |
| g.i. | 4.14± 0.27 | 4.24± 0.45 | 2.86± 0.49 | 2.80± 0.55 | 0.31± 0.11 | 0.90± 0.45 |
| liver | 3.46± 0.16 | 3.01± 0.29 | 1.53± 0.12 | 1.44± 0.21 | 0.25± 0.12 | 0.45± 0.20 |
| adrenals | 0.02± 0.006 | 0.03± 0.006 | 0.006± 0.003 | 0.011± 0.004 | 0.001± <0.001 | 0.004± 0.002 |

**Table 1b**

Distribution of $^{14}$C-Labeled Fipronil Derived Radioactivity in Male and Female Rats (µg equivalents / gram tissue)

| | LDM | LDF | RDM | RDF | HDM | HDF |
|---|---|---|---|---|---|---|
| carcass | 1.72± 0.34 | 1.93± 0.27 | 0.77± 0.14 | 0.68± 0.08 | 3.81± 2.25 | 6.24± 4.27 |
| g.i. | 1.37± 0.02 | 1.69± 0.24 | 1.14± 0.18 | 0.89± 0.08 | 3.67± 1.23 | 10.49± 5.48 |
| liver | 2.53± 0.34 | 2.72± 0.29 | 1.09± 0.04 | 0.97± 0.10 | 6.45± 2.54 | 11.15± 3.45 |
| abdominal fat | 14.70± 3.50 | 18.84± 2.06 | 5.75± 0.36 | 5.76± 0.99 | 29.40± 15.82 | 54.48± 31.10 |
| adrenals | 4.25± 0.41 | 4.66± 0.60 | 1.53± 0.39 | 1.39± 0.33 | 7.60± 3.21 | 14.55± 5.69 |

As the above tables show, the highest percentage of an administered dose of Fipronil was found in the carcass, g.i. tract, liver, and adrenal glands at 168 hours post-dose. The order observed was: carcass >> g.i. tract > liver > adrenals. Repeated low oral dosing or single high oral dosing did appear to result in a loss of the % radioactivity found in these tissues, especially after a single high oral dose.

On a µg/g tissue basis (which is a more accurate depiction of tissue distribution), the same tissues showed significant amounts of residual radioactivity, with the addition of abdominal fat, which showed the highest level of residual radioactivity. Repeated oral dosing again resulted in a decreased amount in tissues. A single high dose resulted in increased amounts of residual radioactivity in all tissues listed above, but was especially prominent in the tissues of female rats.

3. Excretion

The excretion of $^{14}C$-labeled Fipronil in urine and feces at the low and high dose levels in this study (5 mg/kg and 150 mg/kg) is summarized below for male and female rats. Data were obtained from Tables 7.18 and 7.19 for the 4 mg/kg single dose, pages 75-76 of the report; from Tables 7.30-7.31, pages 87-88 of the report for the repeated 4 mg/kg dose; and from Tables 7.42-7.43, pages 99-100 for the single high 150 mg/kg oral dose.

**Table 2**

Excretion of $^{14}C$-Labeled Fipronil Derived Radioactivity in Male and Female Rats[a]

| | LDM | LDF | RDM | RDF | HDM | HDF |
|---|---|---|---|---|---|---|
| urine | 5.63± 2.12 | 5.61± 1.10 | 16.22± 3.38 | 13.80± 1.33 | 29.25± 2.86 | 22.04± 2.80 |
| feces | 45.62± 7.89 | 46.01± 7.16 | 56.06± 4.43 | 61.36± 3.35 | 66.90± 3.72 | 75.10± 3.44 |
| cage wash + debris | 0.904 | 1.19 | 1.64 | 3.08 | 4.48 | 4.00 |
| tissues | 46.05± 8.9 | 45.77± 5.23 | 23.66± 4.27 | 20.16± 2.85 | 2.90± 1.70 | 5.32± 3.47 |
| Total | 98.20± 2.32 | 98.58± 2.18 | 97.58± 0.76 | 98.40± 0.62 | 103.5± 0.25 | 106.5± 3.62 |

[a]data represent the mean percent dose excreted at 168 hours post-dose for all dose groups. Abbreviations used are : LD, 4 mg/kg single low dose; RD, repeated low dose of 4 mg/kg; HD, single high dose of 150 mg/kg.

As the above data show, feces was the major route of excretion for Fipronil derived radioactivity in male and female rats in all dose groups. The percentage excreted in urine, while minor in all dose groups, showed an increase after both repeated oral dosing as well as after a single high dose. Interestingly, feces also showed this trend. Usually, if an increase is observed in excretion by any one route, a corresponding decrease will be observed in the other route. In this case, both urine and feces showed increases in the percent of the dose excreted after repeated low dosing and single high dosing. What compensated for this was an apparent decrease in the percentage of the dose found in tissues after repeated oral dosing and a single high oral dose. Total recoveries among dose groups did not differ

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significantly. According to the report, the differences observed here were based on the presence of a lipophilic phase I metabolite, which at the low dose, would be taken up into tissues, and thus little would be available for excretion in urine. At the high dose or after repeated low dose administration, the tissue compartment would become saturated, resulting in an increased proportion of the dose available for renal excretion. While this may be a plausible explanation, analysis of metabolite data is necessary before a conclusion can be drawn.

d. Plasma Levels of 14-C Fipronil Derived Radioactivity

Pharmacokinetic parameters were calculated in whole blood using dose groups D and E, as described above. The data were presented in summary format in Tables 7.54-7.57, pages 111-114 of the report. The parameters measured are summarized below:

**Table 3**
Pharmacokinetic Parameters in Fipronil Treated Rats

| | LDM | LDF | HDM | HDF |
|---|---|---|---|---|
| Cmax (µg/g) | 0.679± 0.048 | 0.601± 0.123 | 19.56± 2.90 | 19.72± 4.73 |
| $T_{1/2}$ (hr) | 149.4± 10.92 | 200.2± 58.68 | 54.42± 20.10 | 51.22± 10.50 |
| AUC (0-168h) (µg equiv.h/g) | 60.37± 3.62 | 61.21± 9.26 | 1570± 195.3 | 1790± 217.6 |

At the single low dose, Cmax, half life, and AUC (0-168hr) were equivalent between male and female rats. Blood radioactivity reached a maximum value between 4-6 hours post-dose, and subsequently fell slowly, with 40% of Cmax still present after 168 hours post-dose. Elimination half-life was 149.4 hr in males, and 200.2 hr in females.

At the single high dose, pharmacokinetic parameters were again similar between male and female rats but differed from that of the low dose. At the high dose, blood concentrations of radioactivity increased slowly, with a Tmax of between 48-72 hours for male and female rats. Thereafter, blood concentration fell more rapidly than at the low dose, reducing the elimination half-life to 54.42 hr in males and 51.22 hr in females.

It is difficult to determine whether first order or zero order kinetics is operative at the high dose. Half life is not increased at the high dose, and

AUC is almost equivalent to the change in dose (increase of approximately 30-fold vs a 37-fold increase in dose).

2) Metabolite Characterization and Identification

Data were provided showing the retention time and identification of reference standards used in this study. In addition, a summary table was provided showing retention times and identification of metabolites found in urine, feces, and tissue extracts at both the 4 mg/kg and 150 mg/kg dose levels from GC/MS analysis. These are shown below as extracted from the report, pages 132 and 133:

**GC retention times and characteristic ions as derived from mass spectroscopy of reference articles**

| Reference standard | Molecular weight* | GC retention time (min) | Characteristic ions from mass spectroscopy |
|---|---|---|---|
| M&B 46,030 | 437.2 | 14.0 | 367(M-$CF_3$),351(M-$CF_2O$),213 |
| M&B 46,136 | 453.1 | 18.5 | 383 (M-$CF_3$), 255, 213 |
| RPA200766 | 455.2 | 23.1 | 385 (M-$CF_3$), 255, 213 |
| M&B 45,950 | 421.1 | 13.4 | 351 (M-$CF_3$), 213, 255 |
| M&B 45,897 | 321.1 | 13.2 | 320 (M-$CF_3$), 213 |
| M&B 46,513 | 388 | 9.0 | 388, 213 |

* Chlorine taken as 35.5

**GC retention times and characteristic ions as derived from mass spectroscopy of urine, faeces and tissue extracts from male and female rats following a single oral administration of ($^{14}$C)-M&B 46,030 at a nominal dose level of 4.0 or 150 mg/kg body weight**

| Sample | Metabolite code | Retention time (min) | Characteristic ions | Metabolite identification |
|---|---|---|---|---|
| Pooled urine -deconjugated | Ro1 | 5 | 213, 215, 229, 254, 281 | Ring opened metabolite 1 |
| | Ro2 | 8 | 213, 215, 229, 254, 281 | Ring opened metabolite 2 |
| | | 21.8 | 213, 215, 351, 353 | M&B 45,950 |
| | U17 | 22.2 | 213, 215, 367, 369 | M&B 46,030 |
| | | 24.3 | 213, 215, 383, 385 | M&B 46,136 |
| | U16 | 26.5 | 213, 215, 385, 387 | RPA200766 |
| | | 21.8 | 213,215, 320, 322 | M&B 45,897 |
| 48-96h pooled faeces | F9 | 15 | 213, 215, 367, 369 | M&B 46,030 |
| | F10 | 14 | 213, 215, 351, 353, 420 | M&B 45,950 |
| | F11 | 19 | 213, 215, 383, 385 | M&B 46,136 |
| 168h fat | F2 | 19 | 213, 215, 383, 385 | M&B 46,136 |
| 168h liver | L1 | 19 | 213, 215, 383, 385 | M&B 46,136 |
| 168h kidney | K2 | 19 | 213, 215, 383, 385 | M&B 46,136 |
| 168h muscle | M1 | 19 | 213, 215, 383, 385 | M&B 46,136 |

* Retention time shift was apparent, but standards were run (where appropriate) to confirm retention data

It is noted that metabolites were apparently not characterized in terms of the percent of the administered dose, but only in terms of the percent present within the sample at a particular time point. This is derived from information provided in Tables 7.68 and 7.69 for fecal metabolites, in which the report describes an equation used to calculate the percent of radioactivity administered. This equation reads:

$$\%\ \text{of radioactivity administered} = \frac{(\%\ \text{radioactivity in region} \times \text{efficiency})}{\%\ \text{dose in sample}} \times 100$$

This equation shows that the levels of fecal metabolites were calculated as the percent of radioactivity for a particular metabolite based on the amount of radioactivity in the sample for that time point. There is no apparent calculation for the levels of metabolites in terms of percent total dose. If this is the same equation used for urine, then the metabolite levels reported do not reflect the amounts in relation to the total dose, and it is also difficult to tell if all or most of the radioactivity recovered in urine and feces was identified.

a) <u>Urinary Metabolites</u>

Information on urinary metabolites was found within the report in Tables 7.62-7.67, pages 119-124. According to the report, there were low levels of radioactivity present in urine at 24 hours for male rats in the single low oral dose. Therefore, only information from the 48-72 hour collection interval was reported. For other dose groups, additional times were shown for urinary metabolite levels. This format makes comparison of metabolite levels difficult between sexes and among dose groups. To facilitate some comparison, 24 hour post-dose urinary levels are illustrated; however, it must be kept in mind that these may not represent the percentage of the dose found at 24 hours in urine, but only the percentage of radioactivity as the particular metabolite out of the total radioactivity found in the 0-24 hour sample. Thus, because excretion rates may vary among dose groups, this table by itself does not provide conclusive comparisons.

**Table 4**

Urinary Metabolites in $^{14}$C-Fipronil Treated Rats

| | LDM | LDF | RDM | RDF | HDM | HDF |
|---|---|---|---|---|---|---|
| U1 | ND | ND | 0.076 | 0.225 | ND | ND |
| U2 | ND | ND | ND | ND | ND | 0.115 |
| U3 | ND | ND | 0.092 | 0.045 | 0.053 | ND |
| U4 | ND | ND | 0.277 | 0.098 | 0.042 | ND |
| U5 | ND | ND | 0.122 | ND | ND | ND |
| U6 | ND | ND | ND | ND | 0.039 | ND |
| U7 | ND | ND | 0.334 | 0.202 | 0.154 | ND |
| U8 | 0.352[a] | 0.616 | 0.491 | 0.371 | 0.586 | 0.301 |
| U9 | ND | ND | ND | ND | ND | 0.094 |
| U10 | 0.465 | 0.442 | 1.535 | 0.717 | 0.316 | ND |
| U11 | ND | 0.67 | ND | 0.103 | ND | 0.168 |
| U12 | 0.180 | 0.063 | 0.976 | 0.837 | 0.255 | 0.196 |
| U13 | ND | ND | ND | ND | 3.69[a] | 0.135 |
| U14 | ND | ND | ND | 0.118 | ND | ND |
| U15 | ND | 0.075 | 0.214 | ND | ND | ND |
| U16 | 0.351 | ND | ND | 0.107 | 0.208 | ND |
| U17 | 0.121 | 0.026 | 0.373 | 0.326 | 0.196 | 0.141 |
| U18 | ND | ND | ND | ND | ND | ND |
| Total (0-24hr) | 1.117 | 1.147 | 4.490 | 3.185 | 1.449 | 1.150 |

[a]represents 72 hour urine sample.

Although not specified in the report, the 24 hour totals shown above, as calculated by the reviewer, agree well with the 24 hour totals (as % of the total dose) presented in the report for urinary excretion of radioactivity. Thus, it is possible that the metabolite levels shown above actually do represent the percent of the total dose excreted. However, there is still

some limitation with respect to interpretation of the data, in that there is not a total percentage for all of a particular metabolite found over the entire collection period for urine, and rates of excretion may differ among dose groups over time. With this limitation in mind, it is seen overall that the percentage appearing in 0-24 hour urine was small for both male and female rats in all dose regimens (less than 5% of the administered dose), but repeated low dosing did increase the 0-24 hour urinary percentage of radioactivity found.

Single Low Dose

At the single low dose, the following metabolites were observed: U8, U10, U11, U12, U15, U16, and U17. Of these, two (U16 and U17) were tentatively identified by co-chromatography with reference standards. Further work using GC/MS showed that these 2 metabolites represented the conjugate of the metabolite RPA200766 (U16) and parent chemical (U17, also known as M&B 46,030). Mass spectral analysis showed that these 2 metabolites contained the characteristic ions for their structures. The presence of metabolites U8 and U10 were also observed, although U8 was observed only in female 0-24 urine from the low dose group (0.616% of the dose). These 2 metabolites gave mass spectral ion chromatograms corresponding to ring (pyrazole) opened products.

Repeated Low Dose

Compared to the low dose, several additional metabolites were observed in the 0-24 hour urine of rats given a repeated low oral dose of radiolabeled fipronil. A total of 12 components were tentatively identified in 0-24 hour urine, of which 8 were present in 0-24 hour male rat urine. The major components were observed to be U10 and U12, representing 1.535 and 0.976% of the dose excreted in urine 0-24 hours post-dose for male rats. The metabolite U10 has been mentioned as a ring-opened product, but there was no apparent mention of the identity of U12. It is of interest that after repeated oral dosing, the percentages of both U10 and U12 were increased in male and female rat 0-24 hour post-dose urine when compared to a single oral dose. In contrast, the percentage of U8 in female rat urine showed a decrease after repeated oral dosing in comparison to a single oral dose.

For metabolites U3, U4, and U10, male rats showed higher 0-24 hour urine percentages than female rats. For U1, the reverse was true. Metabolites U11 (0.139%) and U14 (0.118%) were found in female 0-24 hour urine only in this dose group. The remaining metabolites (U7, U8, U12, U17) appeared in male and female rat urine in approximately equal percentages.

Single High Dose

In male rat urine, 13 components were observed in 0-24 hour urine. Of these, five could be considered "major" components. These were: U8 (0.586% of the dose), U10 (0.316% of the dose), U12 (0.255% of the dose), U16 (0.208% of the dose), and U17 (0.196% of the dose. As mentioned, U8 and U10 were identified as ring-opened products, while U16 was identified as an oxidative product of the parent chemical, and U17 was shown to be parent chemical. The

levels of the ring-opened products U8 and U10 resembled those seen after a single low oral dose at 24 hours post-dose. Levels of U12, U16, and U17 also did not appear to differ significantly from those seen after a single low dose.

b) **Fecal Metabolites**

Because both male and female feces were analyzed from 24-120 hours post-dose, it is instructive to summarize the time points and present the data for fecal metabolites over the whole period of collection. However, these values do not apparently represent the percent total dose excreted in feces at 120 hours, but only the percent of radioactivity identified as various metabolites based on the radioactivity of the sample. The report noted that "the efficiency for extraction of radioactivity from pooled feces ranged from 68-82%. It was assumed that losses were due to the number of stages in the extraction process and the selective extraction of soluble components. Therefore, the final extract is assumed to represent the total radioactivity present in the sample prior to extraction."

Table 5

Fecal Metabolites in $^{14}$C-Fipronil Treated Rats

| | LDM | LDF | RDM | RDF | HDM | HDF |
|---|---|---|---|---|---|---|
| F1 | 0.388 | 0.597 | 1.58 | 1.31 | 1.76 | 1.08 |
| F2 | 0.911 | 0.796 | 4.06 | 3.77 | 5.99 | 4.56 |
| F3 | 2.03 | 2.72 | 4.51 | 6.14 | 6.88 | 4.80 |
| F4 | 0.193 | 0.069 | 1.27 | 1.56 | 2.17 | 1.76 |
| F5 | ND | ND | 0.921 | 0.278 | 0.703 | 0.652 |
| F6 | 0.249 | 0.153 | 1.68 | 2.69 | 2.83 | 1.38 |
| F7 | ND | ND | 0.127 | ND | 0.776 | ND |
| F8 | ND | ND | 0.212 | ND | 0.262 | 0.253 |
| F9 | 13.13 | 10.50 | 8.33 | 6.44 | 10.60 | 18.57 |
| F10 | 1.55 | 1.15 | 3.02 | 1.03 | 1.29 | 2.12 |
| F11 | 11.67 | 9.08 | 7.16 | 7.76 | 3.82 | 4.43 |
| Total | 30.12 | 25.06 | 32.87 | 30.97 | 37.08 | 39.60 |

Single Low Dose

In feces from rats treated with a single low dose of Fipronil, the most prominent metabolites in the samples obtained over the 120 hour collection period were F3 (~ 2% of the sample; not identified), F9 (10-13% of the sample radioactivity, identified as M&B 46,030, or Fipronil), and F11 (9-11% of the sample radioactivity, identified as M&B 46,136; this is the sulfoxidation product of Fipronil). The other components separated in feces comprised 1% or less of the sample radioactivity. Of all the metabolites identified and/or separated, the metabolite F11 was in fairly constant proportion in fecal samples collected over time within a given dose group. The parent chemical F9 appeared in greatest proportion in samples obtained at 24 and 48 hours, and thereafter tapered off to low or undetectable levels.

Repeated Low Dose

In feces from this dose group, the most prominent metabolites were F2 (3-4% of the sample radioactivity; not identified), F3 (4-6% of the sample radioactivity; not identified) F9 (6-8% of the sample radioactivity; identified as parent chemical as in the single low dose study above), F10 (1-3% of the sample radioactivity, identified as M&B 45,950, or parent chemical with the loss of the sulfur oxygen), and F11 (~ 7% of the sample radioactivity; identified as the sulfoxidation product of Fipronil).

The most noticeable difference in the metabolite profile for this dose group was a reduction in the percent of F9 and F11 compared to the low dose. The time course for appearance of the F9 and F11 metabolites was similar to that observed for the single low dose. In essence, there did not appear to be major differences in the metabolite profile from the low dose with the exception of F2 and F10, which were relatively minor components.

Single High Dose

At the single high oral dose, the most prominent fecal metabolites were again F2, F3, F9, F10, and F11. Differences observed were a slightly higher percentage of F9 in female rats (18% of sample radioactivity), and a slightly lower percentage of F11 (4-4.5% of sample radioactivity) in comparison to the single low and repeated low oral dose.

c. Tissue Extracts

According to the report, extraction efficiency in tissues was variable (generally >50%), but low due to the low levels in tissues and possible matrix effects. It was not believed that selective extraction was operative, but that losses were procedural. Thus, it may be assumed that the results obtained are not quantitative, but indicative of the metabolite(s) present.

Results of tissue extractions were presented in Tables 7.70-7.74, pages 127-131 of the report. These tables show that qualitatively speaking, M&B 46, 136 was the prominent metabolite detected in any tissue. Small amounts of a

polar fraction were observed in male and female fat extracts from the high dose group (stated as comprising ~ 5% of the radioactivity in the extract), and a minor polar component in male kidney extract (~ 12% of the sample radioactivity)..

IV. DISCUSSION

In this study, the disposition of radiolabeled Fipronil was examined in male and female Sprague-Dawley rats as part of a tolerance petition submitted for food/feed use of this chemical. The study involved groups of 5 male and female rats/dose group which received either single oral doses of 4 and 150 mg/kg labeled Fipronil, or repeated low oral doses of 4 mg/kg Fipronil for 14 days followed by a single radiolabeled dose.

According to the report (page 53), absorption at the low dose was concluded to be rapid, while at the high dose, a delay was apparent. This could be inferred from the large increase in Tmax (from approximately 4-6 hours at the low dose to 48 hours at the high dose), which would suggest a change in rate of absorption. This could also be supported from the nature of the blood kinetic curves at the low and high dose.However, it is suggested that the rate of absorption may be similar for both the low and high dose for the first 12 hours after dosing, but that a delay becomes apparent at the high dose betwen 12-48 hours post-dose. This apparent delay or slowing of absorption at 12 hours may be based on either saturation of absorption or an effect on dissolution rate. The fact that the blood kinetic curves for the low and high dose appear the same for the first 12 hours with the high dose curve diverging after that point suggests some sort of equilibrium effect brought on by saturation of absorption or the establishment of a blood/tissue equilibrium with fipronil metabolites. Fractional absorption could not be calculated due to the lack of an intravenous dose group; thus, the fractional absorption (AUC[oral] / AUC[i.v.]) could not be determined to indicate extent of absorption. Examination of urinary excretion data from 0-24 hours shows that the total percentage of the dose excreted was similar for the single low dose and high dose groups in both sexes (1-1.5% of the dose), but was increased to 3-4% of the dose in the repeated low dose group. The increase in 0-24 hour excretion in urine from repeated oral dosing may not be so much an effect on absorption as from an effect on distribution and elimination. A lipophilic phase I metabolite was identified in the urine, feces, and tissues of dosed rats (M&B 46, 136). After a single low oral dose, this metabolite would sequester into fat and would be then slowly excreted. The same could apply at the single high oral dose with the addition that the tissue compartment might become saturated at the single high dose. After a repeated oral dose, however, this metabolite would achieve equilibrium between blood and tissues. Thus, after the last dose, there would be an increased amount in blood for excretion, and hence, the increased percentage in urine could possibly be based on the increased amount available for excretion. However, the report, while stating that this metabolite was identified in urine (page 51), did not identify any of the metabolites detected in urine as this metabolite. Possible candidates include U4, U7, or U12, which were not given identifiers. It is noted that this explanation for metabolism could also

explain the changes seen in half-life at the high dose.

Distribution data at 168 hours post-dose showed significant percentages of residual radioactivity in carcass, g.i. tract (including contents), liver, and adrenals. On a percentage basis, there was a noticeable decrease in the percentage of residual radioactivity found in these tissues after repeated low oral dosing and single high dosing. When examined on a µg/g tissue basis, the same tissues showed significant amounts of residual radioactivity, but the abdominal fat (included here, but not listed on a % basis) showed the highest levels in any dose group. After repeated low dosing, amounts of residual radioactivity decreased in all tissues, but at the single high dose, there was a noticeable increase in the amount in abdominal fat of both sexes, and liver and g.i. tract of female rats, with smaller increases in the adrenals and the carcass. When considered together with the information provided on metabolite identification by the report (which showed the presence of only M&B 46,136 in tissues), it can be concluded that this metabolite has the potential to accumulate in the fat, especially when considering the amounts found on a µg/g tissue basis and the identification of M&B 46,136 as the sole metabolite identified in tissues (Table 1b and page 18 of this review).

Excretion data were summarized in Table 2 of this review, and showed that feces was the major route for excretion of Fipronil derived radioactivity. Of interest is the observation that the percentage of a dose of radiolabeled Fipronil eliminated in urine and feces increased in both routes after repeated low dose and single high dose administration. This was compensated for by an apparent decrease in the percentage recovered in tissues with repeated low oral dosing or single high oral dosing. The report again cited the phase I lipophilic metabolite as evidence for this type of behavior. At a single low dose, this metabolite would be taken up into tissues, and thus little would be available for excretion in urine. At the high dose or after repeated low dose administration, the tissue compartment would become saturated, resulting in an increased proportion of the dose available for renal excretion. The evidence in this report does not fully support this explanation, as urinary excretion data from 0-24 hours does not show a significant difference between the single high and low dose as observed in the repeated dose, and information on the actual amount of M&B 46,136 is lacking.

Pharmacokinetic data presented in the report showed a significant half-life for Fipronil derived radioactivity in whole blood (150-200hr.). At the high dose, half-life was actually decreased (~ 50hr). AUC was approximately proportional to dose, but it is difficult to draw definitive conclusions about first or zero order processes from these data. It is obvious that the kinetics observed involve both parent chemical, rates of metabolite production, and the distribution of radioactivity into tissues and fat. It is likely that the explanation for the change in half-life is in part based on the above discussed material on absorption and distribution. At any rate, the half-life of Fipronil at either a low or high dose is significant, and may come to bear as a significant factor in any toxic manifestations observed after long-term treatment with Fipronil.

Metabolites of Fipronil were identified in urine, feces, and tissues. Although in some cases the levels reported did not reflect the percentage of

the total dose, the information is useful. Pages 16-18 of this review provide relevant information which summarize the data, so a repeat will not be done here. Based on the data provided, the report provided a scheme for metabolism of Fipronil, which appears plausible for the most prominent of the metabolites detected (see attached figure). It would be instructive if the report had actually identified which of the 17 metabolites originally separated in urine was the lipophilic phase I metabolite M&B 46,136 as was shown for feces and tissues.

As a subsequent submission by the registrant, additional information was provided which showed urinary and fecal metabolites of Fipronil as a percentage of the administered dose. This information was requested in order to gain an understanding of whether the registrant identified the majority of recovered radioactivity in urine and feces. The data submitted (see attached Tables) showed...

Core Classification: minimum

The data in this study satisfy the data requirements for a metabolism study in rats under Guideline §85-1.

Page 22 is not included in this copy.

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