

US EPA ARCHIVE DOCUMENT

F8426

[81-5] Metabolism Study

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## DATA EVALUATION RECORD

STUDY TYPE: Metabolism Study - OTS 798.7485 [S81.5]  
DP BARCODE: D222299 Submission Code: S497356  
P.C. CODE: 128712 Tox. Chem. No.: NA

TEST MATERIAL: F8426

SYNONYMS: Carfentrazone-ethyl, FMC 116426

CITATION: Diane Wu, April 6, 1995, "F8426 Rat Metabolism Study"; FMC Corporation; Study No. A842RAT92M1; MRID 43829714; Unpublished.

SPONSOR: FMC Corporation  
Princeton, NJ 08543

EXECUTIVE SUMMARY:

The absorption, distribution, elimination and biotransformation of 14C-F8426 were studied in Cr1:CD(BR) Sprague-Dawley rats, 5/sex/level. The test compound was administered by gavage in corn oil at a volume of 5 ml/kg.

Group F was administered a "single oral low dose" of 14C-F8426 at 5 mg/kg. Group G was administered a "multiple oral low dose" of unlabeled F8426 at 5 mg/kg daily for 14 days and followed with a 5 mg/kg dose of 14C-F8426 on day 15. Group H was administered a "single oral high dose" of 14C-F8426 at 1,000 mg/kg. Group E control rats (2/sex) were administered a dose of corn oil at 5 ml/kg. Excreta (urine and feces) were collected for 7 days from all groups along with tissues and organs at sacrifice. Groups A to D and I to J were dosed in a pilot study and Group E was a vehicle control group.

F8426 was rapidly absorbed as measured indirectly by the rapid excretion in both sexes of radioactivity [>85%] generally within the first 24 hours after dosing. The urine contained the majority of the administered dose (72.4% to 87.0%); feces contained a lesser amount (10.47% to 25.68%). The blood contained the highest

concentration [ $\sigma$  0.002 ppm,  $\varphi$  0.001 ppm]. The total mean percentage of the administered dose found in all tissues and carcasses assayed was very low [ $\sigma$  0.09%,  $\varphi$  0.08%] with the highest concentration found in the residual carcasses [0.08%] of both sexes.

The major metabolites in both the urine and feces were F8426-chloropropionic acid [48.84 to 66.06%] and to a lesser extent 3-hydroxymethyl-F8426-chloropropionic acid [17.97 to 34.03%] followed by 3-hydroxymethyl-F8426-propionic acid [1.73 to 5.83%] and F8426-cinnamic acid [0.34 to 1.50%]. In addition minute quantities of 3-hydroxy-F8426-cinnamic acid and F8426-propionic acid were found in the urine, but the levels were too low to quantify. Extensive metabolism was indicated by only a small amount of the parent (0.06 to 2.78%) found in the feces; no parent was found in the urine by 48 hours. A variety of polar and non-polar metabolites were found in both urine and feces ( $\leq$ 1.83%). No differences in the metabolic profile existed between the sexes.

The proposed metabolic pathway appeared to be the conversion of the parent compound by hydrolysis of the ester moiety to form F8426-chloropropionic acid, followed by oxidative hydroxylation of the methyl group to form 3-hydroxymethyl-F8426-chloropropionic acid, or dehydrochlorination to form F8426-cinnamic acid. Dechlorination of F8426-chloropropionic acid formed F8426-propionic acid, hydroxylation of the methyl group of F8426-propionic acid formed 3-hydroxymethyl-F8426-propionic acid.

The overall recovery of the administered dose was >96% in both sexes.

The study is classified as Acceptable and satisfies the data requirement [§85-1] for a metabolic study.

## I. MATERIALS AND METHODS:

### A. MATERIALS

#### 1. Test Material

Name: F8426  
Chemical Name: Ethyl 2-chloro-3-[2-chloro-4-fluoro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]propanoate

#### a. Uniformly labelled in the phenyl ring with $^{14}\text{C}$

Sp. Act.: 38.7 mCi/mmol  
Radiochem.  
Purity: 99%  
Lot No.: M239:151 used in the pilot study; lot CFQ7036 used in the definitive study  
FMC isotope: 207

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b. Labelled at the carbonyl of the triazole ring with  $^{14}\text{C}$

Sp. Act.: 39.6 mCi/mmol  
Radiochem.  
Purity: 99%  
Lot No.: M239:150; used in the in-life phase  
FMC isotope: No. 206

c. Unlabelled F8426

Lot No.: E8268:12A; used in the in-life phase  
Purity: 95.7%

2. Test Animals

Species: Rat  
Strain: Crl:CD BR  
Source: Charles River Laboratories, Kingston, NY  
Groups: 10 groups of 1 to 7 animals/sex/group [pilot and definitive studies]  
Food: Certified Rodent Chow #5002; Purina Mills, Inc. ad-libitum in powder form during testing period. During the quarantine period the pellet form was used.  
Water: Tap-water ad-libitum by bottle dispensers  
Acclimation: 1 week  
Weight:  $\sigma$  123.7 to 155.8 gm,  $\phi$  145.2 to 175.2 gm (on receipt), except for Group G where  $\sigma$  64.0 to 66.0 gm,  $\phi$  90.1 to 106.0 gm were used (on receipt).  
Age: 5.4 to 7 weeks old (on receipt), except for Group G which were 3.7 to 5 weeks old were used (on receipt).  
Caging: Housed 4-5 in Nalgene cages during quarantine and acclimation. Housed individually during testing in either glass or stainless steel metabolism cages designed to separate the urine and the feces. The multiple oral low dose group were housed in individual Nalgene cages during nonradioactive dosing; individual rats were transferred to stainless steel cages during  $^{14}\text{C}$ -F8426 urine and feces collection.  
Environment: Temp.:  $20^{\circ}\pm 4^{\circ}\text{C}$ .; humidity:  $50\pm 20\%$ ;  
light/dark cycle: 12 hour

## II. METHODS:

### A. Purity Analysis

#### 1. Pilot Study

The radiopurity, stability and concentration of the dosing solutions used in Groups A, B, C and D were checked by HPLC, before and after dosing each group.

#### 2. Definitive Study

The radiopurity, stability and concentration of the dosing solutions used in the low dose (Groups F and G) and the high dose (Group H) were checked by HPLC before and after dosing each group.

### B. Preparation of the Dosing Solutions

An aliquot of either carbonyl or phenyl 14C-F8426 was isotopically diluted with unlabeled F8426. The solvent was removed by evaporation under nitrogen; corn oil was then added to prepare the dosing solutions. The specific activity of the dosing solutions was ascertained by liquid scintillation counting of the samples.

#### 1. Unlabeled Dosing Solution

Unlabeled F8426 (95.7% a.i.) was dissolved in corn oil and divided into 14 vials for use in the 14 day dosing regimen (MOLD).

### C. Experimental Design and Dosing Regimen

#### 1. Pilot Rat Study

Three (3) day pilot metabolism studies were conducted in both male and female rats with either carbonyl-14C-F8426 (Groups A and C) or phenyl-14C-F8426 (Groups B, D, I and J).

##### a. Pilot Study 1 [Group A and B]

Two (2) rats/sex were administered a single dose [carbonyl label] of 14C-F8426 at 5 mg/kg (Group A). Four (4) additional rats consisting of 2 animals/sex were administered a single oral dose of 5 mg/kg of the phenyl label (Group B). Testing and sampling of Groups A and B rats were conducted in a closed system using individual metabolism cages. Expired carbon dioxide was trapped in monoethanolamine/cellosolve (1:1). Urine, feces, and cage rinses [distilled water, except for final cage rinse which was methanol/water (1:1)] were collected every 24 hours for 3 days. Trapped carbon dioxide was collected every 24 hours for 1 (Group B) or 2 (Group A) days (Table 1).

Groups A and B were designed to investigate the differences in the metabolic profiles of different labeling positions at the low dose and to ascertain the amount of organic volatiles exhaled.

b. Pilot Study 2 [Group C and D]

Four rats consisting of 2 rats/sex were administered a single dose of 14C-F8426 in the carbonyl form (Group C) at 250 mg/kg. Four (4) additional rats consisting of 2 animals/sex were administered a single oral dose of the phenyl labelled 14C-F8426 (Group D) at 1,000 mg/kg. Urine, feces, and cage rinses were collected every 24 hours for 3 days (Table 1). Group C samples were chosen to provide samples for analytical method development, metabolite identification, and to investigate the differences in the metabolic profiles of different labeling positions at the high dose (Table 1).

c. Pilot Study 3 [Group I and J]

Two rats consisting of 1 rats/sex were administered a single dose of 14C-F8426 in the phenyl form (Group I) at 5 mg/kg at volume of 1 ml/kg. Two (2) additional rats consisting of 1 animals/sex were administered a single oral dose of the phenyl labeled 14C-F8426 (Group J) at 1,000 mg/kg at a volume of 1 ml/kg. Urine, feces, and cage rinses were collected every 24 hours for 3 days. Group I and J samples were chosen to test if there was any effect on excretion patterns due to the administration of different amounts of corn oil at different doses (Table 1).

2. Definitive Rat Study

Four groups of male and female rats were each dosed by gavage with the phenyl-ring-UL-14C-F8426 or dosing vehicle. All dosing solutions were prepared in corn oil and each rats received  $\approx 5$  ml/kg by gavage (Table 1). Due to the low water solubility of F8426 ( $\approx 22$  ppm at  $\approx 25^\circ$  C), an intravenous dose experiment was not performed. A direct measurement of absorption, therefore, could not be calculated.

a. Group E - Control Group

Four (4) rats (2 animals/sex) were orally administered the dosing vehicle (corn oil) at  $\approx 5$  ml/kg (Table 1).

b. Group F - Single Oral Low Dose (SOLD)

Ten (10) rats (5 animals/sex) were each orally administered a single dose of 14C-F8426 at 5 mg/kg (Table 1).

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c. Group G - Multiple Oral Low Dose (MOLD)

Fourteen (14) rats (7 animals/sex) were each orally administered unlabeled F8426 at 5 mg/kg, once daily for 14 consecutive days. On day 15, 5 rats/sex were administered a single oral gavage dose of 14C-F8426 at 5 mg/kg by gavage (Table 1).

d. Group H - Single Oral High Dose (SOHD)

Ten rats (5 animals/sex) were administered a single oral gavage dose of 14C-F8426 at 1,000 mg/kg by gavage (Table 1).

Table 1. Experimental Design<sup>1</sup>

Group <sup>a</sup>	Stage	Nominal Dose <sup>a</sup> (mg/kg)	Number of Animals	
			♂	♀
A	P	5 [carbonyl 14C] <sup>b</sup>	2	2
B	P	5 [phenyl 14C] <sup>b</sup>	2	2
C	P	250 [carbonyl 14C] <sup>b</sup>	2	2
D	P	1,000 [phenyl 14C] <sup>b,c</sup>	2	2
I	P	5 [phenyl 14C] <sup>b,c</sup>	1	1
J	P	1,000 [phenyl 14C] <sup>b</sup>	1	1
E	D	Corn Oil <sup>b</sup>	2	2
F	D	5 (SOLD) <sup>b</sup>	5	5
G	D	5 (MOLD) <sup>d</sup>	5 <sup>e</sup>	5 <sup>e</sup>
H	D	1,000 (SOHD) <sup>d</sup>	5	5

<sup>1</sup>From p. 53 of the study report (MRID 43829714).

<sup>a</sup>Groups B, D, I and J and F, G and H used phenyl labeled <sup>14</sup>C-F8426; group A and C used carbonyl labeled <sup>14</sup>C-F8426.

<sup>b</sup>Single dose in a volume of 5 ml/kg.

<sup>c</sup>Volume of 1 ml/kg.

<sup>d</sup>Fourteen-day non-radioactive dose, followed by a single radiolabeled dose.

<sup>e</sup>7 animals/sex dosed with F8426; but only 5 animals/dosed with 14C-F8426 used in evaluation.

SOLD = single oral low dose, MOLD = multiple oral low dose, SOHD = single oral high dose.

P = Pilot Study.

D = Definitive Study.

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## C. Clinical Observations

### 1. Mortality/Morbidity

All animals were examined daily for signs of mortality and morbidity and clinical signs. Any animal found moribund was euthanized.

### 2. Body Weights

Body weight were recorded at the time of arrival, at dosing and at sacrifice. Body weights for the multiple dose rats were recorded on days 8 and 15 of dosing.

## D. Excreta and Tissue Sampling

### 1. Pilot Study

After dosing, each rat from Groups A and B was placed in an enclosed glass metabolism cage. Rats from the remaining dose groups were individually housed in open stainless-steel metabolism cages. Urine, feces and cage rinses were collected daily for 3 days. In addition, expired carbon dioxide was collected daily from Groups A and B. Urine was collected in containers surrounded by dry ice. Carbon dioxide was collected by trapping in monoethanolamine/cellosolve (1:1). Feces samples were frozen upon collection prior to analysis. Rats were euthanized by carbon dioxide at the end of the study period. The carcasses were not analyzed in the preliminary groups.

### 2. Definitive Study

The definitive full-range study was conducted in open stainless-steel metabolism cages. All urine, feces and cage rinses were collected at the following time intervals, except controls which were collected at 24 hours; 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144 and 144 to 168 hours after dosing. Urine was freeze-trapped to avoid oxidation, evaporation and bacterial degradation. After urine samples were collected, cages were rinsed with distilled water and collected as cage rinses. At the end of the study cages were washed thoroughly with a methanol/water (1:1) mixture and collected as a final cage rinse. Feces were collected at the same time intervals as the urine and frozen upon collection prior to analysis.

At the end of the 7-day study both control and treated rats were euthanized by carbon dioxide and exsanguinated by cardiac puncture. Blood, brain, bone, liver, kidney, heart, lungs, spleen, fat muscle, skin ovaries, uterus, or testes and the remaining carcass were collected for total radioactive residue analysis.

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### E. Total Radioactive Residue Determination and Material Balance

Levels of radioactivity in urine and cage rinses were determined by directly counting duplicate aliquots using a liquid scintillation counter. Feces were homogenized with  $\approx 5$  times its weight with deionized water, tissues (except bone, skin, and fat which were minced, and carcass which were ground without adding water) were homogenized with 2 times its weight with water prior to combustion. Levels of radioactivity in feces, blood, tissues and post-extraction solids were determined by combustion of  $\approx 0.05$  to  $0.75$  gm subsamples in a biological sample oxidizer. The combustion products were then counted by LS. All combustion samples were assayed in either duplicate or triplicate.

### F. Study Extraction and Isolation of $^{14}\text{C}$ -Residues from Urine from Pilot Study.

#### 1. Urine

Pooled day 1 urine from Group C [ $^{14}\text{C}$ -carbonyl labeled,  $250$  mg/kg] was used for the analytical method development and metabolite identification. The day 1 urine samples were used because they contained the majority of the radioactive residues. The urine was extracted and analyzed by preparative TLC and produced at total of 9 bands (Table 6). The bands were isolated and repurified by HPLC. The metabolites were characterized and identified by GC/MS analyses [either as derivatized or underivatized metabolites] and by comparison to synthetic reference standards (Figure 1).

### F. Study Extraction and Isolation of $^{14}\text{C}$ -Residues from the Urine of the Definitive Study and the Feces from the Pilot and Definitive Studies

#### 1. Urine

The pooled day 1 and day 2 urine from Groups F (SOLD), G (MOLD) and H (SOHD) were analyzed directly by HPLC.

#### 2. Feces

Pooled day 1 and day 2 feces homogenates from Groups F (SOLD), G (MOLD) and H (SOHD) were extracted with acetonitrile and then analyzed by HPLC. The retention times of the radioactive peaks were compared to those of the reference compounds and from Group C, day 1 female urine in the pilot study (Figure 2).

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## H. Pilot and Definitive Study Extraction, Isolation and Quantitation Methodology for <sup>14</sup>C-Residues from the Urine and Feces

### 1. Preparation of Trimethylsilyl Derivatives

Samples were placed in 1 to 4 ml Reacti-Vials and concentrated to dryness under nitrogen. Approximately 25 to 50 µl of N, O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was added to each sample and the samples heated to ≈85° C for about 1 to 3 hours. After they were cooled, the derivatized samples were analyzed directly by GS/MS.

### 2. Preparation of Methyl Derivatives with Diazomethane

Samples were placed in 1 to 4 ml Reacti-Vials and concentrated to dryness under nitrogen. Approximately 1 to 2 ml of ethereal diazomethane generated from Diazald was added to each vial. The reaction was continued until the receiving ether was bright yellow in color. Any reacted samples were then left for about 30 minutes at room temperature unless phenolic moieties were present in which case treatment over a weekend (2 days minimum) was allowed.

### 3. High Performance Liquid Chromatography (HPLC)

Acetonitrile fractions obtained from pooled feces and methanol eluate obtained from C18 solid-phase extraction of pooled urine contained significant levels of the administered dose (on days 1 and 2 post treatment) were subjected to HPLC analyses.

### 4. Thin Layer Chromatography (TLC)

Analytical TLC was used for qualitative comparison and confirmational purposes. Designated fractions and products were chromatographed on the plates and developed in the appropriate solvent systems. TLC plates were developed in one-or two dimensions to 12 to 15 cm from the origin to the solvent front. Reference chemicals when applicable were spotted alongside the samples and co-developed with the appropriate sample extracts.

During the isolation process, preparative TLC was also use. Urine methanol eluate from the C18 SPE columns and HPLC radioactive peaks were collected and streaked on silica gel plates and developed.

#### (i) TLC Radiochromatograms

Developed TLC plates were scanned using the AMBIS Radioanalytical Imaging System.

Other developed TLC plates were exposed to the image plates of the FUJIX Bas 1000 Bio-imaging Analyzer System. Photostimulated luminescence values were, thereby, obtained. This value is

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proportional to disintegrations per minute. It was used because counts per second (cps) cannot be calculated directly using LSC.

#### 5. Gas Chromatography with a Radioactivity Monitor and a Flame Ionization Detector

Gas chromatography analysis was performed on reference compounds, HPLC eluate and extracts using a gas chromatograph equipped with a radioactivity monitor (RAM) and a flame ionization detector (FID).

#### 6. Gas Chromatography/Mass Spectrometry (GC/MS)

Isolated, underivatized, nonpolar metabolites and isolated, derivatized, polar metabolites were subjected to GS/MS analyses.

#### 7. Liquid Scintillation Counting (LSC)

Radioactivity levels in urine, HPLC collections, various feces extracts and eluates of TLC scrapings were determined by counting the samples.

#### 8. Tissue Oxidation

The evolved <sup>14</sup>C carbon dioxide from feces, tissues, or solid samples were combusted using a tissue oxidizer and then counted. HPLC eluates were also oxidized and counted.

#### G. Storage Stability

Urine and feces extracts from the pilot groups were analyzed by HPLC shortly after collection as well as 4 months later. There was no significant differences in the metabolic profiles. Definitive samples were profiled within 4 months of sample collection; therefore, no storage stability problem was expected.

#### H. Regulatory Compliance

A quality assurance statement, statements of compliance with Good Laboratory Practice Standards, and no confidentiality claims were signed and dated.

#### I. Statistical Analysis

Statistical analysis was not performed. Only the arithmetic mean and standard deviation were calculated.

### III. RESULTS:

#### A. Radiochemical Purity, Specific Activity and Stability of the Dosing Solutions

The radiochemical purity of the test materials in the dosing solutions was >96% by HPLC and was considered to be suitable for use in this study (Table 2).

The specific activity of the dosing solutions was noted in Table 2.

Table 2. Specific Activity and Purity of Radiolabeled F8426 Dosing Solution<sup>1</sup>

Group	Description	C-14 Label	Nominal Dose (mg/kg)	Specific Activity (dpm/ $\mu$ g)	Post Dose Purity (%)
A	P	Carbonyl	5	13.84	96.96
B	P	Phenyl	5	1.24	97.16
C	P	Carbonyl	250	0.28	96.97
D	P	Phenyl	1,000	0.07	97.35
I	P	Phenyl	5	12.24	98.56
J	P	Phenyl	1,000	0.07	98.42
B	C	NA	NA	NA	NA
F	SOLD	Phenyl	5	13.90	96.63
G	MOLD	Phenyl	5*	13.90	95.72
H	SOHD	Phenyl	1,000	0.12	97.10

<sup>1</sup>from p. 53 of the study report (MRID 43829714)

\*mg/kg/day for 14 days, 14C label on day 15.

NA = not applicable

SOLD = Single Oral Low Dose

MOLD = Multiple Oral Low Dose

SOHD = Single Oral High Dose

#### B. In-vivo Observations

No observations were documented in the report. this deficiency did not compromise the study.

#### C. Body Weights

Body weights were used for dosing calculations only for the time interval used for body weight determinations was too short for any meaningful toxicological evaluation.

#### D. Pilot Radioanalysis

Results from the pilot study showed that counting of the exhaled 14C was not necessary since only <0.02% of the administered dose was exhaled. The pilot study also showed no differences in the metabolic profile between the labels or dose levels and suggested that the 14C phenyl label [at 5 and 1,000 mg/kg] was suitable for use in the definitive study.

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### E. Definitive Radioanalysis

Values for the items noted below were calculated for 7 days except for the "percent of dose recovered in the urine and feces during the first 24 hours".

#### 1. Single Oral Low Dose - Group F (SOLD)

##### a. Urine

The average percentage of the total dose recovered in urine was 60.09% in the male and 68.19% in the female (Table 3, 4).

##### b. Cage Washes

Approximately 12.33% and 12.98% of the total dose was recovered in the cage washes, respectively, of the male and female (Table 3, 4).

##### c. Feces

Approximately 25.68% and 16.00% of the total dose was recovered in the feces, respectively, of the male and female (Table 3, 4).

##### d. Percent of Dose Recovered in the Urine and feces in the first 24 hours

Data indicated that for both sexes the majority (~88%) of the dose was recovered during the first 24 hours after dosing (Table 3).

##### e. Percentage of Dose Found in the Tissues

The total average percentage of the dose found in all tissues and carcasses assayed were very low being 0.09% for males and 0.08% for females. The highest concentration was found in the residual carcasses being 0.08% in both sexes (Table 4). The mean ppm values for all tissues were low, with the highest amount found in the blood 0.007 ppm in males and 0.009 ppm found in females (Table 5).

##### f. Overall Recovery

The overall recovery of the administered dose was 98.2% in the male and 97.25% in the female during the 7 day study (Table 3, 4).

#### 2. Multiple Oral Low Dose - Group G (MOLD)

##### a. Urine

The average percentage of the total dose recovered in urine was 61.44% in the male and 61.84% in the female (Table 3, 4).

b. Cage Washes

Approximately 7.39% and 12.94% of the total dose was recovered in the cage washes, respectively, of the male and female (Table 3, 4).

c. Feces

Approximately 22.68% and 21.89% of the total dose was recovered in the feces, respectively, of the male and female (Table 3, 4).

d. Percentage of Dose Recovered in the Urine and Feces in 24 hours

Data indicated that for both sexes the majority (>89%) of the dose was recovered during the first 24 hours after dosing (Table 3).

e. Percentage of Dose found in the Tissues

The total average percentages of the dose found in all tissues and carcasses assayed were very low being 0.01% for males and 0.01% for females. The highest concentration was found in the residual carcasses being 0.01% in both sexes. The mean ppm values for all tissues were low, with the highest amount (0.01%) found in the residual carcass for both sexes (Table 4). The mean ppm values for all tissues were low, with the highest amount found in the blood 0.002 ppm in males and 0.001 ppm found in females (Table 5).

f. Overall Recovery

The overall recovery of the administered dose was 98.51% in the male and 96.67% in the female during the 7 day study (Table 4).

3. Single Oral High Dose (SOHD)

a. Urine

The average percentage of the total dose recovered in urine of the male was 73.69% in the male and 75.67% in the female (Table 3, 4).

b. Cage Washes

Approximately 3.89% and 11.34% of the total dose was recovered in the cage washes, respectively, of the male and female (Table 3, 4).

c. Feces

Approximately 19.54% and 10.47% of the total dose was recovered in the feces, respectively, of the male and female (Table 3, 4).

d. Percentage of Dose Recovered in the Urine and Feces in 24 hours

Data indicated that for both sexes the majority (>85%) of the dose was recovered during the first 24 hours after dosing (Table 3).

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e. Percentage of Dose Found in the Tissues

The total average percentages of the dose found in all tissues and carcasses assayed were very low being 0.11% for males and 0.13% for females (Table 4).

Because of the 200 fold increase in dose, the ppm values of the tissues from the high dose group (1,000 mg/kg) were higher than the ppm values for the low dose group. In male rats, other than the residual carcasses, blood showed the highest ppm value (0.485 ppm) followed by the skin (0.439 ppm), liver (0.277 ppm) and kidney (0.272 ppm). In female rats, other than residual carcasses, kidneys contained the highest residue (1.101 ppm) followed by the liver (0.811 ppm), blood (0.627 ppm) and skin (0.475 ppm) [Table 5].

f. Overall Recovery

The overall recovery of the administered dose was 97.22% in the male and 97.61% in the female during the 7 day study (Table 4).



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## D. Metabolite Isolation and Identification

### 1. Preliminary Study

Pooled Group C (day 1) urine from female animals contained nine radioactive bands (Table 6).

Table 6. Isolated and Identified Metabolites from the Pilot Urine Samples (Group C)<sup>a</sup>

TLC Band #	Isolated and Identified Metabolite	% TLC <sup>b</sup>
1	-	0.77
2	-	1.40
3	-	0.62
4	3-OH-F8426-Cl-PAc (III)	19.54
5	3-OH-F8426-CAc (IV)	1.39
6	3-OH-F8426-PAc (V)	2.39
7	F8426-Cl-PAc (II) & F8426-CAc (VI)	72.89
8	F8426-PAc (VII)	0.70
9	-	0.29

<sup>a</sup>Adapted from the original report, p 41 [MRID 43829714].

<sup>b</sup>Percentage of original dose on various bands on the TLC plate.

### 2. Definitive Study HPLC Analyses of Metabolites in Urine and Feces

Urine and feces samples for days 1 and 2 from Groups F, G and H containing the majority of the radioactivity were pooled according to matrix, sex and dosing regimen and then analyzed by HPLC analyses.

#### a. Urine Analysis

The major metabolites were summarized below:

No parent F8426 (Metabolite I) was detected in either male or female rats in Day 1 or Day 2 urine of the SOLD, MOLD or SOHD groups.

##### (i) F8426-chloropropionic acid (Metabolite II)

In male rats, 38.16%, 41.15% and 56.64% of the dose were observed in the day 1 urine of the SOLD, MOLD and SOHD groups, respectively. Day 2 urine contained 0.46%, 0.65% and 0.87% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

In female rats, 43.70%, 42.94% and 60.58% of the dose were observed in the day 1 urine of the SOLD, MOLD and SOHD groups, respectively.

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Day 2 urine contained 0.75%, 0.64% and 2.43%, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

(ii) 3-hydroxymethyl-F8426-chloropropionic acid (Metabolite III)

In male rats, 26.81%, 24.27% and 15.35% of the dose were observed in the day 1 urine of the SOLD, MOLD and SOHD groups, respectively. Day 2 urine contained 0.45%, 0.66% and 0.39%, of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

In female rats, 28.37%, 22.28% and 16.21% of the dose were observed in the day 1 urine of the SOLD, MOLD and SOHD groups, respectively. Day 2 urine contained 0.66%, 0.47% and 1.15% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

(iii) 3-hydroxymethyl-F8426-propionic acid (Metabolite V)

In male rats, 2.33%, 3.79% and 0.9135% of the dose were observed in the day 1 urine of the SOLD, MOLD and SOHD groups, respectively. Day 2 urine contained 0.18%, 0.65% and 0.12% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

In female rats, 2.54%, 3.41% and 1.13% of the dose were observed in the day 1 urine of the SOLD, MOLD and SOHD groups, respectively. Day 2 urine contained 0.36%, 0.48% and 0.24% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

(iv) F8426-cinnamic acid (Metabolite VI)

In male rats, 0.41%, and 0.48% of the dose were observed in the day 1 urine of the SOLD and MOLD groups, respectively. Day 2 urine contained 0.02%, 0.07% and 0.03% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

In female rats, 0.84% and 0.78% of the dose were observed in the day 1 urine of the SOLD and MOLD groups, respectively. Day 2 urine contained 0.07%, 0.12% and 0.07% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 7, 8).

(v) Minor Metabolites

A number of minor polar and non-polar metabolites which were not identified (<1.5% in Groups F, G and H) were detected (Tables 7, 8).

During the preliminary study, 3-hydroxymethyl-F8426-cinnamic acid (Metabolite IV) and F8426-propionic acid (Metabolite VII) were isolated and identified in the rat urine, however, due to the minute quantities, no samples remained after MS analysis. HPLC retention times of the purified metabolites thus could not be determined, and therefore, they were not quantified in the definitive study (Tables 7, 8).

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## b. Feces Analysis

The day 1 and day 2 feces homogenate were pooled according to sex and dosing regimen. The pooled feces homogenate were blended and extracted with acetonitrile which extracted  $\approx 77\%$  to  $95\%$  of the radioactivity in the feces. The major fecal metabolites were summarized below:

### (i) F8426 (Metabolite I)

In male rats, 0.94%, 0.06% and 2.77% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained no significant amount of material (Table 9, 10).

In female rats, 0.22%, 0.08%, and 0.08% of the dose were observed in the day 1 feces of the SOLD and MOLD groups, respectively. Day 2 feces contained 0.01% of the dose in only the SOLD group (Table 9, 10).

### (ii) F8426-chloropropionic acid (Metabolite II)

In male rats, 9.67%, 7.97% and 7.71% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained 0.54%, 1.28% and 0.83% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 9, 10).

In female rats, 4.44%, 9.35% and 1.90% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained 1.04%, 1.43% and 1.13% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Table 9, 10).

### (iii) 3-hydroxymethyl-F8426-chloropropionic acid (Metabolite III)

In male rats, 5.47%, 3.79% and 1.71% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained 1.30%, 1.36% and 0.52% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Table 9, 10).

In female rats, 2.30%, 3.37% and 0.73% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained 2.05%, 1.28% and 0.99% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Table 9, 10).

### (iv) 3-hydroxymethyl-F8426-propionic acid (Metabolite V)

In male rats, 0.86%, 0.74% and 0.67% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained 0.24%, 0.65% and 0.10% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Table 9, 10).

In female rats, 0.34%, 0.51% and 0.23% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained 0.37%, 0.47% and 0.12% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 9, 10).

(v) F8426-cinnamic acid (Metabolite VI)

In male rats, 0.50%, 0.57% and 0.19% of the dose were observed in the day 1 feces of the SOLD, MOLD and SOHD groups, respectively. Day 2 feces contained 0.07%, 0.13% and 0.12% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 9, 10).

In female rats, 0.26%, 0.49, and 0.09% of the dose were observed in the day 1 feces of the SOLD and MOLD groups, respectively. Day 2 feces contained 0.14%, 0.112% and 0.11% of the dose, respectively, in the SOLD, MOLD and SOHD groups (Tables 9, 10).

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(vi) Minor Metabolites and Summary Tables

The following tables denote the metabolites in urine and feces, total of day 1 and 2 (Table 11); metabolites in the excreta, total of days 1 and 2 (Table 12), and the known isolated and identified metabolites (Table 13).

A variety of unknown polar and non-polar metabolites were also found in both urine (<1.5%) and feces ( $\leq 0.94\%$ ). All the identified metabolites were nonconjugated (Tables 11, 12).

The post extraction solid (PES) phase (feces) contained 1.37% to 3.45% of the administered dose and thus was not further analyzed (Tables 11 and 12).

As summarized in Table 13, levels of parent compound and 4 metabolites in urine plus feces accounted for 87.22% to 88.95% of the dose in all groups. Parent compound, F8426, accounted for only 0.06% to 2.78% of the dose. The major metabolites were 3-hydroxymethyl-F8426-chloropropionic acid (17.97% to 34.03% of the dose) and F8426-chloropropionic acid (48.84% to 56.06% of the dose).

**F. Proposed Metabolic Pathway**

The proposed metabolic pathway of F8426 in rats was noted in Fig. 1. The metabolism of the test compound was rapid and extensive and was found to occur through a variety of pathways involving hydrolysis of the ester moiety to form F8426-chloropropionic acid, followed by oxidative hydroxylation of the methyl group to form 3-hydroxymethyl-F8426-chloropropionic acid, or dehydrochlorination to form F8426-cinnamic acid. Dechlorination of F8426-chloropropionic acid formed F8426-propionic acid, hydroxylation of the methyl group of F8426-propionic acid formed 3-hydroxymethyl-F8426-propionic acid. Only a very small amount of F8426 remained after 48 hours. In the preliminary study, 3-hydroxymethyl-F8426-cinnamic acid and F8426-propionic acid were isolated and identified from the rat urine, but due to the minute quantities of these metabolites present, these compounds were not quantified in the definitive study. No significant differences in the metabolite profiles and distributions were found between sexes.



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