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Chemical:	**Fipronil**
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HED File Code	**13000 Tox Reviews**
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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FEB 25 1998

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: I.D. Nos.: **FIPRONIL**. Evaluation of the Data Base for Registration for Use on Rice.

Tox. Chem. No.	None
PC Code No.	129121
DP Barcode Nos.	D237893
Submission Nos.	S524626

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I. CONCLUSIONS

The existing toxicity database supports the registration for fipronil technical and its photodegradate for use on rice.

HED was requested to review several new studies on the parent **MB46030 (fipronil)**, metabolite **MB45897** and **photodegradate MB46513**. DERs are attached. All studies were acceptable except as follows. The acute neurotoxicity study (81-8, MRID 44262808) is classified as unacceptable due the lack of positive control data. This data has been received in HED and will be reviewed.

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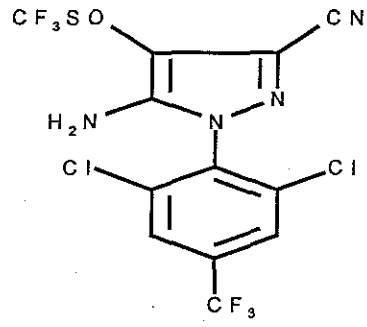
II. ACTION REQUESTED

HED has been requested to review the toxicity data submitted for fipronil and its **photodegradate MB46513** and to determine whether the toxicity data base supports a section 3 registration on rice.

III. BACKGROUND

STRUCTURE



Fipronil, also called **MB 46030**, is an insecticide. The toxicology data base for **fipronil** has previously been evaluated and was considered adequate to support registration for use on corn.

A **photodegradate** has been identified that appears to have greater toxicity than the parent, **fipronil**. This **photodegradate** is not an animal metabolite and, while not present on corn, is potentially present on rice due to the foliar application.

IV. REQUIREMENTS

See Table 1 for the requirements (CFR 158.135) for Food/Feed Use for **Fipronil** Technical.

Table 1.

Test		Technical		Photodegradate
		Required	Satisfied	Acceptable studies
81-1	Acute Oral Toxicity	Y	Y	Y
81-2	Acute Dermal Toxicity	Y	Y	Y
81-3	Acute Inhalation Toxicity	Y	Y	-
81-4	Primary Eye Irritation	Y	Y	-
81-5	Primary Dermal Irritation	Y	Y	-
81-6	Dermal Sensitization	Y	Y	-
81-7	Acute Delayed Neurotox. (Hen)	N	-	-
81-8-ss	Acute Neurotox. Screening Battery (Rat)	Y	Y	N[3]
82-1	Oral Subchronic (Rodent)	Y	Y	Y
82-1	Oral Subchronic (Non-Rodent)	Y	Y	Y
82-2	21-Day Dermal	Y	Y	-
82-3	90-Day Dermal	N[1]	-	-
82-4	90-Day Inhalation	N[2]	-	-
82-5	90-Day Neurotoxicity (Hen)	N	-	-
82-5	90-Day Neurotoxicity (Mammal)	N	-	-
82-7	90 Day Neurotoxicity Screening Battery (Rat)	Y	Y	-
83-1	Chronic Toxicity (Rodent)	Y	Y	-
83-1	Chronic Toxicity (Non-rodent)	Y	Y	-
83-2	Oncogenicity (Rat)	Y	Y	-
83-2	Oncogenicity (Mouse)	Y	Y	-
83-3	Developmental Toxicity (Rodent)	Y	Y	Y
83-3	Developmental Toxicity(Non-rodent)	Y	Y	-
83-4	Reproduction	Y	Y	-
83-5	Chronic/Oncogenicity	Y	Y	-
83-6	Develop. Neurotoxicity Rat)	Y	Y	-
84-2	Mutagenicity—Gene Mutation, Bact.	Y	Y	Y
84-2	Mutagenicity—Gene Mutation, Mam.	Y	Y	Y
84-2	Mutagenicity—Struct. Chrom. Aber.	Y	Y	Y
84-4	Mutagenicity—Other Genotoxic Effects	N	N	-
85-1	General Metabolism	Y	Y	-
85-2	Dermal Penetration	Y	Y	Y
86-1	Domestic Animal Safety	N	-	-

Y yes, N no

[1] Not required based on low dermal toxicity observed in the 21-day dermal study, and based on expected exposure.

[2] Not required since significant exposure via inhalation not expected.

[3] This study needs positive control data to be submitted by the registrant.

V. DATA GAP(S)

There are currently no data gap for **fipronil**, its metabolites or the **photodegradate**. The Registrant has submitted positive control data for the acute rat neurotoxicity study with the **photodegradate**. This has been received in HED and will be reviewed. In addition, the Registrant should submit a chronic study on the **photodegradate (MB 46513)** if one is completed.

VI. SUMMARY OF THE TOXICITY DATA BASE FOR FIPRONIL TECHNICAL, SELECTED METABOLITES AND PHOTODEGRADATE MB46513

A. ACUTE TOXICITY

Adequacy of data base for acute toxicity (Series 81-1 to 81-6): The data base for acute toxicity for technical **fipronil** is considered complete. No additional studies are required at this time.

FIPRONIL PARENT - toxicity categories

§81-1 acute oral - MRID 42918628 , [II] - ♂ 92/♀ 103 mg/kg; ♂+♀ 97 mg/kg
§81-2 acute dermal - MRID 42918629, [III] - > 2000 mg/kg [rat]
§81-2 acute dermal - MRID 42918630, [II] - 354 mg/kg [rabbit]
§81-3 acute inhalation - MRID 43544401, [II] - ♂ 0.36/♀ 0.42 mg/L; ♂+♀ 0.39 mg/L
§81-4 primary eye irritation - MRID 42918632, [III]
§81-5 primary dermal irritation - MRID 42918633, [IV]
§81-6 dermal sensitization - MRID 42918634, [non-sensitizing]

FIPRONIL FORMULATION Icon 6.2FS (Reg.: 264-LTT) - toxicity categories
(data reviewed by Ian Blackwell, RD, 11/17/97, Byron Bacus, 1/13/98)
§81-1 acute oral - MRID 4426192 , [II] - ♂ 217/♀396 mg/kg; ♂+♀275 mg/kg
§81-2 acute dermal - MRID 44261903, [III] - ♂ 802/♀ 865 mg/kg; ♂+♀ 841 mg/kg [rabbit]
§81-3 acute inhalation - MRID 44261904] - study acceptable [II]
§81-4 primary eye irritation - MRID 44261905, [III]
§81-5 primary dermal irritation - MRID 44261906, [III]
§81-6 dermal sensitization - MRID not given, self validated [**SENSITIZER**]

FIPRONIL PHOTODEGRADATE (MB46513) - toxicity categories

§81-1 acute oral - MRID 43235401, [I] - ♂ 18/♀ 15 mg/kg
§81-2 acute dermal - MRID 43235402, [III] - > 2000 mg/kg [rat]
x§81-3 acute inhalation - no study
x§81-4 primary eye irritation - no study
§81-5 primary dermal irritation - no study
§81-6 dermal sensitization - no study

FIPRONIL METABOLITE (MB45897) - toxicity categories

§81-1 acute oral - MRID 44262819, [III] - ♂>2,000 mg/kg/♀>2,000 mg/kg
§81-2 acute dermal - MRID 44262820, [III] - > 2000 mg/kg [rat]

B. SUBCHRONIC TOXICITY

Adequacy of data base for subchronic toxicity (Series 82): The data base for subchronic toxicity is considered complete. No additional studies are required at this time.

1. Studies Conducted with Fipronil

82 1a Subchronic Oral Toxicity Feeding - Rat

Fipronil (95.4% a.i.) was administered in the diet to groups of ten male and ten female CD rats per group at dosages of 0, 1, 5, 30 or 300 ppm daily for thirteen weeks (MRID # 42918643). Overall mean body weight gain was decreased by 9% in the 300 ppm group females as compared to the controls. The 300 ppm group males and females had higher total protein concentrations than the controls in association with higher values for alpha-1, alpha-2 and beta globulins and lower albumin/globulin (A/G) ratios. The 5 and 30 ppm group males and females had similar alterations in protein values but the A/G ratios were not affected. At necropsy, the 300 ppm group males and females had higher absolute and relative thyroid weights. Absolute thyroid weights were increased in the 30 ppm group females. Absolute liver weights were increased in the 300 pm group males and in females which received 5 ppm or greater. Relative liver weights were increased in the 30 and 300 ppm group males and females. On histopathology, there was a significant increase in the incidence of hypertrophy of the follicular epithelium of the thyroid in the 300 ppm group males and females. Liver sections stained with Oil-Red-O showed a higher incidence and distribution of fat in the liver of the 300 ppm group males. **LOEL = 30 ppm for males (1.93 mg/kg/day) and females (2.28 mg/kg/day) based on alterations in serum protein values and increased weight of the liver and thyroid. NOEL = 5 ppm for males (0.33 mg/kg/day) and females (0.37 mg/kg/day).**

Classification: Minimum

82-1a Subchronic Oral Toxicity Feeding - Mouse

In a subchronic toxicity study (MRID 44262804), **MB46030 (fipronil**; 95.4-96.5% a.i.) was administered to CD-1 albino mice (12/sex/dose) in the diet at nominal dose levels of 0, 1, 3, 10, or 25 ppm (13-week measured mean 0, 0.13, 0.38, 1.27, and 3.20 mg/kg/day, respectively, for males, and 0, 0.17, 0.57, 1.72, and 4.53 mg/kg/day, respectively, for females) for 13 weeks. Ophthalmoscopic examinations and blood and urine analyses were not conducted. Liver was the only tissue routinely examined histologically.

There were no deaths, clinical signs of toxicity or effects on food consumption. Male and female mice in the 25 ppm treatment group had mean body weight gains 2.3-3.2 g lower (22 and 34%, respectively) than the controls. Liver abnormalities were observed in all male treatment groups. Minimal to moderate liver cell periacinar hypertrophy with cytoplasmic vacuolation was present in 0/12, 2/12, 3/12, 6/12 and 10/12 rats from controls to high dose group. There was one 10 ppm male with a grossly enlarged liver. Absolute and relative liver weights were increased 23 and 33 % above controls, respectively at 25 ppm. Females did not exhibit a similar response to treatment. One female in the 25 ppm group and two in the 10 ppm group exhibited slight midzonal hepatocytic fatty vacuolation, and mean relative liver weights for both groups were 8-13% higher than the controls. The above treatment induced changes are considered adaptive rather than toxic. No neoplastic tissue was observed. **The LOEL was 25 ppm (3.2 and 4.53 mg/kg/day, for males and females, respectively) based on a possible decreased body weight gain. The NOAEL was 10 ppm (1.27 and 1.72 mg/kg/day, for males and females, respectively). The NOEL is less than**

or equal to 1 ppm (0.13 and 0.17 mg/kg/day for males and females, respectively) based on hepatic hypertrophy at all doses.

This 90-day subchronic toxicity study (dietary) is classified **acceptable (non-guideline)** due to its abbreviated protocol and its design as a range finding study and does not satisfies the Subdivision F guideline requirement for a subchronic toxicity study in rodents (§82-1a).

82-1b Subchronic Oral Toxicity [capsule] - Dog

Fipronil (95.4% a.i.) was administered in capsules to groups of four male and four female beagle dogs per group at dosages of 0, 0.5, 2.0 or 10.0 mg/kg/day for 13 weeks (MRID # 42918642). One male and three females in the 10 mg/kg/day group were euthanized during the second week of treatment due to poor condition.

Extensive clinical signs of toxicity, including those involving the nervous system, were also seen in the surviving animals in this group. The only clinical sign of toxicity in the 2.0 mg/kg/day group was inappetence in two of four females. Abnormal findings in the routine physical and neurological examinations during the course of the study were confined to the 10.0 mg/kg/day group. Mean body weight gain over the course of the study was decreased in females in the 2.0 and 10.0 mg/kg/day groups by 17% and 12%, respectively, in comparison to the controls. (Mean values for females in the 10.0 mg/kg/day group were based on only one animal after Day 14.) No other treatment-related findings were reported. **LOEL = 10.0 mg/kg/day for males (based on clinical signs of toxicity) and 2.0 mg/kg/day for females (based on clinical signs of toxicity and decreased body weight gain). NOEL = 2.0 mg/kg/day for males and 0.5 mg/kg/day for females.**

Classification: Guideline

82-2 Repeated Dose Dermal – Rat

M&B 460430 (96.7% a.i.) was applied in a 0.5% solution of carboxymethylcellulose to the intact skin of 6 New Zealand White rabbits/sex/group at doses of 0, 0.5, 1.0, 5.0 or 10.0 mg/kg/day for six hours per day for 15 doses over a 21-day period (MRID # 42918644). Males and females in the 10 mg/kg/day group had decreased mean body weight gain and food consumption in comparison to the control group. One male and one female in the 10 mg/kg/day group exhibited signs of extreme hyperactivity that may have been treatment-related. **Systemic LOEL = 10 mg/kg/day based on decreased body weight gain and food consumption; Dermal irritation LOEL > 10.0 mg/kg/day. Systemic NOEL = 5.0 mg/kg/day; Dermal irritation NOEL ≥ 10.0 mg/kg/day.**

Classification: Guideline

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2. Studies Conducted with Metabolite MB 46513

82 1a Subchronic Oral Toxicity Feeding - Rat

In this subchronic rat study (MRID # 43559501), MB 46513 was administered in the diet to groups of ten male and ten female CD rats at dosages of 0, 0.5, 3, 10 or 30 ppm (males: 0, 0.029, 0.177, 0.594 and 1.772 mg/kg/day; females: 0, 0.035, 0.210, 0.709, and 2.101 mg/kg/day, respectively) daily for 90 days.

There were four deaths in both sexes of the 30 ppm group during the treatment period. There was an increased incidence of clinical signs of neurotoxicity (aggressivity, irritability to touch, increased motor activity and curling up on handling) in the 10 and 30 ppm group males and females. One male in the 3 ppm group was also observed to display most of these signs. Mean body weights were statistically decreased in the 30 ppm group males and females

and the 10 ppm group males at multiple weekly measurements during the study. Overall mean body weight gains for the 10 and 30 ppm group males was decreased 15.4% and 12.9%, respectively. Mean weekly food consumption and food conversion efficiency for the 30 ppm group males and females were lower than the controls during the first two weeks of the study only. There were no treatment-related changes in hematology or urinalysis parameters. Alterations in clinical chemistry parameters were of no toxicological significance. Treatment-related decreases were seen in T_4 at weeks 2 and 10 in the 30 ppm group males and in the 30 ppm group females at week 10. There was also a decrease in T_3 in the 30 ppm group males at week 10. However, there were no changes in TSH, or the thyroid gland on macroscopic or microscopic examination. Therefore, the toxicological significance of the hormone alterations is questionable. There were no treatment-related macroscopic or microscopic necropsy changes. **The study demonstrates that the metabolite is more toxic than the parent chemical (MB 46030) when administered to rats for 90 days. The Lowest Observed Effect Level (LOEL) was 3 ppm (0.177 and 0.210 mg/kg/day for males and females, respectively) based on the occurrence of agressivity, irritability to touch and increased motor activity in one male (these signs are also observed in the mouse). The No Observed Effect Level (NOEL) was 0.5 ppm (0.029 and 0.035 mg/kg/day for males and females, respectively).**

This study is classified as **Acceptable** and **satisfies** the data requirements for a subchronic rat study (82-1).

82-1a Subchronic Oral Toxicity Feeding - Mouse

In a subchronic toxicity study (MRID 44262811), **MB46513** (a **photodegradate** of fipronil; 96% a.i.) was administered to OF1 mice (10/sex/dose) in the diet at nominal dose levels of 0, 0.5, 2, or 10 ppm (13-week measured mean 0, 0.08, 0.32 or 1.74 mg/kg/day for males; 0, 0.11, 0.43, or 2.15 mg/kg/day for females) for 13 weeks.

In the 10 ppm treatment group, 9/10 males died prematurely (between days 20 and 62) and 1/10 was sacrificed moribund (day 84); 1/10 females died on day 5. On one occasion each, two of the males exhibited excessive jumps, and on several occasions one male exhibited aggressiveness and/or irritability. Diffuse centrilobular hypertrophy of the liver was noted in 6/10 males. The severity of the condition was described as mild in the five males that died prematurely and moderate in the one male that was sacrificed. The liver of the sacrificed animal also had moderate multifocal mitotic figures and mild multifocal extramedullary hematopoiesis. In addition, three males had enlarged livers and four had atrophied thymus glands. The organs of males in the 10 ppm treatment group were not weighed. The organs of females in the 10 ppm treatment group appeared normal. In the 2 ppm treatment group, two males on two occasions each exhibited aggressive and irritable behavior with increased motor activity in one of them. Although 1 male and 1 female in the 0.5 ppm treatment group exhibited aggressive behavior (total of four occasions) this could not be definitively attributed to treatment because; 1) low frequency, 2) only one sign; 3) no effect in females at any higher dose. No differences in organ weights or gross or microscopic pathology were observed between mice in the 2 or 0.5 ppm treatment groups and the controls. Body weights, food consumption, and clinical blood chemistry were not affected in any treatment group. No neoplastic tissue was observed in mice in the treatment and control groups. Hematology, ophthalmoscopic and urine analyses were not conducted during the study. **The LOEL for this study is 2 ppm (0.32 mg/kg/day), based on the aggressive and irritable behavior with increased motor activity in males. The NOEL is 0.5 ppm (0.08 mg/kg/day).**

This 90-day subchronic toxicity study (dietary) is classified **acceptable** and satisfies the Subdivision F guideline requirement for a subchronic toxicity study in rodents (§82-1a).

82-1a Subchronic Oral Toxicity Feeding - Dog

In a subchronic toxicity study (MRID 44262812 - main; 44262810 - range-finding), **MB46513** (96.0% a.i.), a **photodegradate** of fipronil, was administered to 5 beagle dogs/sex/dose by feeding at dose levels of 0, 3.5, 9.5, or 35 ppm (mean achieved dosages of 0, 0.10, 0.27, or 0.95 mg/kg/day for males and 0, 0.10, 0.29, or 1.05 mg/kg/day for females) for 90 days. In a range finding study, 2 dogs/sex/dose (0, 27, 80, or 270 ppm) were treated with MB 46513 for 28 days.

In the **28 day range finding study** ½ males at 27 ppm (1 mg/kg/day) had convulsions at 28 days and animals at 80 ppm had clinical signs as early as day 4 (this group had to be sacrificed early due to extreme toxicity). In the **main study**, 35 ppm group, 1/5 females was sacrificed prematurely after exhibiting increase salivation, prostration, writhing, tremors, absence of rotular reflex, noisy breathing, and dyspnea. Histopathological examination of this female after 28 days revealed multifocal myocardial necrosis associated with intramural coronary arteritis. Behavioral changes were observed in a second female in the 35 ppm treatment group consisting of excessive barking and aggressivity on one occasion and irritability, tremors, and increased salivation on another occasion. No other treatment-related behavioral effects were observed in the 35 ppm treatment group. No treatment-related behavior effects were observed in any dogs in the 9.5 or 3.5 ppm treatment groups. No treatment-related differences in ophthalmology, hematology, clinical blood chemistry or urinalysis parameters or gross pathology were observed between dogs in any treatment group and the controls. No neoplastic tissue was observed in any of the treatment groups. **The LOEL is 35 ppm (1.05 mg/kg/day), based on behavioral changes in 2/5 females. The NOEL is 9.5 ppm (0.29 mg/kg/day).**

This subchronic toxicity study is classified **acceptable** and satisfies the guideline requirements for a subchronic oral study (**§82-1b**) in non-rodents.

C. CHRONIC TOXICITY

Adequacy of data base for chronic toxicity (Series 83-1, 83-5): The data base for chronic toxicity is considered complete. No additional studies are required at this time.

83-1a Chronic Feeding – Rat

Fifteen (15) CD rats/sex/group were administered **fipronil** (95.4%) in the diet for 52 weeks to assess the chronic toxicity of the chemical (MRID # 42918648). An additional 15 rats/sex/group were fed the chemical for 52 weeks and then were untreated for an additional 13 weeks to test the reversibility of treatment-related changes. Fifty rats/sex/group were supposed to be treated for 104 weeks to assess the carcinogenic potential of the chemical. The doses administered in all the phases were 0, 0.5, 1.5, 30 and 300 ppm. The carcinogenic phase of the study was terminated after 89 and 91 weeks in males and females, respectively, due to excessive mortality and to ensure that a sufficient number of animals were available for the terminal sacrifices. No treatment related differences in mortality between the groups were observed.

Evidence of treatment-related toxicity included: 1) neurotoxicity (including seizures which resulted in death) in the 1.5, 30 and 300 ppm group males and females; 2) decreased body weight gain in the 300 ppm group males and females and the 30 ppm group females; 3) decreased food consumption and food conversion efficiency in the 300 ppm group males and females at the beginning of the study; 4) decreased hematology parameters in the 300 ppm group males and females in comparison to the control groups (values were comparable to pretreatment measures); 5) alterations in clinical chemistry (increased cholesterol and calcium values; protein alterations with increased total protein, decreased albumin and increased globulins) mostly in the 30 and 300 ppm group males and females; protein alterations were seen in the 1.5 ppm group males after 76 and 81 weeks of treatment; 6) alterations in thyroid hormones (increased TSH and decreased T_4 levels) in all treated groups at some time points with the 30 and 300 ppm group males and females consistently affected; 7) alterations in urinalysis parameters (lower pH, higher protein, elevated urine volume with decreased specific gravity) in the 30 and 300 ppm groups (predominately males); 8) changes on gross necropsy (large and/or pale kidneys and large livers, adrenals and thyroids) in the 30 and 300 ppm group males and females; 9) increased absolute and relative weights of the liver and thyroids in the 30 and 300 ppm group males and females; 10) increased incidence and severity of progressive senile nephropathy in the 30 and 300 ppm group males and females. **LOEL = 1.5 ppm for males (0.059 mg/kg/day) and females (0.078 mg/kg/day) based on an increased incidence of clinical signs and alterations in clinical chemistry and thyroid parameters. NOEL = 0.5 ppm for males (0.019 mg/kg/day) and females (0.025 mg/kg/day).**

Benign (follicular cell adenoma) and malignant (follicular cell carcinoma) neoplastic changes were observed in the thyroid gland in increased incidences in all the treated animals as compared to the control group. However, only the 300 ppm group males and females exceeded the historical incidence of these tumors, either alone or in combination, for this strain of rat in this laboratory. **The study demonstrated that fipronil is carcinogenic to rats at doses of 300 ppm in males (12.68 mg/kg/day) and females (16.75 mg/kg/day).**

Classification: Minimum

83-1b Chronic Oral Toxicity [capsule] - Dog

Male and female beagle dogs were administered **fipronil** (96.8% a.i.) in capsules at dosages of 0, 0.2, 2.0 or 5.0 mg/kg/day for 52 weeks (MRID # 42918645).

One male in the 2.0 mg/kg/day group and two males in the 5.0 mg/kg/day group were sacrificed during the study due to poor condition. Clinical signs of neurotoxicity were seen in the 2.0 and 5.0 mg/kg/day groups beginning in Week 2. Abnormal neurological examinations were observed in males and females in the 5.0 mg/kg/day group and in females in the 2.0 mg/kg/day group. Body weight gain was decreased in the 5.0 mg/kg/day group females, however the mean decrease was due to reduced gain in one female.
LOEL = 2.0 mg/kg day based on clinical signs of neurotoxicity and abnormal neurological examinations.
NOEL = 0.2 mg/kg/day.

Classification: Guideline

D. CARCINOGENICITY

Adequacy of data base for Carcinogenicity (Series 83-2, 83-5): The data base for carcinogenicity is considered complete. No additional studies are required at this time.

83-2a Carcinogenicity Study - rat

This study (MRID 42918648) is presented in the Chronic Toxicity Section. see 83-5 above.

83-2b Carcinogenicity [feeding] - Mouse

Six groups of 20 male and 20 female CD-1 mice/group were administered **fipronil** (95.4% a.i.) in the diet at dosages of either 0, 0.1, 0.5, 10, 30 or 60 ppm for 52 weeks to test the chronic toxicity of the chemical (MRID # 42918649). An additional six groups of 52 male and 52 female mice were treated at the same dosages of 78 weeks to test the carcinogenic potential of the chemical. Due to excessive mortality, animals in the 60 ppm group were sacrificed during Week 10 of the study.

Signs of toxicity in the remaining groups included: 1) decreased body weight gain in the 30 ppm group males and females at most of the evaluation periods; values for the 10 ppm group were also decreased, although not as consistently; 2) decreased food consumption in the 30 ppm group females; 3) decreased food conversion efficiency in the 10 and 30 ppm group males; 4) altered white blood cell differential counts in the 30 ppm group females; 5) increased incidence of liver pathology on gross examination in the 30 ppm group males in the carcinogenicity phase; 6) increased absolute and/or relative liver weights in the 10 and 30 ppm group males and females in both the toxicity and carcinogenicity phases; 7) increased incidence of periacinar and microvesicular vacuolation in the liver of the 10 and 30 ppm group males at the toxicity and carcinogenicity phase necropsies; 8) increased incidence of hepatocellular hyperplasia and chronic degenerative changes in the liver of the 30 ppm group males which died or

were sacrificed during the treatment period of the carcinogenicity phase. There was an increased incidence of malignant hepatocellular tumors in males in the 30 ppm group as compared to the controls at the carcinogenicity phase necropsy. However, the incidence in the control group was lower than the historical incidence with this species and this laboratory. In addition, the difference in incidence was not statistically significant, and when benign and malignant tumors were considered together, the incidences were similar. **LOEL = 10 ppm (1.181 mg/kg/day for males and 1.230 mg/kg/day for females) based on decreased body weight gain, decreased food conversion efficiency (males), increased liver weights and increased incidence of hepatic histopathological changes. NOEL = 0.5 ppm (0.055 mg/kg/day for males and 0.063 mg/kg/day for females).**

The study demonstrated that Fipronil is not carcinogenic to CD-1 mice when administered at doses of 30 ppm.

Classification: Minimum

E. DEVELOPMENTAL TOXICITY

Adequacy of data base for Developmental Toxicity (Series 83-3): The data base for developmental toxicity is considered complete. No additional studies are required at this time.

1. Studies Conducted with Fipronil

83-3a Prenatal Developmental Study - Rat

Specific Pathogen Free female rats of the Crl:CD^{R} (SD) BR VAF/Plus strain from Charles River, St. Aubin les Elbeuf, France, received either 0, 1, 4, or 20 mg/kg/day **fipronil** (93% a.i.) by oral gavage from gestation days 6 through 15, inclusive (MRID # 429779-03).

Maternal toxicity was noted at 20 mg/kg/day in the form of reduced body weight gain during the dosing period (82.6% of control, gestation days 6-16) and to a lesser extent for the period including the dosing plus post dosing period (90.1% of control, gestation days 6 though 20) and for the entire gestation period (91.8% of control, gestation days 2 through 20). There was an increase in water consumption in the high dose group throughout the study ranging from a 3 to 28% increase as compared to control; there was an 18% increase over control in the high dose group for gestation days 6-15. Food consumption was slightly decreased in the high dose group at the beginning of the dosing period (days 6-11) with an overall reduction of 90% of control for gestation days 6-15, after which no treatment-related effect was noted. There was a slight reduction in the high dose group food efficiency during the dosing period, 27.8, 28.5, 27.0 and 25.3% for the control, low, mid and high dose groups, respectively. No effects were noted in developmental toxicity parameters.

Maternal toxicity LOEL = 20 mg/kg/day based on reduced body weight gain, increased water consumption, reduced food consumption and reduced food efficiency. Maternal toxicity NOEL = 4 mg/kg/day.

Developmental toxicity LOEL = greater than 20 mg/kg/day. Developmental toxicity NOEL = 20 mg/kg/day or higher.

Classification: Minimum

83-3b Prenatal Developmental Study - Rabbit

Sexually mature virgin female New Zealand White rabbits from Ranch Rabbits, Crawley Down, Sussex, England, received either 0, 0.1, 0.2, 0.5 or 1.0 mg/kg/day **fipronil** (95.4% a.i.) by oral gavage from gestation days 6 through 19, inclusive (MRID # 42918646).

Maternal toxicity was noted at all dose levels tested in the form of reduced body weight gain at all gestation day periods evaluated. Body weight gains for the treatment period (gestation days 6-20) were 73, 73, 50 and 30% of control for the 0.1, 0.2, 0.5 and 1.0 mg/kg/day groups, respectively. For gestation days 20-28, weight gains of the treated animals exceeded the controls. For gestation days 0-28, gains were 88, 86, 81 and 67% of control for the 0.1, 0.2, 0.5 and 1.0 mg/kg/day groups. All treated groups consumed less food than that of the control group during the dosing period; the differences were statistically significant for the two highest dose groups. Food efficiency was decreased in all treated groups. No effects were noted in developmental toxicity parameters.

Maternal toxicity LOEL ≤ 0.1 mg/kg/day based on reduced body weight gain, reduced food consumption and efficiency. Maternal toxicity NOEL is < 0.1 mg/kg/day.

Developmental toxicity LOEL > 1.0 mg/kg/day. Developmental toxicity NOEL ≥ 1.0 mg/kg/day.

Classification: Minimum

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2. Studies Conducted with Photodegradate M&B 46513

83-3a Prenatal Developmental Study - Rat

In a developmental toxicity (teratology) study (MRID# 44275001), adult virgin female rats (CD strain, Sprague Dawley Crl: CD (SD) BR from Charles River Laboratories, St Aubin les Elbeuf, France) received either 0, 0.5, 1.0, or 2.5 mg/kg/day of MB 046513 (Purity: 992 g/kg, Batch 805 DAP/DA999) suspended (w/v) in an aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France) at 0.5% by oral gavage from gestation days 6 to 15 inclusive of presumed gestation. Maternal parameters included clinical signs of toxicity, body weights (on gestation day 0, daily from gestation day 6-16 and on gestation day 20) and food consumption (interval gestation day 0-6, daily from gestation days 6-15 and the interval of gestation day 16- 20). All surviving animals were sacrificed on gestation day 20 and each female was subjected to macroscopic examination of the visceral organs, the reproductive tract was weighed (gravid uterine weight), dissected out and the number of corpora lutea in each ovary, the number of implantation sites, number and localization of resorption sites (classified as early and late), the number and distribution of live and dead foetuses in each uterine horn were determined along with the sex of viable fetuses and the individual weights of viable fetuses and placenta were measured. The viable fetuses were sacrificed, subjected to an external examination and the approximately half of the viable fetuses from each litter were prepared for soft tissue examination and the remaining for skeletal examination.

Maternal toxicity was noted as clinical signs in the high dose animals as hair loss on either the paws, limbs, flanks, abdomen and/or thorax. The high dose group had lower body weight gains on study days 6-16 (58.1% of control), study days 0-20 (90.0% of control), study days 6-20 (83.9% of control) and days 0-20 corrected for gravid uterine weights (78.3% of control). The high dose group also consumed less food during the dosing period and there was lower food efficiency relative to the control group except for the post dosing period where an increase was noted which is indicative of a rebound relative to toxicity. **The Maternal Toxicity LOEL was 2.5 mg/kg/day and the NOEL was 1.0 mg/kg/day based an increase in clinical signs of toxicity on reduced body weight gain, food consumption and food efficiency.**

Developmental toxicity was noted as a very slight increase in the fetal and litter incidence of incomplete or reduced ossification in several bones in the high dose group, including the hyoid body, 5th/6th sternebrae, 1st thoracic body, pubic bone and 1 or 2 metatarsi. There was also a slight reduction in fetal body weight (males 97.5% of control, females 97.7% of control) in the high dose group, although statistically significant, the changes are too small to be biologically relevant. **The Developmental Toxicity LOEL was 2.5 mg/kg/day and the NOEL was 1.0 mg/kg/day based on the slight increase in fetal and litter incidence of reduced ossification of several bones.**

This study is classified as <u>Acceptable-Guideline</u> and satisfies the guideline requirements (§ 83-3a) for a teratology study in rats.

F. REPRODUCTIVE TOXICITY

Adequacy of data base for Reproductive Toxicity (Series 83-4): The data base for reproductive toxicity is considered complete. No additional studies are required at this time.

1. Studies Conducted with Fipronil

83-4 Two-Generation Reproduction Study - Rat

Thirty CD rats/sex/group received **fipronil** (95.4% a.i.) continuously in the diet at concentrations of 0, 3, 30 and 300 ppm (equivalent to 0, 0.25, 2.54 and 26.03 and 0.27, 2.74 and 28.40 mg/kg/day for males and females, respectively) (MRID # 42918647). Parental (systemic) toxicity was noted in the form of the following: 1) increased mortality in the 300 ppm group males and females in the F_0 and F_1 generations; 2) decreased body weight gain pre-mating in the 300 ppm group males and females in the F_0 and F_1 generations and in the 300 ppm group females during gestation and lactation in the F_0 generation; 3) food consumption in the 300 ppm group males and females during pre-mating in the F_0 generation; 4) increase in the absolute and relative weights of the thyroid glands and liver in the 30 and 300 ppm group males and females of the F_0 and F_1 generations; decrease in the absolute and relative weights of the ovaries in the 300 ppm group females in the F_0 generation; decrease in the absolute weight of the pituitary gland in the 30 and 300 ppm group females and decrease in the relative weight in all the treated female groups in the F_1 parental animals; decrease in the absolute and relative weights of the testes in the 300 ppm group males in the F_1 parental animals; 5) increased incidence of centriacinar fatty vacuolation in the livers of the 300 ppm group females in both the F_0 and F_1 generations; and 6) increased incidence of follicular epithelial hypertrophy of the thyroid glands in the 300 ppm group males and females in the F_0 generation and in the 30 and 300 ppm group females in the F_1 generation.

Reproductive toxicity was noted in the form of the following findings in the 300 ppm group: 1) clinical signs of toxicity in the F_1 and F_2 offspring; 2) decreased litter size in the F_1 and F_2 litters; 3) decreased body weights in the F_1 and F_2 litters; 4) decrease in the percentage of F_1 parental animals mating; 5) reduction in fertility index in F_1 parental animals; 6) reduced post-implantation survival and offspring postnatal survivability in the F_2 litters; and 7) delay in physical development in the 300 ppm group of the F_1 and F_2 litters.

The Lowest Observed Effect Level (LOEL) for parental (systemic) toxicity was 30 ppm (2.54 mg/kg/day for males and 2.74 mg/kg/day for females) based on increased weight of the thyroid glands and liver in males and females; decreased weight of the pituitary gland in females; and an increased incidence of follicular epithelial hypertrophy in the females. The No Observed Effect Level (NOEL) for parental (systemic) toxicity was 3 ppm (0.25 mg/kg/day for males and 0.27 mg/kg/day for females).

The LOEL for reproductive toxicity was 300 ppm (26.03 mg/kg/day for males and 28.40 mg/kg/day for females) based on clinical signs of toxicity in the F_1 and F_2 offspring; decreased litter size in the F_1 and F_2 litters; decreased body weights in the F_1 and F_2 litters; decrease in the percentage of F_1 parental animals mating; reduction in fertility index in F_1 parental animals; reduced post-implantation survival and offspring postnatal survivability in the F_2 litters; and delay in physical development in the F_1 and F_2 offspring. The NOEL for reproductive toxicity was 30 ppm (2.54 mg/kg/day for males and 2.74 mg/kg/day for females).

Classification: Minimum

G. NEUROTOXICITY

Adequacy of data base for Neurotoxicity (Series 81-8, 82-7, 83-6): The data base for neurotoxicity is considered complete. No additional studies are required at this time.

1. Studies Conducted with Fipronil

81-8 Acute Neurotoxicity - Rat

A single dose of **fipronil** (96.7% a.i.) in corn oil was administered by gavage to four groups of 15 CD rats/sex/group at dosages of either 0, 0.5, 5.0 or 50.0 mg/kg.

Five males and one female in the 50 mg/kg group died during the study (MRID # 42918635). Treatment-related clinical signs of toxicity, including neurotoxicity, were seen with the 50 mg/kg group animals, especially the males. Males in the 50 mg/kg group had decreased body weights in comparison to the controls. During the open field evaluations of the functional observational battery (at 7 hours, 7 days and 14 days post-treatment), effects of both stimulation and depression of the nervous system were seen. Those parameters for which there were statistically significant changes in males included gait, fine tremors (females also), coarse tremors, urination, mean number of rears (females also), approach response, pupil size, muscle tone (females also), air righting and mean hind leg splay (females also). Mean rectal body temperature was also decreased in the males and females of this group. The only treatment-related effects in the 5.0 mg/kg group at this time point were decreased mean body temperature in males and decreased mean hind leg splay in males and females. On Days 7 and 14, the effects were minor in comparison, but females in the 50 mg/kg group had a statistically significant increase in hind leg splay at both evaluations. Mean motor activity was decreased by 90 and 93% in the 50 mg/kg group males and females, respectively, at the 8-hour evaluation. At Day 7, significant increases in mean activity for the 0.5 and 5.0 mg/kg group males were observed. However, supplemental statistical analysis demonstrated that the test substance did not alter motor activity when compared with pretreatment activity. There were no significant differences between the treated and control groups at Day 14. There were no treatment-related gross or microscopic changes on post-mortem examination of the central and peripheral nervous systems. **The No Observed Effect Level (NOEL) = 0.5 mg/kg for males and females. The Low Observed Effect Level (LOEL) = 5.0 mg/kg for males and females based on decreased hind leg splay at the 7 hour post-treatment evaluation in males and females.**

Classification: Minimum

82-7 Subchronic neurotoxicity Screening Battery - rat

In this subchronic neurotoxicity study, male and female Sprague-Dawley rats (15/sex/dose) were fed test diets containing M&B 46030 at 0 (basal diet), 0.5, 5.0, or 150 ppm (equivalent to 0, 0.0297, 0.301, or 8.89 mg/kg/day, males; 0, 0.0354, 0.351 or 10.8 mg/kg/day, females) (MRID No. 43291703). Neurobehavioral screening, consisting of Functional Observational Battery and motor activity evaluations, was performed at pretreatment, and during Weeks 4, 9 and 13. At terminal sacrifice, six animals/sex/dose were anesthetized and perfusion fixed *in situ* for neuropathological evaluation. With the exception of one low-dose female which was found dead on Day 16, all remaining animals survived to terminal sacrifice without the appearance of any treatment-related clinical signs. Decreases in mean body weight, observed in high-dose males and females at Week 1 of treatment, were judged to be slight (6.5%, males; 6.9%, females). The decrease in body weight was accompanied by a concomitant decrease in food consumption, which would suggest a palatability problem, rather than a treatment-related effect. FOB findings revealed minimal effects in high-dose animals at the Weeks 4, 9 and 13 evaluations. High-dose males had a decreased incidence of no urination and an increased incidence of exaggerated tail pinch response. High-dose males and females had an increased incidence startle responses in the manipulative observations. High-dose females had increased forelimb grip strength at Week 13. The mean body weights of treated males were significantly greater than the concurrent control values. Necropsy findings did not reveal any treatment-related gross pathological or histopathological findings. Although histopathological lesions were observed, incidences were low and attributed by the study pathologist to animal variation and artifactual changes. **Based on the results (FOB findings) of this study, the LOEL was established at 150 ppm (8.89 mg/kg/day, males; 10.8 mg/kg/day, females); the NOEL was established at 5.0 ppm (0.301 mg/kg/day, males; 0.351 mg/kg/day, females).**

This study is classified as **Core - Acceptable** and satisfies guideline requirements (§82-7) for a subchronic neurotoxicity screening battery in the rat.

83-6 Developmental Neurotoxicity - Rat

Fipronil (96.1% a.i.) was administered to 30 female Sprague-Dawley rats/group in the diet at dose levels of 0, 0.5, 10 or 200 ppm (0.05, 0.90 or 15 mg/kg/day, respectively) from Gestation Day 6 to Lactation Day 10 (MRID # 44039002).

There was no evidence of a treatment-related effect on maternal survival or clinical signs of toxicity. Two females in the 200 ppm group died during lactation, but there was no evidence that the deaths were treatment-related. Mean maternal body weight values for the 200 ppm group were reduced 15.5%, 10.0% and 8.6% in comparison to the controls on Gestation Days 10, 15 and 20, respectively. Mean body weight gain was statistically decreased for Gestation Day interval 6-10, but increased for interval 10-15. Statistically significant reductions in mean body weight were seen in the 200 ppm group on Lactation Days 0 and 4. Mean body weight gain was statistically increased on Lactation Days 4-11. A statistically significant reduction in group mean food consumption was noted in the 200 ppm group for Gestation Days 6 to 10 but was comparable to the controls for other intervals.

Pregnancy rate and gestation length for treated animals were comparable to the control group. There was no evidence of a treatment-related effect on gross necropsy findings. **The maternal LOEL is 200 ppm (15 mg/kg/day), based on decreased body weight, body weight gain and food consumption. The maternal NOEL was 10 ppm (0.90 mg/kg/day).**

At 200 ppm, litter size was not affected by treatment, but the live birth index was decreased (not statistically significant). The pup viability index (survival from Postnatal Days 0-4) for the 200 ppm group was significantly decreased (98.9% for control vs. 75.5% for 200 ppm group). The weaning index (survival from Postnatal Days 4-21) was decreased for this group, but the difference was not statistically significant. Pup sex distribution was not affected. There was a statistically significant decrease in group mean body weights of both male and female offspring at all recorded intervals during lactation (9.2-34.1% and 8.1-33.8% decrease in males and females, respectively) and for various periods post-weaning. Statistically significant increases in the mean day of achieving pinna detachment, upper and lower incisor eruption, vaginal patency and preputial separation were noted. Auditory startle testing on Postnatal Day 22 demonstrated a statistically significant decrease in the maximum response for males and females. There was no significant difference in the time to maximum response or average response. There were no changes in this parameter on Postnatal Day 60. Motor activity testing on Postnatal Day 17 showed statistically significant increases in motor activity counts for females. Swimming direction scores on Day 6 were reduced for the males and females, although only the males were statistically significant. On Day 14, the scores were comparable. Water "Y" maze time trials for learning and memory showed a statistically significant increase in time required to complete the maze for females in Trials 5 and 6 on Day 24. There were no statistically significant differences for either sex on Days 25, 30, 60, 61 or 65. Statistically significant decreases in absolute brain weights for both sexes, compared to control values, were found on Postnatal Days 11 (20% and 11% decrease in males and females, respectively) and 60 (≈ 7% decrease in males and females). Terminal body weights were also decreased for this group on these days. On Day 11, the relative brain weights for both sexes were significantly increased in comparison to the controls. On Day 60, the values for the control and 200 ppm groups were comparable. There was no evidence of a treatment-related effect on the gross macroscopic or microscopic examinations (including the central and peripheral nervous systems) of the pups sacrificed on Postnatal Days 11 and 60. *At 10 ppm*, group mean weights were significantly reduced for females at all recorded intervals and for males on Days 4 and 17. Post-weaning weights were not affected. There was a statistically significant increase in the time of preputial separation in the 10 ppm group males. Females had a statistically significant increase in mean motor activity counts on Postnatal Day 17. **The developmental LOEL is 10 ppm (0.9 mg/kg/day), based on statistically significant decrease in group mean pup weights during lactation and significant increase in time of preputial separation in males. The developmental neurotoxicity LOEL is 10 ppm (0.9 mg/kg/day) based on a significant increase in mean motor activity counts in females on**

Postnatal Day 17. The NOEL for developmental and developmental neurotoxicity is 0.5 ppm (0.05 mg/kg/day). It is noted that developmental neurotoxicity occurred in the absence of maternal toxicity in this study.

Classification: Acceptable (Guideline)

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2. Studies Conducted with Photodegradate M&B 46513

81-8 Acute Neurotoxicity - Rat

In an acute neurotoxicity study (MRID 44262808), **MB46513, a photometabolite** of fipronil (99.5% a.i.), was administered in corn oil by oral intubation to Crl:CD BR rats (12/sex/dose) at dose levels of 0, 0.5, 2, or 12 mg/kg. The rats were evaluated for reactions in functional observations and motor activity measurements at 6 hours, 7 days, and 14 days post dosing. Clinical signs, body weights, and food consumption were monitored. At study termination, brains were weighed and measured, and neural tissues were processed for microscopic evaluation.

No animals died and there were no treatment-related clinical signs of toxicity. At 12 mg/kg, significant decreases in body weight gains and food consumption were noted for the high-dose males and females during the week following treatment. By the second week, both had returned to the control levels. Body weight gains and food consumption for the low- and mid-dose groups and mean body weights for all treated groups were not significantly different from the controls throughout the study. Food efficiency was not affected by treatment. Behavioral responses were also affected by treatment with MB46513 at 12 mg/kg. At the estimated peak response time, 6 hours post dosing, significant decreases in locomotor activity during the first 30 minutes of observation were noted among high-dose males and females. There were no significant differences in any dose group on days 7 and 14. Treatment-related decreases in hindlimb splay and rectal temperature at 6 hours post dose were observed in high-dose males and females. In addition, decreases in the proportion of high-dose males with an immediate righting reflex on days 7 and 14 were possibly treatment related. Decreased forelimb grip strength in high-dose males on day 7 and increased forelimb grip strength in high-dose females at 6 hours post dosing was possibly related to the treatment, because there were also slight increases in forelimb grip strength in high-dose males at 6 hours and slight decreases in forelimb grip strength in high dose females at 7 days and in high-dose males and females at 14 days. There were no significant differences among groups in neuropathology. **Based on these findings, the neurobehavioral LOEL for rats is 12 mg/kg. The NOEL is 2 mg/kg.**

This study is classified **unacceptable** and does not satisfy the guideline requirement for an acute neurotoxicity study in rodents **(§81-8).** The study may be upgraded after receipt and favorable review of positive control data for the performing laboratory.

H. MUTAGENICITY

Adequacy of data base for Mutagenicity (Series 84): The available studies indicate that **fipronil** and the fipronil **photodegradate, MB46513** are not mutagenic in bacteria and are not clastogenic in vitro or in vivo up to doses that showed clear test material interaction with the target cells.. Based on these considerations, the Committee concluded that there is no concern for mutagenicity.

The submitted test battery for both compounds satisfy the new mutagenicity initial testing battery guidelines. No further studies are required at this time. The data base for Mutagenicity is considered adequate. Based on the available mutagenicity studies, there are no concerns for mutagenicity at this time.

1. Studies Conducted with Fipronil

84-2 Gene Mutation

1) *Salmonella typhimurium* and *Escherichia coli*/mammalian activation gene mutation assay: In two independent experiments (MRID # 42918652), **fipronil** (90.6% a.i.) was not mutagenic in 4 strains of S. typhimurium at concentrations up to 500 µg/plate in the presence or absence of S9 activation.

Classification: Acceptable

2) *In vitro* gene mutation assay in mammalian cells/Chinese hamster V79 cells: In two independent experiments (MRID # 42918651), fiproni (97.2% a.i.) was negative for inducing forward gene mutations at the HGPRT locus in cultured Chinese hamster V79 cells at concentrations up to 385.65 µg/ml both with and without S9 activation.

Classification: Acceptable

84-2 Cytogenetics

1) Cytogenic assay/human lymphocytes: There was no evidence of a clastogenic effect when human lymphocytes were exposed *in vitro* to **fipronil** (90.6% a.i.) at doses of 75, 150 or 300 µg/ml with and without S9 activation (MRID # 42918653).

Classification: Acceptable

2) *In vivo* mouse micronucleus assay (MRID No. 43680801), groups of five male and five female CD-1 mice received single oral gavage dose of 12.5, 25 or 50 mg/kg (actual concentrations based on analytical determinations were 6.95, 16.1 and 48.0 mg/ml) M&B 46030 (96.2%). Bone marrow cells were collected 24, 48 or 72 hours posttreatment from the high-dose males and females and were examined for micronucleated polychromatic erythrocytes (MPEs). Cells, harvested 24-hours postadministration of the low and intermediate doses, were scored for MPEs. The test material was delivered to the test animals as suspensions prepared in 0.5% methyl cellulose.

Clinical signs of toxicity seen in the high-dose group (6 males and 2 females) included hunched posture, hypoactivity, piloerection, convulsions and slow respiration. M&B 46030 was not cytotoxic to the target cell. The positive control induced the expected high yield of MPEs in both sexes. **There was, however, no evidence of a clastogenic or aneugenic effect at any M&B 460030 dose or at any harvest time.**

The study is classified as **Acceptable** and satisfies the guideline requirement for a mouse micronucleus assay (84-2).

84-4 Other Genotoxicity

no studies available

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2. Studies Conducted with Metabolite M&B 46513

84-2 Gene Mutation

1) In two independent microbial gene mutation assays (43291721), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 10, 25, 59, 100 or 250 ug/plate **MB 46513** in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material

was delivered to the test system in dimethyl sulfoxide. Test material insolubility and cytotoxicity were observed at 250 ug/plate with or without S9 activation; compound precipitation was also present at 100 ug/plate +/-S9. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9 activated positive controls.

2) In independently performed mammalian cell gene mutation assays at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus (MRID 44262814), Chinese Hamster Ovary (CHO) cells cultured in vitro were exposed to **MB46513,** a fipronil metabolite (99.5% a.i.) in dimethyl sulfoxide at doses ranging from 5 to 625 µg/mL, with and without S9 activation. Cultures were exposed for 4 hours prior to plating for determination of cytotoxicity, expression, and selection of the mutant phenotype.

MB46513 was tested to insoluble levels (generally ≥60 µg/mL) and reproducible cytotoxic doses (≥125 µg/mL -S9 and ≥250 µg/mL +S9). The positive controls induced the appropriate response. **MB46513 did not, however, induce forward mutations at the HPRT locus in CHO cells at any dose level tested, with or without metabolic activation.**

This study is classified as **acceptable** and satisfies the guideline requirement **(§84-2)** for *in vitro* mammalian forward gene mutation studies.

84-2 Cytogenetics

In an *in vivo* mouse bone marrow micronucleus assay (MRID 44263813), groups of 15 male and female CD-1 mice were dosed by intragastric gavage with **MB46513**, a fipronil metabolite (99.5%, a.i.) in corn oil at 2, 4, 8, and 16 mg/kg. Bone marrow cells were harvested at 24, 48, or 72 hours and scored for micronucleated polychromatic erythrocytes (MPCEs).

Slight piloerection was observed in the 2, 4, 8, and 16 mg/kg groups; slight hunched posture was observed in the 8 mg/kg group. No mortalities occurred in the 2 to 8 mg/kg groups, but 7/38 mice in the 16 mg/kg group died. A significant decrease ($p<0.01$ or 0.001) in the ratio of polychromatic to normochromatic erythrocytes was observed in the 16 mg/kg animals. This finding is indicative of bone marrow cell depression. The positive control induced significant increases in MPCEs. **There was no significant increase in the frequency of MPCEs in bone marrow after any MB46513 treatment time; therefore, the test article is considered negative in this micronucleus assay.**

This study is classified as **acceptable.** It satisfies the requirement for FIFRA Test Guideline **84-2** for *in vivo* cytogenetic mutagenicity data.

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3. Studies Conducted with Metabolite M&B 46136

84-2 Gene Mutation

1) *Salmonella*/mammalian activation gene mutation assay: In two independent experiments (MRID # 42918679), fipronil metabolite (98.7% a.i.) was not mutagenic in 4 strains of S. typhimurium at concentrations of up to 200 µg/plate without S9 activation and up to 500 µg/plate in the presence of S9 activation.

Classification: Acceptable

84-2 Cytogenetics

1) Cytogenic assay/human lymphocytes: There was no evidence of a clastogenic effect when human lymphocytes were exposed *in vitro* to M&B 46136 (98.7% a.i.) at doses of 75, 150 or 300 µg/ml with and without S9 activation (MRID # 42918680).

Classification: Acceptable

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2. Studies Conducted with Metabolite M&B 45897

84-2 Gene Mutation

1) In initial and repeat plate incorporation and initial and repeat preincubation microbial mutagenicity assays (MRID 44262822), *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to MB45897 (99.7% a.i.) in DMSO over dose ranges of 25-2,500 µg/plate in the presence of mammalian metabolic activation (+S9) or 12.5-2,500 µg/plate in the absence of metabolic activation (-S9). Preparations for metabolic activation were made from Aroclor 1254 induced rat livers.

MB45897 was insoluble at 2,500 µg/plate (±S9). Cytotoxicity was also observed in the majority of strains at 2,500 µg/plate +/-S9 under all assay conditions. The positive control substances induced marked increases in revertant colonies in the respective strains. **There were, however, no reproducible, dose-related differences in the number of revertant colonies in any tester strain at any dose level/condition compared to the vehicle controls.**

This study is classified as **Acceptable (Guideline)** and satisfies the requirement for FIFRA Test Guideline **84-2** for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

2) In a microbial mutagenicity assay (MRID 44262823), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to MB45897 (>99% a.i.) in DMSO at concentrations of 8, 40, 200, 1,000, and 5,000 µg/plate in the presence of mammalian metabolic activation (+S9); strain TA100 was exposed at these same levels in the absence of metabolic activation (-S9). Additionally, strains TA98, TA1535, and TA1537 were exposed at 4, 20, 100, 500, and 2,500 µg/plate in the absence of activation. Preparations for metabolic activation were made from Aroclor induced rat livers.

MB45897 (>99% a.i.) was tested to the limit concentration of 5,000 µg/plate. Cytotoxicity was observed in the absence of metabolic activation at 5,000 µg/plate in the TA100 strain and at 2,500 µg/plate in strains TA98, TA1535, and TA1537 and in the presence of metabolic activation in strains TA98, TA1535, and TA1537 at 5,000 µg/plate. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **acceptable** and satisfies the requirement for FIFRA Test Guideline **84-2** for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

3) In a mammalian cell chromosome aberration assay (MRID 44262824), cultured human lymphocytes were exposed to MB45897 (99.7% a.i.), in dimethyl sulfoxide at concentrations of 50, 100, 200, 400, 500, 600, and 800 µg/mL with metabolic activation and 12.5, 25, 50, 75, 100, and 150 µg/mL without metabolic activation. Without metabolic activation, the cells were exposed continuously for 20 or 44 hours; with metabolic activation, the cells were exposed to MB45897 for 3 hours and harvested 17 or 41 hours later. Preparations for metabolic activation were made from induced rat livers. Positive controls (±S9) were included.

Precipitation of MB45897 was apparent in all cultures treated with ≥400 µg/mL. Doses of 600 and 800 µg/mL +S9 were severely cytotoxic. For the metaphase analysis, concentrations of 125 and 150 µg/mL -S9 produced marked cytotoxicity, resulting in decreased mean mitotic index (MIs) of 74-81% compared to vehicle controls. In the first cytogenetic assay, there was a significant increase in aberrant cell frequency compared to the vehicle control value in cultures harvested 20 hour after exposure to 150 µg/mL -S9, both when gaps were included and excluded (8.0% and 6.0%; 0.05>p>0.01); the historical control range excluding gaps (0-5%) was also exceeded. In the second cytogenetic assay, significant increases in the aberrant cell frequency were noted at the 20-hour sampling of cultures dosed with 150 µg/mL -S9, both when gaps were included and excluded (p<0.001) and at 125 µg/mL -S9 at the 44-hour sampling both including (p<0.001) and excluding (0.05>p>0.01) gaps; these values also exceeded the historical control ranges (0-10% with gaps) and (0-5% without gaps). The data are, therefore, indicative of clastogenic activity by MB45897 in the absence of metabolic activation. In the presence of S9 activation, a reproducible increase the number of polyploid cells was seen in the 400-µg/mL group at the 20-hour sampling. A similar increase in polyploidy was noted at 400 µg/mL (44-hour sampling). The positive controls induced the expected responses in the treated lymphocytes. **Under the conditions of the test, MB45897 appears to be clastogenic to human lymphocytes at cytotoxic concentrations (≥125 µg/mL -S9) and induces polyploidy at 400 µg/mL +S9.**

This study is classified as **Acceptable (Guideline)** and satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro cytogenetic mutagenicity data.

84-4 Other Genotoxicity

no studies available for review

I. METABOLISM

Adequacy of data base for metabolism (Series 85-1, 85-2): The data base for metabolism (both 85-1 and 85-2) is considered to be complete. No additional studies are required at this time.

85-1 Metabolism - Rat

1. Studies Conducted with Fipronil

^{14}C-Fipronil (^{14}-C Fipronil, >97.0% radiochemical purity; unlabelled **Fipronil**, >99% a.i.) was administered orally in aqueous methylcellulose to groups (5 sex/dose) of male and female Sprague-Dawley rats at doses of 4 and 150 mg/kg (single dose) and 4 mg/kg x 14 days (repeated dose) (MRID # 42918655). 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. Feces appeared to be the major route of excretion for **fipronil** derived radioactivity, where 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. Major metabo lites 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. Whole blood half-life ranged from 149.4-200.2 hours in male and female rats at 4 mg/kg, with 0-168 hours AUCs approximately equal between sexes. At 150 mg/kg, whole blood half-life was noticeably decreased to 54.4 hours in male rats and 51.2 hours in female rats. Blood AUCs at this dose were approximately proportional to the increase in dose.

Classification: Minimum

2. Studies Conducted with Metabolite M&B 46513

In a rat metabolism study (MRID 44262817), [U-^{14}C-phenyl] MB46513 (>99% a.i.) was administered to five Sprague-Dawley CD strain rats/sex/dose by gavage as a single dose at 1 or 10 mg/kg or as a single dose at 1 mg/kg following a 14-day pretreatment with unlabeled MB46513 at 1 mg/kg.

Within 168 hours of dosing, 93-101% of the administered dose was recovered from both sexes of each dose group, of which 46.4-69.5% was in the feces, 19.9-41.1% was in the tissues and carcass, and 4.4-10.8% was in the urine. In all test groups, fecal excretion was higher for males (60.1-69.5%) than for females (46.4-56.0%), and less radioactivity was retained in the carcasses and tissues of males (19.9-26.6%) than females (30.0-41.1%). Levels of urinary excretion were comparable between sexes. Excretion of the radioactivity was increased slightly by pretreatment and at the high dose level. Within 168 hours of dosing, the single low-dose (SOLD) animals excreted 51.6-67.1% of the dose in feces and urine (including cage wash), whereas the repeated low-dose (ROLD) animals excreted 66.4-73.7%, and the single high-dose (SOHD) animals excreted 69.9-80.6%. Radioactivity was excreted gradually by all dose groups, but the rate of excretion differed between dose groups. Fecal excretion peaked on Day-1 for the SOLD group, on Day-6 for the ROLD group, and on Day-5 for the SOHD group. Urinary excretion showed a similar pattern within dose groups. Maximum concentrations of radioactivity in blood were attained within 46 to 73 hours of dosing and were similar between sexes within dose group (low dose, 0.15 ppm; high dose, 2.03-2.31 ppm). For both dose groups, elimination half-lives were 156-170 hours for males and 210-221 hours for females. The ratio of the areas under the concentration curves (AUC) for high to low-dose animals was 15.2 for males and 10.9 for females, reflecting the difference in dose levels. The general distribution of radioactivity among tissues was the same between dose groups and sexes, although the actual levels differed between dose groups and sexes. Concentrations of radioactivity were highest in fat [fat/plasma ratios: 6.3-12.8 in males and 16.4-25.2 in females], followed usually by the adrenals and liver. Females also had high concentrations associated with the ovaries. The lowest concentrations of radioactivity were generally associated with the brain, spleen, muscle, whole blood, and stomach. With the exception of whole blood and plasma, concentrations of radioactivity in all tissues were generally higher for females than for males, e.g. radioactivity in fat was 1.6-2.8 times higher in females than in males. Among the dose groups, ^{14}C-residues were lowest in tissues from the SOLD group with the exceptions of residual carcass and skin plus fur. Pretreatment with MB46513 increased the residue levels in tissues, and residue levels in tissues from the SOHD group were 10-30 times higher than in tissues from the SOLD group. The major radioactive component identified in excreta was unchanged MB46513 (males, 28.6-44.2%; females, 35.4-39.6%), nearly all of which was found in the feces. Unchanged MB46513 in urine accounted for <0.1% of the dose. The only other components in excreta accounting for >5% of the dose were MB46400 (males, 5.7-10.6%; females, 3.1-7.1%) and the 4-cyano-5-(N)cysteine conjugate of MB46513 (males, 7.2-14.2%; females, 3.8-9.2%). Other minor components identified in excreta included: RPA 105048; the sulfate conjugate of MB46513 (≤2.4%); a 4-cyano-5-(N) cysteine glycine conjugate of MB46513 (0.7-3.8%); and a 5-(N) cysteine conjugate of MB46513 (1.9-3.5%). Within each dose group, the metabolite profile was the same among sexes, although metabolite levels were generally higher in males than females. The metabolic profile was also similar between dose groups, although there were differences in the relative levels of metabolites. Pretreatment resulted in lower levels of MB46513 and higher levels of metabolites than in SOLD animals. Levels of MB46513 were similar in excreta of SOHD and SOLD groups, but levels of metabolites were generally higher for the SOHD group. These data indicate that fecal excretion of unchanged MB46513 is the principal pathway for elimination of MB46513 from rats. The metabolism of MB46513 in rats involves substitution of the trifluoromethyl or cyano groups on the pyrazole ring and/or sulfate, glucuronide, or glutathione conjugation at the amide on the pyrazole ring. The high levels of radioactivity in fat compared to blood and the prolonged elimination half-life indicate that there is a potential for bioaccumulation of MB46513 in fatty tissues.

This metabolism study in the rat is classified **Acceptable (Nonguideline)** as it is not a required guideline study. It is acceptable for the purposes for which it was intended (metabolism information on MB46513, a photolytic metabolite of **fipronil**) as a special study.

85-2 Dermal Absorption - Rat

1. Studies Conducted with Fipronil

In this dermal absorption study (MRID: 43737308; Guideline No.: §85-2), male rats were dosed at 0.071, 0.688 and 3.88 mg/cm^2 for exposures of 0.5, 1, 2, 4, 10 and 24 hours with **fipronil**. Quantity of **fipronil** in washed skin was 1.14-2.45%, 0.60-3.29% and 0.35-0.80% at the respective doses. Quantity of **fipronil** absorbed was <1% at all doses. The system was saturated at 3.88 mg/cm^2. The dermal absorption rate was calculated to be <1% at 24 hours.

2. Studies Conducted with Metabolite M&B 46513

In a dermal absorption study (MRID 44262816), 24 male Crl:CD BR rats/dose group were dosed dermally with [^{14}C]MB4613 (99% a.i.) as a 1% carboxymethylcellulose aqueous suspension at dose levels of 0.081, 0.881, or 7.17 mg/rat (0.006, 0.071, or 0.574 mg/cm^2). Four rats/dose were sacrificed for assessment of dermal absorption after 0.5, 1, 2, 4, 10, and 24 hours of exposure.

Dosed radioactivity was quantitatively recovered from each dose group (92.5-103%). After 24 hours of exposure, dermal absorption of MB46513 was minimal. For all dose groups, the majority of the dose was not absorbed (90.2-102.3%), and only trace amounts (≤0.1%) of radioactivity were excreted in the urine and feces. For the low- and mid-dose groups, radioactivity remaining in/on the skin after washing increased with the duration of exposure, reaching maximums of 3.97% and 1.05% of the dose, respectively, by 24 hours. However, duration of exposure had no effect on accumulation of radioactivity in/on the skin for the high-dose group. For the low-dose group, absorption (measured as amount excreted plus amount retained in the body) increased over time from <0.005% of the dose at 0.5 hours to 2.64% of the dose at 24 hours. Potential absorption (amount absorbed plus amount retained in/on skin) also increased over time from 0.74% of the dose at 0.5 hours to 6.61% of the dose at 24 hours. For the mid-dose group, absorption increased over time from <0.01% of the dose at 0.5 hours to 0.35% of the dose at 24 hours. Potential absorption increased from 0.28% of the dose at 0.5 hours to 1.40% of the dose at 24 hours. For the high-dose group, the maximum amount of absorption was 0.14% of the dose at 0.5 hours, and the maximum potential absorption was 0.66% of the dose at 4 hours.

SELECT DERMAL ABSORPTION VALUES
(Total % adhered to the skin and absorbed)

ave. dose	1 hour	10 hours	24 hours
0.006 mg/cm^2	<1	**2.35**	6.61
0.071 mg/cm^2	<1	<1	1.4
0.574 mg/cm^2	<1	<1	<1

There was 2.35% adhered to the skin and absorbed at the 10 hour time point with the lowest dose applied (0.006 mg/cm^2).

This study is classified as **Acceptable (Guideline)** and satisfies the requirement for a dermal absorption study **(§85-2)** in the rat.

J. SPECIAL STUDIES

1. Studies Conducted with Fipronil

1) Thyroid Function/Rat: Four groups of 27 male Crl:CD (SD) BR rats per group were administered either methylcellulose (vehicle control), 10 mg/kg/day fiproni (95.4% a.i.) l, 200 mg/kg/day propylthiouracil (PTU) or 50 mg/kg/day Noxyflex for 14 days. On Day 15, each animal received $Na^{125}I$ at a dose level of 1 µCi ^{125}I (MRID # 42977904). Six hours later, 9 males per group received either 10 or 25 mg/kg potassium perchlorate or 0.9% saline solution. The treatment with **fipronil** or Noxyflex appeared to result in stimulation of the thyroid glands as evidenced by increased accumulation of ^{125}I in the thyroid glands and by increases in the ratios of radioactive distribution between the blood and thyroid. These changes were accompanied by increases in thyroid weight. Treatment with PTU produced decreases in the amount of ^{125}I incorporated in the thyroid and in the blood:thyroid ratios along with elevated levels of ^{125}I in the blood. However, the weights of the thyroids from these animals were increased by over 2.5 fold compared to the controls and therefore, the ratio of ^{125}I in the blood to thyroid weight was reduced. The administration of perchlorate produced further reductions in the ^{125}I content in the thyroids and in the blood:thyroid ^{125}I radioactivity ratio. There was no evidence of an inhibition of iodide incorporation by either **fipronil** or Noxyflex.

Classification: Supplementary

2) Thyroxine Clearance: Six groups of six male Crl:CD (SD) rats per group were administered either **fipronil** (95.4%) (10 mg/kg/day by gavage), phenobarbital (80 mg/kg/day intraperitoneally) or 0.5% methylcellulose (vehicle control at 5 ml/kg by gavage) for a duration of either one day or fourteen days (MRID # 42918654. Four hours after the final dose of either test substance, each rat received [^{125}I] thyroxine at a dosage of 10 µCi/kg. **Fipronil** had no effect on mortality or other ante mortem parameters. Phenobarbital-treated animals were observed to have collapsed posture, lethargy and shallow breathing on the first day of treatment. There was no effect of **fipronil** on clearance after one day of treatment, however after 14 days, there was a decrease in terminal half life (52% of control level) and increases in clearance and volume of distribution (261% and 137% of control level, respectively). The effects seen with phenobarbital treatment were similar, although quantitatively not as severe and were evident on Day 1 of treatment.

Classification: Supplementary

3) 28-Day Study with Fipronil: Conclusions: Five Crl:CD (SD)BR rats/sex/group were treated with Fiproni (93% a.i.) l in the diet for four weeks at doses of either 0, 25, 50, 100, 200 or 400 ppm (3.4, 6.9, 12.6, 24.5 and 45.3 mg/kg/day, respectively for males; 3.5, 6.7, 12.9, 24.9 and 54.9 mg/kg/day, respectively, for females (MRID Number: 4402801)). One female in the 400 ppm group died during the treatment period; the cause of death could not be determined. Group mean body weight was decreased in both sexes of the 200 and 400 ppm groups the first five days of the study, most likely due to decreased palatability. Overall (weeks 0-4) group mean body weight gain (BWG) was decreased by 18 and 23% in the 200 and 400 ppm group males, respectively. Overall BWG was decreased by 21, 24 and 26% in the 100, 200 and 400 ppm group females, respectively. Group mean food consumption was decreased for both sexes of the 100, 200 and 400 ppm groups at Day 5, but was comparable to the controls after one week for females and three weeks for males. There were no treatment-related changes in hematology parameters. Total protein and globulin were statistically increased in all the treated male and female groups; cholesterol was increased in the 400 ppm group males and all treated females. On gross pathological examination, the liver was enlarged in 5/5 male rats and 3/5 female rats in the 400 ppm group, 2/5 females in the 200 ppm group, 1/5 males in the 100 ppm group and 1/5 females in the 50 ppm group. Increased group mean liver weights were seen in all treated males and females, however the differences in males treated at 100 ppm and below were not statistically significant. Group mean thyroid weights were increased in all the treated females, however the differences were not statistically significant or dose-related. There was an increased incidence of follicular

hypertrophy of the thyroids in all treated male and female rats. The change was graded as moderate in the male rats at 200 and 400 ppm and minimal in all other treated groups. A dose-related increase in generalized hepatocyte enlargement was seen in males and females in the 200 and 400 ppm groups and in males in the 100 ppm group.

The LOEL is ≤ 25 ppm (3.4 mg/kg/day in males; 3.5 mg/kg/day in females) based on clinical laboratory changes, increased absolute liver weights in females and histopathological alterations in the thyroid glands. The NOEL is < 25 ppm.

Classification: Acceptable (Nonguideline)

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2. Studies Conducted with Photodegradate M&B 46513

In a 28-day range-finding study (MRID 44262809), **MB 46513** (a metabolite of fipronil; 97.5% a.i.) was administered to Sprague-Dawley rats (10/sex/dose) in the diet at nominal dose levels of 0, 0.5, 3, 30 or 100 ppm (equivalent to 0, 0.04, 0.23, 2.20 or 3.74 mg/kg body weight/day, respectively, for males; 0, 0.04, 0.24, 2.32 or 3.8 mg/kg body weight/day, respectively, for females). In addition to evaluation of standard study parameters, thyroid hormone levels were measured on days 7 and 23.

No treatment-related effects were observed in the 3 and 0.5 ppm treatment groups. One male in the 30 ppm group was found dead on day 6 and all 100 ppm group animals died within the first 2 weeks of study. Clinical signs at 30 and 100 ppm included piloerection (M 9/10 and 4/10 at 30 and 100 ppm; F 5/10 and 6/10 at 30 and 100 ppm), curling up at handling (M 6/10 and 4/10 at 30 and 100 ppm; F 8/10 and 5/10 at 30 and 100 ppm); thin (M 5/10 and 4/10 at 30 and 100 ppm; F 6/10 and 6/10 at 30 and 100 ppm); increased motor activity (F 1/10 and 2/10 at 30 and 100 ppm) and irritability with convulsions at 100 ppm in 1 female. There was a decrease in body weight (9-18% and 26-36% for 30 and 100 ppm) and food consumption (8-34% and 69-73% for 30 and 100 ppm). Clinical chemistry parameters affected at 30 ppm included bilirubin (decrease - 28-33%) and aspartate aminotransferase (increased F - 22%). At 30 ppm on day 7 or 23, males had decreased T3 and T4 levels (33-49%) and females had decreased T4 levels (61%) compared to the controls. While at 100 ppm T3 levels were decreased 46% (females only), and T4 levels were decreased 50-63%. No treatment-related differences in hematology or urine parameters, organ weights or gross postmortem or microscopic appearance were observed.. No neoplastic tissue was observed. Ophthalmoscopic examinations were not conducted. **The LOEL for this study is 30 ppm (2.20 and 2.32 mg/kg/day for M and F, respectively), based on clinical signs including piloerection, curling up and thin appearance; and decreased body weights in both sexes. The NOEL is 3 ppm (0.23 and 0.24 mg/kg/day for M and F, respectively).**

This 28-day dietary feeding study is classified **acceptable (non-guideline)** as it is not a required guideline study. It is acceptable for the purposes for which it was intended. It is recommended that a maximum treatment rate between 3 and 30 ppm be used in longer term studies.

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3. Studies Conducted with Metabolite M&B 45897

In a subchronic toxicity study (MRID 44262821), MB 45897 (intermediate of fipronil; 99.7 a.i.) was administered to CD rats (5/sex/dose) by gavage at nominal dose levels of 0, 50, 200 or 1000 mg/kg/day for 4 weeks.

In the 1000 mg/kg/day treatment groups, the livers of males and females had increased absolute (32-58%) and relative (53-70%) weights, and the livers of 3/5 males exhibited periacinar hypertrophy with cytoplasmic vacuolation. Total plasma protein levels were increased (10-19%) in both sexes, and alanine aminotransferase activity (48%) was increased in females. All rats salivated excessively (days 2-28), and exhibited hunched posture (days 8, 9, 11, and 12), underactivity (days 8-13), and staggered gaits (day 8). Males (4-5) and females (2-3) had

hair loss (days 3-28). Both sexes had reduced body weight gains (16%), and females were slightly anemic. In the 200 mg/kg/day treatment groups, all rats salivated excessively (days 3-28) and exhibited hunched posture (days 8 and/or 11 and 12). Males had increased (9%) total plasma protein levels. In the 50 mg/kg/day treatment groups, all rats salivated excessively (days 8-15, males; days 8-28, females) This may have been due to local irritation of the test material. No rats died as a result of treatment. Ophthalmoscopic exams and urinalysis were not conducted. No treatment-related differences in food consumption were observed in any treatment group. No neoplastic tissue was observed in treated or control rats. **The LOEL for this study is 200 mg/kg/day, based on hunched posture in all rats treated at this dose level. The NOEL is 50 mg/kg/day.**

This 4-week oral toxicity study is classified **acceptable (non-guideline)** as it is not a required guideline study. It is acceptable for the purposes for which it was intended, that being to assess the systemic toxic effects following 4 weeks of repeated daily administration (gavage) of the test substance.

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4. Studies Conducted with metabolite RPA 200766

28-Day Study with RPA 200766: Conclusions: Ten Sprague-Dawley rats/sex/group were administered RPA 200766 (96.2% a.i.), a fipronil metabolite, in the diet at dose of either 0, 50, 500, 5000 or 15000 ppm for 28 days (mg/kg/day doses respectively: males - 3.80, 38.16, 385.07 and 1087.05; females - 4.44, 43.97, 386.75 and 1062.84)(MRID Number: 44017701). One female in the 15000 ppm group died during the study; the cause of death was attributed to difficulty in blood collection. Body weight was significantly decreased from Day 8 to Day 28 for males and females in the 5000 and 15000 ppm groups. Mean body weight gain over the course of the study was decreased by 27% and 61% in the 5000 and 15000 ppm group males, respectively; it was decreased by 46% and 77% for the 5000 and 15000 ppm group females, respectively. Mean food consumption over the course of the study was decreased by 11% and 25% in the 5000 and 15000 ppm group males, respectively; it was decreased by 22% and 33% in the 5000 and 15000 ppm group females, respectively. Mean hemoglobin values were decreased at 500 ppm and above for males and females; all were statistically significant, except for the 15000 ppm group females. Mean hematocrit values were decreased at 5000 ppm and above; all were statistically significant, except for the 15000 ppm group females. Mean corpuscular hemoglobin values were decreased in the 15000 group males and females and in the 5000 ppm group males. Mean cholesterol values were significantly increased in males and females at 500 ppm and above. Mean triglyceride values were increased in the 5000 and 15000 ppm group males and females. Urea nitrogen was increased in the 5000 and 15000 ppm group females, whereas creatinine values were increased in males at 500 ppm and above. There were no histopathological changes in the kidneys, therefore, these changes are of doubtful toxicological significance. Dose-related increases in absolute and relative liver weights were seen in males and females at 500 ppm and higher. Liver-to-brain weights were also increased in these groups. Significantly increased adrenal weights were seen in all the treated males. Thyroid weights were increased in males at 50 ppm and above, however the increases were not consistently found in all males in the group. In addition, there were no microscopic changes in the thyroid. Therefore, these findings are of questionable toxicological significance, although the parent compound, **fipronil**, is known to be a thyroid toxicant. Microscopic examination of the tissues from males and females in the 15000 ppm were not conducted. Slight to moderate hepatocellular hypertrophy was noted in the adrenals of males and females at 5000 ppm. Slight to moderate extra-medullary hematopoiesis was observed in males and females at 5000 ppm. A fine/coarse vacuolation of the zona fasciculata was observed in males at 50 ppm and higher and in females in the 5000 ppm group. **The NOEL was 50 ppm (3.80 mg/kg/day for males and 4.44 mg/kg/day for females). The LOEL was 500 ppm (38.16 mg/kg/day for males and 43.97 mg/kg/day for females) based on decreased hemoglobin values, increased cholesterol values and increased liver weights in both sexes.**

Classification: Acceptable (Nonguideline)

V. TOXICOLOGY ISSUES

A. **Toxicity of the photodegradate MB46513 as compared to the parent fipronil**

The **photodegradate MB46513** is not an animal metabolite. However in several toxicity studies it has been determined that it is more toxic that the parent **fipronil**. Since this **photodegradate** is present in rice it has been included in the dose response assessment below.

VI. DOSE RESPONSE ASSESSMENT

The HED Hazard Assessment Review Committee met on 10-JUL-1997 to select appropriate endpoints for acute dietary and short-, intermediate-, and long-term occupational exposure (dermal and inhalation) for **fipronil** and on 9-DEC-1997 (document dated 18-DEC-1997)to select appropriate endpoints for acute dietary and short-, intermediate-, and long-term occupational exposure (dermal and inhalation) for the fipronil **photodegradate**. On 22-JAN-1998 (Document dated 29-JAN-1998) the Committee reassessed the potential sensitivity of infants and children and to discuss the uncertainty factors (UF) and/or Margins of Exposure (MOE) for both the parent, **fipronil** and the **photodegradate**.

A. FIPRONIL

a. Special Sensitivity to Infants and Children

EPA generally defines the level of appreciable risk as exposure that is greater than 1/100 of the no observed effect level in the animal study appropriate to the particular risk assessment. This 100-fold uncertainty (safety) factor/margin of exposure (safety) is designed to account for inter-species extrapolation and intra-species variability. FFDCA section 408 provides that EPA shall apply an additional 10-fold margin of safety for infants and children in the case of threshold effects to account for pre-and post-natal toxicity and the completeness of the data unless EPA determines that a different margin of safety will be safe for infants and children. Margins of safety are incorporated into EPA risk assessments either directly through use of a margin of exposure analysis or through using uncertainty (safety) factors in calculating a dose level that poses no appreciable risk to humans.

The following discussion represents the information that was considered and the following conclusions were drawn by the Hazard Identification Assessment Review Committee taking into account both the 2-JUL-1997 and 29-JAN-1998 meetings.

1. Adequacy of data: An acceptable two-generation reproduction study in rats (MRID# 42918647) and acceptable prenatal developmental toxicity studies in rats (MRID#42977903) and rabbits (MRID# 42918646) have been submitted to the Agency, meeting basic data requirements, as defined for a food-use chemical by 40 CFR Part 158.

In addition, an acceptable developmental neurotoxicity study was conducted with fipronil and reviewed by the Agency. There are no data gaps for the assessment of the effects of fipronil on developing animals following *in utero* and/or early postnatal exposure.

2. Susceptibility Issues: The data contained evidence of increased sensitivity of rats to alterations in functional development following pre- and/or postnatal exposure with fipronil. Specifically, in a developmental neurotoxicity study in rats, the developmental and developmental-neurotoxicity NOEL of 0.5 ppm (0.05 mg/kg/day) was lower than the maternal toxicity NOEL of 10 ppm (0.9 mg/kg/day). In the offspring, decreased pup weights, increased time of preputial separation in males, and increased motor activity counts in female pups were observed at the developmental LOEL of 10 ppm (0.9 mg/kg/day), while maternal toxicity (decreased body weight, body weight gain, and food consumption) was observed at the maternal LOEL of 200 ppm (15 mg/kg/day).

It was noted by the Committee that previously conducted studies with fipronil did not identify any issues of increased sensitivity in the fetuses or pups following pre- and/or postnatal exposure. In the prenatal developmental toxicity study in rats, there was no evidence of developmental toxicity at the highest doses tested (20 mg/kg/day). Maternal toxicity (decreased body weight gain, food consumption and/or water consumption) was observed at this dose (20 mg/kg/day) with the maternal NOEL established at 4 mg/kg/day. In the prenatal developmental toxicity study in rabbits, there was also no evidence of developmental toxicity at the highest doses tested (1.0 mg/kg/day). Maternal toxicity (decreased body weight gain, food consumption and/or water consumption) was observed at this same dose (1.0 mg/kg/day). Toxicity was also observed at the lowest does of 0.1 mg/kg/day. Therefore, the maternal NOEL is lower then 0.1 mg/kg/day.

Additionally, in the two-generation reproduction study in rats, offspring toxicity was observed only in the presence of parental toxicity. The offspring NOEL was 30 ppm (2.54-2.74 mg/kg/day), based upon clinical signs of toxicity, decreased litter size, decreased body weights, decreased pre- and postnatal survival, and delays in physical development at the LOEL of 300 ppm (26.0-28.4 mg/kg/day). In the parental animals, reproductive toxicity (reductions in mating and fertility) was also observed at the 30 ppm dietary level. The systemic NOEL for the parental animals was 3 ppm (0.25-0.27 mg/kg/day), based upon increased weight of the thyroid gland and liver in both sexes, decreased weight of the pituitary gland in the females, and increased incidence of thyroid follicular epithelial hypertrophy in the females at the LOEL of 30 ppm.

3. Uncertainty Factor: The Committee noted that the developmental neurotoxicity NOEL of 0.05 mg/kg/day, when adjusted for 1% dermal absorption, yields an equivalent NOEL of 5 mg/kg/day, the value established as the systemic NOEL in the 21-day dermal study in rabbits. This value was selected for use in the short term and intermediate risk assessment calculations for fipronil. The NOEL used for the RfD calculation was 0.019 mg/kg/day from the combined chronic toxicity-carcinogenicity study in the rat, a value

that is even lower than the NOEL used for short- and intermediate-term exposure.

On **22-JAN-1998**, the HIARC, in view of OPP's current Science Policy, re-evaluated the data base for Fipronil and determined that the **10 x factor** to account for the enhanced sensitivity of infants and children (as required by FQPA) **should be retained.** The rationale for this decisions is based on the following weight-of-the-evidence considerations:

1. In the developmental neurotoxicity study in rats, there is evidence of additional susceptibility to the offspring, following pre- and/or postnatal exposure. In that study, developmental toxicity (decreased pup weights and delayed sexual maturation in male pups) and developmental neurotoxicity (increased motor activity counts in female pups) occurred in the absence of maternal toxicity. The developmental and neurodevelopmental NOELs were 0.5 ppm (0.05 mg/kg/day), while the maternal NOEL was 10 ppm (0.9 mg/kg/day). Although though there was no indication of offspring susceptibility in the prenatal developmental toxicity studies in rats or rabbits nor in the two-generation reproduction study in rats, the functional endpoints evaluated in the developmental neurotoxicity study were not examined in the other studies.

2. Evidence of neurotoxicity demonstrated in the acute and subchronic neurotoxicity studies with Fipronil

3. Prenatal toxicity studies in rats and rabbits, a two-generation reproduction study in rats, and a developmental neurotoxicity study in rats have been received by the Agency. There are, therefore, no gaps in the Agency assessment of the effects of Fipronil following *in utero* and/or early postnatal exposure.

4. Recommendation for a Developmental Neurotoxicity Study: A neurotoxicity study is available and has been considered together with the rest of the toxicity data base for fipronil.

b. Reference Dose

The Hazard ID Assessment Review Committee (document dated September 4 1997) assigned an **RfD of 0.0002 mg/kg/day** using a NOEL of 0.019 mg/kg/day and an uncertainty factor of 100; NOEL established from a combined chronic toxicity/ carcinogenicity study in rats; LOEL = 1.5 ppm (M: 0.059 mg/kg/day; F: 0.078 mg/kg/day), based on an increased incidence of clinical signs (seizures and death) and alterations in clinical chemistry (protein) and thyroid parameters (increased TSH, decreased T4) at 1.5 ppm (M: 0.059 mg/kg/day; F: 0.078 mg/kg/day).(MRID# 42918648).

Based on the reevaluation by the HIARC in January 1998, the RfD for **fipronil** is:

$$\frac{0.019 \text{ mg/kg/day (NOEL)}}{1000 \text{ (UF)}} = 0.00002 \text{ mg/kg/day}$$

c. Carcinogenicity Classification

Cancer Classification and Basis: According to the proposed new guidelines, this chemical has been classified by the HED Cancer Peer Review Committee (document dated July 18, 1997) as a Group C - Possible Human Carcinogen, based on increases in thyroid follicular cell tumors in both sexes of the rat, which were statistically significant by both pair-wise and trend analyses. The RfD methodology should be used to estimate human risk because the thyroid tumors appear to be related to a disruption in the thyroid-pituitary status. There was no apparent concern for mutagenicity (no mutagenic activity).

d. Developmental and Reproduction Toxicity

Fipronil is not classified as a developmental or reproductive toxicant.

e. Dermal Absorption

% Absorbed: < 1% at 24 hours based on a dermal absorption study (MRID# 42918635).

f. Other Toxicological Endpoints

I. Acute Dietary Exposure (one day)

Dose and endpoint for use in risk assessment: **NOEL - 0.5 mg/kg** in an acute neurotoxicity study in rats, based on decreased hind leg splay in male and female rats observed at LOEL = 5 mg/kg (MRID# 42918635).

This risk assessment is required.

II. Short Term Dermal Exposure (1 to 7 days) and Intermediate Term Dermal Exposure (1 week to several months)

Dose and endpoint for use in risk assessment: Critical study: 21-day dermal **NOEL = 5 mg/kg/day** based on decreased body weight gain and food consumption in

male and female rabbits observed at the LOEL of 10 mg/kg/day (MRID# 42918644). Supporting study: Developmental toxicity and developmental neurotoxicity **NOEL = 0.5 ppm** (0.05 mg/kg/day). Developmental LOEL was 10 ppm (0.9 mg/kg/day) based on decreased mean pup weights during lactation and a significant increase in time to preputial separation in male rats. The developmental neurotoxicity LOEL was 10 ppm (0.9 mg/kg/day) based on an increase in mean motor activity counts for females on Postnatal Day 17 (MRID# 44039002).

Comments about study and/or endpoint: The NOEL established after dermal administration in the 21-day dermal toxicity study is 5 mg/kg/day. When the co-critical study NOEL based on oral administration in the developmental neurotoxicity study, 0.05 mg/kg/day is corrected for the less than 1% dermal absorption, exposure is essentially the same as the critical study (5 mg/kg/day).

This risk assessment is required.

III. Chronic Dermal Exposure (Several Months to Lifetime)

Dose and endpoint for use in risk assessment: In a combined chronic toxicity/carcinogenicity study [rat], the **NOEL is 0.5 ppm** (M: 0.019 mg/kg/day; F: 0.025 mg/kg/day), based on an increased incidence of clinical signs (seizures and death) and alterations in clinical chemistry (protein) and thyroid parameters (increased TSH, decreased T4) at 1.5 ppm (M: 0.059 mg/kg/day; F: 0.078 mg/kg/day) (MRID# 42918648).

Comments about study and/or endpoint: Since the NOEL identified is from an oral study, a dermal absorption factor of < 1% should be used in risk calculations. NOTE: This study/dose was also used to establish the RfD.

This risk assessment is required.

IV. Inhalation Exposure (Any time period)

There are three critical acute inhalation studies, one with the technical and two with formulations.

Critical Study 1: Acute inhalation study (technical) - Guideline §81-3, MRID No. 43544401

Executive Summary: Sprague-Dawley rats (5/sex) were exposed nose-only to fipronil at doses of 0.33, 0.52 and 0.72 mg/L for four hours. At 0.72 and 0.52 mg/L, 100% mortality was observed. At the 0.33 mg/l exposure, 2 male rats died, one on day 1

post-exposure and 1 on day 6 post-exposure. At the 0.72 mg/l dose, the following were noted in all rats on the day of exposure: urogenital, body and periocular wetness; unkempt fur; fine whole body tremors. In one male at this dose, incoordination was observed on post-exposure days 4 through 7, hyperactivity on post-exposure day 4 and hypoactivity and swollen penis on post-exposure day 7. This rat died on day 8. In one female rat at this dose, perioral, perinasal and periocular red encrustation was observed on post-exposure days 1 through 11, hyperactivity on post-exposure days 2 through 4, hypoactivity on post-exposure days 7 through 10 and incoordination on post-exposure days 6 through 11. This rat died on day 12. At the 0.52 mg/L dose, the same signs as mentioned for all rats at the 0.72 mg/L exposure concentration were observed without any additional signs. At the 0.33 mg/L dose, similar signs were recorded, with the following additions in males: perioral, perinasal, and periocular red encrustation up to day 5 post-exposure; incoordination in 4 of 5 male rats up to post-exposure day 3; hypoactivity in 4 of 5 male rats up to post-exposure day 2. In female rats at this exposure concentration, similar signs were observed as for males. The incoordination did not appear until post-exposure day 3 in 4 of 5 female rats and did not last beyond this point. The LC_{50} in rats was 0.36 mg/L, 0.42 mg/L and 0.39 mg/L in males, females and the combined sexes, respectively (Toxicity Category II).

Critical Study 2: Acute inhalation study (1.6% formulation) - Guideline §81-3, MRID No. 42918638

$LC_{50} > 5.11$ mg/L (Toxicity Category IV)

Critical Study 3: Acute inhalation study (0.242% formulation) - Guideline §81-3, MRID No. 43121106

$LC_{50} > 5.06$ mg/L (Toxicity Category IV)

Dose and Endpoint For Use in Risk Assessment: None

Comments and Rationale: In the acute inhalation study, death occurred at the lowest dose tested. At the next higher dose, there were deaths and clinical signs of toxicity. The lack of a NOEL in an inhalation study with the technical chemical would usually require an additional Uncertainty Factor. However, using the lowest dose in the acute inhalation study (0.33 mg/L) and available exposure data demonstrated an adequate margin of exposure (>68,000).

Therefore a separate risk assessment is not required.

B. PHOTODEGRADATE MB46513

a. Special Sensitivity to Infants and Children

On 9-DEC-1997 and 22-JAN-1998, the Hazard Identification Committee evaluated the chemical **MB46513 a photodegradate** of fipronil for FQPA considerations. The following discussion represents the information that was considered and the following conclusions were drawn by the Committee.

1. Adequacy of data: There are no data gaps for the assessment of the effects of **Fipronil** and/or the **photodegradate** on developing animals following *in utero* and/or early postnatal exposure.

2. Susceptibility Issues: In determining sensitivity, the toxicity of the **photodegradate MB46513** and the parent compound will be considered simultaneously. The oral perinatal and prenatal data with the parent compound **Fipronil** demonstrated no indication of increased sensitivity of rats or rabbits to *in utero* exposures. No additional sensitivity was identified in the prenatal developmental toxicity study in rats following *in utero* exposure to Fipronil **photodegradate MB46513**.

(i) Developmental Toxicity

In a prenatal developmental toxicity study, pregnant Sprague-Dawley rats (25/group) received oral administration of **Fipronil photodegradate MB 46513** (99.2%) in aqueous methylcellulose (10 ml/kg) at dose levels of 0, 0.5, 1.0, or 2.5 mg/kg/day during gestation days 6 through 15. The dams were sacrificed on gestation day 20. For maternal toxicity, the NOEL was 1.0 mg/kg/day and the LOEL was 2.5 mg/kg/day based on an increase in clinical signs of toxicity (hair loss) and on reduced body weight gain, food consumption, and food efficiency. For developmental toxicity, the NOEL was 1.0 mg/kg/day and the LOEL was 2.5 mg/kg/day based on a slight increase in fetal and litter incidence of reduced ossification of several bones (hyoid, 5th/6th sternebrae, 1st thoracic vertebral body, pubic bone, and 1 or 2 metatarsi). [Note that most of the reduced ossification is weak evidence of a developmental effect. Although the minor decrement in fetal weight at 2.5 mg/kg/day has questionable biological relevance, the decrement is supported by the delayed ossification.] (MRID No. 44275001).

In both the prenatal developmental toxicity studies in rats and rabbits with **Fipronil,** there was no evidence of developmental toxicity at the highest doses tested (20 mg/kg/day in rats and 1.0 mg/kg/day in rabbits). Maternal toxicity (decreased body weight gain, food consumption and/or water consumption) was observed at these same doses, with a maternal NOEL of 4 mg/kg/day for rats and <0.1 mg/kg/day for rabbits (MRID Nos.

42977903 and 42918646) .

(ii) Reproduction Toxicity

In the two-generation reproduction study in rats with **Fipronil,** offspring toxicity was observed only in the presence of parental toxicity. In the parental animals, reproductive toxicity (reductions in mating and fertility) was also observed at the 30 ppm dietary level. For parental systemic toxicity, the NOEL was 3 ppm (0.25-0.27 mg/kg/day) and the LOEL was 30 ppm (2.54-2.74 mg/kg/day) based on increased weight of the thyroid gland and liver in both sexes, decreased weight of the pituitary gland in the females, and increased incidence of follicular epithelial hypertrophy in the females. For offspring toxicity, the NOEL was 30 ppm (2.54-2.74 mg/kg/day) and the LOEL was 300 ppm (26.0-28.4 mg/kg/day) based upon clinical signs of toxicity, decreased litter size, decreased body weights, decreased pre- and postnatal survival, and delays in physical development. (MRID No.42918647) .

(iii) Developmental Neurotoxicity

In a developmental neurotoxicity study in rats with **Fipronil,** there was evidence of increased sensitivity of offspring to alterations in functional development following pre- and/or postnatal exposure. Specifically, the developmental and developmental-neurotoxicity, the NOEL of 0.5 ppm (0.05 mg/kg/day) was lower than the maternal toxicity NOEL of 10 ppm (0.9 mg/kg/day). In the offspring, decreased pup weights, increased time of preputial separation in males, and increased motor activity count in female pups was observed at the developmental LOEL of 10 ppm (0.9 mg/kg/day), while maternal toxicity (decreased body weight, body weight gain, and food consumption) were observed at the developmental LOEL of 200 ppm (15 mg/kg/day) (MRID No. 44039002).

3. Uncertainty Factor: On January 22, 1998, the HIARC, in view of OPP's current Science Policy, re-evaluated the data base for Fipronil and determined that the **10 x factor** to account for the enhanced sensitivity of infants and children (as required by FQPA) **should be retained.** The rationale for this decisions is based on the following weight-of-the-evidence considerations:

1. In the developmental neurotoxicity study in rats, there is evidence of additional susceptibility to the offspring, following pre- and/or postnatal exposure. In that study, developmental toxicity (decreased pup weights and delayed sexual maturation in male pups) and developmental neurotoxicity (increased motor activity counts in female pups) occurred in the absence of maternal toxicity. The developmental and neurodevelopmental NOELs were 0.5 ppm (0.05 mg/kg/day), while the maternal NOEL was 10 ppm (0.9 mg/kg/day). Although though there was no indication of offspring susceptibility in the prenatal

developmental toxicity studies in rats or rabbits nor in the two-generation reproduction study in rats, the functional endpoints evaluated in the developmental neurotoxicity study were not examined in the other studies.

2. Evidence of neurotoxicity demonstrated in the acute and subchronic neurotoxicity studies with Fipronil

3. Prenatal toxicity studies in rats and rabbits, a two-generation reproduction study in rats, and a developmental neurotoxicity study in rats have been received by the Agency. There are, therefore, no gaps in the Agency assessment of the effects of Fipronil following *in utero* and/or early postnatal exposure.

4. Recommendation for a Developmental Neurotoxicity Study: A developmental neurotoxicity study was not recommended for **MB46513** (a fipronil photodegradate). A developmental neurotoxicity study was however conducted for the parent, **fipronil**.

b. Reference Dose

The Hazard ID Assessment Review Committee (document dated December 18, 1997) assigned an RfD of 0.00002 mg/kg/day (Study MRID 42918648), based on an adjusted NOEL of 0.019 mg/kg/day for the photodegradate (see discussion below relating to the adjusted NOEL) and an Uncertainty Factor of 100. Effects seen at the LOEL, 0.059 mg/kg/day were an increased incidence of clinical signs indicative of neurotoxicity as well as alterations in clinical chemistry and thyroid parameters. However on 22-JAN-1998, the Committee determined that a UF of 1000 should applied resulting in an RfD of 0.000002 mg/kg/day.

Dose and Endpoint for establishing the RfD: Adjusted NOEL = 0.0019 mg/kg/day for the **photodegradate MB46513.** This adjusted dose was derived by the application of a Potency Adjustment Factor (PAF) of 10 to the chronic NOEL of 0.019 mg/kg/day for the parent compound (i.e., NOEL of 0.019 mg/kg/day ÷10 PAF = 0.0019 mg/kg/day).

Comments about Study, Dose and Endpoint: The toxicity profile of the Fipronil **photodegradate MB46513** indicate this material to be *approximately* 10 times more potent than the parent compound when the NOELs/LOELs are compared. A comparison of the NOELs/LOELs established for the **Photodegradate and Fipronil** are presented below:

STUDY	Photodegradate	Fipronil
Acute Oral	LD_{50}= 16 mg/kg	LD50 = 92 mg/kg
28-Day Oral - Rat	NOEL/LOEL = 0.23 / 2.2 mg/kg/day	NOEL/LOEL = 3.4 mg/kg/day (LDT)
90-Day Oral - Mouse	NOEL/LOEL = 0.08 / 0.32 mg/kg/day	NOAEL/LOEL = 1.7 / 3.2 mg/kg/day
90-Day Oral - Rat	NOEL/LOEL = 0.029 / 0.18 mg/kg/day	NOEL =0.33 / 1.9 mg/kg/day
Developmental -Rat	Maternal NOEL/LOEL = 1 / 2.5 mg/kg/day Develop. NOEL/LOEL = 1 / 2.5 mg/kg/day	Maternal NOEL/LOEL = 4 / 20 mg/kg/day Develop. NOEL/LOEL= 20 mg/kg/day (HDT)

As shown above, results of acute oral as well as the 28 day and 90 day subchronic oral studies and oral developmental studies consistently demonstrated an approximately 10-fold greater potency of the **photodegradate MB46513** as compared to the parent compound, **Fipronil**.

In the acute oral studies the $_{LD50}$ was 16 mg/kg for the **photodegradate** as compared to 92 mg/kg for **Fipronil**. In the 28 day study with rats, the NOEL was 0.23 mg/kg/day for the **photodegradate** based on piloerection, curling up and decreased body weight gain as compared to the LOEL of 3.4 mg/kg/day (low-dose)for **Fipronil** based on marginal increase in liver weights and thyroid hypertrophy.

In the 90-day studies with mice, the NOEL was 0.08 mg/kg/day based on neurotoxic endpoints of aggression, irritability and increased motor activity for the **photodegradate** as compared to a the NOAEL of 1.7 mg/kg/day for **Fipronil** based on decreases in body weight gain. The slight changes in liver hypertrophy were not considered to be toxicologically significant.

In the 90-day studies with rats, the NOEL was 0.029 mg/kg/day for the **photodegradate** based on neurotoxic endpoints of aggression, irritability and increased motor activity for the **photodegradate** as compared to a the NOEL of 3.3 mg/kg/day for **Fipronil** based on liver and thyroid hypertrophy.

In developmental toxicity studies in the rat with the **photodegradate,** for maternal toxicity, the NOEL was 1 mg/kg/day based on increased clinical signs, decreased body weight gain, food consumption and food conversion efficiency. For developmental toxicity, the NOEL 1 mg/kg/day was based on a slight increase in reduced ossification. In comparable developmental studies in the rat with **Fipronil**, for maternal toxicity, the NOEL was 4 mg/kg/day based on reduced body weight gain, food consumption and conversion efficiency and increased water consumption. For developmental toxicity, the NOEL was 20 mg/kg/day (highest dose tested).

The Committee, however, noted that the in the acute neurotoxicity studies, the **photodegradate** with a NOEL of 0.5 mg/kg/day did not appear to be more toxic than **Fipronil** with a NOEL of 2 mg/kg/day. . The Committee was unable to determine the biological significance for this dissimilarity (in relation to the apparat greater toxicity in the acute and other longer term studies) since the studies were conducted in different laboratories, the animals were obtained from different suppliers, and the doses tested (**Fipronil** at 0.5, 5 or 50 mg/kg and the **photodegradate** at 0.5, 2 or 12 mg/kg) were difficult to compare (i.e., dissimilar dose spread).

For **Fipronil,** the RfD was derived from the NOEL of 0.019 mg/kg/day established in a combined chronic toxicity/carcinogenicity study in rats and an Uncertainty Factor (UF) of 100 for inter-and intra-species variation. The 10 was also retained for sensitivity to infents and children. The LOEL of 0.059 mg/kg/day was based on thyroid hypertrophy and neurological signs.

Since no long-term (chronic or carcinogenicity) studies are available for the **photodegradate**, the Committee concluded that there is sufficient experimental evidence to warrant the application of a 10 fold Potency Adjustment Factor (PAF) to the chronic NOEL for the parent compound to calculate a chronic NOEL for the degradate in the absence of test data on the chemical. Thus, the application of the adjustment factor to the chronic rat NOEL of 0.019 mg/kg/day for the parent resulted in a adjusted chronic NOEL of 0.0019 mg/kg/day for the degradate .

Uncertainty Factor (UF): An UF of 100 was applied to account for inter (10 x)-and intra-(10 x) species variation. The 10x was retained to account for sensitivity to infents and children.

$$\textbf{RfD} = \frac{\text{Adjusted NOEL= 0.0019 mg/kg/day)}}{\text{1000 (UF)}} = \textbf{(0.000002 mg/kg/day} \text{ rounded off)}$$

Chronic Dietary Risk Assessment: The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be retained.** For chronic dietary risk assessment, **a UF of 1000 is adequate** for the protection of the general U.S. population including infants and children from exposure to the **photodegradate MB46513.** A UF of 1000 following a 10-fold adjustment of the NOEL for **Fipronil** to account for the increase in potency of the **photodegradate** is required based on the following weight-of-the-evidence considerations:

(a) In the developmental neurotoxicity study in rats, there is evidence of additional susceptibility to the offspring, following pre- and/or postnatal exposure. In that study, developmental toxicity (decreased pup weights and delayed sexual maturation in male pups) and developmental neurotoxicity (increased motor activity counts in female pups) occurred in the absence of maternal toxicity.

The developmental and neurodevelopmental NOELs were 0.5 ppm (0.05 mg/kg/day), while the maternal NOEL was 10 ppm (0.9 mg/kg/day). Although though there was no indication of offspring susceptibility in the prenatal developmental toxicity studies in rats or rabbits nor in the two-generation reproduction study in rats, the functional endpoints evaluated in the developmental neurotoxicity study were not examined in the other studies.

(b) Evidence of neurotoxicity demonstrated in the acute and subchronic neurotoxicity studies with Fipronil

(c) Prenatal toxicity studies in rats and rabbits, a two-generation reproduction study in rats, and a developmental neurotoxicity study in rats have been received by the Agency. There are, therefore, no gaps in the Agency assessment of the effects of Fipronil following *in utero* and/or early postnatal exposure.

c. Carcinogenicity Classification

No carcinogenicity studies are available with the **photodegradate MB46513**. **Fipronil**, the parent compound, was classified as a **Group C Carcinogen** (Possible Human Carcinogen) by the HED's Carcinogenicity Peer Review Committee (CPRC). The CPRC based this classification on statistically significantly (pair-wise and trend analyses) increased incidences of thyroid follicular cell tumors in both sexes of Charles River CD rats. The CPRC recommended that the RfD methodology for the quantification of human risk be used because the thyroid tumors appeared to be related to a disruption in the thyroid-pituitary status and there was no apparent concern for mutagenicity or available information from structurally related analogs (Memorandum: V. Dobozy, HED to R. Keigwin, RD, dated July 18,1997).

d. Dermal Absorption

% Absorbed: Approximately 2% at 10 hours based on a dermal absorption study (MRID# 44262816).

e. Other Toxicological Endpoints

I. Acute Dietary Exposure (one day)

Dose and Endpoint for Risk Assessment: **NOEL=2 mg/kg** in an acute neurotoxicity study in tats (with the photodegradate) based on significant decreases in locomotor activity in both sexes during the first 30 minutes as well as decreases in hindlimb splay and rectal temperature in both sexes at 6 hours post dosing at 12 mg/kg/day (LOEL). (44262808)

Comments about Study and Endpoint: Effects were seen on the day of treatment after a single oral exposure (dose) and thus is appropriate for this risk assessment.

This risk assessment is required.

Although a developmental neurotoxicity study with the parent compound **Fipronil** was available (MRID No. 4403902), the Committee did not use this study for acute dietary risk assessment. In that study, the developmental and developmental neurotoxicity NOEL was 0.05 mg/kg/day and the LOEL was 0.9 mg/kg/day. The developmental LOEL was based on significant decreases in group mean pup weight during lactation and significant increase in time of preputial separation in males and the developmental neurotoxicity LOEL was based on a significant increase in mean locomotor activity counts in females on Postnatal Day 17. The Committee determined that these effects are not attributable to a single exposure (dose) and therefore are not appropriate for acute dietary risk assessments.

Acute Dietary Risk Assessment: The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be retained.** For acute dietary risk assessment, **a Margin of Exposure (MOE) of 1000 is adequate** for the protection of the general U.S. population including infants and children from acute exposure to Fipronil **photodegradate MB 46513.** A MOE of 1000 is adequate based on the following weight-of-the-evidence considerations:

(a) In the developmental neurotoxicity study in rats, there is evidence of additional susceptibility to the offspring, following pre- and/or postnatal exposure. In that study, developmental toxicity (decreased pup weights and delayed sexual maturation in male pups) and developmental neurotoxicity (increased motor activity counts in female pups) occurred in the absence of maternal toxicity. The developmental and neurodevelopmental NOELs were 0.5 ppm (0.05 mg/kg/day), while the maternal NOEL was 10 ppm (0.9 mg/kg/day). Although though there was no indication of offspring susceptibility in the prenatal developmental toxicity studies in rats or rabbits nor in the two-generation reproduction study in rats, the functional endpoints evaluated in the developmental neurotoxicity study were not examined in the other studies.

(b) Evidence of neurotoxicity demonstrated in the acute and subchronic neurotoxicity studies with Fipronil

(c) Prenatal toxicity studies in rats and rabbits, a two-generation reproduction study in rats, and a developmental neurotoxicity study in rats have been received by the Agency. There are, therefore, no gaps in

the Agency assessment of the effects of Fipronil following *in utero* and/or early postnatal exposure.

II. Short Term Dermal Exposure (1 to 7 days) and Intermediate Term Dermal Exposure (1 week to several months)

Dose and Endpoint for Risk Assessment: Adjusted Dose= 0.5 mg/kg/day. This dose was derived by dividing the actual study NOEL of 5 mg/kg/day by the Potency Adjustment Factor (PAF) of 10 (5÷10 = 0.5 mg/kg/day). The LOEL was based on decreases in body weight gain and food consumption. (42918644)

Comments about Study and Endpoint: The Committee selected the dose and endpoint from the 21-day dermal study with the **parent compound** for the following reasons: 1) a 21-dermal toxicity study with the **photodegradate** is not available; 2) low potential for risk from dermal exposure due to minimal dermal absorption as indicated for both the parent (<1%) and the **photodegradate** (2%) materials; and 3) when the developmental/developmental neurotoxicity NOEL of 0.05 mg/kg/day for **Fipronil** (established in the developmental neurotoxicity study) is adjusted for 1% dermal absorption (DA), results in a comparable dermal dose of 5 mg/kg/day (i.e., 0.05 mg/kg/day ÷ 1% DA = 5 mg/kg/day) which essentially is the same as the NOEL for **Fipronil** in the 21-day dermal toxicity study.

A MOE of 1000 is adequate for short and intermediate occupational and/or residential exposures to Fipronil **photodegradate MB 46513.** A MOE of 1000 is adequate for the same reasons stated under Acute and Chronic dietary exposure risk assessments. MOE's for Long-Term dermal and inhalation (anytime period) exposures are not required since use pattern does not indicate a potential for exposures via these routes.

This risk assessment is required.

III. Chronic Dermal Exposure (Several Months to Lifetime)

Dose and Endpoint for Risk Assessment: Not Applicable

Comments about Study and Endpoint: Based on the current use pattern for the **photodegradate** (i.e., 1 application/year), Long-Term exposure via the dermal route is not expected.

This risk assessment is **NOT** required.

IV. Inhalation Exposure (Any time period)

Dose and Endpoint for Risk Assessment: Not Applicable.

Comments about Study and Endpoint: The use pattern (i.e., 1 application/year) and the method of application does not indicate a potential for exposure via the inhalation route.

This risk assessment is **NOT** required.

V, Recommendation for Aggregate Exposure Risk Assessments

An aggregate systemic (oral) and dermal exposure risk assessment is not appropriate due to differences in the toxicity endpoints observed between the oral (neurotoxicity and alterations in clinical chemistry and thyroid parameters) and dermal (decreases in body weight gain and food consumption) routes. An aggregate oral and inhalation risk is not required due to the lack of exposure potential via the inhalation route based on the current use pattern.

TABLE 3 ENDPOINTS FOR FIPRONIL (Parent)

EXPOSURE DURATION	EXPOSURE ROUTE	ENDPOINT AND TOXICOLOGICAL EFFECT
Acute	Dietary	NOEL: 0.5 mg/kg based on decreased hind leg splay in male and female rats in an acute neurotoxicity study in rats. MOE = 1000 (includes FQPA considerations)
Short-Term (1-7 days) Occupational/Residential	Dermal	NOEL: 5 mg/kg/day based on ↑ body weight gain and food consumption in ♂s and ♀s in a 21-day dermal study in rabbits. Supported by a NOEL of 0.05 mg/kg/day in a developmental neurotoxicity rat study based in ↓ pup weight during lactation, an ↑ in time to preputial separation in males, and an ↑ in mean motor activity counts for ♀ on Postnatal Day 17. MOE = 1000 (includes FQPA considerations)
Intermediate-Term (1 week - several months) Occupational/Residential	Dermal	Same as Short Term MOE = 1000 (includes FQPA considerations)
Chronic-Term (several months-lifetime) Occupational/Residential	Dermal	NOEL: 0.019 mg/kg/day based on ↑ incidence of seizures and death, and alterations in clinical chemistry (protein) and ↑ TSH, ↓ T4. MOE = 1000 (includes FQPA considerations)
All time periods	Inhalation	LC50 in rats: ♂0.36 mg/L,♀0.42 mg/L, ♂+♀ 0.39 mg/L, Tox category II MOE = 1000 (includes FQPA considerations)
Cancer	Dietary/Dermal/ Inhalation	**Group C - Possible Human Carcinogen** (increases in thyroid follicular cell tumors with fipronil (M&F)). Use RfD to estimate human risk.
Chronic (non-cancer)	Dietary	RfD: 0.00002 mg/kg/day NOEL: 0.019 mg/kg/day based on ↑ incidence of seizures and death, alterations in clinical chemistry (protein) and ↑ TSH, ↓ T4. UF = 1000 (includes FQPA considerations)

TABLE 3. ENDPOINTS FOR PHOTODEGRADATE (MB46513)

EXPOSURE DURATION	EXPOSURE ROUTE	ENDPOINT AND TOXICOLOGICAL EFFECT
Acute	Dietary	NOEL: 2.0 mg/kg based on decreases in locomotor activity as well as decreases in hindlimb splay and rectal temperature in an acute neurotoxicity study. MOE = 1000 (includes FQPA considerations)
Short-Term (1-7 days) Occupational/Residential	Dermal	*Adjusted NOEL: 0.5 mg/kg/day based on decreases in body weight gain and food consumption in a 21-day dermal study. MOE = 1000 (includes FQPA considerations)
Intermediate-Term (1 week - several months) Occupational/Residential	Dermal	*Adjusted NOEL: 0.5 mg/kg/day based on decreases in body weight gain and food consumption in a 21-day dermal study. MOE = 1000 (includes FQPA considerations)
Chronic-Term (several months-lifetime) Occupational/Residential	Dermal	Not Applicable Use pattern (1 application/year) does not indicate a potential for this exposure; risk assessment not required. MOE = Not Applicable
All time periods	Inhalation	Not Applicable Use pattern (1 application/year) does not indicate a potential for this exposure; risk assessment not required. MOE = Not Applicable
Cancer	Dietary/Dermal/ Inhalation	**Group C - Possible Human Carcinogen** (increases in thyroid follicular cell tumors with fipronil (M&F)). Use RfD to estimate human risk.
Chronic (non-cancer)	Dietary	RfD: 0.000002 mg/kg/day * Adjusted NOEL: 0.0019 mg/kg/day based on neurotoxic clinical signs and alterations in clinical chemistry and thyroid parameter seen in a combined/ chronic toxicity study in the rat. UF = 1000 (includes FQPA considerations)

* = Adjusted NOEL obtained by dividing the actual NOELs established in the studies conducted with the parent compound **Fipronil** and Potency Adjustment Factor of 10. A Potency Adjustment Factor of 10 was determined by the Committee based on the toxicity profiles of the **photodegradate MB41513** and **Fipronil.**

VII. STUDIES REVIEWED FOR THIS ACTION

The following studies were reviewed for this action (DERs are attached). The executive summaries for these studies are in section IV.

The acute neurotoxicity study (81-8 MRID 44262808) is classified as unacceptable due the lack of positive control data. This study may be upgraded to acceptable upon the receipt and evaluation of adequate positive control data from the testing facility.

MB46030 (fipronil)
82-1 - rat MRID 44262821
82-1 - mouse MRID 44262804

MB45897 (metabolite)
81-1 MRID 44262819
81-2 MRID 44262820
84-2 (3 studies) MRID 44262822, -23, -24

MB46513 (photodegradate)
81-8 MRID 44262808
82-1 - rat MRID 44262809
82-1 - mouse MRID 44262811
82-1 - dog MRID 44262810
83-3 - rat MRID 44275001
84-2 (3 studies) MRID 44262813, -14, -15
85-1 MRID 44262817
85-2 MRID 44262816

The NOEL/LOEL for the Subchronic oral toxicity study in rats with MB 46513 (MRID 43559501) was modified by the Hazard ID Assessment Review Committee. The new executive summary is in the toxicity profile section. An amended DER is attached.

DATA EVALUATION RECORD

012509

MB46030 (Fipronil)

Study Type: 82-1a; Subchronic Oral Toxicity (Feeding) Study in Mice

Work Assignment No. 3-23F (MRID 44262804)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
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Primary Reviewer:
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Date: 10/1/97

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Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date:

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 10/1/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. 12/6/97
Registration Action Branch 1(7509C)

Work Assignment Manager: Marion Copley, D.V.M., D.A.B.T. 12/6/97
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 90-Day subchronic toxicity [dietary]- mouse
OPPTS Number: 870.3100 OPP Guideline Number: §82-1a

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46030 (95.4-96.5% a.i.)

SYNONYMS: Fipronil

CITATION: Broadmeadow, A. (1991) M&B 46030: Preliminary toxicity study by dietary administration to CD-1 mice for 13 weeks. Final Report. Life Science Research Limited, Eye, Suffolk IP23 7PX, England. LSR Report 90/0860. October 31, 1991. MRID 44262804. Unpublished.

SPONSOR: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet, BP 9163, 69263 Lyon Cedex 09, France.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44262804), MB46030 (fipronil; 95.4-96.5% a.i.) was administered to CD-1 albino mice (12/sex/dose) in the diet at nominal dose levels of 0, 1, 3, 10, or 25 ppm (13-week measured mean 0, 0.13, 0.38, 1.27, and 3.20 mg/kg/day, respectively, for males, and 0, 0.17, 0.57, 1.72, and 4.53 mg/kg/day, respectively, for females) for 13 weeks. Ophthalmoscopic examinations and blood and urine analyses were not conducted. Liver was the only tissue routinely examined histologically.

There were no deaths, clinical signs of toxicity or effects on food consumption. Male and female mice in the 25 ppm treatment group had mean body weight gains 2.3-3.2 g lower (22 and 34%, respectively) than the controls. Liver abnormalities were observed in all male treatment groups. Minimal to moderate liver cell periacinar hypertrophy with cytoplasmic vacuolation was present in 0/12, 2/12, 3/12, 6/12 and 10/12 rats from controls to high dose group. There was one 10 ppm male with a grossly enlarged liver. Absolute and relative liver weights were increased 23 and 33 % above controls, respectively at 25 ppm. Females did not exhibit a similar response to

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treatment. One female in the 25 ppm group and two in the 10 ppm group exhibited slight midzonal hepatocytic fatty vacuolation, and mean relative liver weights for both groups were 8-13% higher than the controls. The above treatment induced changes are considered adaptive rather than toxic. No neoplastic tissue was observed. **The LOEL was 25 ppm (3.2 and 4.53 mg/kg/day, for males and females, respectively) based on a possible decreased body weight gain. The NOAEL was 10 ppm (1.27 and 1.72 mg/kg/day, for males and females, respectively). The NOEL is less than or equal to 1 ppm (0.13 and 0.17 mg/kg/day for males and females, respectively) based on hepatic hypertrophy at all doses.**

This 90-day subchronic toxicity study (dietary) is classified **acceptable (non-guideline)**due to its abbreviated protocol and its design as a range finding study and does not satisfies the Subdivision F guideline requirement for a subchronic toxicity study in rodents (§82-1a).

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Quality Assurance, and Flagging statements were provided.

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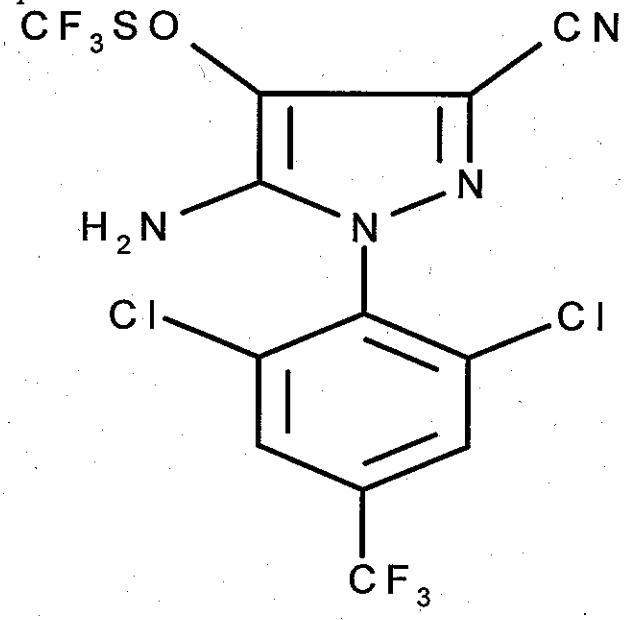
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB46030 (Fipronil)
   Description: Cream yellow-colored powder
   Lot/Batch #: PGS 963
   Purity: 95.4-96.5% a.i.
   Stability of compound: Stable for a minimum of 9 months when stored in the dark at 13 C
   CAS #: Not reported
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Mouse
   Strain: CD-1
   Age and weight at initiation of treatment: 35 to 42 days of age; males - 26-34 g; females - 20-27 g
   Source: Charles River U.K. Limited, Margate, Kent, England
   Housing: Housed in groups of four of similar sex and treatment group in polypropylene cages (33 x 15 x 13 cm) with stainless steel lids and wood shavings bedding
   Diet: Laboratory Animal Diet No. 2 (powdered; Biosure, Manea, Cambridgeshire, England), ad libitum
   Water: Municipal tap water, ad libitum, via polyethylene bottles
   Environmental conditions:
   Temperature: 18-25 C

Humidity: 40-70%
Air Changes: Approximately 15 per hour
Photoperiod: 12-Hour light/dark cycle
Acclimation period: 14 Days

B. STUDY DESIGN

1. In life dates - Start: 4/11/90 End: 7/17/90

2. Animal assignment

Mice were assigned to the test groups in Table 1 on arrival using computer-generated random allocation. Prior to the initiation of treatment, five mice with body weights at the extreme of the weight range were discarded and replaced with more typical animals from the same lot.

Table 1: Study design.[a]

Test Group	Nominal dose to animal (ppm)	Animals assigned	
		Male	Female
1 Control	0	12	12
2 Low	1	12	12
3 Low Mid	3	12	12
3 High Mid	10	12	12
4 High	25	12	12

[a] The rationale for the dose selection was not specified. It was reported that in a 6-week study (LSR Report No. 90/RHA299/0325), treatment at 40 ppm or more resulted in increased mortality.

3. Treatment preparation

The test diet was prepared fresh weekly and stored at room temperature in transparent polyethylene bags. MB46030 was premixed manually with a small amount of feed, then milled through a 2 mm screen using an ultracentrifugal mill. The premix was mixed for 15 minutes in a small planetary mixer with sufficient feed to produce a final diet concentration of 25 ppm. A portion of the 25 ppm mix was set aside for use as the high dose treatment; the remaining mix was mixed with additional feed in a Hobart

A200 mixer to prepare the 1, 3, and 10 ppm feeds. Samples (≥100 g) of each diet preparation were collected for concentration analyses. Samples from weeks 1 and 13 were analyzed.

It was reported that the homogeneity and stability of MB46030 in the diet at 1 and 300 ppm was established in LSR Report No. 90/RHA298/0781. This report was not appended to the study for review; however, detailed results were provided.

Results:
Homogeneity:
1 ppm: 91.6-100% of nominal (mean 96%)
300 ppm: 88.3-95.7% of nominal (mean 91%)
Stability analysis (storage at 21 C):
1 ppm:
0 days: 95.6% of nominal
7 days: 97.9% of nominal
14 days: 97.0% of nominal
300 ppm:
0 days: 90.7% of nominal
7 days: 87.3% of nominal
14 days: 90.8% of nominal
Concentration analysis (for weeks 1 and 13):
1 ppm: 91 and 100% of nominal
3 ppm: 80 and 104% of nominal
10 ppm: 88 and 109% of nominal
25 ppm: 92 and 109% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

Mean body weight changes were analyzed using Student's t-tests with a pooled within-group error variance. Organ weights were tested for homogeneity of variance using Bartlett's test. If variance was homogeneous, Behren's-Fisher test was used for pairwise comparison. If variance was heterogeneous, data were analyzed using Dunnett's test. Macroscopic and microscopic changes were analyzed using Fisher's Exact Test (two-tailed). All comparisons were performed at the two-tailed 5% level.

C. METHODS

1. Observations

Animals were observed once or twice daily for mortality, moribundity, and clinical signs of toxicity. Observations included skin, fur, eye and mucous membranes, respiration, circulation autonomic and central nervous system, somatomotor activity, behavior, and excreta. Animals were given a detailed physical examination including palpation each week.

2. Body weight

Body weights were measured prior to the initiation of treatment, on days 0, 3, and 7, weekly during treatment, and prior to necropsy.

3. Food and compound consumption and food efficiency

Food consumption was measured weekly during treatment. Food scatted from the hopper was weighed/estimated twice each week. Food consumption was reported as g food/animal/week. Weekly mean food efficiency for each group was calculated using the following formula:

[food consumption (g/week) ÷ body weight gain (g/week)]

Mean compound intake for each group was calculated on a weekly basis and was reported as mg/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted during the study.

5. Blood

Hematological and clinical blood chemistry parameters were not measured during the study.

6. Urinalysis

Urinalysis was not performed during the study.

7. Sacrifice and Pathology

Animals were killed by carbon dioxide asphyxiation. It was not stated if the animals were fasted prior to sacrifice. The bodies were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. It should be noted that while the "X" tissues were stored in preservative, only the liver and macroscopic abnormalities were prepared for and subsequently under went histologic examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Sciatic nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord*
X	Stomach*		(femur)	X	Pituitary*
X	Duodenum*	X	Lymph nodes*	X	Eyes (optic nerve)
X	Jejunum*	XX	Spleen*		
X	Ileum*	X	Thymus*		
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
X	Rectum*				
XX	Liver*+	XX	Kidneys*+	X	Adrenal gland*
X	Pancreas*	X	Urinary bladder*		Lacrimal gland
X	Gall bladder	XX	Testes*+	X	Mammary gland
		X	Epididymides	X	Thyroids*++ with parathyroids*
		X	Prostate		
		X	Seminal vesicle	X	Harderian gland
	RESPIRATORY	X	Ovaries*+		
		XX	Uterus* with cervix		
X	Trachea*	X	Vagina		
XX	Lungs*				
	Muzzle				
	Pharynx				
	Larynx				OTHER
				X	Bone* (femur, sternum)
				X	Muscle* (skeletal)
				X	Skin*
				X	All gross lesions and masses*

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic studies.
++ Organ weight required for non-rodent studies. T = required only when toxicity or target organ.

II. RESULTS

A. Observations

1. Mortality - No animals died prematurely.

2. Clinical Signs - All clinical signs occurred randomly and sporadically in all treatment groups, and were not attributed to treatment effects.

B. Body weight and weight gain

Female mice in the 25 ppm treatment group had mean body weight gains 34% ($p<0.05$) lower than the control groups after 13 weeks of treatment (Table 2). This depression of mean body weight gains was due, in part, to the low weight gains of two high dose females (0 and 1 g). Removal of these two females from the group calculations results in mean weight gain of 5 g, which is similar to the mean body weight gains of the 3 ppm treatment group.

Male mice in the 25 ppm treatment group had body weight gains 22% lower than the controls; the difference was not statistically significant. Body weight gains of mice in the 1, 3, and 10 ppm treatment groups were lower but not statistically different from the controls.

Table 2. Mean body weights and body weight gains (g) of mice before and during treatment with MB46030.[a]

Treatment rate (ppm)	Body weight (g)			13-Week body weight gain	
	0 Week	4 Weeks	13 Weeks	Total[b] (g)	% of Control gain
Males					
0	30.4	38.8	45.0	14.6	---
1	30.4	36.5	42.6	12.2	-16
3	31.0	38.5	44.9	13.9	-5
10	30.5	36.0	43.7	13.2	-10
25	31.6	38.8	43.0	11.4	-22
Females					
0	22.3	26.0	29.0	6.7	---
1	22.6	27.0	28.3	5.7	-15
3	22.5	25.0	27.3	4.8	-28
10	22.8	26.6	30.3	7.5	+12
25	22.8	24.8	27.2	4.4*	-34

[a] Data obtained from Table 2, pages 30 and 31, in the study report.
[b] Calculated by the reviewer.
* Significantly different from the control, $p<0.05$.

C. Food and compound consumption and food efficiency

Mean food consumption was similar to the controls in all treatment groups, ranging from 93 to 100% of the controls for the 13-week treatment period.

Weekly food efficiencies were variable, ranging from a net loss to 442.0. For the 13-week treatment period, the efficiencies of the male and female 25 ppm treatment groups were more than 26 and 51% higher, respectively, than the controls.

Mean compound consumption (13-week period) for the 1, 3, 10, and 25 ppm treatment groups were 0.13, 0.38, 1.27, and 3.20 mg/kg/day, respectively, for males, and 0.17, 0.57, 1.72, and 4.53 mg/kg/day, respectively, for females.

D. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted during the study.

E. Blood work

Hematological and clinical blood chemistry parameters were not measured during the study.

F. Urinalysis

Urinalysis was not performed during the study.

G. Sacrifice and Pathology

1. Organ weight - Absolute liver weights of males in the 25 ppm treatment group were 28% higher ($p<0.01$) than the controls. Relative liver weights of males and females in the 25 ppm treatment group were 33 and 13% higher ($p<0.01$), respectively, than the controls. Relative liver weights of mice in the 10 ppm treatment group were higher (8-12%) than the controls; the difference was not statistically significant. No other differences in organ weights were observed between mice in the treated and control groups.

Table 3. Mean absolute (g) and relative (organ weight/body weight x 100) liver weights of mice following 13 weeks of treatment with MB46030.[a]

Treatment rate (ppm)	Liver	
	Absolute	Relative
Males		
0	2.20	5.005
1	2.18	5.120
3	2.16	4.910
10	2.41	5.606
25	2.82**	6.637**
Female		
0	1.66	5.616
1	1.64	5.541
3	1.63	5.817
10	1.90	6.059
25	1.75	6.352**

[a] Data obtained from Tables 5A and 5B, pages 34-37, in the study report.

** Statistically different from the control, $p<0.01$.

2. Gross pathology - One male in the 10 ppm treatment group had an enlarged liver. This was considered to be treatment-related because of observed histological differences in the livers of treated mice that were clearly associated with treatment. No other treatment-related gross postmortem differences were observed between rats in the treated and the control groups. All other abnormalities appeared to occur randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Periacinar hypertrophy with cytoplasmic vacuolation was observed in the livers of male mice in all treatment groups. The incidence increased with increasing treatment rate, with 2/12 mice affected in the 1 ppm group, 3/12 in the 3 ppm group, 6/12 in the 10 ppm group, and 10/12 in the 25 ppm group. The severity of the condition ranged from minimal to moderate, and did not appear to be related to treatment. No female mice exhibited hepatocytic hypertrophy. Sight

midzonal hepatocytic fatty vacuolation was observed in 2/12 females in the 10 ppm treatment group and 1/12 females in the 25 ppm group.

No other treatment-related postmortem differences were observed between mice in the treated and the control groups. All other abnormalities appeared to occur randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in mice in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that a NOEL was not established in this study, since hepatocelluar hypertrophy was observed in male mice at all treatment levels. The study author attributed the observed changes in the liver tissues to adaptive rather than toxic responses, especially at treatment levels ≤10 ppm.

B. Reviewer's Discussion

We disagree with the study author that a NOEL was not established in this study. Although hepatocellular hypertrophy with cytoplasmic vacuolation, a response that is both treatment-related and concentration-dependent, was observed in 2/12 of the male mice at 1 ppm, the lowest dose studied. This is an adaptive response (see below) and is not used, by itself, to establish a NOEL and LOEL.

Although male mice in all treatment groups exhibited hepatocelluar hypertrophy in response to treatment, this response does not appear to be toxicologically significant at the dose levels tested in this subchronic study. In the male 25 ppm treatment group, there were no observed clinical signs of toxicity, and the body weight gains were slightly but not significantly lower than the control gains. Female mice in the 25 ppm treatment group exhibited no clear histopathological responses to treatment. Although female body weight gains were 34% lower than the control gains, the magnitude of the depression is misleading since the mean body weight gains differed by only 2.3 g (4.4 g in the 25 ppm group, compared to 6.7 g in the control), which does not appear to be life-threatening. Therefore, an upper dose level of at least 25 ppm (approximately 4.5 mg/kg/day) or greater should be used in long term studies.

IV. STUDY DEFICIENCIES

Histopathological and clinical blood chemistry parameters were not measured during the study since this was a range finding study.

DATA EVALUATION RECORD

012508

MB46513 - (PHOTODEGRADATE OF FIPRONIL)

Study Type: 81-8; Acute Neurotoxicity Study - Rats
Work Assignment No. 3-23H (MRID 44262808)

Prepared for

Health Effects Division
Office of Pesticide Programs
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Robert F. Fricke, Ph.D.
Reregistration Branch II (7509C)

Work Assignment Manager: Marion Copley, DVM, DABT
Registration Action Branch (2002)

Robert F. Fricke 12 Nov 97.

Mcopley 12 Nov 12/97

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Oral Gavage Study in Rats
OPPTS Number: 870.6200 OPP Guideline Number: §81-8

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (99.5% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole

CITATION: Hughes, E.W. (1996) MB 46513; Neurotoxicity to Rats by Acute Oral Administration (Including a dose range finding study). Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Project Number RNP/471. January 11, 1996. MRID 44262808. Unpublished.

SPONSOR: Rhône-Poulenc Secteur Agro, 355 rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France.

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 44262808), MB46513, a photometabolite of fipronil (99.5% a.i.), was administered in corn oil by oral intubation to Crl:CD BR rats (12/sex/dose) at dose levels of 0, 0.5, 2, or 12 mg/kg. The rats were evaluated for reactions in functional observations and motor activity measurements at 6 hours, 7 days, and 14 days post dosing. Clinical signs, body weights, and food consumption were monitored. At study termination, brains were weighed and measured, and neural tissues were processed for microscopic evaluation.

No animals died and there were no treatment-related clinical signs of toxicity. At 12 mg/kg, significant decreases in body weight gains and food consumption were noted for the high-dose males and females during the week following treatment. By the second week, both had returned to the control levels. Body weight gains and food consumption for the low- and mid-dose groups and mean body weights for all treated groups were not significantly different from the controls throughout the study. Food efficiency was not affected by treatment.

Behavioral responses were also affected by treatment with MB46513 at 12 mg/kg. At the estimated peak response time, 6 hours post dosing, significant decreases in locomotor activity during the first 30 minutes of observation were noted among high-dose males and females. There were no significant differences in any dose group on days 7 and 14. Treatment-related decreases in hindlimb splay and rectal temperature at 6 hours post dose were observed in high-dose males and females. In addition, decreases in the proportion of high-dose males with an immediate righting reflex on days 7 and 14 were possibly treatment related. Decreased forelimb grip strength in high-dose males on day 7 and increased forelimb grip strength in high-dose females at 6 hours post dosing was possibly related to the treatment, because there were also slight increases in forelimb grip strength in high-dose males at 6 hours and slight decreases in forelimb grip strength in high dose females at 7 days and in high-dose males and females at 14 days.

There were no significant differences among groups in neuropathology.

Based on these findings, the neurobehavioral LOEL for rats is 12 mg/kg. The NOEL is 2 mg/kg.

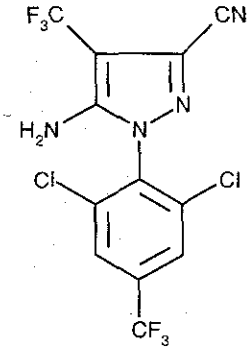
This study is classified **unacceptable** and does not satisfy the guideline requirement for an acute neurotoxicity study in rodents **(§81-8)**. The study may be upgraded after receipt and favorable review of positive control data for the performing laboratory.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MB46513
   Description: White powder
   Lot/Batch #: CH089
   Purity: 99.5% a.i.
   Stability of compound: Reported to expire November 1996
   CAS #: 120068-37-3
   Structure:



2. Vehicle: Corn oil

3. Test animals: Species: Rat
   Strain: Crl:CD BR
   Age (approximate) at study initiation: 48 days
   Weight at study initiation: Males, 187-238 g; females, 144-185 g
   Source: Charles River Breeding Laboratories, Margate, Kent, England
   Housing: Individually housed in suspended cages with wire mesh floors
   Diet: SDS Rat and Mouse Maintenance Diet, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
      Temperature: 21 ± 2 C
      Humidity: 44 ± 18%
      Air changes: Not reported
      Photoperiod: 12-Hour light/dark cycle
   Acclimation period: 13 Days for males; 20 days for females

B. STUDY DESIGN

1. In-life dates - Males: 3/28/95 to 4/14/95
   Females: 4/4/95 to 4/21/95

2. Animal assignment

Animals were selected based on health and body weights and were randomly assigned to the test groups shown in Table 1. The groups were stratified by body weight so that initial group means were approximately equal.

Table 1: Study design

Test Group	Dose (mg/kg)	Males	Females
Control	0	12	12
Low (LDT)	0.5	12	12
Mid (MDT)	2	12	12
High (HDT)	12	12	12

Rats were orally administered, via gastric intubation, a single dose of MB46513 as a suspension in corn oil at a dosing volume of 10 mL/kg body weight (adjusted according to the most recently recorded body weight and to the nearest 0.1 mL). Animals were fasted overnight prior to dosing. Control animals received the vehicle alone. During dosing, the suspensions were stirred with a magnetic stirrer. Animals were dosed using a graduated syringe and a rubber catheter inserted into the stomach.

The doses selected were based on the results of a dose range finding study (RNP/450), during which rats were given a single oral dose of MB46513 at 0.5, 2, 10, or 13.5 mg/kg by gavage. A functional observational battery (FOB) and motor activity assessment were performed prior to treatment and 2, 4, 6, and 24 hours after dosing. After treatment, body weight was recorded daily for 7 days until necropsy. At 13.5 mg/kg clinical signs (piloerection, salivation, hunched posture, cold to the touch), behavioral changes (clonus of jaws, clonic convulsions, decreased arousal, decreased rearing and activity counts, changes in posture, gait and motor patterns), reduced rectal temperature, and decreased body weight gains were noted. Piloerection, decreased arousal, rearing and activity counts, changes in motor patterns, and slight initial weight losses were observed at 10 mg/kg. Several behavioral changes were noted at 2 and 0.5 mg/kg; however, a negative control group was not included, so an association between these changes and the treatment could not be established. The peak time of effect was established as between 4 and 6 hours after dosing. Based on these findings, dose levels of 0.5, 2, and 12 mg/kg were selected by the sponsor for the acute neurotoxicity study, and 6 hours post dosing was selected for the first

post-dosing FOB and motor activity assessment.

3. Dosing solution preparation and analysis

The test substance was prepared for oral dosing by grinding the powder in a mortar with a small amount of corn oil to form a paste, and adding additional vehicle to bring the suspension to the desired volume. The suspension was mixed using a high shear homogenizer. A series of suspensions (0.05, 0.2, and 1.2 mg/mL) were made by serial dilution to yield a constant dose volume of 10 mL/kg body weight. Fresh dosing suspensions were prepared on the day prior to use.

In order to establish the adequacy of the preparation methods, homogeneity, concentration, and stability analyses were conducted prior to the start of the study. Bulk formulations containing the test substance at nominal concentrations of 0.01 or 12.5 mg/mL were thoroughly mixed by shaking and magnetic stirring. To determine physical stability (homogeneity) samples from the top, middle, and bottom of each formulation were collected after 5 minutes (0 hour), 0.5, and 1 hour of magnetic stirring. Samples were also collected for analysis after the formulation was stored (without magnetic stirring) in the dark at ambient temperature for 3 hours, and at ambient temperature during the day and 4 C overnight for 24 hours. At 3 and 24 hours each formulation was re-mixed and sampled for analysis as above. To determine chemical stability, additional samples were collected for analysis after the formulation was stored in the dark at ambient temperature for 3 hours, and at ambient temperature during the day and 4° C overnight for 24 hours. Actual concentrations were reported as the means of duplicate samples from each of 4 dosing dates during the rangefinding study and 2 dosing dates during the acute study.

Results:
Homogeneity (0 hour):
0.01 mg/mL: 90.2-92.4% of nominal
12.5 mg/mL: 97.6-103.2% of nominal

The homogeneity of the dosing suspensions was confirmed at 0.5 and 1 hour of magnetic stirring and after storage for 3 and 24 hours; recoveries were 89.3-103% of nominal (the laboratory excluded one value at the 0.5 hour time point that was 26% above nominal) for the 0.01 mg/mL suspension and 94.4-104% of nominal for the 12.5 mg/mL suspension.

Stability:
3 hours:
0.01 mg/mL: 90.2-92.7% of nominal
12.5 mg/mL: 101.6-102.4% of nominal
24 hours:

0.01 mg/mL: 90.1-102% of nominal
12.5 mg/mL: 104.8-109.6% of nominal

The stability of the dosing suspensions was confirmed up to 24 hours.

Concentration:
range finding study
0.05 mg/mL: 89-92.8% of nominal
0.20 mg/mL: 95.5-99.5% of nominal
1.00 mg/mL: 87.7-98% of nominal
1.35 mg/mL: 103% of nominal
acute study
0.05 mg/mL: 89.6-99.4% of nominal
0.20 mg/mL: 85-93% of nominal
1.20 mg/mL: 90-97.5% of nominal

All concentrations were within 15% of nominal. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dose to the animals was acceptable.

4. Statistics

Body weight, weight gain, and food consumption recorded during the functional observation battery, forelimb grip strength, hindlimb splay, activity and rearing counts, rectal temperature, and brain measurements were analyzed using analysis of variance. With the exception of predose data, analyses of variance were followed by the Student's *t* test and Williams' *t* test for a dose-related response. Kruskal-Wallis analyses were followed by the nonparametric equivalents of the *t* test and Shirley's test. For predose data, analyses of variance were followed by the Student's *t* test. When a difference between the control and treated groups was indicated, the data were analyzed using the Linear by Linear Association test. A one-tailed test was applied for abnormal gait, palpebral closure and tremors. A two-tailed test was applied for all other parameters.

C. METHODS

1. Observations

Animals were observed and palpated at least once daily for signs of treatment-related toxicity, reaction to treatment or ill health. Animals were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality.

2. Body weight

Animals were weighed one week prior to dosing, on the day of dosing, and at weekly intervals following dosing. Animals were also weighed on each occasion that the functional

observational battery and motor activity assessment was performed.

3. Food consumption

Food consumption for each animal was measured weekly beginning at Week -1, (7 day interval for males or 6 day interval for females) prior to dosing through Week 2 following dosing. Food intake (g/rat/week) was calculated based on the amount of food given to and left by each rat during each recording period. Food efficiency (food consumption/body weight gain) was calculated for each test group for weeks 1 and 2.

4. Neurobehavioral Studies

Motor Activity - Motor activity of all animals was measured at approximately the same time of day, before initiation of treatment (not further defined), and on days 0 (6 hours after dosing), 7, and 14 after dosing. Six hours was the estimated time of peak effect based on the range finding study. The placement of each animal within a cage was balanced as much as possible across groups. The test session was initiated when all animals were placed in cages, and lasted one hour for each animal. Motor activity was monitored using a Colbourn Infra-Red Activity Monitoring System which uses an infra-red detector. For each animal, the time and number of events spent in no movement, locomotor, and non-locomotor activity were recorded (only locomotor activity was reported). Data were collected every 2 minutes.

Functional Observational Battery - An FOB was performed on all animals at approximately the same time of day, before initiation of treatment and on study days 0 (6 hours post dosing), 7, and 14. The parameters in the following table were evaluate.

Positive Control Studies: Valid positive control studies were not submitted for the performing laboratory, Huntingdon Life Sciences Ltd.

	HOME CAGE OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture in cage	X	Convulsions, tremors,
X	Convulsions, tremors, twitches	X	Level of activity in arena
X	Spontaneous vocalizations	X	Level of arousal
X	Palpebral closure	X	Rearing count
		X	Grooming
	HAND-HELD OBSERVATIONS	X	Assessment of gait
X	Ease of removal from cage	X	Presence of fecal boluses, urine
X	Ease of handling rat		QUANTITATIVE/MEASUREMENTS
X	Salivation/lacrimation	X	Tail pinch response
X	Palpebral closure	X	Grip strength; fore and hindlimb
X	Exophthalmus	X	Landing foot splay
X	Piloerection	X	Body temperature
X	Vocalization on handling	X	Body weight
	REFLEXES/RESPONSES		
X	Approach response		
X	Touch response		
X	Startle response		
X	Righting reflex		
X	Pupil response		

5. Sacrifice and Pathology

All animals were sacrificed on day 15 by intraperitoneal injection of sodium pentobarbital and perfused *in situ* with heparinized 0.7% sodium nitrite followed by a glutaraldehyde/paraformaldehyde solution. Neuropathological examination was initially conducted on tissues from five rats/sex from the control and high-dose groups. However, because axonal lesions were detected in the high-dose males, tissues from the remaining seven control and high-dose males were also examined. The following tissues were examined:

	CENTRAL NERVOUS TISSUES	X	Cervical dorsal root ganglia
X	Forebrain[a]	X	Lumbar dorsal root ganglia
X	Midbrain[a]	X	Dorsal and ventral fibers (cervical level)[c]
X	Cerebellum[a]	X	Dorsal and ventral root fibers (lumbar level)[c]
X	Pons[a]		
X	Pons[a]		
X	Medulla oblongata[a]		PERIPHERAL NERVOUS TISSUE
X	Lumbar spinal cord[b]	X	Sciatic nerve[b]
X	Cervical spinal cord[b]	X	Sural nerve[b]
X	Gasserian ganglia	X	Tibial nerve[b]

a Cross sections of these tissues were evaluated.
b Cross and longitudinal sections of these tissues were evaluated.
c Longitudinal sections of these tissues were evaluated.

Brains, spinal cords, ganglia, and dorsal and ventral root fibers were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Peripheral nerves from the right side were embedded in epon, sectioned, and stained with toluidine blue.

III. **RESULTS**

A. Observations

1. Mortality - No animals died during the study.

2. Clinical signs - No treatment-related differences in clinical signs were observed in any of the treatment groups immediately following dosing or during the 2-week observation period.

B. Body weight and body weight gain

Significant decreases (↓22-24%, $p<0.01$) in body weight gains were noted for the high-dose males and females compared to the controls within one week following treatment. By the second week, body weight gains had recovered to control levels. Body weight gains for the low- and mid-dose groups and mean body weights for all treated groups were not statistically different from the controls throughout the study.

C. Food consumption

Food consumption was significantly decreased (↓13-19%, $p<0.01$) for the high-dose males and females compared to the controls

during the first week following treatment. By the second week, food consumption had returned to control levels. Food consumption for the low- and mid-dose groups were comparable to the controls. Food efficiency was not affected by treatment.

Table 2. Body weight and food consumption (g) following a single oral dose of MB 46513 at 0.5, 2, or 12 mg/kg.[a]

Observation/study week	Dose (mg/kg)			
	0	0.5	2	12
Males				
Body weight (g)/Week 0	209	215	215	215
Body weight (g)/Week 1	282	292	289	273
Body weight (g)/Week 2	319	328	328	320
Weight gain (g)/Week 0-1	73	76	74	57**
Weight gain (g)/Week 1-2	37	36	39	48*
Food consumption (g)/Week 0-1	223	230	230	194**
Food consumption (g)/Week 1-2	230	239	242	237
Females				
Body weight (g)/Week 0	163	167	161	168
Body weight (g)/Week 1	209	211	207	202
Body weight (g)/Week 2	228	230	222	221
Weight gain (g)/Week 0-1	45	44	45	34**
Weight gain (g)/Week 1-2	20	19	15	19
Food consumption (g)/Week 0-1	179	177	178	145**
Food consumption (g)/Week 1-2	175	182	179	180

a Data extracted from study report Tables 1 and 2, pages 37-38.
* Significantly different from control value, $p<0.05$.
** Significantly different from control value, $p<0.01$.

D. Motor Activity Measurements

At the 6-hour post-dosing observation period, significant decreases (↓54-57%, $p<0.01$) in locomotor activity during the first 30 minutes were observed among high-dose males and females. No other significant differences were observed for the lower dose groups at 6 hours post dosing or for any group on days 7 and 14, although a dose-related increase was apparent for the females on day 7 (Table 3).

E. Functional Observational Battery

Significant decreases in hindlimb splay (↓17-20%, $p<0.05$ or 0.01) and rectal temperature (↓1-2%, $p<0.05$ or 0.01) at 6 hours post dose were observed in high-dose males and females. These findings were considered to be treatment related. In addition, a decrease in the proportion of high-dose males with an immediate righting reflex on days 7 (5/12 vs 7/12 for the controls) and 14 (5/12 vs 9/12 for the controls) may have been related to the treatment. Significant findings that were not considered to be related to treatment included decreased forelimb grip strength (↓23%, $p<0.01$) in high-dose males on day 7, mainly due to low values in 3/12 rats; an increase in forelimb grip strength (↑17%, $p<0.05$) in high-dose females at 6 hours post dose; an increase in mean rearing counts (↑44%, $p<0.05$) in high-dose females on day 14; and increases in mean activity counts in high-dose females (↑44-54%, $p<0.05$) on days 7 and 14 and in mid-dose females (↑54%, $p<0.05$) on day 7. The increased activity and rearing counts among females were not supported by the quantitative assessment of activity by the Coulbourn system; therefore, they were not considered treatment related. For all treatment groups, no other differences in FOB parameters were considered to be treatment related following dosing. Selected FOB results are presented in Table 3.

Table 3. Locomotor activity and selected functional observational battery results following a single oral dose of MB 46513 at 0.5, 2, or 12 mg/kg.[a]

Behavioral Endpoint	Dose (mg/kg)			
	0	0.5	2	12
Males				
Motor activity Mean Large Movements (Total sec of activity)				
Predose	784	730	747	835
6 hour[b]	288	362	300	133**
7 day	438	412	439	424
14 day	435	552	482	496
Activity counts				
Predose	12	12	11	11
6 hour	5	7	5	4
7 day	9	10	7	11
14 day	9	9	7	11
Rearing counts				
Predose	12	13	14	11
6 hour	5	7	4	3
7 day	9	9	7	10
14 day	7	10	6	10
Forelimb grip strength (kg)				
Predose	0.59	0.56	0.54	0.56
6 hour	0.64	0.67	0.66	0.69
7 day	0.75	0.78	0.76	0.58**
14 day	0.74	0.85	0.85	0.69
Hindlimb splay (cm)				
Predose	7.8	8.9	7.9	8.2
6 hour	8.3	9.2	7.7	6.9*
7 day	8.4	9.4	9.0	8.6
14 day	8.5	9.8	9.8	8.5
Rectal temperature (C)				
Predose	37.9	38.0	38.1	37.8
6 hour	38.5	38.5	38.7	38.1*
7 day	38.4	38.3	38.2	38.1
14 day	37.8	37.8	37.7	37.9
Immediate righting reflex (# of rats)				
Predose	10/12	10/12	11/12	8/12
6 hour	9/12	9/12	11/12	8/12
7 d	7/12	10/12	7/12	5/12
14 day	9/12	11/12	10/12	5/12[c]

Behavioral Endpoint	Dose (mg/kg)			
	0	0.5	2	12
Females				
Motor activity Mean Large Movements (Total sec of activity)				
Predose	895	875	775	864
6 hour[b]	283	301	337	121**
7 day	660	704	710	809
14 day	712	765	688	769
Activity counts				
Predose	13	16	13	16
6 hour	12	14	11	9
7 day	13	20	20*	20*
14 day	18	24	20	26*
Rearing counts				
Predose	10	14	11	15*
6 hour	10	11	11	8
7 day	11	15	18	17
14 day	16	21	19	23*
Forelimb grip strength (kg)				
Predose	0.62	0.57	0.57	0.58
6 hour	0.63	0.65	0.65	0.74*
7 day	0.67	0.65	0.62	0.61
14 day	0.71	0.69	0.63	0.57
Hindlimb splay (cm)				
Predose	8.2	8.6	8.4	8.0
6 hour	8.5	8.8	8.3	6.8**
7 day	8.8	9.2	8.8	8.4
14 day	8.7	9.1	9.2	8.2
Rectal temperature (C)				
Predose	38.2	38.6	38.5	38.7*
6 hour	38.7	38.7	38.5	37.9**
7 day	38.8	38.9	39.0	39.0
14 day	39.0	39.1	39.1	39.3
Immediate righting reflex (# of rats)				
Predose	12/12	11/12	9/12	11/12
6 hour	11/12	11/12	10/12	10/12
7 day	9/12	10/12	11/12	10/12
14 day	9/12	11/12	9/12	9/12

a Data obtained from Tables 4, 5, 7, 8-10, and 12; pages 41-45, 47-50, and 52-54 of the study report.
b 30-minute observation, 60-minute observation at all other time points.
c Significantly different from the control, p=0.004 (Linear by Linear Association Test).

* Significantly different from the control, $p<0.05$.
** Significantly different from the control, $p<0.01$.

F. Sacrifice and Pathology

There were no significant differences among groups in brain weights or measurements. Trace axonal degeneration of the lumbar dorsal root fibers and/or sciatic nerve was observed in 6/12 high-dose and 4/12 control males (Table 4). One high dose male showed minimal axonal degeneration of the lumbar dorsal root fibers. The difference between the control and high-dose group was not statistically significant and, because trace axonal degeneration was not considered to be biologically significant, the increased incidence was not thought to be related to the treatment. Trace axonal degeneration of the sciatic nerve was also observed in 2/5 high-dose and 1/5 control females, however, it was not considered to be of toxicological significance. There were no other microscopic findings considered to be related to treatment with MB46513.

Table 4. Axonal degeneration in control and high-dose male rats[a]

Observation	Dose Group (mg/kg)	
	0	12
	Incidence (# affected/# examined)	
Spinal cord (L1-4) axonal degeneration in nerve root		
Trace	0/12	2/12
Total	0/12	2/12
Dorsal root fibers (L) axonal degeneration		
Trace	1/12	3/12
Minimal	0/12	1/12
Total	1/12	4/12
Sciatic nerve (sciatic-notch) axonal degeneration		
Trace	1/12	3/12
Total	1/12	3/12
Sciatic nerve (mid-thigh) axonal degeneration		
Trace	3/12	5/12
Total	3/12	5/12

[a] Data obtained from Table 14, pages 56-58 of the study report.

III. **DISCUSSION**

A. Investigator's Conclusions

The study author concluded that a single oral dose of MB46513 at 0.5, 2, or 12 mg/kg was associated with decreased body weight gains and lower food consumption in male and female rats at 12 mg/kg during the week following treatment. Decreased hindlimb foot splay, rectal temperature, and locomotor activity in both sexes at 12 mg/kg were limited to observations performed 6 hours after dosing. At 7 and 14 days after dosing, an increased incidence of slowed righting reflex in males at 12 mg/kg was possibly related to treatment. There was no evidence of neuropathology at any dose level. The behavioral LOEL is 12 mg/kg. The NOEL is 2 mg/kg.

B. Reviewer's Discussion

MB46513 in corn oil was administered orally by gavage as a single dose to rats (12/sex/dose) at 0.5, 2, or 12 mg/kg. A functional observation battery and motor activity testing were

performed at 6 hours, 7 days and 14 days post dosing.

No animals died and there were no treatment-related clinical signs of toxicity. Both body weight gains and food consumption were affected by treatment with MB46513 at 12 mg/kg. There were significant decreases (↓22-24%, $p<0.01$) in body weight gains for the high-dose males and females during the week following treatment. By the second week, body weight gains had recovered to control levels. Body weight gains for the low- and mid-dose groups and mean body weights for all treated groups were not significantly different from the controls throughout the study. Food consumption was significantly decreased (↓13-19%, $p<0.01$) for the high-dose males and females during the first week following treatment. By the second week, food consumption had returned to control levels. Food consumption for the low- and mid-dose groups were comparable to the controls, and food efficiency was not affected by treatment.

Neurobehavioral responses were also affected by treatment with MB46513 at 12 mg/kg. At the estimated peak response time, 6 hours post dosing, significant decreases (↓54-57%, $p<0.01$) in locomotor activity during the first 30 minutes of observation were noted among high-dose males and females. No significant differences were observed for the lower dose groups at 6 hours post dosing or for any group on days 7 and 14, although there was an apparent dose-related increase in locomotor activity in females on day 7. Treatment-related decreases in hindlimb splay (↓17-20%, $p<0.05$ or 0.01) and rectal temperature (↓1-2%, $p<0.05$ or 0.01) at 6 hours post dose were observed in high-dose males and females. In addition, a decrease in the proportion of high-dose males with an immediate righting reflex on days 7 (5/12 vs 7/12 for the controls) and 14 (5/12 vs 9/12 for the controls) may have been related to the treatment. Decreased <u>forelimb grip strength</u> (↓23%, $p<0.01$) in high-dose males on day 7, mainly due to low values in 3/12 rats and increased <u>forelimb grip strength</u> (↑17%, $p<0.05$) in high-dose females at 6 hours post dose may have been related to the treatment, because there were also slight increases in forelimb grip strength in high-dose males at 6 hours and slight decreases in forelimb grip strength in high-dose females at 7 days and in high-dose males and females at 14 days. An increase in <u>mean rearing counts</u> (↑44%, $p<0.05$) in high-dose females on day 14 and increases in <u>mean activity counts</u> in high-dose females (↑44-54%, $p<0.05$) on days 7 and 14 and in mid-dose females (↑54%, $p<0.05$) on day 7 were not considered to be treatment related, because they were not supported by the quantitative assessment of activity using the Coulbourn system. Although the mean activity and rearing counts for the females on day 7 were increased at all dose levels compared to the controls, and a dose-related increase in locomotor activity monitored by the Coulbourn system was apparent in females on day 7, the effect was not large enough to be considered treatment related.

There were no significant differences among groups in brain weights or measurements. Trace axonal degeneration of the lumbar dorsal root fibers and/or sciatic nerve was observed in 6/12 high-dose and 4/12 control males. One high-dose male showed minimal axonal degeneration of the lumbar dorsal root fibers. The difference between the control and high-dose group was not statistically significant and, because the axonal degeneration was only given a trace grade (except for one rat with a minimal grade), it was not considered to be biologically significant and not related to the treatment. Trace axonal degeneration of the sciatic nerve observed in 2/5 high-dose and 1/5 control females was also not considered to be of toxicological significance.

Based on these findings, the neurobehavioral LOEL was established at 12 mg/kg. The NOEL was established at 2 mg/kg.

IV. STUDY DEFICIENCIES

Positive control data were not provided by the laboratory to document the reliability of the FOB methods or the motor activity measurements in the testing laboratory. Subdivision F guidelines require that positive control data be collected at the time the study was conducted, unless the laboratory can demonstrate the adequacy of historical data for this purpose. Positive control data provide evidence of the ability of the observational methods to detect major neurotoxic endpoints, and demonstrate the sensitivity and reliability of the activity-measuring device and testing procedures. Therefore, positive control data must be submitted in order for this study to be considered acceptable.

DATA EVALUATION RECORD

012509

MB 46513 (Photodegradate of Fipronil)

Study Type: N/A; 28-Day Dietary Range-finding Study in Rats

Work Assignment No. 3-23D (MRID 44262809)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 1 (7509C)

11/4/97

11/4/97

DATA EVALUATION RECORD

STUDY TYPE: 28-Day dietary administration (range-finding) - rat
OPPTS Number: N/A OPP Guideline Number: 82-1a

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB 46513 (97.5% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl-phenyl)-4-trifluoromethylpyrazole; RPA097801; RPA591085

CITATION: Dange, M. (1995) MB 46513. Preliminary 28-day toxicity study in the rat by dietary administration. Rhone-Poulenc Agrochimie, Centre de Recherche, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France. Study SA 93138. November 6, 1995. MRID 44262809. Unpublished.

SPONSOR: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet, BP 9173, F-69263 Lyon Cedex 0960, France.

EXECUTIVE SUMMARY:

In a 28-day range-finding study (MRID 44262809), MB 46513 (a metabolite of fipronil; 97.5% a.i.) was administered to Sprague-Dawley rats (10/sex/dose) in the diet at nominal dose levels of 0, 0.5, 3, 30 or 100 ppm (equivalent to 0, 0.04, 0.23, 2.20 or 3.74 mg/kg body weight/day, respectively, for males; 0, 0.04, 0.24, 2.32 or 3.8 mg/kg body weight/day, respectively, for females). In addition to evaluation of standard study parameters, thyroid hormone levels were measured on days 7 and 23.

No treatment-related effects were observed in the 3 and 0.5 ppm treatment groups. One male in the 30 ppm group was found dead on day 6 and all 100 ppm group animals died within the first 2 weeks of study. Clinical signs at 30 and 100 ppm included piloerection (M 9/10 and 4/10 at 30 and 100 ppm; F 5/10 and 6/10 at 30 and 100 ppm), curling up at handling (M 6/10 and 4/10 at 30 and 100 ppm; F 8/10 and 5/10 at 30 and 100 ppm); thin (M 5/10 and 4/10 at 30 and 100 ppm; F 6/10 6/10 at 30 and 100 ppm); increased motor activity (F 1/10 and 2/10 4/10 at 30 and 100 ppm)

and irritability with convulsions at 100 ppm in 1 female. There was a decrease in body weight (9-18% and 26-36% for 30 and 100 ppm) and food consumption (8-34% and 69-73% for 30 and 100 ppm). Clinical chemistry parameters affected at 30 ppm included bilirubin (decrease - 28-33%) and aspartate aminotransferase (increased F - 22%). At 30 ppm on day 7 or 23, males had decreased T3 and T4 levels (33-49%) and females had decreased T4 levels (61%) compared to the controls. While at 100 ppm T3 levels were decreased 46% (females only), and T4 levels were decreased 50-63%. No treatment-related differences in hematology or urine parameters, organ weights or gross postmortem or microscopic appearance were observed.. No neoplastic tissue was observed. Ophthalmoscopic examinations were not conducted. **The LOEL for this study is 30 ppm (2.20 and 2.32 mg/kg/day for M and F, respectively), based on clinical signs including piloerection, curling up and thin appearance; and decreased body weights in both sexes. The NOEL is 3 ppm (0.23 and 0.24 mg/kg/day for M and F, respectively).**

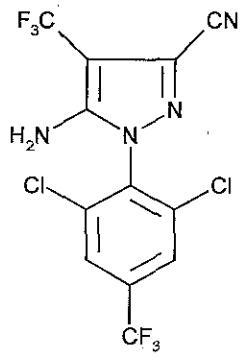
This 28-day dietary feeding study is classified **acceptable (non-guideline)** as it is not a required guideline study. It is acceptable for the purposes for which it was intended. It is recommended that a maximum treatment rate between 3 and 30 ppm be used in longer term studies.

COMPLIANCE: Signed and dated Data Confidentiality and GLP statements were provided. Quality Assurance and Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB 46513
   Description: Cream-colored solid
   Lot/Batch #: 10 DGM 22
   Purity: 97.5% a.i.
   Stability of compound: Reported to expire October 1, 1993
   CAS #: None
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Rat
   Strain: Sprague-Dawley
   Age and weight at initiation of treatment: 6-7 Weeks of age; males - 223-260 g; females - 168-206 g
   Source: Charles River France, St Aubin-les-Elbeuf, France
   Housing: Housed individually in suspended stainless steel wire mesh cages
   Diet: Certified rodent diet AO4C P1 (UAR, Villemoisson-sur-Orge, France), ad libitum
   Water: Municipal tap water, ad libitum
   Environmental conditions:
      Temperature: 20-24 C
      Humidity: 40-70%
      Air Changes: 15 per hour
      Photoperiod: 12-Hour light/dark cycle
   Acclimation period: 6 Days

B. STUDY DESIGN

1. In life dates - Start: 5/19/93 End: 6/18/93

2. Animal assignment

Rats were assigned to the test groups in Table 1 one day before treatment using a randomization procedure that ensured a similar body weight distribution among groups for each sex.

Table 1: Study design.[a]

Test Group	Nominal dose to animal (ppm)	Animals assigned	
		Male	Female
1 Control	0	10	10
2 Low	0.5	10	10
3 Mid low	3	10	10
4 Mid high	30	10	10
5 High	300	10	10

[a] No rationale for dose selection was provided.

3. Treatment preparation

MB 46513 was dissolved in acetone and mixed with sufficient feed to achieve the desired dietary concentrations. The control diet was prepared by adding acetone to untreated feed in a similar manner. Two diet preparations were prepared during the course of the study.

The concentration of each treated diet was confirmed. To determine homogeneity, duplicate samples (40 g) were collected from the top, middle, and bottom of the 0.5 and 100 ppm dietary mixes, and were analyzed on the day of preparation. Stability was determined for both freezer and room temperature storage. Samples from the 0.5 and 100 ppm diet preparations were stored at -15 C for up to 35 days prior to analysis. Additional 0.5 or 100 ppm diet mixture were stored at room temperature for 7 days, then stored frozen until Day 52 prior to being analyzed. To confirm the stability of treated feed in the cages, pools of the 0.5 and 100 ppm diet mixtures remaining in the feeders were constituted after being in the cage for 7 days, then stored frozen until Day 35. No sample was taken for the 100 ppm diet since all 100 ppm group animals died.

Results:
Homogeneity:
0.50 ppm: 96-102 of nominal (mean 98.6%)
100 ppm: 83-149% of nominal (mean 100.7%)
Concentration analysis:
0.5 ppm: 99% of nominal
3 ppm: 97% of nominal
30 ppm: 101% of nominal
100 ppm: 101% of nominal

The methodology presented in the text for the stability analyses did not agree with the storage dates reported in Table A2 of Appendix M. However, based on the data, the test substance was stable under freezer and ambient temperature storage; recoveries were 90-110% of nominal.

The analytical data indicated that the mixing procedure was adequate. All results were within acceptable ranges except for a 149% recovery for a 100 ppm homogeneity sample.

4. Statistics

Data for body weights, body weight changes, and food consumption were compared between the treated and control groups using Dunnett's test. The equality of means for hematology, clinical chemistry, and urinalysis parameters and absolute and relative organ weight data for each treatment group was established using Bartlett's test of homogeneity of variances. If variances were equal, the data were analyzed by standard one-way ANOVA followed by Dunnett's t-test. If variances were unequal, the data were analyzed using a modified t-test. The tests were conducted at the 5 and 1% levels.

C. METHODS

1. Observations

Animals were observed twice daily (once on weekends and holidays) for mortality, moribundity, and clinical signs of toxicity. Animals were given a detailed physical examination at least once each week.

2. Body weight

Body weights were measured once during acclimatization, on the first day of treatment, weekly during treatment, and prior to necropsy.

3. Food and compound consumption

Food consumption was measured weekly during treatment. Food consumption was reported as g food/animal/day. Mean compound consumption for each group was calculated on a weekly basis and was reported as mg/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted only during the acclimatization period.

5. Blood

Blood was collected from all surviving animals prior to necropsy. Animals were fasted overnight prior to the collection of blood from the reto-orbital venous plexus under ether anesthesia. The CHECKED (X) hematology and clinical blood chemistry parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride		Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Cholesterol
X	Potassium		Globulin
X	Sodium	X	Glucose
		X	Total bilirubin
		X	Total serum protein (TP)
	ENZYMES	X	Triglycerides
X	Alkaline phosphatase		
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
	Gamma glutamyl transferase (GGT)		

6. Thyroid hormone assays

On study days -1, 7, and 23, all surviving rats were anesthetized by ether vapor inhalation, and blood samples were collected from the retro-orbital venous plexus of each rat. Blood was collected from nonfasted animals on days -1 and 7 and from overnight fasted animals on days 23. Plasma was separated from the blood samples and analyzed for total triiodothyronine (T3), total thyroxine (T4), and thyroid stimulating hormone (TSH).

7. Urinalysis

Urine was collected on days 29, 30, and 31, in the morning, prior to sacrifice. Feed and water were inaccessible to the test animals during urine collection. The CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific Gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrite
X	Protein	X	Urobilinogen

8. Sacrifice and Pathology

Animals were fasted overnight, anesthetized using pentobarbital and sacrificed by exsanguination. The bodies were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Only tissues from animals in the 0 and 30 ppm treatment groups were examined. Target organs observed in the 30 ppm group were to be examined in the intermediate groups to determine a NOEL The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain
X	Salivary glands	XX	Heart*	X	Sciatic nerve
X	Esophagus	X	Bone marrow*	X	Spinal cord
X	Stomach		(sternum)	XX	Pituitary
X	Duodenum	X	Lymph nodes*	X	Eyes (optic nerve)
X	Jejunum	XX	Spleen*		
X	Ileum	XX	Thymus*		
X	Cecum				
X	Colon		UROGENITAL		GLANDULAR
X	Rectum				
XX	Liver	XX	Kidneys*$^{+}$	XX	Adrenal gland
X	Pancreas	X	Urinary bladder*	X	Lacrimal gland
	Gall bladder	XX	Testes*$^{+}$	X	Mammary gland
		XX	Epididymides	X	Thyroids*$^{++}$ with parathyroids
		XX	Prostate		
		X	Seminal vesicle	X	Harderian gland
	RESPIRATORY	XX	Ovaries*$^{+}$		
		XX	Uterus*		
X	Trachea	X	Vagina		
X	Lungs				
	Muzzle				
	Pharynx				
X	Larynx				OTHER
				X	Bone* (femur, sternum)
				X	Muscle (skeletal)
				X	Skin
				X	All gross lesions and masses*

II. **RESULTS**

A. Observations

1. Mortality - All rats (10/sex) in the 100 ppm treatment groups were found dead on the following study days: one male on day 5; three males and three females on day 6; two males and one female on day 7; one male and three females on day 8; one female on day 9; one male and one female on both days 12 and 14; and, one male on day 15. One male in the 30 ppm treatment group was found dead on day 6.

2. Clinical Signs - In the 100 ppm treatment groups, four males exhibited piloerection, curled up at handling, and appeared thin. Six females exhibited piloerection and appeared thin, and five of these females curled up at handling. Piloerection was observed more often in males than females, affecting one male on 2 days and three males on 5-8 days, compared to five females on 1 or 2 days, and one female on 5 days. Two females had increased motor activity on 1 or 4 days; one of these females was irritable to touch and exhibited tonic/clonic convulsions on 1 day. In the 30 ppm treatment groups, nine males and five females exhibited piloerection, six males and eight females curled up at handling, and five males and six females appeared thin. Piloerection was observed more often in males than females; seven males were affected on 8-11 days and two males were affected on 1 or 4 days, whereas females were affected on 1 or 2 days only. Males and females curled up on handling on 1 or 2 days (3 days for one male), and appeared thin on 1-5 days (10 days for one male). One female also had increased motor activity on 12 days. No other clinical signs could be attributed to treatment effects in any of the treatment groups. Clinical signs observed in the 3 and 0.5 ppm treatment groups were infrequent, were not concentration-related, and were similar to background incidences.

B. Body weight and weight gain

In the 100 ppm treatment groups, the four males and three females that were alive on day 8 had mean body weights 36 and 26% lower, respectively, than the corresponding controls. Body weights and body weight gains of the 0.5 and 3 ppm group males and females did not differ significantly from the respective control groups throughout the study. Final body weight gains of males were 22.83-23.68 g for the 0.5 and 3 ppm treatment and control groups, compared to 15.95 g for the 30 ppm treatment group. Final body weight gains of females were 10.80-11.08 g for the 0.5 and 3 ppm treatment and control groups, compared to 7.32 g for the 30 ppm treatment group. The surviving males and females in the 100 ppm treatment groups lost 6.20 and 4.53 g, respectively, by the end of week 1. In the 30 ppm treatment groups, both sexes had significantly ($p<0.05$) lower body weights throughout the study compared to the corresponding controls. Body weights of the males were 17-18% lower during weeks 1-2 and 14-15% lower during the weeks 3-4 compared to the controls. Body weights of the females were 13% lower during week 1 and 9-10% lower during weeks 2-4 compared to the controls. Body weights of rats in the 3 and 0.5 ppm treatment groups were similar to the controls. (See table 1)

TABLE 1 Body Weight (g) at 28 Days

DOSE	0 ppm	0.5 ppm	3 ppm	30 ppm	100 ppm
MALE					
weight (g)	404.3	402.7	409.0	345.6*	--
FEMALE					
weight (g)	263.5	260.6	251.7	239.9*	--

There were no survivors at 100 ppm
* p=0.05 with Dunnett's test

C. Food and compound consumption

In the 100 ppm treatment groups, the four males and six females that survived study week 1 consumed 73 and 68% (each at $p<0.05$) less food, respectively, than the corresponding controls. Food consumption by the 0.5 and 3 ppm group rats was unaffected by treatment. Food consumption by males and females in the 30 ppm treatment groups was significantly ($p<0.05$) reduced during weeks 1-2 compared to the controls, and was lower but not statistically significant during weeks 3-4. Food consumption by males was 34% lower during week 1, 18% lower during week 2, and 7-8% lower during weeks 3-4 compared to the controls. Food consumption by females was reduced 30% during week 1, and 2-9% during weeks 2-4 compared to the controls. Food consumption by rats in the 3 and 0.5 ppm treatment groups was similar to the controls.

Mean compound consumption (4-week period) for the 0.5, 3, and 30 ppm treatment groups was 0.04, 0.23 and 2.20 mg/kg/day, respectively, for males, and 0.04, 0.24, and 2.32 mg/kg/day, respectively, for females. Compound consumption for the 100 ppm group rats that were alive after week 1 was 3.74 and 3.8 mg/kg/day for males and females, respectively.

TABLE 2 Mean Food Consumption/Day (g) for Week 2

DOSE	0 ppm	0.5 ppm	3 ppm	30 ppm	100 ppm
MALE					
consumption	27.5	27.7	27.8	22.5*	--
FEMALE					
consumption	19.6	19.7	19.8	18.1*	--

There were no survivors at 100 ppm
* p=0.05 with Dunnett's test

D. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted during or after treatment.

E. Blood work

1. Hematology - No differences in hematology parameters between the 0.5, 3, and 30 ppm treatment and control groups were observed.

2. Clinical Chemistry - In the 30 ppm treatment groups, males and females had total bilirubin levels 28 and 33% lower (each at $p<0.01$), and females had aspartate aminotransferase activity 22% higher ($p<0.05$) than the controls. No other differences in clinical chemistry parameters between the treatment and control groups were observed.

F. Thyroid hormone assays

In the 100 ppm treatment groups, females had T3 levels 46% lower, and males and females had T4 levels 63 and 50% lower ($p<0.05$ or 0.01) than the controls on day 7. In the 30 ppm treatment groups, males had T3 and T4 levels 40 and 49% lower (each at $p<0.01$), respectively, and females had T4 levels 61% lower ($p<0.001$) than the controls on day 23. The 30 ppm group males also had T4 levels 33% lower ($p<0.05$) than the controls on day 7. The decreased levels in the 30 ppm treatment groups were reported to be within the normal range of physiological variation for rats of this age and strain. No differences in thyroid hormone levels between the 3 or 0.5 ppm treatment and control groups were observed.

G. Urinalysis

No differences in urine parameters between the 0.5, 3, and 30 ppm treatment and control groups were observed.

H. Sacrifice and Pathology

1. Organ weight - Mean absolute thymus weight for the 30 ppm group females was 29% lower ($p<0.01$) than the control weight. No other differences in organ weights in any of the treatment groups were considered to be treatment-related.

2. Gross pathology

No treatment-related gross postmortem differences between rats in the treatment and control groups were observed. All abnormalities appeared to occur randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - In the 30 ppm female treatment group, the uteri of 2/10 females had minimal endometrial hyperplasia, and the mammary glands exhibited minimal hyperplasia in 2/10 females and increased tissue in 1/10 females. In the 3 ppm female treatment group, 1/10 females had minimal endometrial hyperplasia of the uterus, and 2/10 females had increased mammary gland tissue. No other treatment-related postmortem differences between rats in the treatment and the control groups were observed. All other abnormalities were considered to be of spontaneous origin and of no toxicological significance.

b) Neoplastic - No neoplastic tissue was observed in any treated or control rats.

III. **DISCUSSION**

A. Investigator's Conclusions

The study author concluded that treatment-related effects were observed in rats in the 30 and 100 ppm treatment groups. Effects included piloerection, curling up at handling, decreased body weight, and reduced food consumption. Other effects observed at 30 ppm were decreased total bilirubin in both sexes, and decreased absolute thymus weights in females. One female in the 100 ppm treatment group exhibited convulsions. The NOEL was established at 3 ppm (approximately 0.25 mg/kg/day) for both sexes.

B. Reviewer's Discussion

We agree with the study author that treatment-related effects were observed at 30 and 100 ppm, and that the NOEL for this study is 3 ppm for both sexes.

Rats in the 3 and 0.5 ppm treatment groups did not appear to be affected by treatment.

One 30 ppm group male died during the study. Clinical signs observed at the 30 ppm treatment level were piloerection (9 males, 5 females), curling up at handling (6 males, 8 females), and thin appearance (5 males, 6 females). Males exhibited piloerection in males on more study days than in females; most males were affected on 8-11 days, whereas females were affected on 1-2 days only. In general, curling up on handling was observed on 1 or 2 days, and thin appearance was observed on 1-5 days. Increased motor activity was observed in one female for 12 days. Mean weekly body weights were lower in males

(14-18%) and females (9-13%) compared to the controls. Both sexes had decreased total bilirubin (males, 28%; females, 33%), and females had increased aspartate aminotransferase activity (22%) compared to the controls. Both sexes consumed less food than the controls throughout the study; the differences were significant ($p<0.05$) during weeks 1-2 (males, 18-34%; females, 8-30%) but not during weeks 3-4 (both sexes, ≤9%). Males had T3 and T4 levels 40 and 49% lower (each at $p<0.01$), respectively, and females had T4 levels 61% lower ($p<0.001$) than the controls on day 23. The 30 ppm group males also had T4 levels 33% lower ($p<0.05$) than the controls on day 7. The decreased T3 and T4 levels were reported to be within the normal range of physiological variation for rats of this age and strain. The decreased mean absolute thymus weight in females ($p<0.01$; 29% lower) lacked associated histological changes, and therefore, cannot be attributed to treatment.

Rats in the 100 ppm treatment groups exhibited behavioral changes and died prematurely. All rats (10/sex) in the 100 ppm treatment groups were found dead between study days 5 and 15. Observed clinical signs were piloerection (4 males, 6 females), curling up at handling (4 males, 5 females), and thin appearance (4 males, 6 females). As with the 30 ppm treatment group, piloerection was observed in males on more study days than in females, occurring on 5-8 days in most affected males compared to 1 or 2 days in most affected females. Two females had increased motor activity on 1 or 4 days; one of these females was also irritable to touch and exhibited tonic/clonic convulsions on 1 day. Body weights of the surviving rats were decreased (males, 36%; females, 26%), as was food consumption (69-73%) during the first week of treatment. Decreased T3 levels in the surviving females and decreased T4 levels in both sexes on day 7 were attributed by the study author to the general toxicity of MB 46513. Based on 100% mortality observed at this dose level by study day 15, we agree that MB 46513 exerts a generalized toxic effect.

No other differences were considered to be treatment-related in any of the treatment groups. Minimal changes in the uteri and mammary glands of the 3 and 30 ppm group females were of too low an incidence (1-2 females/group) to conclude a treatment relationship.

The upper dose level of MB 46513 that should be used in longer term studies appears to be between 3 and 30 ppm. This conclusion is based on the apparent lack of life-threatening effects at this dose level, and the distinct potential for life-threatening effects at the 30 ppm treatment level, as demonstrated by significant decreases in food consumption and body weights observed in rats treated at this dose level.

IV. STUDY DEFICIENCIES

No scientific deficiencies were noted in this rangefinding study.

DATA EVALUATION RECORD

MB46513 (PHOTODEGRADATE OF FIPRONIL)

Study Type: 82-1b; Subchronic Toxicity (Feeding) Study in Dogs

Work Assignment No. 3-23G (MRIDs 44262812 and 44262810)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by
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Primary Reviewer William Spangler, Ph.D.	Signature: William J. Spangler Date: 9/17/97
Secondary Reviewer Joan Harlin, M.S.	Signature: Joan E Harlin Date: 10/1/97
Project Manager Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date:
Quality Assurance Reto Engler, Ph.D.	Signature: Date:

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T.
Reregistration Action Branch 1 (7509C)

Work Assignment Manager: Marion Copley, D.V.M., D.A.B.T.
Reregistration Action Branch 1 (7509C)

11/21/97

11/21/97

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [feeding] - dog
OPPTS Number: 870.3151 OPP Guideline Number: §82-1b

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (96.0% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole

CITATION: Dange M. (1996) MB46513. 90-Day toxicity study in the dog by dietary administration. Rhône-Poulenc Agrochimie, Centre de Recherche, 355, rue Dostoïevski, BP153, F-06903 Sophia Antipolis Cedex, France. Laboratory Study SA 95100. May 14, 1996. MRID 44262812. Unpublished.

Dange M. (1995) MB46513. Preliminary 28-day toxicity study in the dog by dietary administration. Rhône-Poulenc Agrochimie, Centre de Recherche, 355, rue Dostoïevski, BP 153, F-06903 Sophia Antipolis Cedex, France. Laboratory Study SA 94143. September 5, 1995. MRID 44262810. Unpublished.

SPONSOR: Rhône-Poulenc Agrochimie, 14-20, rue Pierre Baizet, BP 9163, F-69263 Lyon Cedex 09, France

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44262812 - main; 44262810 - range-finding), MB46513 (96.0% a.i.), a photodegradate of fipronil, was administered to 5 beagle dogs/sex/dose by feeding at dose levels of 0, 3.5, 9.5, or 35 ppm (mean achieved dosages of 0, 0.10, 0.27, or 0.95 mg/kg/day for males and 0, 0.10, 0.29, or 1.05 mg/kg/day for females) for 90 days. In a rengefinding study, 2 dogs/sex/dose (0, 27, 80, or 270 ppm) were treated with MB 46513 for 28 days.

In the **28 day rangefinding study** ½ males at 27 ppm (1 mg/kg/day) had convulsions at 28 days and animals at 80 ppm had clinical signs as early as day 4 (this group had to be sacrificed early due to extreme toxicity). In the **main study**, 35 ppm group, 1/5 females was sacrificed

prematurely after exhibiting increase salivation, prostration, writhing, tremors, absence of rotular reflex, noisy breathing, and dyspnea. Histopathological examination of this female after 28 days revealed multifocal myocardial necrosis associated with intramural coronary arteritis. Behavioral changes were observed in a second female in the 35 ppm treatment group consisting of excessive barking and aggressivity on one occasion and irritability, tremors, and increased salivation on another occasion. No other treatment-related behavioral effects were observed in the 35 ppm treatment group. No treatment-related behavior effects were observed in any dogs in the 9.5 or 3.5 ppm treatment groups. No treatment-related differences in ophthalmology, hematology, clinical blood chemistry or urinalysis parameters or gross pathology were observed between dogs in any treatment group and the controls. No neoplastic tissue was observed in any of the treatment groups. **The LOEL is 35 ppm (1.05 mg/kg/day), based on behavioral changes in 2/5 females. The NOEL is 9.5 ppm (0.29 mg/kg/day).**

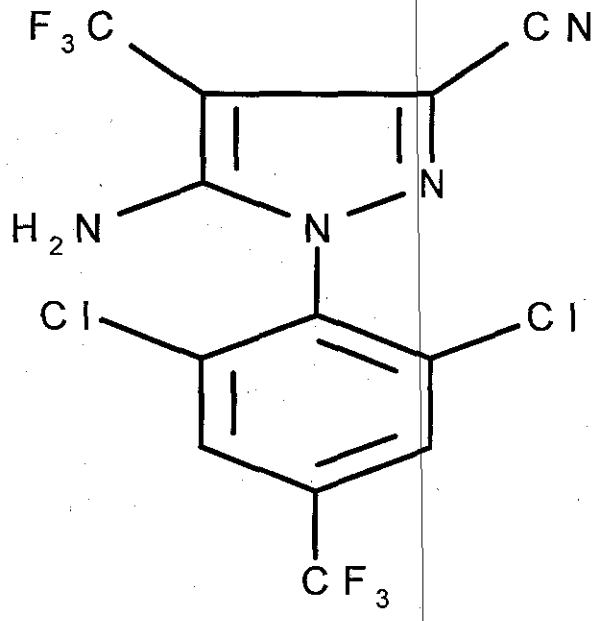
This subchronic toxicity study is classified **acceptable** and satisfies the guideline requirements for a subchronic oral study (**§82-1b**) in non-rodents.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MB46513
   Description: Yellow solid
   Lot/Batch #: 805 DAP
   Purity: 96.0% a.i.
   Stability of compound: The undiluted test article was shown to be stable for at least 2 years at ambient temperature and for at least 35 days in feed either frozen or stored at room temperature
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Dog
   Strain: Beagle (pedigreed)
   Age and weight at study initiation: 35 weeks of age;
   body weights 9.1-12.3 kg for males; 6.7-9.6 kg for females
   Source: Harlan France, 03800 Gannat, France
   Housing: Individually housed in stainless steel kennels of 1.2 m^2
   Diet: Certified Canine Meal JAPE 21 (Pietrement-Extra Labo, 77 650 Sainte Colombe, France), 300 g of diet/animal was moistened with 500 mL of water at feeding and presented each morning for 2 hours
   Water: Municipal tap water, ad libitum
   Environmental conditions:
   Temperature: 18-21 C
   Humidity: 40-70%
   Air changes: 10-15 per hour
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 29 days

B. STUDY DESIGN:

1. In life dates - Start: 05/03/95 End: 08/04/95

2. Animal assignment

Animals (40/sex) were assigned to the test groups in Table 1 to insure similar body weight distribution among groups for each sex.
Table 1: Study design.

Test Group	Dose to Animal (mg/kg/day)	Animals Assigned	
		Male	Female
1 Control	0	5	5
2 Low	3.5	5	5
3 Mid	9.5	5	5
4 High	35	5	5

3. Dose selection rationale

In a 28-day range-finding study (MRID 44262810), MB46513 (97.5% a.i.) was administered in the diet to 2 beagle dogs/sex at 0, 27 (1 mg/kg/day), 80, or 270 ppm.

High toxicity at 80 and 270 ppm resulted in early termination of these treatment groups (days 10-15). Clinical signs at both treatment levels of toxicity occurred as early as day 4 (80 ppm) and day 2 (270 ppm) and included absence of feces, emaciation, decreased food consumption, and weight loss. In addition, 80 ppm beagles exhibited reduced motor activity, irritability, staggering gait, and increased salivation. Thymuses were reduced in size. Livers were pale, whitish, or mottled, and histopathological examination revealed diffuse sinusoidal leukocytosis, centrilobular hepatocytic enlargement, mild multifocal hepatocytic hydropic degeneration, and chronic hepatitis with periportal fibrosis. At 27 ppm, small thymuses and pale livers indicative of thymic atrophy and liver damage were observed in the absence of histopathological findings. One/2 males had clonic convulsions on the last day of the study. There were no changes in ophthalmologic, clinical chemistry, or urinalysis parameters at any treatment level. A NOEL was not established (<27 ppm) in this study.

Based on these results, dose levels of 3.5, 9.5, and 35 ppm were selected for the definitive study.

4. Diet preparation and analysis

Diets were prepared seven times (periods unspecified) throughout the study by dry-mixing appropriate amounts of MB46513 with JAPE 21 diet and were stored at room temperature in polyethylene containers until used. Controls received untreated diet.

Prior to the study, the homogeneity and stability of the 4 and 40 ppm diet preparations were determined. Samples were collected from the top, middle, and bottom of each preparation to determine homogeneity. Stability was determined for both freezer storage (<-15 C) and room temperature (20 C) for 35 and 65 days.

In addition, concentration of test diets prepared for use in the study were determined. The homogeneity of the pre-mixture prior to and during the study was confirmed. The samples collected from each diet preparation were analyzed for concentration.

Results - Homogeneity Analysis:
The concentration of MB46513 in the pre-test feeds were:
4 mg/kg: 80-98% of nominal
40 mg/kg: 92-102% of nominal

Stability Analysis:
Storage at 20 C:
4 mg/kg/35 days: 85% of nominal
4 mg/kg/65 days: 105 of nominal
40 mg/kg/35 days: 96% of nominal
40 mg/kg/65 days: 107% of nominal
Storage at <-15 C:
4 mg/kg/35 days: 88% of nominal
4 mg/kg/65 days: 95% of nominal
40 mg/kg/35 days: 104% of nominal
40 mg/kg/65 days: 98% of nominal

Concentration Analysis:
3.5 ppm - 86-114% of nominal
9.5 ppm - 93-106% of nominal
35 ppm - 89-105% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Body weights, body weight gains, food consumption, hematology, clinical chemistry and urinalysis variables for exposed groups were compared to controls by use of the Bartlett test for homogeneity of variances and analysis of variance (ANOVA). If Bartlett's test indicated homogeneous variances and the ANOVA was significant, the data were analyzed using Dunnett's test. If Bartlett's test indicated heterogeneous variances, non-parametric analysis was performed using the Kruskal-Wallis non-parametric one way analysis of variance by ranks. If the Kruskal-Wallis test was significant, the Mann-Whitney test was used to compare each group to the control.

Terminal body weights, organ weights, organ-to-body weight ratio and organ-to-brain weight ratios were analyzed using Dunnett's test when Bartlett's test indicated homogeneous variances. If Bartlett's test indicated nonhomogeneous variances, variables were analyzed using the Kruskal-Wallis non-parametric one way analysis of variance by ranks. If the Kruskal-Wallis test was significant, the data were analyzed using the Mann-Whitney test. The tests were conducted at the 1 and 5% levels of significance.

C. METHODS:

1. Observations:

All animals were observed for mortality, health effects, and behavioral changes twice daily during the weekdays and once daily on weekend days and holidays. Kennels were examined daily for vomitus, blood, or diarrhea. Each dog was given a weekly physical examination with emphasis on:

- Fur and skin
- Eyes, ears, teeth, and gums
- Mucous membranes
- Rectal temperature
- Gait, stance, and general behavior
- Chest including heart and respiratory rate
- Abdomen including palpation
- External genitalia and mammary glands

A weekly neurological examination was also performed which included:

- Cranial nerves reflexes: Pupillary light and consensual light, palpebral blink and corneal reflex.
- Segmental reflexes: Flexor (withdrawal) including crossed extensor.
- Postural reactions: Placing reactions, visual, and tactile.

2. Body weight

Animals were weighed twice prior to the initiation of dosing, weekly during treatment, and before necropsy.

3. Food consumption and compound intake

Food consumption (g) for each animal was measured daily during the treatment period. Mean food consumption for each test group was calculated weekly during the study period.

4. Ophthalmoscopic examination

Ophthalmological examinations were performed on both eyes of each dog using focal illumination and indirect ophthalmoscopy prior to dose initiation and during weeks 6 and 13 of the study. Mydriasis was induced with an atropinic agent and examination was performed using a SOLA binocular indirect ophthalmoscope.

5. Blood

Twice prior to dosing and during weeks 6 and 13 of treatment, blood was collected from the jugular vein of each dog. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
			Direct bilirubin
	ENZYMES	X	Total serum protein (TP)*
		X	Triglycerides
X	Alkaline phosphatase (ALK)		Serum protein electrophoresis
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Gamma glutamyl transpeptidase		

* Required for subchronic toxicity studies.

6. Urinalysis*

Urine was collected from all test animals in the morning 8 days prior to treatment and on study days 42 and 85. Access to water was not restricted during urine collection. The CHECKED (X) parameters were examined.

X	Appearance	X	Protein
	Volume	X	Glucose
	Specific Gravity	X	Ketone bodies
X	pH	X	Bilirubin
X	Sediment (microscopic)	X	Occult Blood
X	Refractive Index	X	Urobilinogen

*· Urinalysis is not required for subchronic toxicity studies.

7. Sacrifice and Pathology

The single female found in distress was euthanized and necropsied. The remaining test animals were sacrificed by exsanguination and were subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examinations, which were performed on all animals from the control and high-dose groups and on the kidney, liver, lung, heart, and gallbladder only from the mid- and low-dose groups. The (XX) organs, in addition, were weighed. For low- and mid-dose animals, only the kidneys, liver, lungs, heart, and gallbladder were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		Pituitary*
X	Duodenum*	XX	Spleen*	XX	Eyes (optic n.)*
X	Jejunum*	XX	Thymus*	X	
X	Ileum*				
X	Cecum*				GLANDULAR
X	Colon*		UROGENITAL		
X	Anus				
X	Rectum*	XX	Kidneys*+	XX	Adrenal gland*
XX	Liver*+	X	Urinary bladder*		Harderian gland
XX	Gall bladder*	XX	Testes*+		Lacrimal gland[T]
X	Pancreas*	XX	Epididymides	X	Mammary gland[T]
		XX	Prostate	XX	Thyroids with
		X	Seminal vesicles		parathyroids*
	RESPIRATORY	XX	Ovaries*+		
		X	Oviducts		OTHER
	Trachea*	XX	Uterus*		
X	Lung*			X	Bone (femur and
X	Nose				sternum)*
	Pharynx			X	Skeletal muscle*
X	Larynx			X	Skin*
				X	All gross lesions and masses*

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic toxicity studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations:

1. Mortality - One 35 ppm female was sacrificed *in extremis* on day 28 of the study.

2. Clinical Signs - The 35 ppm female that was sacrificed exhibiting increased

salivation, prostration, writhing, tremors, absence of rotular reflex, noisy breathing and dyspnea prior to sacrifice. A second female treated at 35 ppm exhibited excessive barking and aggressivity on day 84 and increased salivation, irritability, and tremors on day 86. These changes in behavior were considered to be treatment-related because they were typical of the effect of fipronil in related toxicological studies. All other changes in behavior and appearance were considered to be normal for the beagle and unrelated to treatment.

B. Body weight and weight gain

No significant differences in body weight and body weight gain were observed between treated and control animals of either sex.

C. Food consumption and compound intake

1. Food consumption - No significant differences in food consumption were noted for any treatment group.

2. Compound consumption - The average compound consumption for MB46513 is presented in Table 2. During the study, male and female dogs ingested 105-121% of the nominal dose of MB46513.

Table 2. Average consumption of MB46513 in dogs during 90-day feeding study.[a]

Test Group	Treatment rate (ppm)	Measured Dose (mg/kg/day)	
		Male [mean]	Female [mean]
Control	0	0	0
Low	3.5	0.09-0.10 [0.10]	0.09-0.11 [0.10]
Mid	9.5	0.27 [0.27]	0.27-0.30 [0.29]
High	35	0.90-0.99 [0.95]	0.91-1.15 [1.05]

[a] Data extracted from table on page 67 of the study report.

D. Ophthalmoscopic examination

There were no treatment-related ophthalmoscopic findings at any treatment level.

E. Blood work

1. Hematology - No treatment related differences in hematology parameters were observed between the treated and control groups.

2. Clinical Chemistry - No treatment-related differences in clinical blood chemistry parameters were observed between the treated and control groups.

F. Urinalysis - The urine pH in 35 ppm males was 7.10, compared to 6.30 in controls ($p<0.01$) at 85 days. No other effects were observed in urine parameters of dogs at any treatment level.

G. Sacrifice and Pathology:

1. Organ weight - No treatment-related differences in organ weights were observed in any of the treatment groups.

2. Gross pathology - No treatment-related differences in gross pathology were observed between dogs in the treated and control groups. No gross pathological changes were observed at necropsy in the 35 ppm female sacrificed *in extremis* after 28 days of treatment.

3. Microscopic pathology

a) Non-neoplastic - No treatment-related microscopic changes were observed in tissues examined. Histopathological examination of organs and tissues from the high-dose female sacrificed after 28 days of treatment revealed moderate acute multifocal myocardial necrosis associated with acute intramural coronary arteritis.

b) Neoplastic - No neoplastic tissue was observed in dogs in the treatment or control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the LOEL for MB46513 is 35 ppm (1.05 mg/kg/day) based on behavioral effects in one female. The NOEL is 9.5 ppm. The only changes that the author believed were a result of treatment were behavioral changes observed in one female consisting of excessive barking and aggressivity on one occasion and irritability, tremors, and increased salivation on another occasion. One other 35 ppm female, exhibiting signs of toxicity including salivation, prostration, writhing, tremors, absence of rotular reflex, noisy breathing, and dyspnea, was sacrificed after 28 days of treatment. The author believed that the clinical signs observed in the sacrificed female were due to coronary arteritis and myocardial necrosis based on histopathological findings. He also

believed that the clinical signs and histopathological findings were not treatment-related.

B. Reviewer's Discussion

We agree with the study author's conclusion that the behavioral effects observed in 1/5 females in the 35 ppm treatment group resulted from treatment. However, his conclusion that the signs of toxicity observed in the 35 ppm female sacrificed *in extremis* after 28 days of treatment were not treatment-related is equivocal. The signs of toxicity including salivation, prostration, writhing, tremors, absence of rotular reflex, noisy breathing, and dyspnea were similar to the behavioral changes observed in another 35 ppm female whose behavior was attributable to treatment. That this female also exhibited coronary arteritis and myocardial necrosis may be incidental.

No treatment-related differences in body weight, body weight changes, food consumption, hematology, clinical chemistry, urinalysis, organ weights, gross morphological changes, and histopathology were observed in any treatment group compared to controls. No treatment-related effects were observed in males at the highest dose tested. The LOEL is 35 ppm (1.05 mg/kg/day) for females, based on behavioral changes in 2/5 females, and the NOEL is 9.5 ppm (0.29 mg/kg/day).

C. Study deficiencies

No deficiencies were noted in this study.

DATA EVALUATION RECORD

MB46513 (Photodegradate of Fipronil)

Study Type: 82-1a; Subchronic Oral Toxicity (Feeding) Study in Mice

Work Assignment No. 3-23E (MRID 44262811)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 1 (7509C)

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4/4/97

4/5/97

DATA EVALUATION RECORD

STUDY TYPE: 90-Day subchronic toxicity [dietary]- mouse
OPPTS Number: 870.3100 OPP Guideline Number: §82-1a

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (96% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl-phenyl)-4-trifluoromethylpyrazole; RPA097801; RPA591085

CITATION: Bigot, D. (1996) MB 46513. 90-Day toxicity study in the mouse by dietary administration. Rhone-Poulenc Agrochimie, Centre de Recherche, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France. Study SA 95055. January 12, 1996. MRID 44262811. Unpublished.

SPONSOR: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet, BP 9163, 69263 Lyon Cedex 09, France.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44262811), MB46513 (a photodegradate of fipronil; 96% a.i.) was administered to OF1 mice (10/sex/dose) in the diet at nominal dose levels of 0, 0.5, 2, or 10 ppm (13-week measured mean 0, 0.08, 0.32 or 1.74 mg/kg/day for males; 0, 0.11, 0.43, or 2.15 mg/kg/day for females) for 13 weeks.

In the 10 ppm treatment group, 9/10 males died prematurely (between days 20 and 62) and 1/10 was sacrificed moribund (day 84); 1/10 females died on day 5. On one occasion each, two of the males exhibited excessive jumps, and on several occasions one male exhibited aggressiveness and/or irritability. Diffuse centrilobular hypertrophy of the liver was noted in 6/10 males. The severity of the condition was described as mild in the five males that died prematurely and moderate in the one male that was sacrificed. The liver of the sacrificed animal also had moderate multifocal mitotic figures and mild multifocal extramedullary hematopoiesis. In addition, three males had enlarged livers and four had atrophied thymus glands. The organs of

males in the 10 ppm treatment group were not weighed. The organs of females in the 10 ppm treatment group appeared normal. In the 2 ppm treatment group, two males on two occasions each exhibited aggressive and irritable behavior with increased motor activity in one of them. Although 1 male and 1 female in the 0.5 ppm treatment group exhibited aggressive behavior (total of four occasions) this could not be definitively attributed to treatment because; 1) low frequency, 2) only one sign; 3) no effect in females at any higher dose. No differences in organ weights or gross or microscopic pathology were observed between mice in the 2 or 0.5 ppm treatment groups and the controls. Body weights, food consumption, and clinical blood chemistry were not affected in any treatment group. No neoplastic tissue was observed in mice in the treatment and control groups. Hematology, ophthalmoscopic and urine analyses were not conducted during the study. **The LOEL for this study is 2 ppm (0.32 mg/kg/day), based on the aggressive and irritable behavior with increased motor activity in males. The NOEL is 0.5 ppm (0.08 mg/kg/day) .**

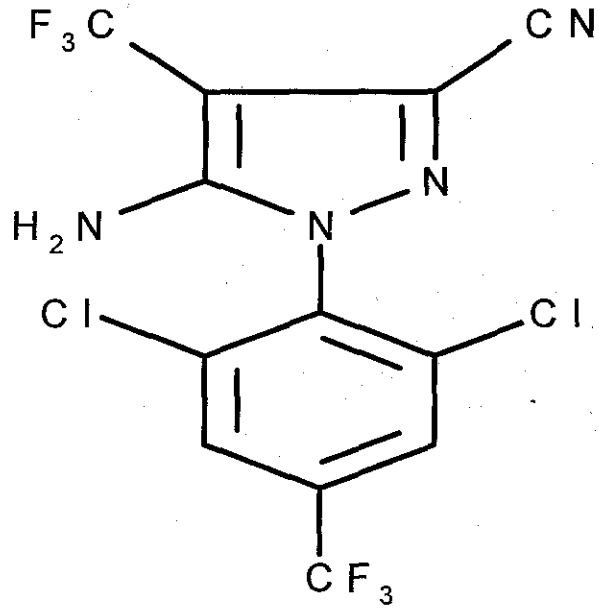
This 90-day subchronic toxicity study (dietary) is classified **acceptable** and satisfies the Subdivision F guideline requirement for a subchronic toxicity study in rodents (§82-1a).

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Quality Assurance, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB46513
   Description: Yellow solid
   Lot/Batch #: 805-DAP
   Purity: 96% a.i.
   Stability of compound: Stable during 2 years of storage at room temperature (page 266 of the study report)
   CAS #: None
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Mouse
   Strain: OF1
   Age and weight at initiation of treatment: 6-7 weeks of age; males - 28.2-32.6 g; females - 22.2-24.8 g
   Source: Iffa-Credo, 69210 L'Arbresle, France
   Housing: Housed individually in suspended stainless steel wire mesh cages
   Diet: Certified rodent diet AO4C P1 (UAR, Villemoisson-sur-Orge, France), ad libitum

Water: Municipal tap water, ad libitum
Environmental conditions:
Temperature: 20-24 C
Humidity: 40-70%
Air Changes: 10-15 per hour
Photoperiod: 12-Hour light/dark cycle
Acclimation period: 8 Days

B. STUDY DESIGN

1. In life dates - Start: 3/23/95 End: 6/22/95

2. Animal assignment

Mice were assigned to the test groups in Table 1 one day prior to the initiation of treatment using a weight-biased computerized randomization procedure.

Table 1: Study design.[a]

Test Group	Nominal dose to animal (ppm)	Animals assigned	
		Male	Female
1 Control	0	10	10
2 Low	0.5	10	10
3 Mid	2	10	10
4 High	25	10	10

[a] Doses were selected on the basis of a 28-day toxicity study with the mouse using MB46513. The 28-day study was not provided to review, and no information about the study methodology or results were included in this MRID.

3. Treatment preparation

The test diet was prepared at 0 and 7 weeks and stored at -18 C until use. MB46513 was dissolved in acetone and mixed with sufficient feed to achieve a 20 ppm premix. The premix was then diluted with additional feed to obtain the desired dose levels. Samples of each diet preparation were collected for concentration analyses.

The homogeneity and stability of MB46030 in the diet at 0.1 and 10 ppm was established in prestudy SA 95055-AO. This report was appended to the study for review. The treated feed was prepared as described. Samples (30 g) were collected from the top, middle, and bottom of the feed mixes. Homogeneity samples were analyzed on the day of preparation. Stability samples were stored at -15 C or ambient temperatures for up to 94 days prior to analysis.

Results:
Homogeneity:
- 0.1 ppm: 100-110% of nominal (mean 105%)
- 10 ppm: 86-96% of nominal (mean 90.0%)
- 20 ppm: 88-93% of nominal (mean 90.3%)

Stability analysis:
- Storage at ambient temperature (approximately 20 C)
  - 0.1 ppm:
    - 0 days: 105% of nominal
    - 60 days: 120% of nominal
    - 94 days: 100% of nominal
  - 10 ppm:
    - 0 days: 90% of nominal
    - 60 days: 88% of nominal
    - 94 days: 84% of nominal
- Storage at less than -15 C
  - 0.1 ppm:
    - 0 days: 105% of nominal
    - 60 days: 110% of nominal
    - 94 days: 100% of nominal
  - 10 ppm:
    - 0 days: 90% of nominal
    - 60 days: 89% of nominal
    - 94 days: 90% of nominal

Concentration analysis (for weeks 0 and 7):
- 0.5 ppm: 86 and 78% of nominal
- 2 ppm: 105 and 100% of nominal
- 10 ppm: 88 and 82% of nominal

The analytical data indicated that the mixing procedure was adequate. Variance between nominal and actual dosage to the animals was >10% for the 0.5 and 10 ppm treatment diets. The study author considered a variance of ≤25% acceptable for concentrations ≤10 ppm.

4. Statistics

The equality of means for data from the treatment groups was established using Bartlett's test of homogeneity of variances. If the variances were found to be equal, the data were analyzed by standard one-way ANOVA followed by Dunnett's t-test. If variances proved to be unequal, the data were analyzed by the Kruskal-Wallis non-parametric one-way analysis of variance by ranks. If the Kruskal-Wallis test was significant, the data were analyzed using the Mann-Whitney test. All statistical analyses were performed using SAS programs. The tests were conducted at the 5 and 1% levels.

C. METHODS

1. Observations

Animals were observed twice daily (once on weekends) for mortality, moribundity, and clinical signs of toxicity. Animals were given a detailed physical examination at least once each week.

2. Body weight

Body weights were measured once during acclimatization, on the first day of treatment, weekly during treatment, and prior to necropsy.

3. Food and compound consumption

Food consumption was measured weekly during treatment. Food consumption was reported as g food/animal/day. Mean compound intake for each group was calculated on a weekly basis and was reported as mg/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted only during the acclimatization period.

5. Blood

Blood was collected from all surviving animals prior to necropsy. Animals were fasted overnight prior to the collection of blood from the retro-orbital venous plexus under ether anesthesia. The CHECKED (X) parameters were examined in all samples analyzed.

a. Hematology

No hematological parameters were measured during the study.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
	Chloride*		Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*	X	Cholesterol
	Potassium*		Globulin
	Sodium*		Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES		Triglycerides
X	Alkaline phosphatase		
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
	Gamma glutamyl transferase (GGT)		

* Required for subchronic toxicity studies.

6. Urinalysis

Urinalysis was not conducted during the study.

7. Sacrifice and Pathology

Animals were fasted overnight, anesthetized using pentobarbital and sacrificed by exsanguination. The bodies were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. All tissues from animals in the 0, 2, and 10 ppm treatment groups were examined; the liver, lung, and kidneys were examined in the 0.5 ppm treatment groups. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Sciatic nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord*
X	Stomach*		(sternum)	X	Pituitary*
X	Duodenum*	X	Lymph nodes*	X	Eyes (optic nerve)
X	Jejunum*	XX	Spleen*		
X	Ileum*	X	Thymus*		
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
X	Rectum*				
XX	Liver*+	XX	Kidneys*+	X	Adrenal gland*
X	Pancreas*	X	Urinary bladder*		Lacrimal gland
X	Gall bladder	XX	Testes*+	X	Mammary gland
		X	Epididymides	X	Thyroids*++ with
		X	Prostate		parathyroids*
		X	Seminal vesicle	X	Harderian gland
	RESPIRATORY	X	Ovaries*+		
		X	Uterus*		
X	Trachea*	X	Vagina		
X	Lungs*				
	Muzzle				
	Pharynx				
X	Larynx				OTHER
				X	Bone*
					(sternum)
				X	Articular surface
					(femoro-tibial)
				X	Muscle*
					(skeletal)
				X	Skin*
				X	All gross lesions and masses*

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic studies.
++ Organ weight required for non-rodent studies. T = required only when toxicity or target organ.

II. RESULTS

A. Observations

1. Mortality - Ten mice died prematurely, and an eleventh mouse was sacrificed moribund. All premature deaths were in the 10 ppm treatment groups. One female died on day 5. Nine males died on days 20, 28 (4 males), 39, 48, 52, and 62. The moribund male was sacrificed on day 84.

2. Clinical Signs - One male and 1 female in the 0.5 ppm treatment group and 2 males in the 2 ppm treatment group exhibited aggressiveness often accompanied by irritability to touch and/or increased motor activity (males only). Two of the males in the 10 ppm treatment group that died prematurely exhibited excessive jumps; one male that was sacrificed moribund exhibited aggressiveness, irritability, and increased motor activity. One female in the 10 ppm treatment group exhibited excessive vocalization. In general cases, the behavior was observed on only one or two occasions. No animals in the control groups exhibited similar behavior.

   No other clinical signs could be attributed to treatment effects.

B. Body weight and weight gain

Body weights of males and females in all treatment groups were not significantly different from the control groups throughout the study. At the end of the treatment period, mean body weights of males in the 0, 0.5, and 2.0 ppm treatment groups ranged from 41.62 to 42.71 g. Body weights for males in the 10 ppm treatment group were within 6% of the control throughout the study, with the exception of the final weighing interval for this group (day 78) when the weight of the single surviving mouse was 10% lower than the control group. Mean body weights of females in all treatment groups ranged from 29.40 to 30.24 g at the end of the treatment period.

C. Food and compound consumption

Mean food consumption was not affected by treatment.

Mean compound consumption (13-week period) for the 0.5, 2, and 10 ppm treatment groups were 0.08, 0.32 and 1.74 mg/kg/day, respectively, for males, and 0.11, 0.43, and 2.15 mg/kg/day, respectively, for females.

D. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted during or after treatment.

E. Blood work

1. Hematology - No hematological parameters were measured during the study.

2. Clinical Chemistry - No significant treatment-related differences in clinical blood chemistry were observed between the treated and control animals. Although females in the 10 ppm treatment group had a higher (61%, $p<0.05$) mean alkaline phosphatase (AP) activity compared to the controls, this difference could be attributed to one female in the 10 ppm treatment group that had a very high AP activity of 258 IU/L and

one female in the control group that had a very low AP activity of 17 IU/L. If these two mice are eliminated from the calculations of the means, mean AP activities for the 0, 0.5, 2, and 10 ppm female treatment groups are 80, 95, 102, and 90 IU/L, respectively.

F. Urinalysis

Urinalysis was not performed during the study.

G. Sacrifice and Pathology

1. Organ weight - No differences in organ weights were observed between male mice in the 0, 0.5, and 2 ppm treatment groups. Organs of the male mice in the 10 ppm treatment group were not weighed. No differences in organ weights were observed between female mice in the treated and control groups.

2. Gross pathology - In the 10 ppm treatment group, three male mice had enlarged livers and four had atrophied thymus glands. No other treatment-related gross postmortem differences were observed between mice in the treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Diffuse centrilobular hypertrophy of the liver was noted in 6/10 males in the 10 ppm treatment group. The severity of the condition was described as mild in the five males that died at 28 or 39 days and moderate in the sixth male, which was sacrificed moribund at 84 days. The liver of the male that was sacrificed moribund also had moderate multifocal mitotic figures and mild multifocal extramedullary hematopoiesis. The liver of the female in the 10 ppm treatment group that died prematurely was autolytic and could not evaluated. Liver abnormalities that could be attributed to treatment were not observed in the controls or any treated animals surviving until the termination of the study.

No other treatment-related postmortem differences were observed between mice in the treated and the control groups. All other abnormalities appeared to occur randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in mice in the treatment and control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the LOEL for this study is 10 ppm, based on the premature deaths and histopathological changes in the liver of males in this treatment group. The NOEL was reported to be 2 ppm. The study author suggested that since the observed aggressiveness/irritability to touch/increased motor activity observed in all treatment groups was not concentration-dependent and of low incidence, it should not be used to establish the LOEL and NOEL.

B. Reviewer's Discussion

We disagree with the study author that the LOEL for this study is 10 ppm.

Although the aggressive behavior does not appear to be concentration-dependent since it is observed at approximately the same frequency at all treatment levels, the premature deaths of all male mice in the 10 ppm treatment group beginning after only 3 weeks of treatment confounds interpretation of the response data.

The premature deaths of all males in the 10 ppm treatment groups clearly indicate that this dose level is too high for long term clinical studies done with males. Although aggressive behavior was observed at the 2 and 0.5 ppm treatment levels, this response does not appear to be toxicologically significant at these dose levels. There were no other treatment-related responses in the male 2 and 0.5 ppm treatment groups. There were no signs of toxicity in any female treatment group, including the 10 ppm group. Although 1/10 females in the 10 ppm treatment group died on day 5, this death does not appear to be treatment-related since females as a group did not respond to treatment and since males, clearly more sensitive to MB46513 than females, did not begin to die until day 20. None of the nine surviving females in the high dose group exhibited any response to treatment, including changes in body weights, blood chemistry, organ weights, and histopathology. Therefore, the upper dose level of MB46513 that should be used in long term studies appears to be about 6 ppm (1 mg/kg/day) for males and greater than 10 ppm (2.15 mg/kg/day) for females.

On the basis of the high mortality and occurrence of liver abnormalities in the 10 ppm male treatment group, and the lack of significant toxicity responses to treatment at 2 ppm, the maximum dose level for MB46513 should be slightly less than 10 ppm.

In summary: Two animals of the same sex at 2 ppm displayed multiple signs on two or three occasions of findings observed in other fipronil and fipronil photodegradate MB 46513 studies. In addition, these signs were observed in males at higher doses but not in controls. Therefore effects at 2 ppm can be attributed to treatment even though there are

not other signs of toxicity at this dose. At 0.5 ppm however, the signs can not be definitively attributed to treatment due to low incidence, only a single sign and in females there were no occurances of signs at higher doses. Therefore, the LOEL for this study should be 2 ppm.

IV. STUDY DEFICIENCIES

Limited blood analyses were conducted during the study but this is not expected to alter the conclusions of the study.

DATA EVALUATION RECORD

Fipronil Metabolite MB45897

Study Type: Acute Oral Toxicity (§81-1)

Work Assignment No. 3-23A (MRID 44262819)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

Pesticide Health Effects Group
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Signature: Mary L Menetrez
Date: 9/18/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 1 (7509C)

Work Assignment Manager: M. Copley, DVM, DABT
Registration Action Branch (7509C)

11/6/97

11/6/97

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS Number: 870.1100 OPP Guideline Number: §81-1

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Fipronil Metabolite MB45897 (purity not specified)

SYNONYMS: 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl-phenyl)pyrazole

CITATION: Hayes, G. (1988) MB 45897 (a metabolite of fipronil) acute oral toxicity study in the rat. Toxicol Laboratories Limited, Ledbury, Herefordshire, England. Laboratory Project Identification A/O/4855. June 22, 1988. MRID 44262819. Unpublished.

SPONSOR: Rhone-Poulenc Limited, Rainham Road South, Dagenham, Essex, RM10 7XS, England.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44262819), five young adult Sprague Dawley-derived albino rats/sex were given a single oral dose of Fipronil Metabolite MB45897 (purity not specified) at 2,000 mg/kg. The test substance was administered in sesame oil. Animals were observed for clinical signs of toxicity and mortality for up to 14 days postdosing.

Oral LD_{50} Males = >2,000 mg/kg (observed)
Females = >2,000 mg/kg (observed)

Fipronil Metabolite MB45897 is classified as **TOXICITY CATEGORY III** based on the observed LD_{50} values for both sexes.

All animals survived the 14-day observation period. Hypoactivity was observed between 1 and 4 hours in all animals, and was no longer evident by day 1. No other effects were observed during the 14-day observation period. No treatment-related effects on body weight were observed, and gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

This study is classified **acceptable (§81-1)** and satisfies the guideline requirement for an acute

oral study in the rat.

COMPLIANCE: Signed and dated GLP (1988, United Kingdom), Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MB45897
   Description: Brown powder
   Ref #: 8JJW 1888
   Purity: Not specified
   CAS #: Not provided

2. Vehicle: The test material was formulated in sesame oil to achieve a dose volume of 10 mL/kg.

3. Test animals: Species: Albino rat
   Strain: Sprague Dawley derived [Crl:CD(SD)BR]
   Age: Young adult (approximately 5-8 weeks)
   Weight: 150-179 g males; 126-141 g females
   Source: Charles River Limited, Margate, Kent, UK
   Acclimation period: ≥5 Days
   Diet: Special Diet Services SQC R and M No. 1, ad libitum
   Water: Tap water, ad libitum
   Housing: Five/cage, separated by sex

B. STUDY DESIGN and METHODS:

1. In-life dates: December 21, 1987 - February 2, 1988

2. Animal assignment and treatment: Dose selection for the definitive study was based on the results of a preliminary test conducted with two animals/sex and dose levels of 313, 625, 1,250, or 2,500 mg/kg (reportedly the highest attainable concentration based on the physical nature of the material). All animals survived the 14-day preliminary test.

   Following an overnight fasting period, five young adult rats/sex were given a single oral dose of Fipronil Metabolite MB45897 at 2,000 mg/kg by gavage; the study author reported that this was the limit dose. The test substance was formulated in sesame oil prior to administration. The rats were observed for

signs of gross toxicity and/or mortality frequently on the day of dosing and once daily thereafter for up to 14 days. Body weights were recorded at 0 (prior to dosing), 7, and 14 days. At 14 days, all animals were sacrificed, necropsied, and examined for gross pathological changes.

3. Statistics: Not applicable to this study.

II. RESULTS AND DISCUSSION:

A. Mortality: All animals survived the 14-day observation period.

Oral LD_{50} Males = >2,000 mg/kg (observed)
Females = >2,000 mg/kg (observed)

B. Clinical observations: Hypoactivity was observed between 1 and 4 hours in all animals, and was no longer evident by day 1. No other effects were observed during the 14-day observation period.

C. Body Weight: No significant effect on body weight was observed during the study. Animals exhibited overall (0-14 days) average increases of 61% for males and 44% for females.

D. Necropsy: Gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

E. Deficiencies: Although initial body weights for female animals were below the desired 150 g, this deficiency does not significantly effect the results of the study and is considered minor.

DATA EVALUATION RECORD

Fipronil Metabolite MB45897

Study Type: Acute Dermal Toxicity (§81-2)

Work Assignment No. 3-23B (MRID 44262820)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
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Date: 9-16-97

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Project Manager:
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Date: 9/18/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: M. Copley, DVM, DABT
Registration Action Branch 1 (7509C)

Work Assignment Manager: M. Copley, DVM, DABT
Registration Action Branch 1 (7509C)

M Copley 11/6/97

M Copley 11/6/97

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat
OPPTS Number: 870.1200 OPP Guideline Number: §81-2

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Fipronil Metabolite MB45897 (purity not specified)

SYNONYMS: 5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl-phenyl)pyrazole

CITATION: Hayes, G. (1988) MB 45897 (a metabolite of fipronil) acute dermal toxicity study in the rat. Toxicol Laboratories Limited, Ledbury, Herefordshire, England. Laboratory Project Identification A/D/4856. April 22, 1988. MRID 44262820. Unpublished.

SPONSOR: May and Baker Limited, Rainham Road South, Dagenham, Essex, RM10 7XS, England.

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44262820), five young adult Sprague Dawley-derived albino rats/sex were dermally exposed to Fipronil Metabolite MB45897 (purity not specified) at 2,000 mg/kg (limit dose) for 24 hours. Animals were observed for clinical signs of toxicity and mortality for up to 14 days following administration.

Dermal LD_{50} Males = >2,000 mg/kg (observed)
Females = >2,000 mg/kg (observed)

Fipronil Metabolite MB45897 is classified as **TOXICITY CATEGORY III** based on the observed LD_{50} values in both sexes.

All animals survived the 14-day observation period. Perinasal staining was observed in 8/10 animals 4 hours following dosing. No other effects were observed during the 14-day observation period. No dermal irritation was reported. No treatment-related effects on body weight were observed, and gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

Since the actual size of the application sites were not specified, this study may not currently fulfill guideline requirements for an acute dermal study in the rat and is classified **unacceptable**

(§81-2). This study may be upgraded to acceptable status if additional information is provided verifying that the application sites comprised at least 10% of the total body surface area.

COMPLIANCE: Signed and dated GLP (1988, United Kingdom), Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MB45897
   Description: Brown powder
   Ref #: 8JJW 1888
   Purity: Not specified
   CAS #: Not provided

2. Vehicle: The test material was moistened with distilled water prior to application.

3. Test animals: Species: Albino rat
   Strain: Sprague Dawley derived [Crl:CD(SD)BR]
   Age: Young adult
   Weight: 183-198 g males; 153-173 g females
   Source: Charles River Limited, Margate, Kent, UK
   Acclimation period: 13 Days
   Diet: SQC R and M No. 1 expanded, Special Diet Services, ad libitum
   Water: Tap water, ad libitum

B. STUDY DESIGN and METHODS:

1. In-life dates: February 4-18, 1988

2. Animal assignment and treatment: Fur from the dorsal trunk areasof 5 animals/sex was clipped 1 day prior to dermal administration of Fipronil Metabolite MB45897 at 2,000 mg/kg (limit dose). The test substance was moistened with distilled water, and evenly applied to the prepared site (the size of the application area was not specified). Each treatment site was covered with a 4-ply gauze pad and overlaid with a strip of aluminum foil secured with Elastoplast elastic adhesive bandage. The coverings were removed 24 hours following application, and the test sites were gently wiped with water-moistened cotton wool. The rats were observed for signs of toxicity and/or mortality frequently on the day of dosing and once daily

thereafter for up to 14 days. Body weights were recorded at 0 (prior to dosing), 8, and 14 days. At 14 days, all animals were sacrificed, necropsied, and examined for gross pathological changes.

3. Statistics: Not applicable to this study.

II. RESULTS AND DISCUSSION:

A. Mortality: All animals survived the 14-day observation period.

Dermal LD_{50} Males = >2,000 mg/kg (observed)
Females = >2,000 mg/kg (observed)

B. Clinical observations: Perinasal staining was observed in 8/10 animals 4 hours following dosing. No other effects were observed during the 14-day observation period. No dermal irritation was reported, and it was not clear if dermal irritation was included as part of the clinical observations.

C. Body Weight: No significant effect on body weight was observed during the study. Animals exhibited overall (0-14 days) average increases of 48% for males and 23% for females.

D. Necropsy: Gross necropsy of animals sacrificed after 14 days revealed no visible lesions.

E. Deficiencies: The actual size of the application sites were not reported. Since Subdivision F guidelines specify that the application site cover at least 10% of the total body surface area (or approximately 28 cm^2 for a 170-g rat), this study may not fulfill guideline requirements and is currently deemed unacceptable. This study may be upgraded to acceptable status if additional information is provided verifying that the application sites comprised at least 10% of the total body surface area.

Although initial body weights for all animals were below the desired 200-300 g, this deficiency does not significantly effect the results of the study and is considered minor.

012509

DATA EVALUATION RECORD

MB 45897 (Intermediate of Fipronil)

Study Type: N/A; 4-Week Oral (Gavage) Toxicity Study in Rats

Work Assignment No. 3-23C (MRID 44262821)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Work Assignment Manager: Marion Copley, D.V.M., D.A.B.T.
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mcopley 11/6/97

mcopley 11/6/97

DATA EVALUATION RECORD

STUDY TYPE: 4-Week oral toxicity [gavage]- rat
OPPTS Number: N/A OPP Guideline Number: N/A

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB 45897 (99.7% a.i.)

SYNONYMS: 5-amino-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carbonitrile; Pyrazole; Intermediate of Fipronil (MB46030); RPA097920; RPA591060

CITATION: Johnson, I.R. (1995) Pyrazole/MB45897/RPA09720 intermediate of fipronil (MB46030): Four-week oral toxicity study in the rat. Amended Final Report. Pharmaco LSR Ltd, Eye, Suffolk IP23 7PX, England. Amended Final Report No. 95/RHA535/0684. June 28, 1995. MRID 44262821. Unpublished.

SPONSOR: Rhone-Poulenc Agrochimie, Centre de Recherche, 355 Rue Dostoievski, B.P. 153, F-06903 Sophia Antipolis Cedex, France

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 44262821), MB 45897 (intermediate of fipronil; 99.7 a.i.) was administered to CD rats (5/sex/dose) by gavage at nominal dose levels of 0, 50, 200 or 1000 mg/kg/day for 4 weeks.

In the 1000 mg/kg/day treatment groups, the livers of males and females had increased absolute (32-58%) and relative (53-70%) weights, and the livers of 3/5 males exhibited periacinar hypertrophy with cytoplasmic vacuolation. Total plasma protein levels were increased (10-19%) in both sexes, and alanine aminotransferase activity (48%) was increased in females. All rats salivated excessively (days 2-28), and exhibited hunched posture (days 8, 9, 11, and 12), underactivity (days 8-13), and staggered gaits (day 8). Males (4-5) and females (2-3) had hair loss (days 3-28). Both sexes had reduced body weight gains (16%), and females were slightly anemic. In the 200 mg/kg/day treatment groups, all rats salivated excessively (days 3-28) and exhibited hunched posture (days 8 and/or 11 and 12). Males had increased (9%) total plasma

protein levels. In the 50 mg/kg/day treatment groups, all rats salivated excessively (days 8-15, males; days 8-28, females) This may have been due to local irritation of the test material. No rats died as a result of treatment. Ophthalmoscopic exams and urinalysis were not conducted. No treatment-related differences in food consumption were observed in any treatment group. No neoplastic tissue was observed in treated or control rats. **The LOEL for this study is 200 mg/kg/day, based on hunched posture in all rats treated at this dose level. The NOEL is 50 mg/kg/day.**

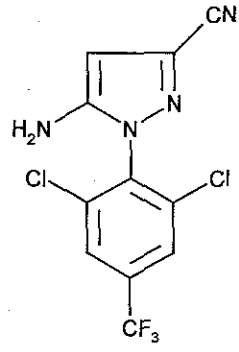
This 4-week oral toxicity study is classified **acceptable (non-guideline)** as it is not a required guideline study. It is acceptable for the purposes for which it was intended, that being to assess the systemic toxic effects following 4 weeks of repeated daily administration (gavage) of the test substance.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB 45897
   Description: Beige powder
   Lot/Batch #: 94 3026 DA 942
   Purity: 99.7% a.i.
   Stability of compound: Stable for a minimum of 9 months when stored in the dark at 13 C
   CAS #: 120068-79-3
   Structure:



2. Vehicle and/or positive control: Maize oil

3. Test animals: Species: Rat
   Strain: CD
   Age and weight at initiation of treatment: 34 to 41 days of age; males - 112-133 g; females - 98-128 g
   Source: Charles River (UK) Limited, Margate, Kent, England
   Housing: Housed in groups of five of similar sex and treatment group in suspended stainless steel grid cages with mesh floors and lids
   Diet: Laboratory Rodent Diet (RM1(E) SQC, Special Diet Services, Witham, Essex, England), ad libitum
   Water: Municipal tap water, ad libitum, via water bottles
   Environmental conditions:
      Temperature: 19-25 C
      Humidity: 40-70%
      Air Changes: ≥10 per hour
      Photoperiod: 12-Hour light/dark cycle
   Acclimation period: ≥5 Days

B. STUDY DESIGN

1. In life dates - Start: 8/30/94 End: 9/27/94

2. Animal assignment

Rats were assigned to the test groups in Table 1 on arrival using computer-generated random allocation.

Table 1. Study design.[a]

Test Group	Nominal dose to animal (mg/kg/day)	Animals assigned	
		Male	Female
1 Control	0	5	5
2 Low	50	5	5
3 Mid	200	5	5
4 High	1000	5	5

[a] Dose levels were selected based on the results of a preliminary study in which rats were treated with MB 45897 by gavage at 0, 10, 50, 200 or 1000 mg/kg/day for 7 consecutive days. Clinical signs and body weights were recorded. Rats in the 1000, 200, and 50 mg/kg/day salivated excessively throughout the treatment period. In addition, rats in the 1000 mg/kg/day treatment groups had reduced body weight gains. Based on these results, dose levels of 50, 200 and 1000 mg/kg/day were selected for the definitive study.

3. Treatment preparation

Prior to the initiation of treatment, batches of low and high concentrations (10.0 and 200 mg/mL) of MB 45897 were prepared in maize oil. To determine homogeneity, six aliquots were collected from locations evenly spaced throughout each bulk preparation. To determine stability, samples of each solution were stored at room temperature and duplicate aliquots were analyzed after 24 or 48 hours of storage.

Dosing solutions were prepared fresh daily by diluting appropriate amounts of MB 45897 to yield solution concentrations of 10.0, 40.0, and 200.0 mg/mL. The test solutions were administered daily by gavage. Rats were given a constant dosing volume of 5 mL/kg body weight. Rats in the control group were given a similar volume of untreated maize oil. Doses were adjusted daily to reflect body weight changes, and were administered at a similar time each day. To confirm the

concentration of MB 45897 in the treatment solutions used in the study, duplicate samples of each formulation prepared on Days 1 and 25 of treatment were analyzed.

Results:
Homogeneity:
10.0 mg/mL: 93.7-95.7% of nominal (mean 94.6%)
200 mg/mL: 97.0-101% of nominal (mean 99.0%)
Stability analysis (storage at 21 C):
10.0 mg/mL:
0 hours: 94.6% of nominal
24 hours: 88.9-94.0% of nominal
48 hours: 92.5-94.6% of nominal
200 mg/mL:
0 hours: 99.0% of nominal
24 hours: 97.5-100% of nominal
48 hours: 98.8% of nominal
Concentration analysis (weeks 1 and 4):
10 mg/mL: 91.4 and 86.7% of nominal
40 mg/mL: 100 and 93.8% of nominal
200 mg/mL: 100 and 99.0% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

Mean body weight changes, hematology and blood chemistry parameters were analyzed using Student's t-tests using a pooled variance. Organ weights were tested for homogeneity of variance using Bartlett's test. If variance was homogeneous, Behren's-Fisher test was used for pairwise comparison. If variance was heterogeneous, data were analyzed using Dunnett's test. Macroscopic and microscopic histopathological changes were analyzed using Fisher's Exact Probability Test. All tests were conducted at the two-tailed level. Confidence intervals were 0.1, 1, and 5% for the Student's t-test, and 1 and 5% for all other tests.

C. METHODS

1. Observations

Animals were observed once daily for mortality and moribundity. Immediately before and after dosing (twice daily), animals were checked for systemic toxicity or ill-health; a third check for systemic toxicity or ill-health was performed on all full work days. Animals were given a detailed physical examination including palpation each week.

2. Body weight

Each animal was weighed on the day of treatment and twice weekly throughout the treatment period.

3. Food consumption and food efficiency

Food consumption by each group of rats was determined weekly during treatment by measuring the amount of food given and the amount of food remaining in the hopper, and combining the total amount with an estimate of the amount of food scattered. Food consumption was reported as g food/animal/week. Weekly mean food conversion ratios for each group were calculated using the formula:

[food consumption (g/week) ÷ body weight gain (g/week)]

It was stated that food conversion ratios for treatment week 4 were not presented due to the disturbance in food consumption during routine blood sample collection.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted during the study.

5. Blood

Blood was collected from the retro-orbital sinus of each animal after 4 weeks of treatment. The blood samples were collected following overnight food withdrawal. The CHECKED (X) hematology and clinical blood chemistry parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Iron	X	Blood urea nitrogen
	Magnesium		Total Cholesterol
	Phosphorus	X	Globulins
X	Potassium	X	Glucose
X	Sodium		Phospholipids
		X	Protein electrophoresis
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)
		X	A/G Ratio
	Alkaline phosphatase (AP)		
	Cholinesterase		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)		
X	Serum aspartate aminotransferase (also AST, SGOT)		
	Gamma glutamyl transferase (GGT)		

6. Urinalysis

Urinalysis was not performed during the study.

7. Sacrifice and Pathology

Animals were killed by carbon dioxide asphyxiation. The bodies were subjected to gross pathological examination. The adrenals, heart, kidneys, liver, spleen, and testes were collected for histological examination. In addition, the adrenals, kidneys, liver, and testes were weighed. Samples were also taken of all macroscopic abnormalities.

II. RESULTS

A. Observations

1. Mortality - No animals died prematurely.

2. Clinical Signs - Post-dose excessive salivation was observed in all treated rats (5/sex/group); the onset of occurrence was concentration-dependent. Salivation was

observed daily on days 2-28 in the 1000 mg/kg/day treatment groups; days 3-28 in the 200 mg/kg/day treatment groups; and days 8-28 in the 50 mg/kg/day group (days 8-15, males; days 8-28 females). In the 1000 mg/kg/day treatment groups, all rats had hunched posture on days 8, 9, 11, and 12, were underactive on days 8-13, and had staggering gaits on day 8. In addition, hair loss was observed in 4-5 males and 2-3 females on days 3-28. In the 200 mg/kg/day treatment groups, hunched posture was observed in all males (5/5) on days 8, 11, and 12, and in all females (5/5) on days 11 and 12. No other differences in clinical signs were observed between any of the treatment and control groups.

B. Body weight and weight gain

Body weight gains for the male 1000 mg/kg/day treatment group were 16% ($p<0.01$) lower than the control weight gains after 4 weeks of treatment. Body weights and body weight gains for the male 50 and 200 mg/kg/day treatment groups were similar to the controls. Body weight gains for the 50, 200 and 100 mg/kg/day female treatment groups, respectively were 16, 12 and 16% lower ($p<0.01$; 14-19 g) than the control gains after 4 weeks (Table 2).

Table 2. Mean body weights and body weight gains (g) of rats before and after treatment with MB 45897.[a]

Treatment rate (mg/kg/day)	Body weight (g)		4-Week body weight gain	
	0 Week	4 Weeks	Total (g)	% of Control gain
Males				
0	127	330	203	---
50	121	325	204	0
200	121	316	195	-4
1000	126	297	171**	-16
Females				
0	118	234	116	---
50	109	206	97**	-16
200	114	216	102*	-12
1000	119	217	98**	-16

[a] Data obtained from Table 2, pages 29 and 30, in the study report.
* Statistically different from the control, $p<0.05$.
** Statistically different from the control, $p<0.01$.

C. Food consumption and food efficiency

Mean food consumption for all treatment groups was similar to the controls throughout the study, ranging from 90 to 105% of the control values.

Weekly food conversion ratios (weeks 1-3) for the male 1000 mg/kg/day treatment group were 8-23% higher than the controls, and for the male 50 and 200 mg/kg/day treatment groups were similar to the controls. Weekly food conversion ratios for all female treatment groups were variable; the overall mean (weeks 1-3) was 12% higher for the 50 and 200 mg/kg/day treatment groups, and 17% higher for the 1000 mg/kg/day treatment group compared to the controls.

D. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted during the study.

E. Blood work

1. Hematology - The 1000 mg/kg/day treatment group males and females had hemoglobin concentrations 7 and 10% ($p<0.05$ or 0.001) lower, respectively, compared to the controls (Table 3). The 1000 mg/kg/day group females also had 6-7% ($p<0.05$) lower hematocrits and RBC counts relative to the controls. No other differences in any of the treatment groups were considered toxicologically or biologically significant.

Table 3. Hemoglobin, hematocrits, and RBC counts of male and female rats after 4 weeks of treatment with MB 45897.[a]

Test Group (mg/kg/day)	Hematocrit(%)	Hemoglobin (g%)	RBC (mil/cm^2)
Males			
0	47	15.7	7.99
50	45	14.9	7.60
200	46	15.2	7.74
1000	45	14.6*	7.63
Females			
0	43	14.6	7.47
50	43	14.9	7.70
200	43	14.8	7.69
1000	40*	13.1**	6.99*

[a] Data obtained from Table 4, pages 32 and 33, in the study report.
* Statistically different from the control, $p<0.05$.
** Statistically different from the control, $p<0.001$.

2. Clinical Chemistry - In the 1000 mg/kg/day treatment groups, total plasma protein levels in males and females were 19 and 10% (each at $p<0.001$) higher, respectively, and alanine aminotransferase (ALAT) activity in females was 48% ($p<0.05$) higher compared to the controls. In the 200 mg/kg/day treatment groups, total plasma protein levels in males were increased 9% ($p<0.01$) compared to the controls. No other differences in any of the treatment groups were considered to be treatment-related or biologically significant.

F. Urinalysis

Urinalysis was not performed during the study.

G. Sacrifice and Pathology

1. Organ weight - Both sexes in the 1000 mg/kg/day treatment groups had increased ($p<0.01$) absolute and relative (organ weight/body weight) liver weights compared to the controls (Table 4). Absolute liver weights for males and females were 32 and 58% higher, respectively, and corresponding relative liver weights were 53 and 70% higher than the control liver weights.

Table 4. Mean absolute (g) and relative (organ weight/body weight x 100) liver weights of rats following 4 weeks of treatment with MB 45897.[a]

Treatment rate (mg/kg/day)	Liver	
	Absolute	Relative
Males		
0	12.1	3.83
50	13.3	4.24
200	12.8	4.31
1000	16.0*	5.86*
Females		
0	8.8	3.97
50	8.4	4.24
200	8.7	4.28
1000	13.9*	6.76*

[a] Data obtained from Tables 6A and 6B, pages 36 and 37, in the study report.

* Statistically different from the control, $p<0.01$.

2. Gross pathology

No treatment-related gross postmortem differences were observed between treated and control rats.

3. Microscopic pathology

a) Non-neoplastic - Periacinar hypertrophy with cytoplasmic vacuolation was observed in the livers of 3/5 males in the 1000 mg/kg/day treatment group. No females exhibited hepatocytic hypertrophy.

No other treatment-related postmortem differences were observed between rats in the treated and the control groups. All other abnormalities appeared to occur randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in any treated or control rats.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that slightly lower RBC counts and hematocrits and slightly increased ALAT activities in the 1000 mg/kg/day treatment groups were the only treatment-related effects observed. It was concluded that no toxicologically significant functional disturbance or morphological change occurred at dose levels ≤1000 mg/kg/day. The NOEL was reported to be 200 mg/kg/day.

B. Reviewer's Discussion

We disagree with the study author and believe that the LOEL for this study is 200 mg/kg/day and that the NOEL should be 50 mg/kg/day, based on clinical signs observed in the 200 mg/kg/day treatment groups that appear to be treatment-related.

At the 200 mg/kg/day treatment level, hunched posture was observed in all rats on several study days, in addition to salivation during days 2-28. That this behavior is treatment-related is supported by similar findings at the highest dose level of 1000 mg/kg/day. No 50 mg/kg/day or control rats exhibited hunched posture. Although increased total plasma protein levels in males ($p<0.01$; 9%) cannot be unquestionably attributed to treatment in the absence of corroborative findings, this conclusion is supported by increased total protein levels observed in the 1000 mg/kg/day group animals. In addition to hunched posture and salivation, the 1000 mg/kg/day group rats exhibited hair loss, underactivity, and staggering gait. Both sexes had increased absolute liver weights (males, 32%; females, 58%) and relative liver weights (males, 53%; females, 70%), and the livers of 3/5 males exhibited periacinar hypertrophy with cytoplasmic vacuolation. Both sexes had reduced body weight gains ($p<0.01$; each 16%), and females were slightly anemic. In both sexes, increased total plasma protein levels (males, 19%; females, 10%), and in females, increased ALAT activity (48%) were possibly indicative of liver damage.

At the lowest dose level of 50 mg/kg/day, excessive salivation was the only effect that could be considered treatment-related. Excessive salivation may have been a response to the test substance's irritant properties that increased in severity with increasing concentration and/or a response to dosing. The onset of occurrence was concentration-dependent, with initial observations of excessive salivation on day 2 in the 1000 mg/kg/day group rats, day 3 in the 200 mg/kg/day group rats, and day 8 in the 50 mg/kg/day group rats. The fact that salivation occurred earliest at the highest dose level and later at the lowest dose level could also indicate an irritant response rather than a toxic reaction to the test substance.

The decreased body weights in the female treatment groups were not clearly treatment-related since the decreases were not concentration-dependent, and body weights for the corresponding male treatment groups were similar to the control weights. All control females had high body weights and all treated females had low body weights. Therefore, the control female body weights may be an aberration.

The data demonstrate that this intermediate of fipronil is clearly much less toxic than the parent compound or MB 46513, the photodegradate of fipronil.

IV. STUDY DEFICIENCIES

No scientific or guideline deficiencies were noted in this study.

DATA EVALUATION RECORD

MB45897-FIPRONIL METABOLITE

Study Type: §84-2; *Salmonella typhimurium*/Mammalian Activation Gene Mutation Assay

Work Assignment No. 3-23M (MRID 44262822)

Prepared for

Health Effects Division
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U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Toxicology Branch I (7509C)

Nancy E. McCarroll 11/12/97

EPA Work Assignment Manager: M. Copley, DVM, DABT
Registration Action Branch I (7509C)

M Copley

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella typhimurium* mammalian activation gene mutation assay
OPPTS Number: 870.5265 OPP Guideline Number: §84-2

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB45897 (99.7% a.i.)

SYNONYMS: 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carbonitrile; RPA097920; RPA591060

CITATION: Percy A. (1996): *Salmonella Typhimurium* Reverse Mutation Assay (Ames Test). Rhône-Poulenc Agrochimie, Sophia Antipolis Cedex, France. Laboratory Report Study SA 95345, February 5, 1996. MRID 44262822. Unpublished

SPONSOR: Rhône-Poulenc Agrochimie, 14-20, Rue Pierre Baizet, BP 9163, F-69263 Lyon Cedex, France

EXECUTIVE SUMMARY: In initial and repeat plate incorporation and initial and repeat preincubation microbial mutagenicity assays (MRID 44262822), *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to MB45897 (99.7% a.i.) in DMSO over dose ranges of 25-2,500 µg/plate in the presence of mammalian metabolic activation (+S9) or 12.5-2,500 µg/plate in the absence of metabolic activation (-S9). Preparations for metabolic activation were made from Aroclor 1254 induced rat livers.

MB45897 was insoluble at 2,500 µg/plate (±S9). Cytotoxicity was also observed in the majority of strains at 2,500 µg/plate +/-S9 under all assay conditions. The positive control substances induced marked increases in revertant colonies in the respective strains. **There were, however, no reproducible, dose-related differences in the number of revertant colonies in any tester strain at any dose level/condition compared to the vehicle controls.**

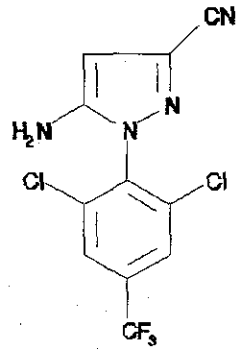
This study is classified as **Acceptable (Guideline)** and satisfies the requirement for FIFRA Test Guideline **84-2** for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB45897
   Description: Beige powder
   Lot/Batch #: 94 3026
   Purity: 99.7%
   Stability of compound: Not provided
   CAS #: 120068-79-3
   Structure:



   Vehicle used: Dimethyl sulfoxide (DMSO)
   Other comments: The test article was stored at room temperature in a light-resistant container.

2. Control Materials
   Negative: Vehicle served as negative control
   Vehicle/final concentration: DMSO/0.1 mL/plate

   Positive:
   Nonactivation:

2-Nitrofluorene	1 µg/plate	*S. typhimurium* TA98
9-Aminoacridine	50 µg/plate	*S. typhimurium* TA1537
Sodium azide	1 µg/plate	*S. typhimurium* TA100 and TA1535
Cumene hydroperoxide	200 µg/plate	*S. typhimurium* TA102

   Activation:

2-Aminoanthracene	2 µg/plate	*S. typhimurium* TA98, TA100, TA1535, and TA1537
2-Aminoanthracene	5 µg/plate	*S. typhimurium* TA102

3. Metabolic Activation
S-9 was derived from:

X	Aroclor 1254	X	Induced	X	Rat	X	Liver

The S9 mix was prepared immediately prior to use and contained: S-9 fraction (10%, v/v), $MgCl_2$ (8 mM), KCl (33 mM), NADP (4 mM), glucose-6-phosphate (5 mM), and sodium phosphate buffer (100 mM). The final concentration of S9 in culture was approximately 2%.

4. Test organisms

S. typhimurium strains:

	TA97	X	TA98	X	TA100	X	TA102
	TA104	X	TA1535	X	TA1537		TA1538

Properly maintained? **Yes**
Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Preliminary cytotoxicity test
Nine dose levels (1, 10, 50, 100, 250, 500, 1,000, 2,500, and 5,000 µg/plate) were evaluated in duplicate with strain TA100 in both the presence and absence of S9 activation. The assay included duplicate vehicle controls and no positive controls.

Mutagenicity assays
First Mutagenicity test:
Experiment 1: 100, 250, 500, 1,000, and 2,500 µg/plate (±S9)
Experiment 2: 25, 50, 100, 250, 500, 1,000, and 2,500 µg/plate (-S9) and 100, 250, 500, 1,000, and 2,500 µg/plate (+S9)

Second Mutagenicity test: Included a pre-incubation step.
Experiment 1: 50, 100, 250, 500, 1,000, and 2,500 µg/plate (-S9) and 100, 250, 500, 1,000, and 2,500 µg/plate (+S9)
Experiment 2: 12.5, 25, 50, 100, 250, 500, 1,000, and 2,500 µg/plate (-S9) and 25, 50, 100, 250, 500, and 1,000 µg/plate (+S9)

The assays included positive and vehicle controls in triplicate.

B. TEST PERFORMANCE

1. Type of Salmonella assay

X	standard plate test *

* The second mutagenicity test included a liquid pre-incubation step.

2. Protocol

Cultures of the tester strains were grown for 10 hours in nutrient broth prior to plating. The stock cultures of TA102 were supplemented with ampicillin (25 µg/mL) and tetracycline (2 µg/mL). The test substance and the positive controls (except sodium azide) were diluted in DMSO to specified concentrations; vehicle for sodium azide was ultrapure water. Bacteria (0.1 mL), test substance, vehicle, or positive control (0.1 mL), and 0.5 mL of S9 mix (or sodium phosphate buffer for tests without metabolic activation) were added to 2.0 mL melted top agar (supplemented with 0.5 mM biotin and 0.5 mM histidine). The mixture was mixed thoroughly and poured on plates containing a layer of minimal agar medium. After the top agar solidified, the plates were inverted and incubated at 37° C for 72 hours. For the liquid pre-incubation step in the second mutagenicity assay, bacteria (0.1 mL), test substance, vehicle, or positive control (0.1 mL), and 0.5 mL of S9 mix or sodium phosphate buffer were pre-incubated at 37° C for 60 minutes prior to the addition of the melted top agar (2.0 mL) and subsequent plating over minimal agar and incubation at 37° C for 72 hours.

For the preliminary cytotoxicity assay, duplicate plates were prepared for each dose, strain, and condition, including duplicate vehicle controls, but no positive controls. For the mutagenicity assays, triplicate plates were prepared for each dose, strain, and condition, including vehicle and positive controls. The plates were evaluated for gross toxicity (reduction of background lawn relative to vehicle controls) and total revertant colony numbers. Revertant colonies were counted by an automatic colony counter.

3. Evaluation Criteria

(a) Assay validity: The assay was considered valid if the following criteria were met: (i) the S9 mix and the solution containing the highest concentration of the test substance were not contaminated, (ii) the number of revertants in the vehicle controls were within the range of provided historical controls, (iii) the number of revertants in the positive controls were in concordance

with values previously observed, (iv) the genetic characteristics of the tester strains were confirmed, and (v) overnight culture viabilities were within the range of 10^9-10^{10} bacteria/mL.

(b) Positive response: The test material was considered mutagenic if there was a reproducible, dose related biologically significant increase in the number of revertants in ≥1 strain, with or without S9 activation. Statistical methods could be used to aid in evaluating the test.

(c) Negative response: The test material was considered non-mutagenic if there was no biologically significant increase in the number of revertants in any strain at any dose tested in two independent mutagenicity assays.

II. REPORTED RESULTS

A. Analytical determinations:
Data regarding concentration and stability analyses were not provided.

B. Preliminary cytotoxicity assay
Nine concentrations of the test substance ranging from 1 to 5,000 µg/plate were evaluated in duplicate with and without S9 activation using strain TA100. Cytotoxicity, apparent as inhibition of bacterial lawn growth, was observed at 2,500 and 5,000 µg/plate (-S9) and at 5,000 µg/plate (+S9). Test material precipitation occurred at ≥2,500 µg/plate (±S9).

C. Mutagenicity assay

Plate incorporation tests: Two trials of the plate incorporation assay were conducted. The initial trial was conducted with 100-2500 µg/plate +/-S9 and the repeat trial was performed with 25-2500 µg/plate -S9 and 100-2500 µg/plate +S9. The repeat trial was performed to insure that an adequate number of noncytotoxic doses were evaluated with S. typhimurium strains TA1535 and TA100. Results from both trials were in good agreement and indicated that the test article was insoluble at 2,500 µg/plate (±S9), induced a reproducible cytotoxic effect in strains TA1535 and TA100 at ≥1000 µg/plate -S9, and was cytotoxic for all strains at 2,500 µg/plate -S9, and for the majority of the strains at 2,500 µg/plate +S9. There was, however, no indication that MB45897 induced a mutagenic response in any strain either in the presence or absence of S9 activation. Slight increases (1.3-to 1.4-fold) in revertant colonies of strain TA102 were seen at 250 to 1,000 µg/plate +S9 (initial trial). However, the increases were not reproducible and the concurrent revertant colony count for DMSO (289) was below the historical control range (383-500).

Results of the initial and repeat experiments for the plate incorporation assay are presented as Attachment I to this DER (Study Report, Legend and Tables 3.3-3.6 and 4.3-4.6 pp. 28, 34, 35, 38, and 39).

Pre-incubation tests: Doses of 50-2500 µg/plate -S9 or 100-2500 µg/plate +S9 (initial trial) or 12.5-2500 µg/plate -S9 or 25-1000 µg/plate +S9 (repeat trial) were investigated using the preincubation modification to the standard assay. The repeat trial was undertaken to ensure that a sufficient number of noncytotoxic concentrations of the test material were assayed. As expected, MB45897 was more cytotoxic under pre-incubation conditions, causing a thinning of the background lawn of growth and a decrease in revertant colonies of strains TA1535 and TA100 at ≥250 µg/plate -S9. Cytotoxicity was apparent for all stains at the highest nonactivated dose tested (2,500 µg/plate) and at ≥1,000 µg/plate +S9. In agreement with the findings from the plate incorporation assay, however, MB45897 was not mutagenic. Results of the initial and repeat experiments of the pre-incubation assay are presented as Attachment II to this DER (Study Report, Tables 5.3-5.6 and 6.3-6.6 pp. 42, 43, 46, and 47).

Under all conditions, the positive control substances induced marked increases in revertant colonies in their respective strains.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions: The study author concluded that, under the conditions of this study, MB45897 was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 with or without metabolic activation.

B. Reviewer's Discussion: The reviewers agree with the study author's conclusions that MB45897 was assayed over an appropriate dose range as it was tested to cytotoxic concentrations (≥2,500 µg/plate +/-S9) with five *S. typhimurium* strains and failed to induce a genotoxic response. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. The study is, therefore, classified as **acceptable**.

IV. **STUDY DEFICIENCIES**: Data on the analysis of dose formulations for actual concentration and stability were not submitted. These deficiencies do not alter the conclusions of this review because cytotoxicity was observed.

ATTACHMENT I

Study Report Legend and Tables 3.3-3.6 and 4.3-4.6
pp. 28, 34, 35, 38, and 39

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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LEGENDS

a : Slight to moderate thinning of the bacterial background lawn
b : Extreme thinning of the bacterial background lawn
c : Extreme thinning of the bacterial background lawn with microcolony formation making the reading of the plate difficult
d : Presence of noticeable macroscopic precipitate on the plate
e : Large amount of macroscopic precipitate on the plate making the reading of the plate difficult
C : Contaminated plate
U : Plate unreadable
N.R. : No result obtained
S.D. : Standard deviation

3.3 - Mean number of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500	Positive controls
TA98	36	33	36	43	41	24 ad	290
S.D.	*2.9*	*5.5*	*5.5*	*3.0*	*4.6*	*7.9*	*24.2*
TA100	144	140	136	120 a	132 a	N.R. cd	596
S.D.	*3.1*	*5.2*	*3.8*	*17.1*	*14.4*		*22.3*
TA1535	12	13	8	8 a	13 a	10 bd	429
S.D.	*3.2*	*0.6*	*2.6*	*3.1*	*1.5*	*3.5*	*4.0*
TA1537	17	13	18	16	10	6 bd	633
S.D.	*2.5*	*2.1*	*4.5*	*1.5*	*2.6*	*2.0*	*167.3*
TA102	377	409	277	319	310	343 ad	1 158
S.D.	*30.3*	*9.5*	*25.0*	*5.5*	*9.5*	*49.7*	*264.1*

3.4 - "R" ratio calculated from numbers of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500
TA98	0.92	1.00	1.19	1.14	0.67
TA100	0.97	0.94	0.83	0.92	N.R.
TA1535	1.08	0.67	0.67	1.08	0.83
TA1537	0.76	1.06	0.94	0.59	0.35
TA102	1.08	0.73	0.85	0.82	0.91

Positive controls: **2-nitrofluorene 1 µg/plate (TA98);**
sodium azide 1 µg/plate (TA100 and TA1535);
9-aminoacridine 50 µg/plate (TA1537);
cumene hydroperoxyde 200 µg/plate (TA102).

3.5 - Mean number of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500	Positive controls
TA98	39	46	37	41	39	40 ad	1 056
S.D.	*8.5*	*5.8*	*5.3*	*15.6*	*4.7*	*10.1*	*36.9*
TA100	161	160	171	165	153	129 bd	1 229
S.D.	*27.6*	*9.1*	*15.6*	*8.7*	*9.1*	*7.8*	*179.1*
TA1535	16	14	15	10	9	8 bd	164
S.D.	*1.5*	*2.3*	*2.3*	*2.6*	*5.5*	*1.0*	*23.5*
TA1537	22	22	18	17	12	13 ad	79
S.D.	*1.7*	*3.2*	*3.1*	*1.5*	*2.0*	*1.2*	*15.1*
TA102	289	277	376	401	412	370 d	1 028
S.D.	*17.6*	*21.7*	*16.0*	*20.8*	*31.0*	*31.8*	*158.1*

3.6 - "R" ratio calculated from numbers of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500
TA98	1.18	0.95	1.05	1.00	1.03
TA100	0.99	1.06	1.02	0.95	0.80
TA1535	0.88	0.94	0.63	0.56	0.50
TA1537	1.00	0.82	0.77	0.55	0.59
TA102	0.96	1.30	1.39	1.43	1.28

Positive control: 2-aminoanthracene 2 µg/plate (TA98, TA100, TA1535, TA1537) and 5 µg/plate (TA102).

4.3 - Mean number of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 25	50	100	250	500	1 000	2 500	Positive controls
TA98	19	23	26	26	25	24	26	32 ad	250
S.D.	*3.1*	*0.6*	*6.0*	*5.1*	*3.0*	*4.0*	*5.2*	*5.0*	*42.9*
TA100	130	133	120	128	131	117	158 a	107 bd	538
S.D.	*16.4*	*9.8*	*17.2*	*21.0*	*15.5*	*3.2*	*5.1*	*5.7*	*55.8*
TA1535	15	18	17	16	14	17	13 a	13 bd	477
S.D.	*3.6*	*10.1*	*6.7*	*4.4*	*6.0*	*5.6*	*2.5*	*4.9*	*31.3*
TA1537	11	10	12	13	10	12	15 a	8 b	230
S.D.	*4.4*	*1.2*	*4.2*	*1.7*	*3.1*	*2.5*	*3.5*	*3.5*	*78.0*
TA102	314	322	333	331	360	341	312	345 ad	1 183
S.D.	*5.5*	*7.0*	*25.0*	*10.4*	*8.3*	*14.7*	*11.0*	*19.9*	*89.2*

4.4 - "R" ratio calculated from numbers of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 25	50	100	250	500	1 000	2 500
TA98	1.21	1.37	1.37	1.32	1.26	1.37	1.68
TA100	1.02	0.92	0.98	1.01	0.90	1.22	0.82
TA1535	1.20	1.13	1.07	0.93	1.13	0.87	0.87
TA1537	0.91	1.09	1.18	0.91	1.09	1.36	0.73
TA102	1.03	1.06	1.05	1.15	1.09	0.99	1.10

Positive controls: **2-nitrofluorene 1 µg/plate (TA98);**
sodium azide 1 µg/plate (TA100 and TA1535);
9-aminoacridine 50 µg/plate (TA1537);
cumene hydroperoxyde 200 µg/plate (TA102).

4.5 - Mean number of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500	Positive controls
TA98	34	37	37	40	42	39 d	2 134
S.D.	*5.5*	*7.8*	*6.6*	*9.8*	*8.9*	*5.1*	***161.0***
TA100	135	126	147	133	135	126 ad	1 885
S.D.	*5.0*	*6.0*	*13.9*	*9.6*	*7.2*	*9.6*	***160.6***
TA1535	11	10	12	9	15	10 ad	233
S.D.	*3.6*	*0.6*	*4.2*	*1.5*	*1.2*	*0.6*	***11.8***
TA1537	10	15	16	12	14	8 ad	193
S.D.	*2.0*	*4.0*	*1.7*	*4.2*	*4.7*	*2.1*	***19.7***
TA102	351	365	389	338	371	364 d	1 124
S.D.	*35.3*	*19.7*	*22.5*	*12.4*	*27.6*	*33.2*	***234.0***

4.6 - "R" ratio calculated from numbers of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500
TA98	1.09	1.09	1.18	1.24	1.15
TA100	0.93	1.09	0.99	1.00	0.93
TA1535	0.91	1.09	0.82	1.36	0.91
TA1537	1.50	1.60	1.20	1.40	0.80
TA102	1.04	1.11	0.96	1.06	1.04

Positive control: 2-aminoanthracene 2 µg/plate (TA98, TA100, TA1535, TA1537) and 5 µg/plate (TA102).

ATTACHMENT II

Study Report Tables 5.3-5.6 and 6.3-6.6
pp. 42, 43, 46, and 47

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5.3 - Mean number of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 50	100	250	500	1 000	2 500	Positive controls
TA98	37	31	27	34	22	38	N.R. e	310
S.D.	*6.7*	*3.5*	*2.5*	*7.2*	*9.6*	*8.5*		*8.0*
TA100	147	147	143	133 a	N.R. c	94 b	N.R. be	556
S.D.	*13.0*	*20.9*	*8.7*	*12.1*		*12.8*		*21.5*
TA1535	13	11	17	10	N.R. c	12 b	N.R. be	455
S.D.	*1.2*	*3.2*	*4.0*	*4.7*		*1.0*		*64.6*
TA1537	16	15	13	12 a	4 b	8 b	N.R. be	339
S.D.	*2.6*	*6.1*	*4.4*	*2.0*	*0.0*	*1.0*		*160.5*
TA102	302	304	303	319	324 b	353	N.R. e	1 067
S.D.	*16.5*	*8.7*	*6.7*	*2.3*	*12.7*	*18.8*		*224.2*

5.4 - "R" ratio calculated from numbers of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 50	100	250	500	1 000	2 500
TA98	0.84	0.73	0.92	0.59	1.03	N.R.
TA100	1.00	0.97	0.90	N.R.	0.64	N.R.
TA1535	0.85	1.31	0.77	N.R.	0.92	N.R.
TA1537	0.94	0.81	0.75	0.25	0.50	N.R.
TA102	1.01	1.00	1.06	1.07	1.17	N.R.

Positive controls: **2-nitrofluorene 1 µg/plate (TA98);**
sodium azide 1 µg/plate (TA100 and TA1535);
9-aminoacridine 50 µg/plate (TA1537);
cumene hydroperoxyde 200 µg/plate (TA102).

5.5 - Mean number of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500	Positive controls
TA98	36	43	35	45	34 bd	N.R. be	1 333
S.D.	*2.6*	*3.6*	*6.6*	*13.5*	*11.8*		*82.9*
TA100	126	134	143	136 a	108 bd	N.R. be	1 138
S.D.	*23.4*	*8.5*	*18.7*	*5.9*	*10.5*		*203.2*
TA1535	9	9	10	9 a	10 bd	10 d	201
S.D.	*1.5*	*3.8*	*2.6*	*3.6*	*4.0*	*6.0*	*40.7*
TA1537	18	16	13	13	8 bd	10 bd	150
S.D.	*3.1*	*2.3*	*5.6*	*2.3*	*4.0*	*2.1*	*67.9*
TA102	412	455	449	446	437 a	N.R. e	847
S.D.	*6.5*	*21.4*	*40.2*	*14.0*	*9.2*		*89.0*

5.6 - "R" ratio calculated from numbers of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 100	250	500	1 000	2 500
TA98	1.19	0.97	1.25	0.94	N.R.
TA100	1.06	1.13	1.08	0.86	N.R.
TA1535	1.00	1.11	1.00	1.11	1.11
TA1537	0.89	0.72	0.72	0.44	0.56
TA102	1.10	1.09	1.08	1.06	N.R.

Positive control: 2-aminoanthracene 2 µg/plate (TA98, TA100, TA1535, TA1537) and 5 µg/plate (TA102).

6.3 - Mean number of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 12.5	25	50	100	250	500	1 000	2 500	Positive controls
TA98	26	26	25	30	25	30	10 b	14 bd	26 ad	283
S.D.	*2.9*	*2.1*	*5.5*	*6.1*	*11.3*	*2.5*	*2.5*	*4.2*	*6.5*	***18.8***
TA100	98	106	113	107	105	71 a	7 b	62 b	N.R. be	474
S.D.	*3.6*	*5.9*	*19.6*	*13.6*	*8.1*	*6.1*	*3.2*	*0.0*		***18.9***
TA1535	10	10	12	13	12	8 a	N.R. c	8 b	N.R. be	442
S.D.	*2.9*	*3.6*	*2.0*	*0.6*	*2.1*	*8.5*		*1.7*		***22.5***
TA1537	11	13	14	15	12	7 a	N.R. c	8 b	N.R. be	700
S.D.	*2.5*	*1.5*	*3.2*	*2.5*	*2.6*	*3.0*		*4.0*		***191.4***
TA102	320	302	318	360	289	293	285 a	319	N.R. ae	1221 a
S.D.	*19.7*	*19.3*	*3.2*	*39.1*	*21.0*	*6.7*	*7.4*	*24.2*		***15.6***

6.4 - "R" ratio calculated from numbers of revertant colonies recorded in the absence of a mammalian metabolic activation system

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 12.5	25	50	100	250	500	1 000	2 500
TA98	1.00	0.96	1.15	0.96	1.15	0.38	0.54	1.00
TA100	1.08	1.15	1.09	1.07	0.72	0.07	0.63	N.R.
TA1535	1.00	1.20	1.30	1.20	0.80	N.R.	0.80	N.R.
TA1537	1.18	1.27	1.36	1.09	0.64	N.R.	0.73	N.R.
TA102	0.94	0.99	1.13	0.90	0.92	0.89	1.00	N.R.

Positive controls: **2-nitrofluorene 1 µg/plate (TA98);**
sodium azide 1 µg/plate (TA100 and TA1535);
9-aminoacridine 50 µg/plate (TA1537);
cumene hydroperoxyde 200 µg/plate (TA102).

6.5 - **Mean number of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)**

STRAIN	Solvent controls	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 25	50	100	250	500	1 000	Positive controls
TA98	29	39	33	31	31	38	26 b	1 226
S.D.	*6.0*	*1.7*	*8.7*	*3.6*	*5.1*	*1.2*	*2.5*	*28.4*
TA100	104	118	108	114	124	135 a	84 b	1 048
S.D.	*6.5*	*3.1*	*9.3*	*9.1*	*30.1*	*11.1*	*22.9*	*47.9*
TA1535	11	15	11	11	8	11	7 b	201
S.D.	*2.6*	*2.5*	*7.2*	*2.6*	*1.0*	*0.6*	*0.6*	*26.6*
TA1537	18	20	14	19	14	11	10 b	114
S.D.	*3.2*	*2.6*	*1.7*	*5.1*	*6.1*	*1.5*	*2.1*	*7.0*
TA102	343	356	336	412	312	423	387 a	626
S.D.	*21.0*	*11.6*	*35.2*	*48.0*	*12.6*	*19.5*	*43.1*	*25.2*

6.6 - **"R" ratio calculated from numbers of revertant colonies recorded in the presence of a mammalian metabolic activation system (S9 mix)**

STRAIN	CONCENTRATION OF TEST SUBSTANCE (µg/plate) 25	50	100	250	500	1 000
TA98	1.34	1.14	1.07	1.07	1.31	0.90
TA100	1.13	1.04	1.10	1.19	1.30	0.81
TA1535	1.36	1.00	1.00	0.73	1.00	0.64
TA1537	1.11	0.78	1.06	0.78	0.61	0.56
TA102	1.04	0.98	1.20	0.91	1.23	1.13

Positive control: 2-aminoanthracene 2 µg/plate (TA98, TA100, TA1535, TA1537) and 5 µg/plate (TA102).

DATA EVALUATION RECORD

MB45897-FIPRONIL METABOLITE

Study Type: §84-2; *Salmonella typhimurium*/Mammalian Activation Gene Mutation Assay

Work Assignment No. 3-23N (MRID 44262823)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
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Signature: Mary L Menetrez
Date: 10/12/97

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Signature: Steven Brecher
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Project Manager:
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Signature: Mary L Menetrez
Date: 10/17/97

Quality Assurance:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 10/14/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C)

EPA Work Assignment Manager: M. Copley, DVM, DABT
Registration Action Branch 1/HED (7509C)

Nancy E. McCarroll 10/28/97
Marion Copley 11/5/97

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella typhimurium* mammalian activation gene mutation assay
OPPTS Number: 870.5265 OPP Guideline Number: §84-2

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB45897 (>99% a.i.)

SYNONYMS: None

CITATION: Kennelly, J.C. (1988): Study to Determine the Ability of MB 45897 to Induce Mutation in Four Histidine-Requiring Strains of Salmonella Typhimurium. Microtest Research Limited, Heslington, York, England. Laboratory Project ID/Report No. MAB19/S, March 8, 1988. MRID 44262823. Unpublished

SPONSOR: May and Baker Ltd. Rainham Road South, Dagenham, Essex, England

EXECUTIVE SUMMARY:
In a microbial mutagenicity assay (MRID 44262823), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to MB45897 (>99% a.i.) in DMSO at concentrations of 8, 40, 200, 1,000, and 5,000 µg/plate in the presence of mammalian metabolic activation (+S9); strain TA100 was exposed at these same levels in the absence of metabolic activation (-S9). Additionally, strains TA98, TA1535, and TA1537 were exposed at 4, 20, 100, 500, and 2,500 µg/plate in the absence of activation. Preparations for metabolic activation were made from Aroclor induced rat livers.

MB45897 (>99% a.i.) was tested to the limit concentration of 5,000 µg/plate. Cytotoxicity was observed in the absence of metabolic activation at 5,000 µg/plate in the TA100 strain and at 2,500 µg/plate in strains TA98, TA1535, and TA1537 and in the presence of metabolic activation in strains TA98, TA1535, and TA1537 at 5,000 µg/plate. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

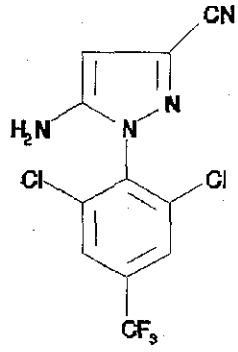
This study is classified as **acceptable** and satisfies the requirement for FIFRA Test Guideline **84-2** for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB45897
   Description: Yellow brown powder
   Lot/Batch #: 6 JJW 1887
   Purity: >99%
   Stability of compound: Not provided
   CAS #: 120068-37-3
   Structure:



   Vehicle used: Dimethyl sulfoxide (DMSO)
   Other comments: The test article was stored in the dark at room temperature.

2. Control Materials
   Negative: Vehicle served as negative control
   Vehicle/final concentration: DMSO/0.1 mL/plate

   Positive:
   Nonactivation:

2-Nitrofluorene	50 µg/plate	*S. typhimurium* TA98
9-Aminoacridine	50 µg/plate	*S. typhimurium* TA1537
Sodium azide	2 µg/plate	*S. typhimurium* TA100 and TA1535

   Activation:

2-Aminoanthracene	5 µg/plate	*S. typhimurium* TA98 and TA100

3. Metabolic Activation
   S-9 was derived from:

X	Aroclor 1254	X	Induced	X	Rat	X	Liver

The S9 mix contained: S-9 fraction (10%, v/v), $MgCl_2$ (250 Mm), NADP (25 mg/Ml), glucose-6-phosphate (60 mg/mL), and phosphate buffer (500 mM), histidine (1 mg/mL), D-biotin (1 mg/mL; 1.0 mL of the S9 mix was used per culture plate. The final concentration of S9 in culture was approximately 2.8%.

4. Test organisms

S. typhimurium strains:

	TA97	X	TA98	X	TA100		TA102
	TA104	X	TA1535	X	TA1537		TA1538

Properly maintained? **Yes**
Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Preliminary cytotoxicity test
Five dose levels (8, 40, 200, 1,000, and 5,000 µg/plate) were evaluated in triplicate with strain TA100 in both the presence and absence of S9 activation. The assay included vehicle and positive controls in quintuplicate.

Mutagenicity assay
The above preliminary cytotoxicity test (±S9) with strain TA100 was also used to evaluate mutagenicity in that strain. With strains TA98, TA1535, and TA1537, five dose levels (4, 20, 100, 500, and 2,500 µg/plate) were evaluated in the absence of S9 activation and five dose levels (8, 40, 200, 1,000, and 5000 µg/plate) were evaluated in the presence of S9 activation. All doses were tested in triplicate. The assays included positive and vehicle controls in quintuplicate.

B. TEST PERFORMANCE

1. Type of Salmonella assay

X	standard plate test

2. Protocol
Overnight cultures of the tester strains were grown in nutrient broth prior to plating. The test substance and the positive controls except sodium azide) were diluted in DMSO to specified concentrations; solvent for sodium azide was distilled water. Bacteria (0.1 mL), test substance, vehicle, or positive control (0.1 mL), and 1.0

mL of S9 mix added to 2.5 mL melted top agar. The mixture was mixed thoroughly and poured on plates containing a layer of minimal agar medium. After the top agar solidified, the plates were inverted and incubated at 37 C for ≥2 days. Triplicate plates were prepared for each dose, strain, and condition. Vehicle and positive controls were prepared in quintuplicate. The plates were evaluated for gross toxicity (reduction of background lawn relative to vehicle control) and total revertant colony numbers. Revertant colonies were counted either by an automatic colony counter or by hand.

3. Evaluation Criteria

(a) Assay validity: The assay was considered valid if the following were met: (i) the number of revertants in the vehicle controls were within the range of historical controls; (ii) the positive controls produced unambiguous increases in the number of revertants allowing for discrimination between the different strains and an active S9 fraction; and (iii) ≤5% of the plates were not evaluated due to contamination or some other unanticipated incident.

(b) Positive response: The test material was considered mutagenic if (i) the assay was valid and (ii) there was a dose related, 2-3 fold increase in the number of revertant numbers accompanied by significant F-statistics (ANOVA and regression analysis) in ≥1 strain.

II. REPORTED RESULTS

A. Analytical determinations:
Data regarding concentration and stability analyses were not presented.

B. Preliminary cytotoxicity assay
Five dose levels of the test substance ranging from 8 to 5,000 µg/plate were evaluated in triplicate with and without S9 activation using strain TA100. A slight inhibition of growth was observed in the cultures treated at 5,000 µg/plate in the absence of S9 activation.

C. Mutagenicity assay
The results of the mutagenicity assay are presented as an attachment to this DER (study report, Tables 1 and 2, pages 19 and 20). Five doses of the test substance ranging from 4 to 2,500 µg/plate were evaluated without S9 activation in strains TA98, TA1535, and TA1537. Five doses ranging from 8 to 5,000 µg/plate were used to evaluate strain TA100 (±S9) and the other three strains with S9 activation. There were no significant differences in the number of revertant colonies

in any tester strain at any dose level/condition compared to the vehicle controls. Cytotoxicity, apparent as inhibition of growth and presence of microcolonies/or decrease in the number of revertant colonies/plate, was observed in the absence of metabolic activation at 5,000 µg/plate in the TA100 strain and at 2,500 µg/plate in strains TA98, TA1535, and TA1537 and in the presence of metabolic activation in strains TA98, TA1535, and TA1537 at 5,000 µg/plate. The positive control substances induced marked increases in revertant colonies in their respective strains.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study author concluded that, under the conditions of this study, MB45897 was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA157 with or without metabolic activation.

B. Reviewer's Discussion

The reviewers agree with the study author's conclusions that MB45897 was assayed over an appropriate dose range as it was tested to the limit concentration with the S. typhimurium strains and failed to induce a genotoxic response. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. The study is classified as **acceptable**.

IV. STUDY DEFICIENCIES

Data on the analysis of dose formulations for actual concentration and stability were not submitted. These deficiencies do not alter the conclusions of this review because cytotoxicity was observed.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

Study Number: MAB 19/S v.1

TABLE 1 M&B 45897

Mean revertants/plate (+/- SD) for treatments in the absence of S-9

Treatment (ug/plate) TA100	Other strains	Strain TA98	TA100	TA1535	TA1537
0	0	26.8 (6.5)	109.5 (7.0)	19.2 (2.2)	14.2 (3.1)
8	4	35.3 (12.9)	112.7 (20.8)	17.7 (8.6)	13.0 (ND)
40	20	29.3 (5.5)	113.3 (3.5)	24.7 (4.0)	14.7 (6.1)
200	100	31.3 (10.0)	110.0 (1.7)	45.3 (18.3)	15.7 (4.7)
1000	500	36.3 (4.9)	114.3 (17.9)	19.7 (9.0)	14.3 (2.5)S
5000	2500	14.3 (4.7)V	69.7 (29.1)S	22.0 (9.6)V	T
Max. increase over solvent control		1.4	1.0	2.4	1.1
Variance ratio		NC	NC	NC	NC
Max. correlation coefficient inc. controls		NC	NC	NC	NC
Positive control		1405.6 (121.9)	753.8 (52.2)	609.8 (41.0)	1520.8 (314.9)
Increase over solvent control		52.4	6.9	31.8	107.1

ND = not determined, less than three plates.

NC = not calculated, maximum increase in revertants less than twice (TA98, TA100) or three times (TA1535, TA1537) solvent control.

V = very thin background lawn and presence of microcolonies.

S = slight thinning of background lawn or presence of microcolonies.

T = toxic, no revertant colonies observed.

Study Number: MAB 19/S

v.1

TABLE 2

M&B 45897

Mean revertants/plate (+/- SD) for treatments in the presence of S-9

Treatment (ug/plate)	Strain			
	TA98	TA100	TA1535	TA1537
0	35.2 (6.6)	145.5 (3.5)	20.2 (6.9)	19.0 (4.6)
8	38.0 (5.6)	116.7 (26.4)	17.7 (1.5)	12.3 (3.5)
40	37.3 (6.1)	129.0 (16.8)	18.7 (4.5)	45.3 (17.8)
200	42.7 (7.5)	122.7 (15.4)	20.3 (2.5)	15.0 (4.4)
1000	34.3 (6.0)	145.7 (16.3)	19.3 (1.5)	14.7 (4.0)
5000	5.7 (5.5)A	127.3 (22.8)	4.7 (2.1)A	3.0 (1.0)A
Max. increase over solvent control	1.2	1.0	1.0	2.4
Variance ratio	NC	NC	NC	NC
Max. correlation coefficient inc. controls	NC	NC	NC	NC
Positive control	1148.8 (63.8)	974.5 (98.1)	NT	NT
Increase over solvent control	32.6	6.7	NT	NT

NC = not calculated, maximum increase in revertants less than twice (TA98, TA100) or three times (TA1535, TA1537) solvent control.

NT = not tested.

A = absence of background lawn and presence of microcolonies.

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DATA EVALUATION RECORD

MB45897-FIPRONIL METABOLITE

Study Type: §84-2; *In vitro* Chromosome Aberration Assay in Cultured Human Lymphocyte Cells

Work Assignment No. 3-230 (MRID 44262824)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Date: 10/12/97

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Project Manager:
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Signature: Mary L Menetrez
Date: 10/23/97

Quality Assurance:
William Spangler, Ph.D.

Signature: William J Spangler
Date: 10/17/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Toxicology Branch 1 (7509C)

Nancy E. McCarroll 11/12/97

EPA Work Assignment Manager: Marion Copley, DVM., DABT
Registration Action Branch 1 (7509C)

11/25/97

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian chromosome aberrations in cultured human lymphocytes
OPPTS Number: 870.5375 OPP Guideline Number: §84-2

DP BARCODE: D223789 SUBMISSION CODE: S24626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB45897 (Fipronil Metabolite; 99.7% a.i.)

SYNONYMS: Pyrazole; RPA097920; 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carbonitrile

CITATION: Johnson, A.L. (1995) Pyrazole/MB45897/RPA097920: An *in vitro* test for induction of chromosome damage: Cytogenetic study in cultured human peripheral lymphocytes. Pharmaco LSR Ltd. Eye Suffolk, England. LSR 94/RHA534/1034, March 21, 1995. MRID 44262824. Unpublished.

SPONSOR: Rhône Poulenc Agrochimie, Centre De Recherche 355, rue Dostoievski B.P. 153, Sophia Antipolis Cedex, France.

EXECUTIVE SUMMARY:

In a mammalian cell chromosome aberration assay (MRID 44262824), cultured human lymphocytes were exposed to MB45897 (99.7% a.i.), in dimethyl sulfoxide at concentrations of 50, 100, 200, 400, 500, 600, and 800 µg/mL with metabolic activation and 12.5, 25, 50, 75, 100, and 150 µg/mL without metabolic activation. Without metabolic activation, the cells were exposed continuously for 20 or 44 hours; with metabolic activation, the cells were exposed to MB45897 for 3 hours and harvested 17 or 41 hours later. Preparations for metabolic activation were made from induced rat livers. Positive controls (±S9) were included.

Precipitation of MB45897 was apparent in all cultures treated with ≥400 µg/mL. Doses of 600 and 800 µg/mL +S9 were severely cytotoxic. For the metaphase analysis, concentrations of 125 and 150 µg/mL -S9 produced marked cytotoxicity, resulting in decreased mean mitotic index (MIs) of 74-81% compared to vehicle controls. **In the first cytogenetic assay, there was a significant increase in aberrant cell frequency compared to the vehicle control value in cultures harvested 20 hour after exposure to 150 µg/mL -S9, both**

when gaps were included and excluded (8.0% and 6.0%; 0.05>p>0.01); the historical control range excluding gaps (0-5%) was also exceeded. In the second cytogenetic assay, significant increases in the aberrant cell frequency were noted at the 20-hour sampling of cultures dosed with 150 µg/mL -S9, both when gaps were included and excluded (p<0.001) and at 125 µg/mL -S9 at the 44-hour sampling both including (p<0.001) and excluding (0.05>p>0.01) gaps; these values also exceeded the historical control ranges (0-10% with gaps) and (0-5% without gaps). The data are, therefore, indicative of clastogenic activity by MB45897 in the absence of metabolic activation. In the presence of S9 activation, a reproducible increase the number of polyploid cells was seen in the 400-µg/mL group at the 20-hour sampling. A similar increase in polyploidy was noted at 400 µg/mL (44-hour sampling). The positive controls induced the expected responses in the treated lymphocytes.

Under the conditions of the test, MB45897 appears to be clastogenic to human lymphocytes at cytotoxic concentrations (≥125 µg/mL -S9) and induces polyploidy at 400 µg/mL +S9.

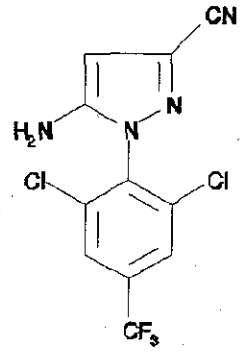
This study is classified as **Acceptable (Guideline)** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality provided

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB45897
   Description: Beige powder
   Lot/Batch #: 94 3026 DA 942
   Purity: 99.7% a.i.
   Stability of compound: Not reported.
   CAS #: 120068-79-3
   Structure:



   Solvent used: Dimethyl sulfoxide (DMSO)
   Other comments: Test material was stored at ambient temperature until use. Dosing solutions were not analyzed for actual concentration.

2. Control Materials:
   Negative: Vehicle control
   Solvent/final concentration: DMSO (10 µl/ml)
   Positive: Nonactivation: Chlorambucil (2 µg/mL in ethanol)
   Activation: Cyclophosphamide (6 µg/mL in sterile water)

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

   The S9 mix was prepared by the study laboratory.
   S9 mix composition: S9 fraction (15% v/v), 1.65 M KCl, $MgCl_2$(0.4M), Na_2HPO_4-KH_2PO_4 buffer pH 7.4 (0.1M), glucose-6-phosphate (0.1M), NADP (0.1M) The final S9 concentration in the culture was 1.5% (v,v).

4. Test compound concentrations used: For the preliminary test and the two cytogenetic tests, the cultures were sampled at 20 and 44 hours

Preliminary toxicity test:

Nonactivated and activated conditions: 7.04, 35.2, 176, 880, and 4400 µg/mL with 20- and 44-hour harvest times

First cytogenetic test:

20 hour sampling interval
Nonactivated conditions: 25, 50, 100, and 150 µg/mL
Activated Conditions: 50, 100, 200, 400, 600, and 800 µg/mL

44 hour sampling interval
Nonactivated conditions: 12.5, 25, 50, 75, 100, and 150 µg/mL
Activated Conditions: 50, 100, 200, 400, 600, and 800 µg/mL

Second cytogenetic test:

20 hour sampling interval
Nonactivated conditions: 25, 50, 100, and 150 µg/mL
Activated Conditions: 100, 200, 400, and 500 µg/mL

44 hour sampling interval
Nonactivated conditions: 100, 125, and 150 µg/mL
Activated Conditions: 400, 500, and 600 µg/mL

5. Test cells: Peripheral blood was obtained from a healthy, non-smoking male volunteer. Whole blood was grown in RPMI 1640 medium (supplemented with 10% fetal calf serum, 1% heparin, and 0.4% penicillin/streptomycin) and phytohemagglutinin to stimulate lymphocytes to divide. Cultures were incubated for 48 hours at 37° C prior to treatment.

Properly maintained? Yes

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Each 48-hour culture was pelleted by centrifugation and resuspended in medium (supplemented with 10% fetal calf serum, 1% heparin, and 0.4% penicillin/streptomycin), S-9 activation mix (10%, v/v), if appropriate, and test solution or solvent (1%, v/v). Duplicate cultures were established for each treatment. The highest level tested was determined by the limit of solubility of the test compound in DMSO. Following an initial 3-hour incubation at 37° C in a shaking water bath, nonactivated cultures were placed in an incubator for the remainder of the 20 or 44 hour exposure period. Activated cultures were washed to remove test compound and S-9 mix. The cells were then resuspended in medium (supplemented with 10% fetal calf serum, 1% heparin, and 0.4% penicillin/streptomycin) and incubated for a further 17 or 41 hours. Cultures were harvested and

slides prepared as described in Section B.2. The number of metaphases observed per 1000 cells (mitotic index) in each culture was determined.

2. Cytogenetic Assay:

a. Cell treatment:
Cultures were grown and treated as described for the preliminary toxicity test, except that positive controls were included.

b. Spindle inhibition
Inhibitor used/concentration: Colcemid (0.4 µg/mL)
Administration time: 3 hours (before cell harvest)

c. Cell harvest:
Cells exposed to test material, solvent, or positive control were harvested 20 or 44 hours after initiation of treatment. The cells were swollen with 0.56% KCl, and fixed with methanol:glacial acetic acid (3:1, v/v).

d. Details of slide preparation:
The cells suspended in fresh fixative were dropped onto microscope slides and air-dried. The slides were stained with Giemsa (1:10 in Sorensen's buffer), air-dried and mounted in DPX. Two slides per culture in each group of the preliminary test and four slides per culture for each group in the cytogenetic tests were made.

e. Metaphase analysis
No. of cells examined per dose: 200
Vehicle control: 200
Positive control: 200

Scored for structural: Yes

Scored for polyploidy: Yes

Coded prior to analysis: Yes

f. Evaluation criteria: No criteria were provided to evaluate assay validity, a positive response or the biological significance of the findings. Historical control data for DMSO without S9 activation were included in the study report.

g. Statistical analysis: The frequency of aberrant metaphases (with and without gaps) were evaluated for statistical significance at $0.05>p>0.01$ or $p<0.001$ using the Fisher Exact Probability Test and a one-sided test.

II. **REPORTED RESULTS:** Precipitation of MB45897 occurred at concentrations ≥400 µg/mL.

A. Preliminary cytotoxicity assay: Cytotoxicity was indicated by reduced mitotic indices (MIs). MIs were not scored in cultures treated with 880 µg/mL ±S9 (both sampling times) because of a lack of cells. At the 20-hour sampling, reductions in the mean MI compared to vehicle control values of approximately 80 and 92% were recorded for cultures exposed to 176 and 4400 µg/mL MB45897 -S9, respectively. Under S9-activated conditions (20-hr harvest), a 63% reduction in the MI was observed at 4400 µg/mL. Few, if any cells were recovered in cultures treated with 880 µg/mL ±S9 or 176 or 4400 µg/mL -S9 at the 44-hour sampling. No appreciable reductions in the MI were observed at lower concentrations (7.04 or 35.2 µg/mL -S9; 7.04-176 µg/mL +S9). Based on these results, concentrations of 150 µg/mL without S9 and 800 µg/mL +S9 were selected as the highest test levels for the initial cytogenetic assay.

B. Cytogenetic assays: The mean mitotic index and the frequency of chromosomal aberrations were determined. The results of the cytogenetic assays are presented as an attachment to this DER (Study Report Tables 4 and 9, pages 37, 38, 49, and 50).

First cytogenetic assay:

Nonactivated conditions: At the 20-hour sampling, reductions in the mean MI compared to vehicle control of 26, 44, or 74% were recorded for cultures exposed to nonactivated 50, 100, or 150 µg/mL MB45897, respectively. Accordingly, lymphocytes treated with these nonactivated doses were analyzed for chromosomal aberrations. As shown in Study Report Summary Table 4, p.37, the mean frequency of aberrant metaphases in solvent control cultures was 2.5% including gaps (2.0% excluding gaps); corresponding values for MB45897 treated cultures were 3.5% (2.5%) at 50 µg/mL, 2.0% (1.5%) at 100 µg/mL, and 8.0% (6.0%) at 150 µg/mL. The response was significant ($p<0.05$) at 150 µg/mL whether gaps were included or excluded from the analysis. The mean frequency of aberrant cells (6%) was also outside of the provided historical control range (0-5%) for DMSO. The most frequently observed chromosome damage was chromatid breaks.

At the 44-hour sampling, reductions in the mean MIs of 31 or 85% were recorded for cultures exposed to 100 or 150 µg/mL of the nonactivated test substance, respectively. Since many metaphases were reported to be unscoreable at 150 µg/mL, cultures dosed with 100 µg/mL were examined for abnormal chromosomes. The results from this analysis, summarized in Study Report Table 4, p. 38, showed a slight, nonsignificant increase in cells with structural chromosome aberrations.

S9-activated conditions: Few, if any cells survived treatment with 600 or 800 µg/mL +S9 (20-hr harvest). A 54% reduction in the MI was observed at 400 µg/mL and the investigators stated that many metaphases were unscoreable in one of the replicate 400-µg/mL cultures. Nevertheless, lymphocytes in the single replicate culture at 400 µg/mL as well as cells treated with 100 and 200 µg/mL were analyzed for chromosomal aberrations. As shown in Study Report Table 4, p.37, there was no evidence of a clastogenic effect in lymphocytes exposed to 100-400 µg/mL MB45897. It was noted, however, that a dose-related increase in polyploid cells was seen at 100-400 µg/mL; the increase ranged from 5 polyploid cells at 100 µg/mL to 23 polyploid cells at 400 µg/mL versus 1 polyploid cell in the solvent control group.

Following the prolonged recovery period (44-hr cell harvest), no cytotoxicity or clastogenicity was observed at 400 µg/mL. However, the incidence of polyploid cells was again increased at this dose (13 in the experimental group vs. 1 in the control).

Second cytogenetic assay: A second cytogenetic assay was performed because an appropriate level of cytotoxicity was not achieved at the 44-hour sampling in the first assay performed with S9 activation. Results from this assay were as follows:

Nonactivated conditions: Summarized results presented in Study Report Table 9, pp. 49 and 50 were in good agreement with the 20-hour harvest data from the first cytogenetic assay. As shown, 150 µg/mL MB45897 was cytotoxic (causing an ≈76% reduction in the MI) and clastogenic as indicated by the significant (<0.001) increase in the frequency of aberrant cells. The response was also dose related. The mean frequency of aberrant metaphases in solvent control cultures was 1.5% (excluding gaps); corresponding values for MB45897 treated cultures were 3.0% at 50 µg/mL, 4.0% at 100 µg/mL, and 11.5% at 150 µg/mL. As noted for the initial test, chromatid breaks were the most frequently scored structural aberration. By contrast to the results from the 44-hour cell harvest in the first assay, a significant ($p<0.05$) clastogenic response was seen at the only scored concentration (125 µg/mL). It was noted, however, that the MI at this dose was ≈80% lower than the solvent control value.

S9-activated conditions: Findings from the 20-hour harvest of the second trial under S9-activated conditions (see Study Report Table 9, pp 49 and 50) indicated that the MI was reduced by 67% at 400 µg/mL and by 80% in one replicate culture at 500 µg/mL; the other 500-µg/mL culture had few cells and no metaphases. Results from the analysis of cultures treated with 100, 200, and 400 µg/mL were negative

for structural chromosomal aberrations. In agreement with the earlier findings, numerical chromosome aberrations were increased at 400 µg/mL. For the 44-hour sampling time, no cytotoxicity was at 400 µg/mL; however, few cells and/or no metaphases were observed at 500 and 600 µg/mL. Since no cytotoxicity was apparent at 400 µg/mL, and a marked cytotoxic response was induced in the 500- and 600-µg/mL treatment groups, no cultures were analyzed for chromosomal aberrations.

In both assays, the positive control agents (2 µg/mL Chlorambucil -S9 or 6 µg/mL Cyclophosphamide +S9) induced significant clastogenic activity ($p<0.05$).

The study author concluded that under the conditions of the test, MB45897 induced a clastogenic response in the absence of metabolic activation at concentrations where cytotoxicity was apparent; the clastogenic activity may have been caused by indirect effects. In the presence of metabolic activation, no evidence of clastogenic activity was observed.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. We agree with the author's conclusion that MB45897 was clastogenic in cultured human lymphocytes only at concentrations eliciting marked cytotoxicity. However, the degree of cytotoxicity (MIs reduced by 74-81% of controls) is acceptable for a high dose in this test system. The clastogenic response was reproducible, at 150 µg/mL (-S9) in the first assay and at both 125 and 150 µg/mL (-S9) in the second assay. Additionally, the most predominant type of structural damage seen in both assays was chromatid breaks. MB45897 at 400 µg/mL +S9, although relatively non-cytotoxic, induced a reproducible increase in polyploidy.

We assess, therefore, that MB45897 appears to be clastogenic to human lymphocytes at cytotoxic concentrations (≥125 µg/mL without S9 activation) and induces polyploidy at 400 µg/mL with S9 activation.

B. Study deficiencies

Dose preparations were not analyzed for actual concentration. This deficiency would not be expected to alter the conclusions of this *in vitro* mutagenicity study.

ATTACHMENTS

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

STUDY REPORT TABLES 4 AND 9, PP. 37, 38, 49 AND 50

TABLE 4

First cytogenetic test - group totals - 20 hour sampling time

Compound (ug/ml)	Numbers of cells scored	Mean mitotic index	Cells with aberrations			Cells with aberrations other than gaps		
			Total	Indiv. values %	+ Mean %	Total	Indiv. values %	+ Mean %
Non-activated cultures								
DMSO (-)	200	12.9	5	2, 3	2.5	4	2, 2	2.0
RPA097920 (50)	200	9.5	7	3, 4	3.5	5	2, 3	2.5
RPA097920 (100)	200	7.2	4	2, 2	2.0	3	1, 2	1.5
RPA097920 (150)	200	3.3	16	7, 9	8.0	12	5, 7	6.0
Chlorambucil (2)	200	9.5	56	27, 29	28.0	45	22, 23	22.5
Activated cultures								
DMSO (-)	200	15.4	8	3, 5	4.0	2	1, 1	1.0
RPA097920 (100)	200	14.1	4	2, 2	2.0	1	0, 1	0.5
RPA097920 (200)	200	12.6	2	1, 1	1.0	1	0, 1	0.5
RPA097920 (400)	186	7.1	8	3, 5.8	4.3	5	2, 3.5	2.7
Cyclophosphamide (6)	200	14.9	33	13, 20	16.5	24	11, 13	12.0

+ Mean % = $\frac{\text{No. aberrant metaphases}}{\text{Total cells scored}} \times 100$

TABLE 4 - continued

First cytogenetic test - group totals - 44 hour sampling time

Compound (ug/ml)	Numbers of cells scored	Mean mitotic index	Cells with aberrations			Cells with aberrations other than gaps		
			Total	Indiv. values %	+ Mean %	Total	Indiv. values %	+ Mean %
Non-activated cultures								
DMSO (-)	200	8.0	7	3,4	3.5	3	1,2	1.5
RPA097920 (100)	200	5.5	11	3,8	5.5	8	2,6	4.0
Chlorambucil (2)	200	9.8	37	17,20	18.5	26	10,16	13.0
Activated cultures								
DMSO (-)	200	9.6	3	1, 2	1.5	3	1, 2	1.5
RPA097920 (400)	200	13.3	9	1, 8	4.5	2	1, 1	1.0
Cyclophosphamide (6)	200	10.0	18	9, 9	9.0	11	5, 6	5.5

+ Mean % = $\frac{\text{No. aberrant metaphases}}{\text{Total cells scored}} \times 100$

TABLE 9

Second cytogenetic test - group totals - 20 hour sampling time

Compound (ug/ml)	Numbers of cells scored	Mean mitotic index	Cells with aberrations			Cells with aberrations other than gaps		
			Total	Indiv. values %	+ Mean %	Total	Indiv. values %	+ Mean %
Non-activated cultures								
DMSO (-)	200	11.4	6	3,3	3.0	3	1,2	1.5
RPA097920 (50)	200	8.8	12	6,6	6.0	6	2,4	3.0
RPA097920 (100)	200	5.1	11	5,6	5.5	8	4,4	4.0
RPA097920 (150)	200	2.7	27	13,14	13.5	23	11,12	11.5
Chlorambucil (2)	200	8.9	79	38,41	39.5	69	33,36	34.5
Activated cultures								
DMSO (-)	200	16.8	6	3, 3	3.0	2	0, 2	1.0
RPA097920 (100)	200	14.8	6	3, 3	3.0	3	1, 2	1.5
RPA097920 (200)	200	14.6	3	1, 2	1.5	1	0, 1	0.5
RPA097920 (400)	200	5.6	10	3, 7	5.0	3	1, 2	1.5
Cyclophosphamide (6)	200	12.7	34	16, 18	17.0	25	11, 14	12.5

+ Mean % = $\frac{\text{No. aberrant metaphases}}{\text{Total cells scored}} \times 100$

TABLE 9 - continued

Second cytogenetic test - group totals - 44 hour sampling time

Compound (ug/ml)	Numbers of cells scored	Mean mitotic index	Cells with aberrations			Cells with aberrations other than gaps		
			Total	Indiv. values %	+ Mean %	Total	Indiv. values %	+ Mean %
Non-activated cultures								
DMSO (-)	200	9.7	7	2,5	3.5	6	2,4	3.0
RPA097920 (125)	200	1.8	26	10,16	13.0	15	5,10	7.5
Chlorambucil (2)	200	11.1	37	18,19	18.5	29	14,15	14.5

+ Mean % = $\frac{\text{No. aberrant metaphases}}{\text{Total cells scored}} \times 100$

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Acting Branch Senior Scientist, Toxicology Branch II/HED (7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat Guideline: §83-3a

EPA ID No.s: EPA MRID No. 44275001
EPA Pesticide Chemical Code: 129121
Toxicology Chemical Code: None
EPA DP Barcode: D236364
EPA Submission Barcode: S524626

Test Material: MB 046513

Synonyms: a metabolite of Fipronil

Citation: O. Foulon (1997): MB 046513, DEVELOPMENTAL TOXICOLOGY STUDY IN THE RAT BY GAVAGE, Rhone-Poulenc Agrochimie Centre de Recherche for Rhone-Poulenc Agrochimie, Study SA 96227, April 10, 1997; EPA MRID 44275001.

Executive Summary: In a developmental toxicity (teratology) study (MRID# 44275001), adult virgin female rats (CD strain, Sprague Dawley Crl: CD (SD) BR from Charles River Laboratories, St Aubin les Elbeuf, France) received either 0, 0.5, 1.0, or 2.5 mg/kg/day of MB 046513 (Purity: 992 g/kg, Batch 805 DAP/DA999) suspended (w/v) in an aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France) at 0.5% by oral gavage from gestation days 6 to 15 inclusive of presumed gestation.

Maternal parameters included clinical signs of toxicity, body weights (on gestation day 0, daily from gestation day 6-16 and on gestation day 20) and food consumption (interval gestation day 0-6, daily from gestation days 6-15 and the interval of gestation day 16- 20). All surviving animals were sacrificed on gestation day 20 and each female was subjected to macroscopic examination of the visceral organs, the reproductive tract was weighed (gravid uterine weight), dissected out and the number of corpora lutea in each ovary, the number of implantation sites, number and localization of resorption sites (classified as early and late), the number and distribution of live and dead foetuses in each uterine horn were determined along with the sex of viable fetuses and the individual weights of viable fetuses and placenta were measured. The viable fetuses were sacrificed, subjected to an external examination and the approximately half of the viable

fetuses from each litter were prepared for soft tissue examination and the remaining for skeletal examination.

Maternal toxicity was noted as clinical signs in the high dose animals as hairloss on either the paws, limbs, flanks, abdomen and/or thorax. The high dose group had lower body weight gains on study days 6-16 (58.1% of control), study days 0-20 (90.0% of control), study days 6-20 (83.9% of control) and days 0-20 corrected for gravid uterine weights (78.3% of control). The high dose group also consumed less food during the dosing period and there was lower food efficiency relative to the control group except for the post dosing period where an increase was noted which is indicative of a rebound relative to toxicity. **The Maternal Toxicity LOEL was 2.5 mg/kg/day and the NOEL was 1.0 mg/kg/day based an increase in clinical signs of toxicity on reduced body weight gain, food consumption and food efficiency.**

Developmental toxicity was noted as a very slight increase in the fetal and litter incidence of incomplete or reduced ossification in several bones in the high dose group, including the hyoid body, 5th/6th sternebrae, 1st thoracic body, pubic bone and 1 or 2 metatarsi. There was also a slight reduction in fetal body weight (males 97.5% of control, females 97.7% of control) in the high dose group, although statistically significant, the changes are too small to be biologically relevant. **The Developmental Toxicity LOEL was 2.5 mg/kg/day and the NOEL was 1.0 mg/kg/day based on the slight increase in fetal and litter incidence of reduced ossification of several bones.**

This study is classified as <u>Acceptable-Guideline</u> and satisfies the guideline requirements (§ 83-3a) for a teratology study in rats.

Compliance: A signed and dated STATEMENT OF NO CONFIDENTIALITY CLAIM, GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT, FLAGGING STATEMENTS (according to the study authors the study neither meets nor exceeds any of the applicable criteria) and QUALITY ASSURANCE STATEMENT was provided.

THIS REVIEW CONTAINS TEXT INFORMATION SCANNED BY THE REVIEWER INTO ELECTRONIC FORMAT (USED IN MATERIALS AND METHODS, STUDY DESIGN AND CONCLUSIONS-INVESTIGATORS SUMMARY SECTIONS).

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A. Materials and Methods:

Test Compound: MB 046513
Purity: 992 g/kg
Description: A yellow solid
Batch 805 DAP/DA999
Other provided information:
The test substance was stored in an air-tight, light-resistant container at room temperature (as specified in test substance specifications).

Vehicle(s): (from page 13 of the study report)

The appropriate amount of test substance was periodically suspended (w/v) in an aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France) at 0.5% and stored at approximately 5°C (i 3°C). Homogeneity of the suspensions was checked during the first formulation for the lowest and the highest concentrations. Stability of the test substance in suspension in the vehicle was determined before the start of the study. All concentrations were checked for each new formulation.

From page 19 of the study report: All the results concerning stability, homogeneity and concentration checks of MB 046513 in suspensions were acceptable since all values measured were within 90-110% of the nominal values. The data provided supports this conclusions.

Test Animal(s): Species: Adult virgin female rats
Strain: CD strain, Sprague Dawley Crl: CD (SD) BR
Source: Charles River Laboratories, St Aubin les Elbeuf, France
Age: "adult"
Body Weight: between 234 and 298 g at mating.
The animals were acclimatized to laboratory conditions for 11 days prior to mating.

B. Study Design

From page 12 of the study report:

The objectives of this study were to assess the potential effects of MB 046513 on pregnancy and embryo-foetal development of the CD rat, when administered by gavage to presumed pregnant females during the period of organogenesis.

This study was designed to meet the following guidelines:
- Organization for Economic Cooperation and Development (O.E.C.D.), O.E.C.D. Guidelines for Testing of Chemicals 414 (1981).
- International guidelines recommended in Directive 94/79 E.C. E.E.C. 92/69 - Annex V - Method B31 (1992).

- US Environmental Protection Agency (E.P.A.), Pesticide Assessment Guidelines, Subdivision F. November 1984. Hazard Evaluation: Human and Domestic Animals, Washington DC (E.P.A./FIFRA 83-3 (1984).

The study protocol was provided as an attachment.

The study time schedule was as follows:

Study initiation date	June 27, 1996
Study sponsor representative protocol approval date	June 27, 1996
First sperm-positive day	July 02, 1996
Experimental start date	July 08, 1996
Last cesarean sections	July 30, 1996
Experimental termination date	September 12, 1996

Mating Procedure: (from page 13 of the study report)

Females were mated on a one-to-one basis (when possible) with stock males of the same strain and same supplier. Each morning following pairing, rats showing spermatozoa in a vaginal smear or sperm plug *in situ* were considered pregnant. The day where evidence of mating was found, was designated as gestation day 0 (GD0).

The females were assigned to control and treated groups at the end of each week of mating using body weight dependent procedure. Permanent identification numbers were assigned to animals within each group. Each animal was identified by a cage card and an ear tag bearing a unique sequential number.

Animal Husbandry: (from pages 13-14 of the study report)

Pregnant females were individually housed in suspended stainless steel wire mesh cages.

The laboratory conditions in the study room were monitored and controlled by an automatic system The target specifications were:

* temperature: 20°C - 24°C
* humidity: 40% - 70%
* lighting: 12-hour light, 12-hour dark cycles (7am - 7pm)
* ventilation: approximately 15 air changes per hour (not monitored)

There were no deviations from target specifications which could have compromised the study. Housing data are placed in the study file.

Certified Rodent pellet diet A04C (Usine d'Alimentation Rationnelle, 91360 Villemoisson-sur-Orge, France) was distributed *ad libitum*. Water from the municipal supply was provided ad libitum with an automatic watering system. Filters servicing the watering system were changed regularly

and sterilization of the system was periodically performed. Routine analyses of feed and water indicated that there was no contamination which could have compromised the study.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0 (Vehicle)	25
Low Dose	0.2	25
Mid Dose	1.0	25
High Dose	2.5	25

From page 14 of the study report: Dose levels have been selected in agreement with the sponsor representative and based on the results obtained in a previous range finding study in the rat (Foulon O., report n° SA 96100) (7). This was not provided in the study report.

Dose Administration:

From page 15 of the study report: Doses were administered daily by gavage to each female on Days 6 to 15 inclusive of presumed gestation, based on the animal's most recent body weight, and at a volume of 10 ml/kg. Control animals received an equivalent volume of vehicle alone (methylcellulose).

The suspensions were mixed continuously before and during treatment with an electromagnetic stirrer.

Observations:

From pages 15-16 of the study report: All the surviving females were examined daily for obvious signs of illness. Their weights were recorded on Day 0, then daily from Days 6 to 16 and on Day 20 of gestation. Food consumption was recorded during the interval Day 0-Day 6, daily from Days 6 to 15 and during the interval Day 16-Day 20 of gestation.

Animals killed in extremis by inhalation of carbon dioxide or found dead, were autopsied. The number of implantations and corpora lutea were noted when present. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulfide(1)

On Day 20 of gestation, surviving females were killed by inhalation of carbon dioxide, for examination of uterine content. Each female was first subjected to macroscopic examination of the visceral organs.

The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded:

- Number of corpora lutea in each ovary.
- Number of implantation sites in each uterine horn.

- Number and localization of resorption sites (classified as early and late) in each uterine horn.
- Number and distribution of live and dead foetuses in each uterine horn.
- Sex of viable foetuses.
- Individual weights of viable foetuses and placenta.

Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulfide.

Intra uterine death was classified as:

- Early resorptions: only visible placental remnants.
- Late resorptions: visible placental and foetal tissues.
- Dead foetuses: a dead foetus was defined as a term foetus not demonstrating marked to extreme autolysis. Foetuses showing marked to extreme autolysis were considered as late resorptions.

All the viable foetuses were killed by subcutaneous injection (0.02 ml/foetus) of Dolethal® (Sanofi, Libourne, France) and subjected to an external examination. Approximately half of the viable foetuses from each litter was immersed in Bouin fluid for subsequent internal examination following free-hand sectioning. The remaining half was eviscerated and then placed in absolute ethanol before staining. A modification of the STAPLES and SCHNELL staining technique (2) was used and a subsequent skeletal examination was performed.

Historical control data were not provided to allow comparison with concurrent controls (only a reference to historical control data, see references below).

Statistical Analysis:

The following statistical analysis methods were employed (from pages 16-18 of the study report):

Data from non-pregnant, dead or killed "in extremis" animals were not included in group mean calculations of all maternal parameters.

Corrected body weight (CBW) and corrected body weight change (CBWC) were calculated as follows:

CBW = Body weight on Day 20 - gravid uterine weight
CBWC = (Body weight on Day 20 - Body weight on Day 0) - (gravid uterine weight)

Litter data (for each female):

Pre-implantation loss was calculated as a percentage according to the formula:

$$\frac{\text{Number of corpora lutea - Number of implantations}}{\text{Number of corpora lutea}} \times 100$$

Post-implantation loss was calculated as a percentage according to the formula:

$$\frac{\text{Number of implantations - Number of viable foetuses}}{\text{Number of implantations}} \times 100$$

Male sex ratio

$$\frac{\text{Number of male foetuses}}{\text{Total number of foetuses}} \times 100$$

Variables

Maternal body weights
Maternal body weight changes
Corrected body weight
Corrected body weight change
Food consumption
Gravid uterine weights
Individual foetal weights
Individual placental weights

Litter data:
- number of corpora lutea
- number of implantation sites
- number of resorptions
- number of male foetuses
- number of female foetuses
- number of live and dead foetuses
- pre-implantation loss
- post-implantation loss

Group mean values and standard deviations (S.T.D.) were calculated for each parameter.

Results of maternal body weight changes, corrected body weight and corrected body weight change, food consumption, individual foetal weights, individual placental weights were intercompared between the treated groups and the control group by use of:

- Bartlett's test (3) for homogeneity of variances
- Analysis of variance (ANOVA) when Bartlett's test indicated homogeneous variances
- Dunnett's test (4) if Anova was significant.

When Bartlett's test indicated heterogeneous variances, non parametric tests were performed using the Kruskal-Wallis (5) non parametric one-way analysis of variance followed by the Mann-Whitney test (6) when the Kruskal-Wallis test was significant.

Litter data were statistically treated using the Kruskal-Wallis test followed by the Mann-Whitney test when the Kruskal-Wallis test was significant.

- When the mean and variance of the control group equal to zero, means of treated groups only (different from zero) were compared with the control group using the Mann-Whitney test.
- When the means and the variances of the control group and the treated groups equal to zero, no statistical analysis of the variable was performed.
- Statistical analysis was performed on all pregnant females and on pregnant females with viable foetuses. As the number of pregnant females equalled the number of pregnant females with viable foetuses, one statistical analysis was included in this report (litter data for pregnant females, presented in Table 5).

A statistical analysis was performed concerning the foetal skeletal observation: "5th and 6th sternebrae not ossified" between the treated groups and the control, for the foetal (chi-square) and the litter (Fisher's exact test, 2-tail) incidence.

The alpha levels for each statistical comparison were 0.05 and 0.01.

Statistical analyses were performed using SAS programs.

From page 24 of the study report: PROTOCOL DEVIATIONS

1. During the storage of the last formulation, the suspensions (which were 5 days old) were put in a refrigerator, kept inadvertently opened for about 22 hours.
After this period, the temperature inside the refrigerator was recorded and found to be at 13 °C. After this incident, these suspensions were administered to few animals for the last 1, 4 or 5 days of the dosing period (in accordance to the animals stage of gestation).

Since MB046513 was demonstrated to be stable in methylcellulose for 15 days which includes a daily 2 hour-period of stirring at room temperature (20 °C), this deviation was not considered to have affected the integrity of the study.

2. A statistical analysis was performed on the foetal skeletal observation "5th and 6th sternebrae not ossified" between the treated groups and the controls for the foetal and litter incidence.

It is the opinion of the study director that these deviations did not affect the results of the study. The reviewer agrees with this assessment.
From page 25 of the study report:
REFERENCES

1: SALEWSKI E., Farbemethode zum Makroskopischen Nachweis von Implantations-Stellen am Uterus der Ratte, Nauvyn, Schmiedebergs, Exp. Pathology Pharmakol, 247, pp. 367.

2: STAPLES and SCHNELL, V.L. Refinements in rapid clearing technique in the KOH - alizarin red S method for fetal bone stain technol., 1964, 39:63.

3: BARTLETT'S test, in SOKAL R.R. and ROHLF F.J.(1981): Biometry, W.H. Freeman, New York, pp. 403-407.

4: DUNNETT C.W. (1955): A multiple comparison procedure for comparing several treatments with a control, Journal American Statistics Association, 50, pp. 1096- 1121.

5: KRUSKAL W.H. and WALLIS W.A. (1952): Use of ranks in one criterion variance analysis, J. Amer. Statist. Ass., 47, pp. 583-621.

6: MANN H.B. and WHITNEY D.R. (1947): On a test of whether one of two random variables is stochastically larger than the other, Ann. Math. Statist., 18, pp. 50-60.

7: FOULON O., Range-finding study for developmental toxicology in the rat, Rhone-Poulenc Agrochimie, Report N° 96100, 1997.

8: Fisher's exact test, in Sokal R.R. and Rohlf F.J. (1981): Biometry, W.H. Freeman, New York, pp. 738-743.

9: Historical control data for Developmental Toxicology in the rat, Press-book from March 1996, Rhone-Poulenc Agrochimie, Sophia-Antipolis, France.

NOTE FROM THE REVIEWER: THE PROTOCOL DESCRIBED ABOVE IN THE MATERIALS AND METHODS SECTION IS ACCEPTABLE TO FULFILL THE GUIDELINE §83-3A.

C. Results

Maternal Toxicity:

Mortality:

The investigators provided group summary and individual animal data. No animals were reported to have died.

Clinical Observations:

The investigators provided group summary and individual animal data. The only treatment related observation reported was 7 high dose animals with hairloss on either the paws, limbs, flanks, abdomen and/or thorax. Three of these animals also presented with scabs and 2 animals had complete depilation on the abdomen and hindlimbs. One control, 1 low dose (2 with scabs) and 2 mid dose animals had some hairloss.

Body Weight:

The investigators provided group summary and individual animal data. The following table presents body weights and body weight gains (from Table 2 and 3, pages 33-38 of the study report:

Table I: Body Weights and Body Weight Gains (grams±sd)

	BODY WEIGHTS				
DAY:	0	6	16	20	C20[1]
Dose (mg/kg/day):					
Control	263.2±13.5	294.2±17.1	341.5±22.4	405.5±26.1	323.83±21.65
0.2	263.9±12.6	294.1±17.2	340.3±21.9	403.3±25.3	323.46±23.41
1.0	264.0±13.0	297.8±15.4	343.0±18.2	406.9±20.9	326.32±11.09
2.5	263.3±14.1	297.9±17.1	325.3±32.5	391.3±35.7	310.71±28.77
HDT % of Control			95.3%	96.5%	96.0%

	BODY WEIGHTS GAINS					
DAY:	0-6	6-16	16-20	0-20[2]	6-20[2]	C0-20[3]
Dose (mg/kg/day):						
Control	30.96±8.59	47.29±8.60	64.08±6.45	142.3	111.3	60.63±14.63
0.2	30.25±6.84	46.21±7.27	62.92±8.27	139.4	109.2	59.58±14.58
1.0	33.80±6.07	45.24±8.52	63.84±8.80	142.9	109.1	62.32±11.09
2.5	34.63±7.83	27.46**±20.43	65.92±8.12	128.0	93.4	47.46*±20.59
HDT % of Control		58.1%		90.0%	83.9%	78.3%

[1] = body weight on day 20 minus gravid uterine weight; [2] = calculated by the reviewer from the mean body weight data; [3] = body weight on day 20 minus body weight on day 0 minus gravid uterine weight.

The high dose group had lower body weights on study days 16 through 20 and lower body weight gains on study days 6-16 (the

period of organogenesis), study days 0-20 (the entire gestation period), study days 6-20 (the dosing period and days following the dosing period) and body weights on day 20 and days 0-20 corrected for gravid uterine weights.

Food Consumption:

The investigators supplied group summary and individual animal data. The following table presents the food consumption data as presented by the investigators (from Table 4, page 39 of the study report):

Table II: Food Consumption Data (g/day±sd)

DAYS:	1-6	6-9	9-12	12-16	16-20
Dose (mg/kg/day):					
Control	26.38±3.05	25.81±2.73	25.56±2.52	25.92±3.00	28.17±2.51
0.2	26.33±2.55	25.38±2.76	25.35±2.75	25.65±3.09	27.88±3.30
1.0	27.08±2.38	25.99±2.87	25.64±2.49	25.50±2.12	27.84±2.03
2.5	27.17±3.07	23.90±3.80	21.19**±5.50	21.82**±5.35	27.29±3.51

The high dose group consumed less food during the dosing period (period of organogenesis) relative to the control group.

Table III: Food Efficiency Data (%)

DAYS:	0-6	6-16	16-20	0-20	6-20
Dose (mg/kg/day):					
Control	19.6	16.7	45.5	26.8	27.9
0.2	19.2	16.5	45.1	26.5	27.7
1.0	20.8	16.9	45.9	26.9	27.5
2.5	21.2	11.2	48.3	25.9	26.9

Food efficiency calculated by the reviewer (body weight gain over a given time period expressed in grams divided by the food consumption in grams over the same time period X 100).

Food efficiency data showed that the high dose animals had lower food efficiency relative to the control group at the 6-16 day time period. At the post dosing period (days 16-20) where an increase was noted which is indicative of a rebound relative to toxicity noted during the dosing period.

Gross Pathological Observations:

The investigators supplied group summary and individual animal data, no treatment related effects were noted.

Cesarean Section Observations:

The investigators provided group summary and individual animal data. The following table presents the cesarean section observations (from Tables 1, 5, and 6, pages 31-32, 40-46 and Appendix C, pages 65-68):

Table IV: Cesarean Section Observations (some values±sd)

Dose (mg/kg/day):	Control	0.2	1.0	2.5
#Animals Assigned	25	25	25	25
#Animals Mated/Inseminated	25	25	25	25
#Animals Pregnant	24	24	25	24
Pregnancy Rate (%)	96	96	100	95
Maternal Wastage				
#Died	0	0	0	0
#Non pregnant	1	1	0	1
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea[1]	433	416	435	422
Corpora Lutea/dam	18.0±2.7	17.3±2.0	17.4±2.5	17.6±2.0
Total Implantations[1]	383	380	402	391
Implantations/Dam	16.0±1.9	15.8±2.0	16.1±1.6	16.3±2.1
Total Live Fetuses[2]	365	355	369	365
Live Fetuses/Dam	15.2±1.8	14.8±2.6	14.8±2.0	15.2±2.2
Total Resorptions[1]	18	24	33	26
Early[1]	16	23	29	25
Late[1]	2	1	4	1
Resorptions/Dam	0.75±0.74	1.00±1.14	1.32±1.28	1.08±1.35
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm) M	3.64±0.29	3.57±0.26	3.62±0.32	3.55*±0.27
F	3.43±0.26	3.44±0.24	3.47±0.25	3.35**±0.25
Preimplantation Loss(%)[1]	11.6	8.7	7.6	7.4
Postimplantation Loss(%)[1]	4.7	6.6	8.2	6.7
Sex Ratio (% Male)	42	48	51	48

[1] = calculated by the reviewer; [2] = from Table 7, page 48 of the study report.

The only effect noted in the above data was a slight

reduction in fetal body weight (males 97.5% of control, females 97.7% of control) in the high dose group, although statistically significant, the changes are too small to be biologically relevant.

2. Developmental Toxicity

The investigators provided group summary and individual animal data. The following table provide the external, visceral and skeletal findings (from Tables 7-9, pages 47-52 of the study report):

a. External Examinations

Table V: External Examinations

Observations[a]	Control	0.2	1.0	2.5
#pups/litters examined	365/24	355/24	369/25	365/24
3rd & 4th digits partially joined - hindpaw (unilateral)	0/0[b]	0/0	0/0	1(0.3)[1]/1(4.2)
Runt	3(0.8)/3(12.5)	0/0	2(0.5)/1(4.0)	4(1.1)/4(16.7)

[a] = one fetus may have more than one observation; [b] = fetal/litter incidence; [1] = percent affected

b. Visceral Examinations

Table VI: Visceral Examinations

Observations[a]	Control	0.2	1.0	2.5
#pups/litters examined	178/24	173/24	177/25	179/24
Thymus enlarged (unilateral or bilateral)	6(3.4)/5(20.8)[b]	3(1.7)/3(12.5)	2(1.1)/2(8.0)	3(1.7)/3(12.5)
Dilation of ureter (unilateral or bilateral)	10(5.6)/8(33.3)	13(7.5)/7(29.2)	12(6.8)/7(28.0)	4(2.2)/3(12.5)
Ureter convoluted (unilateral or bilateral)	18(10.1)/11(45.8)	29(16.8)/16(66.7)	20(11.3)/12(48.0)	15(8.4)/9(37.5)
Dilatation of renal pelvis(es) (unilateral or bilateral)	4(2.2)/3(12.5)	8(4.6)/6(25.0)	4(2.3)/3(12.0)	2(1.1)/1(4.2)
Ectopic kidney (unilateral)	0/0	1(0.6)/1(4.2)	0/0	0/0
Adrenal glands enlarged (bilateral)	0/0	0/0	1(0.6)/1(4.0)	0/0
Slight dilatation of cerebral lateral ventricle (unilateral)	1(0.6)/1(4.2)	0/0	0/0	0/0
Slight dilatation of 3rd ventricle	0/0	1(0.6)/1(4.2)	0/0	0/0
Slight dilatation of 4th ventricle	0/0	1(0.6)/1(4.2)	0/0	0/0

[a] = one fetus may have more than one observation; [b] = fetal/litter incidence; [1] = percent affected

c. Skeletal Examinations

Table VII: Skeletal Examinations

Observations[a] #pups/litters examined	Control 187/24	0.2 182/24	1.0 192/25	2.5 186/24
Nasal/frontal/parietal bones incompletely ossified (uni-/bilateral)	1(0.5)[1]/1(4.2)[b]	0/0	3(1.6)/2(8.0)	3(1.6)/3(12.5)
Anterior fontanelle enlarged	1(0.5)/1(4.2)	2(1.1)/2(8.3)	0/0	1(0.5)/1(4.2)
Interparietal/occipital bones incompletely ossified	3(1.6)/3(12.5)	4(2.2)/4(16.7)	5(2.6)/3(12.0)	3(1.6)/3(12.5)
Occipital bone bipartite	0/0	0/0	2(1.0)/1(4.0)	0/0
Hyoid body - ossification:				
incomplete	13(7.0)/5(20.8)	10(5.6)/6(25.0)	6(3.1)/5(20.0)	3(1.6)/3(12.5)
not	3(1.6)/2(8.3)	4(2.2)/4(16.7)	12(6.3)/9(36.0)	8(4.4)/4(16.7)
Superior and/or inferior incisors absent	1(0.5)/1(4.2)	4(2.2)/2(8.3)	2(1.0)/1(4.0)	3(1.6)/3(12.5)
Sternebra(e) in region 1-4:				
incomp.oss.	7(3.7)/5(20.8)	5(2.7)/5(20.8)	10(5.2)/3(12.0)	8(4.3)/7(29.2)
misaligned	0/0	1(0.5)/1(4.2)	0/0	3(1.6)/3(12.5)
bipartite	0/0	0/0	1(0.5)/1(4.0)	0/0
not oss.	2(1.1)/2(8.3)	0/0	3(1.6)/2(8.0)	2(1.1)/2(8.3)
5th sternebra(e) misaligned	0/0	1(0.5)/1(4.2)	0/0	0/0
5th/6th sternebrae:				
incomp.oss.	169(90.4)/24(100)	152(83.5)/23(95.8)	158(82.3)/25(100)	149(80.1)/24(100)
not oss.	54(28.9)/21(87.5)	66(36.3)/18(75.0)	73(38.0)/24(96.0)	82**(44.1)/21(87.5)
13th rib(s)				
short (uni-/bilateral)	7(3.7)/5(20.8)	5(2.7)/4(16.7)	3(1.6)/3(12.0)	2(1.1)/1(4.2)
slightly wavy (uni-/bilateral)	19(10.2)/10(41.7)	20(11.0)/9(37.5)	15(7.8)/10(40.0)	16(8.6)/7(29.2)
14th thoracic ribs:				
short/extra oss. point on 14th thoracic vertebra (uni-/bilateral)	7(3.7)/4(16.7)	9(4.9)/5(20.8)	8(4.2)/5(20.0)	4(2.3)/4(16.7)
Presence of 27 pre-sacral vertebrae	0/0	1(0.5)/1(4.2)	2(1.0)/1(4.0)	0/0
Ribs wavy (unilateral) (except 14th thoracic rib)	0/0	0/0	1(0.5)/1(4.0)	0/0
Cervical vertebral arches incompletely ossified (uni-/bilateral) (except atlas and axis)	2(1.1)/1(4.2)	1(0.5)/1(4.2)	4(2.1)/3(12.0)	2(1.1)/2(8.3)

continued

Table VII: Skeletal Examinations continued

Observations[a] #pups/litters examined	Control 187/24	0.2 182/24	1.0 192/25	2.5 186/24
Extra ossification point on 7th cervical vertebra (unilateral)	0/0	0/0	1(0.5)/1(4.0)	0/0
1st thoracic body - ossification:				
not	1(0.5)/1(4.2)	1(0.5)/1(4.2)	3(1.6)/2(8.0)	4(2.2)/4(16.7)
incompletely	5(2.7)/5(20.8)	2(1.1)/2(8.3)	5(2.6)/4(16.0)	7(3.8)/5(20.8)
≤ 6 thoracic vertebral bodies (except 1st body):				
incom.oss./dumbbell	5(2.7)/5(20.8)	3(1.6)/3(12.5)	8(4.2)/5(20.0)	3(1.6)/3(12.5)
bipartite	4(2.1)/3(12.5)	3(1.6)/2(8.3)	3(1.6)/2(8.0)	3(1.6)/3(12.5)
≤ 3 thoracic vertebral bodies (except 1st body):				
not ossified	0/0	0/0	1(0.5)/1(4.0)	0/0
hemibodies	0/0	0/0	0/0	1(0.5)/1(4.2)
≤ 3 lumbar vertebral bodies:				
bipartite	0/0	0/0	1(0.5)/1(4.0)	0/0
dumbbell	0/0	1(0.5)/1(4.2)	0/0	0/0
Pubic bone(s) (uni- or bilateral):				
incomp.oss.	3(1.6)/3(12.5)	3(1.6)/3(12.5)	2(1.0)/2(8.0)	6(3.2)/5(20.8)
not ossified	0/0	0/0	2(1.0)/1(4.0)	0/0
Ischii incompletely ossified (bilateral)	0/0	0/0	2(1.0)/1(4.0)	0/0
< 5 caudal vertebrae	2(1.1)/2(8.3)	0/0	3(1.6)/2(8.0)	8(4.3)/6(25.0)
At least 2 distal phalanges of forepaw(s)/hindpaw(s) not ossified (uni-/bilateral) (except pollex)	3(1.6)/3(12.5)	0/0	2(1.0)/1(4.0)	4(2.2)/4(16.7)
1st distal phalanges of forepaws not ossified (bilateral)	1(0.5)/1(4.2)	0/0	2(1.0)/1(4.0)	3(1.6)/3(12.5)
Metacarpi not ossified (uni-/lateral)	1(0.5)/1(4.2)	0/0	1(0.5)/1(4.0)	1(0.5)/1(4.2)
1 or 2 metatarsi not ossified (bilateral)	2(1.1)/2(8.3)	0/0	2(1.0)/1(4.0)	3(1.6)/3(12.5)
Metacarpi/metatarsi incompletely ossified (uni-/bilateral)	1(0.5)/1(4.2)	0/0	2(1.0)/1(4.0)	1(0.5)/1(4.2)

[a] = one fetus may have more than one observation; [b] = fetal/litter incidence; [1] = percent affected

No treatment related effects were noted in external or visceral examinations. There was a very slight increase in the fetal and litter incidence of incomplete ossification or not ossified in several bones in the high dose group, including the hyoid body, 5th/6th sternebrae, 1st thoracic body, pubic bone and 1 or 2 metatarsi.

D. Discussion/Conclusions

1. Investigators Conclusions

From page 11 of the study report:

This study was conducted according to the OECD 414 (1981), E.E.C. 92/69 - Annex V - method B31 (1992) and EPA/FIFRA 83-3 (1984) guidelines.

Sperm-positive female CD rats were exposed to MB 046513 (batch 805 DAP/DA999: a yellow solid, 992 g/kg purity) by gavage from Days 6 to 15 of gestation. The doses given were 0.2, 1.0 and 2.5 mg/kg/day in suspension in aqueous solution of 0.5% methylcellulose 400. The sperm-positive females were allocated to groups (25 females per group); the sperm-positive day being Day 0 of gestation. The volume of administration was 10 ml/kg based on the most recent body weight recorded.

Maternal body weights were recorded for all the females on Days 0, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 20. Food consumption was also measured for all the females during the interval Day 0-Day 6, daily from Days 6 to 15 and during the interval Day 16-Day 20. Clinical observations were recorded daily. At scheduled sacrifice, on gestation Day 20, the gravid uterine weight was recorded and the dams were evaluated for number of corpora lutea, number and status of implantations (resorptions, dead and live foetuses). Live foetuses were removed from the uteri, counted, weighed, sexed and examined externally. Placental weights of live foetuses were also recorded. Approximately half of the live foetuses from each litter was fixed in Bouin's solution and subsequently dissected for internal examination. The remaining half was eviscerated, fixed in absolute ethanol and stained with alizarin red S for skeletal examination.

Maternal toxicity was observed at 2.5 mg/kg/day, as indicated by the reduced body weight changes, reduced food consumption and the increased incidence of hairloss,. At 1.0 mg/kg/day, a transient but statistically significant reduction in body weight change was observed during the middle dosing period. No maternal toxicity was observed at 0.2 mg/kg/day. Litter parameters showed no biologically relevant findings in any group. At 2.5 mg/kg/day, foetal body weights were significantly reduced correlating with slight ossification delays.

Under the conditions of this study, no adverse effects on foetal development were detected in absence of maternal toxicity.

On the basis of this study, the No Observed Effects Levels (NOEL) for maternal effects is 0.2 mg/kg/day and the NOEL for embryo-foetal development is 1.0 mg/kg/day

2. Reviewer's Conclusions

a. Maternal Toxicity:

Clinical signs were limited to high dose animals with hairloss on either the paws, limbs, flanks, abdomen and/or thorax. The high dose group had lower body weight gains on study days 6-16 (58.1% of control), study days 0-20 (90.0% of control), study days 6-20 (83.9% of control) and days 0-20 corrected for gravid uterine weights (78.3% of control). The high dose group also consumed less food during the dosing period and there was lower food efficiency relative to the control group except for the post dosing period where an increase was noted which is indicative of a rebound relative to toxicity.

b. Developmental Toxicity:

i. Deaths/Resorptions:

No treatment related effects were noted.

ii. Altered Growth:

The only effect noted was a slight reduction in fetal body weight (males 97.5% of control, females 97.7% of control) in the high dose group, although statistically significant, the changes are too small to be biologically relevant.

iii. Developmental Anomalies:

There was a very slight increase in the fetal and litter incidence of incomplete ossification or not ossified in several bones in the high dose group, including the hyoid body, 5th/6th sternebrae, 1st thoracic body, pubic bone and 1 or 2 metatarsi.

iv. Malformations:

No treatment related effects were noted.

Maternal Toxicity NOEL = 1.0 mg/kg/day
Maternal Toxicity LOEL = 2.5 mg/kg/day
Developmental Toxicity NOEL = 1.0 mg/kg/day
Developmental Toxicity LOEL = 2.5 mg/kg/day

E. Study Deficiencies:

No relevant study deficiencies were noted.

DATA EVALUATION RECORD

MB46513-FIPRONIL METABOLITE

Study Type: §84-2; *In vivo* Mammalian Cytogenetics - Micronucleus assay

Work Assignment No. 3-23J (MRID 44262813)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice
OPPTS Number: 870.5395 OPP Guideline Number: §84-2

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (Fipronil metabolite; 99.5% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylphenylpyrazole

CITATION: Proudlock, R.J. (1996) MB46513: Mouse Micronucleus Test. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England. Laboratory Project ID. RNP 453, January 11, 1996. MRID 44262813. Unpublished

SPONSOR: Rhône-Poulenc Agro, Product Safety & Risk Evaluation Department, 355 Rue Dostoïevski, BP 153, 06903 Sophia Antipolis, France.

EXECUTIVE SUMMARY:

In an *in vivo* mouse bone marrow micronucleus assay (MRID 44263813), groups of 15 male and female CD-1 mice were dosed by intragastric gavage with MB46513, a fipronil metabolite (99.5%, a.i.) in corn oil at 2, 4, 8, and 16 mg/kg. Bone marrow cells were harvested at 24, 48, or 72 hours and scored for micronucleated polychromatic erythrocytes (MPCEs).

Slight piloerection was observed in the 2, 4, 8, and 16 mg/kg groups; slight hunched posture was observed in the 8 mg/kg group. No mortalities occurred in the 2 to 8 mg/kg groups, but 7/38 mice in the 16 mg/kg group died. A significant decrease ($p<0.01$ or 0.001) in the ratio of polychromatic to normochromatic erythrocytes was observed in the 16 mg/kg animals. This finding is indicative of bone marrow cell depression. The positive control induced significant increases in MPCEs. **There was no significant increase in the frequency of MPCEs in bone marrow after any MB46513 treatment time; therefore, the test article is considered negative in this micronucleus assay.**

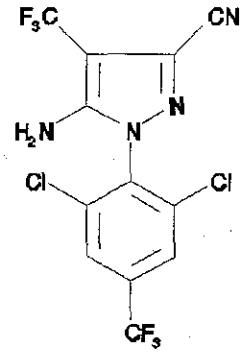
This study is classified as **acceptable**. It satisfies the requirement for FIFRA Test Guideline **84-2** for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB46513
   Description: White powder
   Lot/Batch #: CHO89
   Purity: 99.5 %
   Stability of compound: Corn oil formulations of MB46513 were stable during storage for up to 24 hours (ambient temperature during the day and under refrigeration overnight)
   CAS #: 120068-37-3
   Structure:



   Vehicle used: Corn oil

2. Control Materials:
   Vehicle/Final volume/Route of administration: Corn oil/10 mL/kg/intragastric gavage

   Positive/Final dose(s)/Route of administration: Mitomycin C in 0.9% saline/12 mg/kg/intragastric gavage

3. Test compound administration:
   Volume of test substance administered: 10 mL/kg

   Route of administration: intragastric gavage

   Dose levels used:

   Preliminary Toxicity Tests:
   First: 8, 20, 50, and 125 mg/kg
   Second: 4.1, 5.12, 6.4, and 8 mg/kg
   Third: 4.05, 5.4, 7.2, 9.6, and 12.8 mg/kg

   Micronucleus Assay:
   2, 4, 8, and 16 mg/kg

   Rationale for dose selection: Initial dose levels of 2 to 8

mg/kg used initially in the micronucleus assay were based on the results of the three preliminary toxicity tests. Because dosing at 8 mg/kg bodyweight resulted in minimal toxicity, a supplementary test was performed using a dose level of 16 mg/kg.

4. Test animals:
   a. Species Mouse Strain Swiss CD-1 Age approximately 6 weeks

      Weight: approximately 22-24 grams on arrival
      Source: Charles River UK Limited, Margate, Kent, England

   b. No. animals used per dose:

      Toxicity Study: 2/sex

      Micronucleus Assay: 15 males and 15 females per dose group and the vehicle control, and 5/sex for the positive control. An additional 3-5/sex were dosed at 8 and 16 mg/kg to replace any animals that might die.

   c. Properly maintained? **Yes**

B. TEST PERFORMANCE

1. Treatment and Sampling Times:
   a. Test compound and vehicle controls:
      Dosing: __x__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): _____ 6 hr _____ 12 hr
      __x__ 24 hr __x__ 48 hr __x__ 72 hr

   b. Positive controls:
      Dosing: __x__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): _____ 6 hr _____ 12 hr
      __x__ 24 hr ____ 48 hr ____ 72 hr

2. Tissues and Cells Examined:
   __x__ bone marrow _____ other (list):

   No. of polychromatic erythrocytes (PCEs) examined per animal: 1,000

   No. of normochromatic erythrocytes (NCE) examined per animal; 1,000 erythrocytes were counted and the ratio of PCEs to NCE's was calculated.

3. Details of slide preparation: At 24, 48, and 72 hours after dosing, vehicle controls and animals from each dose group were sacrificed via cervical dislocation. The positive control group was sacrificed 24 hours after dosing. The bone marrow from each femur was removed and suspended in fetal calf serum. The marrow was then smeared on glass slides, fixed in methanol, air-dried, stained in 10% Giemsa, rinsed in distilled H_2O, air-dried, and mounted. The slides were coded prior to scoring.

4. Statistical methods: Non-parametric methods were used for analysis of the results. Wilcoxon's sum of ranks test was used to compare dosed groups with concurrent controls. The Kruskal-Wallis test was used for multiple inter-group comparisons and Jonckheere's test was used to analyze for significance of dose-related trends.

5. Evaluation Criteria: A positive response was a statistically significant ($p<0.01$) increase in MPCEs compared to concurrent controls for ≥1 sampling interval; historical control ranges should be exceeded. A negative response was observed when the values for MPCEs compared to concurrent controls were not significantly greater than the concurrent controls and they fell within the historical control range. A cytotoxic effect was observed when a very large decrease in the ratio of PCEs to NCEs occurred.

II. REPORTED RESULTS

A. Analytical Determinations: Mean concentrations of MB6513 in the test formulations used for the third preliminary toxicity test and the micronucleus assay, including the supplementary test, had relative mean errors (RME) of -12.4 to +1.6% from nominal. Mean concentrations of the formulations used during the first two preliminary toxicity tests had RMEs of -30.0 to +7.4% from nominal. Corn oil formulations of MB46513 at 0.01 and 12.5 mg/mL were stable (RME, -1.6 to +6.3%) during storage for up to 24 hours (ambient temperature during the day and under refrigeration overnight); this time period represented the maximum time from preparation to completion of dosing.

B. Toxicity Study: Three preliminary studies were performed in which groups of 2 mice/sex were dosed at 8 to 125 mg/kg, 4.1 to 8 mg/kg, or 4.05 to 12.8 mg/kg and examined daily for mortality and clinical signs of toxicity during the next 72 hours. Slight to moderate piloerection was observed in all the dosed animals and slight hunched posture was observed in all dose groups, except for the 4.05 mg/kg group. Deaths occurred in 1/4 animals in the 7.2, 8, and 20 mg/kg groups. All animals treated at ≥50 mg/kg died within 24 hours of dosing.

Based on these results, 8 mg/kg was selected as the high dose for the initial micronucleus assay. However, because of minimal clinical signs and the absence of mortalities at 8 mg/kg, a supplementary micronucleus test using 16 mg/kg was also performed.

C. Micronucleus Assay:

1. Animal observations: Groups of mice (15-20/sex/dose) were administered MB46513 by intragastric gavage at 2 to 16 mg/kg. Pharmacotoxic signs and mortality were monitored beginning one hour after dosing. Slight piloerection was observed in the 2, 4, 8, and 16 mg/kg groups; slight hunched posture was observed in the 8 mg/kg group. No mortalities occurred in the 2 to 8 mg/kg groups, but 7/38 mice in the 16 mg/kg group died.

2. Micronucleus assay: The results of the micronucleus assay are presented as an attachment to this DER (study report Tables 1 and 2, pages 23 and 24). The recorded incidence of micronuclei per 1,000 polychromatic erythrocytes varied between 0.4 and 1.3 in all dosed animals. MB46513 did not cause a significant increase in MPCEs compared to vehicle controls in bone marrow cells collected from male or female mice 24, 48, or 72 hours after dosing at 2 to 16 mg/kg. A decrease ($p<0.01$ or 0.001) in the ratio of polychromatic to normochromatic erythrocytes, indicative of bone marrow cell depression, was observed at 48 and 72 hours in animals dosed at 16 mg/kg. The positive control, mitomycin C, induced significant ($p<0.001$) increases in MPCEs.

   The study author concluded that MB46513 was negative in this *in vivo* mouse micronucleus assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. We concur with the study author that MB46513 was negative in this *in vivo* micronucleus assay. We also agree with the study author that the decrease ($p<0.01$ or 0.001) in the ratio of polychromatic to normochromatic erythrocytes observed in the 16 mg/kg animals is indicative of bone marrow cell depression. The sensitivity of this test to detect a genotoxic response was demonstrated by the significant ($p<0.001$) increase in MPCEs induced by the positive control. We conclude that MB46513 was adequately tested and found non-genotoxic in this *in vivo* micronucleus assay.

B. STUDY DEFICIENCIES: None noted.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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TABLE 1

Initial micronucleus test

Summary of results and statistical analysis

Sampling time	Treatment	Dose (mg/kg)	Ratio p/n (mean)†‡	Incidence mnp (mean)†	Incidence mnn (total)
24 Hour	Vehicle control	-	0.761	0.5	0.5
	MB46513	2	0.863 ns	0.5 ns	0.5
		4	0.844 ns	1.3 ns	0.9
		8	0.794 ns	1.0 ns	0.0
	Mitomycin C	12	0.514 *	34.6 **	1.6
48 Hour	Vehicle control	-	0.597	1.2	0.4
	MB46513	2	0.734 ns	0.7 ns	0.5
		4	0.621 ns	0.7 ns	0.1
		8	0.584 ns	0.6 ns	0.3
72 Hour	Vehicle control	-	0.764	1.3	0.5
	MB46513	2	0.968 ns	1.2 ns	0.9
		4	0.821 ns	0.8 ns	0.5
		8	0.746 ns	0.9 ns	1.0

p/n Ratio of polychromatic to normochromatic erythrocytes
mnp Number of micronucleated cells observed per 1000 polychromatic erythrocytes
mnn Number of micronucleated cells observed per 1000 normochromatic erythrocytes
‡ Any small apparent errors of ±0.001 are due to rounding of individual values for presentation in tables

† Results of statistical analysis using Kruskal-Wallis', Jonckheere's and Wilcoxon's tests as appropriate:

ns	P>0.01	one-sided probabilities
*	P<0.01	
**	P<0.001	

TABLE 2

Supplementary test

Summary of results and statistical analysis

Sampling time	Treatment	Dose (mg/kg)	Ratio p/n (mean)†‡	Incidence mnp (mean)†	Incidence mnn (total)
24 hour	Vehicle control	-	0.796	0.4	0.0
	MB46513	16	0.801 ns	0.4 ns	0.0
	Mitomycin C	12	0.581 **	31.8 **	1.2
48 hour	Vehicle control	-	0.822	0.6	0.5
	MB46513	16	0.667 *	0.7 ns	0.5
72 hour	Vehicle control	-	0.777	0.5	1.1
	MB46513	16	0.630 **	0.2 ns	0.4

p/n Ratio of polychromatic to normochromatic erythrocytes
mnp Number of micronucleated cells observed per 1000 polychromatic erythrocytes
mnn Number of micronucleated cells observed per 1000 normochromatic erythrocytes
‡ Any small apparent errors of ±0.001 are due to rounding of individual values for presentation in tables

† Results of statistical analysis using Kruskal-Wallis', Jonckheere's and Wilcoxon's tests as appropriate:

ns	$P>0.01$	one-sided probabilities
*	$P<0.01$	
**	$P<0.001$	

DATA EVALUATION RECORD

MB46513-FIPRONIL METABOLITE

Study Type: §84-2; CHO/HPRT/Mammalian Activation Gene Mutation Assay

Work Assignment No. 3-23K (MRID 44262814)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
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Date: 10/12/97

Quality Assurance:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 10/14/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

MB46513-Fipronil Metabolite Mammalian Cells in Culture; Gene Mutation (§84-2)

EPA Reviewer: Nancy E. McCarroll
Toxicology Branch 1 (7509C)

EPA Work Assignment Manager:
Marion Copley, DVM, DABT
Registration Action Branch 1 (2002)

Nancy E. McCarroll 10/21/97

Marion Copley 11/5/97

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster ovary (CHO) cells
OPPTS Number: 870.5300 OPP Guideline Number: §84-2

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (Fipronil metabolite; 99.5% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylphenylpyrazole

CITATION: Adams, K. (1996) MB46513 CHO Mammalian Cell Mutation Assay. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Study No. RNP 452/950622, January 11, 1996. MRID 44262814. Unpublished.

SPONSOR: Rhône-Poulenc Agro, Product Safety & Risk Evaluation Department, 355 Rue Dostoïevski, BP 153, 06903 Sophia Antipolis, France.

EXECUTIVE SUMMARY:

In independently performed mammalian cell gene mutation assays at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus (MRID 44262814), Chinese Hamster Ovary (CHO) cells cultured *in vitro* were exposed to MB46513, a fipronil metabolite (99.5% a.i.) in dimethyl sulfoxide at doses ranging from 5 to 625 µg/mL, with and without S9 activation. Cultures were exposed for 4 hours prior to plating for determination of cytotoxicity, expression, and selection of the mutant phenotype.

MB46513 was tested to insoluble levels (generally ≥60 µg/mL) and reproducible cytotoxic doses (≥125 µg/mL -S9 and ≥250 µg/mL +S9). The positive controls induced the appropriate response. **MB46513 did not, however, induce forward mutations at the HPRT locus in CHO cells at any dose level tested, with or without metabolic activation.**

This study is classified as **acceptable** and satisfies the guideline requirement **(§84-2)** for *in vitro* mammalian forward gene

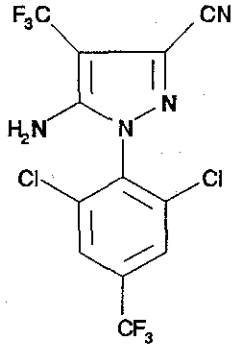
mutation studies.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB46513
   Description: White powder
   Lot/Batch #: CHO89
   Purity: 99.5 %
   Stability of compound: Dimethyl sulfoxide (DMSO) formulations of MB46513 at 0.5 and 100 mg/mL were stable (RME, -1.2 to +2.0%) during storage for up to 24 hours at ambient temperature.
   CAS #: 120068-37-3
   Structure:



   Vehicle used: DMSO
   Other comments: The test article was stored at room temperature in the dark.

2. Control Materials:
   Negative: Vehicle served as negative control
   Vehicle/final concentration: DMSO/1% (v/v)
   Positive:
   Nonactivation: Ethyl methanesulphonate (EMS)/250 µg/mL
   Activation: 20-Methylcholanthrene (MC)/5 µg/mL

   DMSO was also used as solvent for the positive controls

3. Activation: The S9 was derived from Aroclor 1254-induced rat liver. The S9 mixture was prepared in Ham's F12 medium supplemented with 2 mM glutamine and 50 µg/mL gentamicin and contained 25% S9 (v/v), 8 mM NADP and 43.5 mM isocitric acid. The final S9 concentration in the cultures was 4% (v/v).

4. Test Cells: Chinese hamster ovary (CHO) cell line K_1-BH4 was the test system used. The cells were maintained in Ham's F12 medium supplemented with 5% heat-inactivated bovine fetal calf serum. Cultures were incubated at 37 C in a humidified atmosphere containing 5% CO_2 for ≥20 hours prior

to treatment.

Properly maintained? **Yes**
Periodically checked for mycoplasma contamination? **Not reported**
Periodically checked for karyotype stability? **Not reported**
Periodically "cleansed" against high spontaneous background? **Yes**

Media: Ham's F12 supplemented with 2mM glutamine and 50 μg/mL gentamicin was used during the treatment period. The treatment media supplemented with 5% heat-inactivated bovine fetal calf serum was used as growth medium. The selection medium was the growth medium containing 6-thioguanine. The growth medium supplemented with 15 μg/mL hypoxanthine, 0.3 μg/mL amethopterin, and 4 μg/mL thymidine was used to check against spontaneous mutants.

5. Locus Examined: Hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: 10 μg/mL 6-thioguanine (6-TG)

6. Test compound concentrations used

a.Cytotoxicity Assays:

1. Preliminary Assay: 5, 10, 15, 30, 60, 125, 250, 500, and 625 μg/mL (±S9)

2. Main Assay 1: 5, 10, 15, 30, 60, 80, 100, and 125 μg/mL (-S9) and 15, 30, 60, 80, 100, 125, 250, and 625 μg/mL (+S9)

3. Main Assay 2: 5, 10, 15, 30, 60, 80, 100, and 125 μg/mL (-S9) and 30, 60, 80, 100, 125, 175, 250, and 625 μg/mL (+S9)

b.Mutation assays:

Main Trial 1: 15, 30, 60, 80, and 100 μg/mL (-S9) and 30, 60, 80, 100, 125, and 625 μg/mL (+S9)

Main Trial 2: 5, 10, 15, and 30 μg/mL (-S9) and 30, 60, 80, and 100 μg/mL (+S9)

B.TEST PERFORMANCE

1. Cell treatment:
a.Cells were exposed to the test compound, vehicle or the positive controls for:
4 hours (nonactivated); 4 hours (activated).

b.After washing, cells (200/plate) were cultured for 7 days prior to cytotoxicity evaluation. Cells (10^6/plate)

plated in the mutation assay (expression period) were subcultured once during the expression period on Day 4 or 5 and harvested after a total of 7 days.

c. After expression, 2 x 10^5 cells/dish (5 dishes/culture) were cultured for 7 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/culture) were cultured for 7 days in growth medium to determine cloning efficiency.

Note: Duplicate cultures were prepared for each test material treatment group and the positive controls; four replicate cultures were used with the solvent control (+/-S9).

2. Statistical Methods: A weighted analysis of variance was used to determine the statistical significance of the data.

3. Evaluation Criteria: The test article was considered positive if it produced the following: (i) a statistically significant (not specified) increase in mutant frequency following treatment; (ii), a dose-dependent increase in mutant frequencies over ≥2 dose levels; (iii) the mutant frequency increase was reproducible and (iv) the mean mutant frequency was >20 mutants/10^6 survivors (the upper limit of the historical control range) and the survival rate was ≥20%.

II. REPORTED RESULTS

A. Analytical Determinations: Mean concentrations of MB46513 in the three formulations (0.5, 25, and 62.5 mg/mL) analyzed had relative mean errors (RME) of -0.8 to +1.9% from nominal. DMSO formulations of MB46513 at 0.5 and 100 mg/mL were stable (RME, -1.2 to +2.0%) during storage for up to 24 hours at ambient temperature; this time period represented the maximum time from preparation to completion of dosing.

B. Cytotoxicity assay: The results of the cytotoxicity assay are presented as an attachment to this DER (study report Table 1, page 20). MB46513 was evaluated for cytotoxicity at nine concentrations ranging from 5 to 625 µg/mL with and without S9 activation. Cytotoxicity was estimated by cell survival relative to the vehicle controls. At concentrations ≥125 µg/mL (±S9), the test article precipitated from solution. Cell survival under nonactivated conditions was 0-9% of the controls at these insoluble levels. With S9 activation, no cells survived at 500 or 625 µg/mL and survival at ≤250 µg/mL was ≥21%. Based on these results, 625 µg/mL was selected as the highest concentration with S9 and 125 µg/mL was selected as the highest concentration without S9 for the initial mutation assay.

C. Mutagenicity assay: Two trials of the mutation assay were performed. In both trials, MB46513 precipitated at doses ranging from 60-125 μg/mL -S9 or 125-625 μg/mL +S9. Results were as follows:

Nonactivated conditions: Results from the cytotoxicity phase of testing in Trial 1 were in general agreement with the preliminary data and indicated that relative percent survival (RPS) was 9% at the highest dose tested (125 μg/mL) and ≥24% at ≤100 μg/mL. Accordingly, doses of 15-100 μg/mL were selected for cloning. In contrast, the test material was more cytotoxic in Trial 2, causing lethality at ≥80 μg/mL and severe cytotoxicity (3% RPS) at 60 μg/mL. Lower doses (5-30 μg/mL) were not cytotoxic. The study author did not comment on the increases cytotoxic response seen in this trial. Nevertheless, the mutation frequencies (MFs) for all cloned doses were within the generally accepted background range for CHO cells (≤20 mutants/10^6). In both trials, the nonactivated positive control (250 μg/mL EMS) induced the expected significant ($p<0.001$) increase in the MF.

S9-activated conditions: In Trial 1, RPS was 18% at the two highest doses (250 and 625 μg/mL) and ≥77% at ≤125 μg/mL. Based on these data, cells exposed to 30-625 μg/mL were plated for mutant selection. As shown in Study Report Table 7, p.26 (see Attachment), MFs for all treatment groups were lower than the solvent control value. As noted for the second trial of the nonactivated series, MB46513 induced a more severe cytotoxic response in Trial 2 of the S9-activated phase of testing. For this trial, no cells were recovered following treatment with doses ≥175 μg/mL. For the remaining levels, RPS ranged from 54% at 125 μg/mL to 111% at 30 μg/mL. There were, however, no statistically significant or dose-dependent increases in the MFs observed for treated cultures at any dose level. By contrast, mean MFs for the S9-activated positive control (5 μg/mL MC) ranged from 103-230 mutants/10^6 survivors for both trials.

The results of the mutagenicity assays are presented as an attachment to this DER (Study Report Tables 3, 5, 7, and 9, pages 22, 24, 26, and 28).

III. DISCUSSION/CONCLUSIONS:

A. Investigator's Conclusions: The test article was negative for the induction of forward gene mutations at the HPRT locus in CHO cells under the conditions of this study.

B. Reviewer's Discussion: MB46513 was tested to insoluble levels (generally ≥60 μg/mL) and reproducible cytotoxic doses [≥125 μg/mL -S9; ≥250 μg/mL +S9) but failed to induce a

mutagenic response in this mammalian cell forward gene mutation assay. Although the study author did not comment on the inconsistency of the cytotoxicity data, we conclude in agreement with the study author that the test article was not mutagenic at the HPRT locus with and without metabolic activation. The sensitivity of the assay system to detect forward gene mutations at this locus was adequately demonstrated by the results obtained with the positive controls. We assess, therefore, that the results of this study provide adequate evidence to consider MB46513 negative in this *in vitro* system.

C. Study deficiencies: No deficiencies that would be expected to alter the conclusions of the study were identified.

ATTACHMENT

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY
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TABLE 1

Preliminary toxicity test

(i) In the absence of S-9 mix

Concentration of MB46513 (μg/ml)	Viable colony counts				Cell survival (% control)
	1	2	3	Total	
0	58	38	43	139	100
5	81	70	88	239	123
10	91	82	95	268	137
15	95	87	77	259	133
30	70	61	60	191	98
60	90	77	75	242	124
#125	7	2	8	17	9
#250	0	0	0	0	0
#500	0	0	0	0	0
#625	0	0	0	0	0

(ii) In the presence of S-9 mix

Concentration of MB46513 (μg/ml)	Viable colony counts				Cell survival (% control)
	1	2	3	Total	
0 (DMSO control)	51	38	43	132	100
	36	42	44	122	
	59	62	53	174	
	65	66	62	193	
5	43	54	55	152	98
10	54	53	C	(161)	104
15	68	58	66	192	124
30	61	62	63	186	120
60	45	44	40	129	83
#125	19	20	11	50	32
#250	9	11	12	32	21
#500	0	0	0	0	0
#625	0	0	0	0	0

C Contaminated. Figure in brackets is adjustment made for contaminated plates and is used in all calculations

Precipitate observed

TABLE 3

Test 1. Mutant frequency in the absence of S-9 mix

Concentration of MB46513 (μg/ml)	No. of colonies on non-selective plates				Plating efficiency (%)	No. of colonies on selective plates						Mutant frequency per 10^6 survivors	Mean mutant frequency per 10^6 surivors
	1	2	3	Total		1	2	3	4	5	Total		
0 (DMSO control)	129	130	138	397	66	0	0	1	0	1	2	3	
	115	133	124	372	62	0	0	0	0	0	0	0	
	124	168	127	419	70	0	0	0	0	0	0	0	1
	133	110	101	344	57	0	0	0	0	0	0	0	
15	114	111	103	328	55	0	0	0	0	0	0	0	1
	108	116	112	336	56	0	1	0	0	0	1	2	
30	132	118	117	367	61	7	2	2	1	0	12	20	13
	129	113	133	375	63	0	1	1	0	2	4	6	
60	126	111	123	360	60	1	2	3	2	2	10	17	10
	131	120	119	370	62	0	0	0	0	1	1	2	
80	157	117	158	432	72	0	0	1	0	0	1	1	1
	110	120	C	(345)	58	0	0	0	0	0	0	0	
100	127	117	104	348	58	1	0	0	0	0	1	2	1
	109	125	120	354	59	0	0	0	0	0	0	0	
EMS (250 μg/ml)	106	94	103	303	51	21	19	24	C	C	(107)	212	215
	121	119	114	354	59	30	27	26	21	24	128	217	***

C Contaminated. Figure in brackets is adjustment made for contaminated plates and is used in all calculations

*** Significantly different from control $P < 0.001$

TABLE 5

Test 2. Mutant frequency in the absence of S-9 mix

Concentration of MB46513 (μg/ml)	No. of colonies on non-selective plates				Plating efficiency (%)	No. of colonies on selective plates						Mutant frequency per 10^6 survivors	Mean mutant frequency per 10^6 surivors
	1	2	3	Total		1	2	3	4	5	Total		
0 (DMSO control)	159	142	114	415	69	1	0	1	0	1	3	4	
	168	141	138	447	75	1	0	1	0	0	2	3	
	151	169	145	465	78	1	1	2	1	0	5	6	7
	130	119	128	377	63	2	3	0	2	1	8	13	
5	153	110	139	402	67	0	0	0	0	0	0	0	1
	133	111	138	382	64	0	0	0	0	1	1	2	
10	138	123	119	380	63	0	1	0	1	0	2	3	3
	108	115	117	340	57	0	1	0	0	0	1	2	
15	104	118	113	335	56	0	0	0	0	2	2	4	3
	99	119	112	330	55	0	0	1	0	0	1	2	
30	87	112	99	298	50	0	0	0	0	0	0	0	2
	118	109	84	311	52	0	0	0	0	2	2	4	
EMS (250 μg/ml)	113	103	108	324	54	38	30	34	33	45	180	333	504
	54	52	40	146	24	40	32	32	35	25	164	674	***

*** Significantly different from control $P < 0.001$

TABLE 7

Test 1. Mutant frequency in the presence of S-9 mix

Concentration of MB46513 (μg/ml)	No. of colonies on non-selective plates				Plating efficiency (%)	No. of colonies on selective plates						Mutant frequency per 10^6 survivors	Mean mutant frequency per 10^6 surivors
	1	2	3	Total		1	2	3	4	5	Total		
0 (DMSO control)	128	124	146	398	66	2	C	C	C	C	(10)	15	
	129	134	105	368	61	1	0	0	0	C	(1)	2	
	134	138	126	398	66	0	1	0	1	4	6	9	9
	141	142	134	417	70	1	0	1	1	3	6	9	
30	126	119	108	353	59	1	0	1	1	4	7	12	7
	121	114	111	346	58	0	0	0	1	0	1	2	
60	134	119	111	364	61	0	0	0	0	0	0	0	0
	126	117	117	360	60	0	0	0	0	0	0	0	
80	123	130	107	360	60	0	0	0	0	0	0	0	0
	113	112	119	344	57	0	0	0	0	0	0	0	
100	147	109	107	363	61	0	0	0	0	0	0	0	1
	107	116	118	341	57	0	1	0	0	0	1	2	
125	109	86	120	315	53	0	0	0	0	1	1	2	4
	118	125	108	351	59	0	0	0	0	3	3	5	
625	77	57	69	203	34	0	0	0	0	0	0	0	2
	71	84	67	222	37	0	0	0	0	1	1	3	
MC (5 μg/ml)	118	114	106	338	56	26	29	25	31	C	(139)	247	230
	96	126	104	326	54	26	19	18	24	28	115	212	***

C Contaminated. Figure in brackets is adjustment made for contaminated plates and is used in all calculations

*** Significantly different from control $P < 0.001$

TABLE 9

Test 2. Mutant frequency in the presence of S-9 mix

Concentration of MB46513 (μg/ml)	No. of colonies on non-selective plates				Plating efficiency (%)	No. of colonies on selective plates						Mutant frequency per 10^6 survivors	Mean mutant frequency per 10^6 surivors
	1	2	3	Total		1	2	3	4	5	Total		
0 (DMSO control)	110	124	102	351	59	0	1	0	3	0	4	7	
	148	143	149	440	73	2	1	0	0	0	3	4	
	184	145	128	457	76	1	0	0	0	2	3	4	4
	130	142	119	391	65	0	0	0	0	0	0	0	
30	110	159	114	383	64	2	1	1	2	0	6	9	7
	127	156	144	427	71	0	2	1	0	0	3	4	
60	137	162	134	433	72	2	2	0	0	2	6	8	8
	111	127	139	377	63	2	0	2	1	0	5	8	
80	129	101	117	347	58	3	2	0	0	0	5	9	6
	132	110	123	365	61	2	0	0	0	0	2	3	
100	111	83	88	282	47	4	0	0	0	0	4	9	7
	105	103	101	309	52	1	1	0	0	0	2	4	
MC (5 μg/ml)	111	100	95	306	51	18	20	18	21	18	95	186	188
	116	104	116	336	56	21	16	27	18	24	106	189	***

*** Significantly different from control $P<0.001$

012509

DATA EVALUATION RECORD

MB46513-FIPRONIL METABOLITE

Study Type: §84-2; *In vitro* Chromosome Aberration Assay in Cultured Human Lymphocyte Cells

Work Assignment No. 3-23L (MRID 44262815)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

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Date: 10/29/97

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DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian chromosome aberrations in cultured human lymphocytes
OPPTS Number: 870.5375 OPP Guideline Number: §84-2

DP BARCODE: D237893 SUBMISSION CODE: S24626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (Fipronil Metabolite; 99.5% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)4-trifluoromethylphenylpyrazole

CITATION: Adams, K., (1996) MB46513: Metaphase Chromosome Analysis of Human Lymphocytes Cultured *In Vitro*. Huntingdon Life Sciences, Huntingdon Cambridgeshire, England. Ltd Project Id. RNP 451/951219, January 11, 1996. MRID 44262815. Unpublished.

SPONSOR: Rhône Poulenc Agro, Product Safety & Risk Evaluation Department, 355 Rue Dostoievski, BP. 153, Sophia Antipolis, France.

EXECUTIVE SUMMARY:

In independently performed mammalian cell chromosome aberration assays (MRID 44262815), cultured human lymphocytes were exposed to MB46513 (99.5% a.i.), in dimethyl sulfoxide over concentration ranges of 5-625 µg/mL +S9 or 1-625 µg/mL -S9. Cells treated with 5, 15, 30, 40 or 60 µg/mL +S9 or 5, 10, 15 or 30 µg/mL -S9 were subjected to cytogenetic analysis. With metabolic activation, the cells were exposed to MB46513 for 3 hours and harvested 15 or 29 hours later. Without metabolic activation, the cells were exposed continuously for 18 or 32 hours. Preparations for metabolic activation were made from induced rat livers.

MB46513 was insoluble at ≥500 µg/mL and severely cytotoxic at doses ≥125 µg/mL -S9 or ≥250 µg/mL +S9. For the first metaphase analysis, 30 µg/mL -S9 produced a 71% decrease in the mitotic index (MI) while 60 µg/mL +S9 caused a 66% decrease in the MI. For the second metaphase analysis, an ≈36% decreases in the MI was calculated at 15 µg/mL -S9 or 40 µg/mL +S9. The positive controls induced the expected high yield of cells with chromosome aberrations. **There was, however, no indication that MB46513**

induced a clastogenic response at any dose with or without S9 activation.

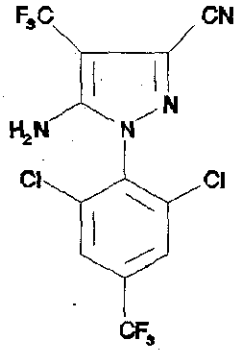
This study is classified as **Acceptable (Guideline)** and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MB46513
   Description: White powder
   Lot/Batch #: CHO89
   Purity: 99.5% a.i.
   Stability: Formulations of 0.5 or 100 mg/mL MB46513 prepared in dimethyl sulfoxide (DMSO) were stable (RME, -1.2 to +2.0%) during storage at ambient temperature for up to 24 hours.
   CAS #: Not provided
   Structure:



   Vehicle used: DMSO
   Other comments: The test article was stored at room temperature in the dark.

2. Control Materials:
   Negative: Vehicle control
   Solvent/final concentration: DMSO (10 µl/mL)
   Positive: Nonactivation: Ethylmethane sulphonate (in DMSO at 500 and 750 µg/mL, first test and 250 and 500 µg/mL, second test)
   Activation: Cyclophosphamide (in sterile distilled water at 10 and 15 µg/mL, first test and 2.5 and 5 µg/mL, second test)

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobartital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

   The S9 mix was prepared by the study laboratory.
   S9 mix composition: S9 fraction (10% v/v), $MgCl_2$ (8 mM), glucose-6-phosphate (5 mM), NADP (4 mM) and sodium ortho-

phosphate buffer pH 7.4 (100 mM). The final S9 concentration in the treatment medium was 2% (v,v).

4. Test compound concentrations used: For the first cytogenetic test, the cultures were harvested after 18 hours. For the second cytogenetic test, the cultures were harvested at 18 and 32 hours.

Preliminary cytotoxicity test: Cytotoxicity was tested in conjunction with the cytogenetic tests.

First cytotoxicity/cytogenetic test:

18-hour sampling interval
Nonactivated and activated conditions: 5, 15, 30, 60, 125, 250, 500, and 625 µg/mL were tested.
Metaphase analyses were performed on the 5, 15, and 30 µg/mL treatments.

Second cytotoxicity/cytogenetic test:

18-hour sampling interval
Nonactivated conditions: 1, 2.5, 5, 10, 15, 25, 30, 50, and 75 µg/mL. Metaphase analyses were performed on the 5, 10, and 15 µg/mL treatments.
Activated Conditions: 5, 10, 15, 30, 40, 60, 75, and 100 µg/mL. Metaphase analyses were performed on the 15, 30, and 40 µg/mL

32-hour sampling interval
Nonactivated conditions: 1, 2.5, 5, 10, 15, 25, and 50 µg/mL. Metaphase analysis was performed on the 10 µg/mL treatment.
Activated Conditions: 15, 30, 40, 60, 75, and 100 µg/mL. Metaphase analysis was performed on the 60 µg/mL treatment.

5. Test cells: Peripheral blood was collected and pooled from healthy, male volunteers. Whole blood was diluted in RPMI 1640 culture medium and lymphocytes were separated by centrifugation. The lymphocytes were then suspended in RPMI 1640 medium, supplemented with 20% fetal calf serum and containing 175 µg/mL phytohemagglutinin to stimulate lymphocytes to divide. Cultures were incubated for 48 hours at 37°C prior to treatment.

Properly maintained? **Yes**

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Not performed

2. Cytogenetic Assay:

a. Cell treatment: After the 48-hour incubation, S-9 activation mix (20%, v/v), if appropriate, and dosing solutions, vehicle, or positive control (1%, v/v) were added to the cultures. Duplicate cultures were established for each test dose and positive control; vehicle controls were established in quadruplicate. The highest level tested was determined by the limit of solubility of the test compound dissolved in DMSO in the culture medium. In the presence of S9 activation, cultures were treated for 3 hours, centrifuged, resuspended in fresh medium, and incubated for a further 15 or 29 hours. Under nonactivated conditions, lymphocytes were continuously exposed to the test material, vehicle or positive control for 18 or 32 hours. Cultures were harvested and slides prepared as described below. The mitotic index (MI) was determined by examining 1000 cells per culture.

b. Spindle inhibition
Inhibitor used/concentration: Colchicine (0.25 µg/mL)
Administration time: 2 hours (before cell harvest)

c. Cell harvest:
Cells exposed to test material, solvent, or positive control were harvested 18 or 32 hours after initiation of treatment. The cells were swollen with 20% Hanks balanced salt solution and fixed with methanol:glacial acetic acid (3:1, v/v).

d. Details of slide preparation:
The cells suspended in fresh fixative were dropped onto microscope slides and air-dried. The slides were stained with Giemsa (1:9 in buffered distilled water), air-dried and mounted in DPX.

e. Metaphase analysis
No. of cells examined per dose: 200
Vehicle control: 400
Positive control: 200

Scored for structural chromosome aberrations: Yes

Scored for polyploidy: No

Coded prior to analysis: Yes

f. Evaluation criteria: The test was considered positive if the incidence of aberrant cells at one or more treatment levels was significantly higher that the solvent control group, exceeded the provided historical control range and was accompanied by a dose response.

g. Statistical analysis: Data were evaluated for statistical significance at $0.01>p<0.001$ using Fisher's Test.

II. REPORTED RESULTS

A. Analytical Determinations: Actual concentrations of MB46513 in the dosing solutions prepared for the first assay (0.5, 25 or 62.5 mg/mL) had relative mean errors (RME) of -0.8 to +1.9% from nominal. DMSO formulations of 0.5 and 100 mg/mL MB46513 were stable (RME, -1.2 to +2.0%) during storage for up to 24 hours at ambient temperature.

B. Cytotoxicity and cytogenetic assays: The results of the cytogenetic assays are presented as an attachment to this DER (Study Report Tables 3, 4, 7, 8, 11, and 12, pages 24, 25, 28, 29, 32, and 33). Results were as follows:

First cytotoxicity/cytogenetic assay: Concentrations ranging from 5-625 µg/mL +/-S9 were processed in the first cytogenetic assay. Precipitation of MB46513 occurred at doses ≥500 µg/mL. No cells were recovered following treatment with ≥125 µg/mL -S9 or ≥250 µg/mL +S9. Reductions in relative MIs of 56, 71 or 81% were recorded for cultures exposed to nonactivated MB46513 at 15, 30 or 60 µg/mL, respectively. In the S9-activated series, relative MIs of 34 or 21% were observed at 60 or 125 µg/mL, respectively. It was also reported that chromosome morphology was poor at 60 and 125 µg/mL +S9. Based on these findings, cells treated with 5, 15 or 30 µg/mL ±S9 were analyzed for chromosomal aberrations. As shown in Study Report Tables 3 and 4, no statistically significant increases in the percentage of aberrant cells was observed at any dose.

Second toxicity/cytogenetic assay: Doses selected for evaluation in the second assay were 1-75 µg/mL -S9 and 5-100 µg/mL +S9. Effects on the MI in the nonactivated phase of testing were not dose related. Relative MIs were 75% at 50 and 75 µg/mL and 63, 42 or 58% at 15, 25 or 30 µg/mL. It was reported that chromosome morphology was poor at ≥25 µg/mL. In the presence of S9 activation, the relative MI was 9% at the highest dose tested (100 µg/mL), 34% at 75 µg/mL and 19% at 60 µg/mL. The lack of dose dependency under nonactivated and S9-activated conditions was not explained. Lower S9-activated levels (≤40 µg/mL) had relative MIs ≥57% of control. The study author stated, however, that the highest analyzable S9-activated dose was 40 µg/mL. Accordingly, cultures treated with 5, 10 or 15 µg/mL (-S9) or 15,

30 and 40 µg/mL (+S9) were examined for chromosomal aberrations. As shown in Study Report Tables 7 and 8, the findings from the second cytogenetic assay confirm the earlier results indicating that MB46513 was not clastogenic using the 18-hour harvest protocol.

A 32-hour harvest was included in the second trial with dose ranges comparable to the 18-hour harvest and metaphase analysis of the 10-µg/mL -S9 or 60-µg/mL +S9 treatment groups. It was reported that these doses were the highest analyzable concentrations based on 32-40% reductions in the MIs. Without S9 activation, the mean frequency of aberrant metaphases (including and excluding gaps) in vehicle control cultures was 0.75%. The corresponding values for the cultures treated with 10 µg/mL -S9 were 4.0% (including gaps; $p<0.001$) and 3.5% (excluding gaps; not significant). Both values were within the historical control range (0-6.5%) whether gaps were or were not considered. In the presence of S9 activation, the aberrant cell frequencies (including and excluding gaps) were 2.75% in the vehicle control cultures and 4% in the cultures treated with 60 µg/mL.

In both assays, the nonactivated (ethylmethane sulphonate) and S9-activated (cyclophosphamide) positive controls induced significant ($p<0.001$) increases in cells with aberrations. From the overall results, the study author concluded that under the conditions of the test, MB46513 showed no evidence of clastogenic activity.

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

MB46513 was tested to concentrations that produced ≥50% cytotoxicity [≥15 µg/mL (-S9); and ≥60 µg/mL (+S9)] but failed to induce an increase in the frequency of cells with abnormal chromosome morphology. Hence, we agree with the study author's conclusion that the test article was not clastogenic in cultured human lymphocytes with or without metabolic activation. The sensitivity of the assay system to detect chromosome aberrations was demonstrated by the results obtained with the positive controls. We conclude, therefore, that the results of this study provide sufficient evidence to consider MB46513 negative in this *in vitro* system.

B. Study deficiencies

None noted.

ATTACHMENTS

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY SEE THE FILE COPY

Study Report Tables 3, 4, 7, 8, 11, and 12,
pages 24, 25, 28, 29, 32, and 33

TABLE 3

Metaphase analysis data - first test, 18 hour harvest

Without S-9 mix

Test agent	Concentration μg/ml	No. cells examined	Aberrations								No. of aberrant cells			
			Chromatid type		Chromosome type		Others	Gaps			Exc. gaps	Mean %	Inc. gaps	Mean %
			ctb	cte	csb	cse		ctg	csg					
Dimethyl sulphoxide (solvent control)	10 μl/ml	100			1						1	1.25	1	1.25
		100			1						1		1	
		100			1						1		1	
		100	1		1						2		2	
MB46513	5	100			1						1	1.0	1	1.0
		100			1						1		1	
	15	100									0	2.0	0	2.0
		100	2		4						4		4	
	30	100	2		1						2	3.5	2	3.5
		100	5		2						5		5	
Ethyl methanesulphonate	500	100	7		12						14	14.0***	14	14.0***
		100	6	1	11						14		14	

*** P<0.001
Otherwise P>0.01

ctb Chromatid break
cte Chromatid exchange
csb Chromosome break
cse Chromosome exchange
ctg Chromatid gap
csg Chromosome gap
others Cells with greater than 10 aberrations, pulverised cells and pulverised chromosomes

TABLE 4

Metaphase analysis data - first test, 18 hour harvest

With S-9 mix

Test agent	Concentration μg/ml	No. cells examined	Aberrations						No. of aberrant cells			
			Chromatid type		Chromosome type		Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %
			ctb	cte	csb	cse		ctg csg				
Dimethyl sulphoxide (solvent control)	10 μl/ml	100	4						3		3	
		100							0	2.5	0	2.5
		100	4		1			1	4		4	
		100	2		1				3		3	
MB46513	5	100	1		1				2	1.5	2	1.5
		100			2				1		1	
	15	100	1						1	1.0	1	1.0
		100	1						1		1	
	30	100	1					1	1	3.0	1	3.0
		100	5		1				5		5	
Cyclophoshamide	10	100	35	1	9		1	1	21	21.5***	21	21.5***
		100	29	1	14		2		22		22	

*** P<0.001
Otherwise P>0.01

ctb Chromatid break
cte Chromatid exchange
csb Chromosome break
cse Chromosome exchange
ctg Chromatid gap
csg Chromosome gap
others Cells with greater than 10 aberrations, pulverised cells and pulverised chromosomes

TABLE 7

Metaphase analysis data - second test, 18 hour harvest

Without S-9 mix

Test agent	Concentration μg/ml	No. cells examined	Aberrations						No. of aberrant cells			
			Chromatid type		Chromosome type		Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %
			ctb	cte	csb	cse		ctg csg				
Dimethyl sulphoxide (solvent control)	10 μl/ml	100							0		0	
		100			1				1	0.75	1	0.75
		100							0		0	
		100			2				2		2	
MB46513	5	100			3				2	1.5	2	1.5
		100	1		1				1		1	
	10	100			3				2	2.5	2	2.5
		100	1		3				3		3	
	15	100							0	0.5	0	0.5
		100			1				1		1	
Ethylmethane sulphonate	500	100	7		9				14	13.0***	14	13.0***
		100	9		5				12		12	

*** P<0.001
Otherwise P>0.01

ctb Chromatid break
cte Chromatid exchange
csb Chromosome break
cse Chromosome exchange
ctg Chromatid gap
csg Chromosome gap
others Cells with greater than 10 aberrations, pulverised cells and pulverised chromosomes

TABLE 8

Metaphase analysis data - second test, 18 hour harvest

With S-9 mix

Test agent	Concentration μg/ml	No. cells examined	Aberrations						No. of aberrant cells			
			Chromatid type		Chromosome type		Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %
			ctb	cte	csb	cse		ctg csg				
Dimethyl sulphoxide (solvent control)	10 μl/ml	100							0		0	
		100	3						3	2.3	3	2.3
		100	3						3		3	
		100	3						3		3	
MB46513	15	100	1						1	2.0	1	2.0
		100	3						3		3	
	30	100	3						2	4.5	2	4.5
		100	6		1				7		7	
	40	100	5		1				6	4.5	6	4.5
		100	3		1				3		3	
Cyclophosphamide	10	97	25	2	3				26	20.8***	26	20.8***
		100	17	1				1	15		15	

*** P<0.001
** P<0.01
Otherwise P>0.01

ctb Chromatid break
cte Chromatid exchange
csb Chromosome break
cse Chromosome exchange
ctg Chromatid gap
csg Chromosome gap
others Cells with greater than 10 aberrations, pulverised cells and pulverised chromosomes

TABLE 11

Metaphase analysis data - second test, 32 hour harvest

Without S-9 mix

Test agent	Concentration μg/ml	No. cells examined	Aberrations							No. of aberrant cells			
			Chromatid type		Chromosome type		Others	Gaps		Exc. gaps	Mean %	Inc. gaps	Mean %
			ctb	cte	csb	cse		ctg	csg				
Dimethyl sulphoxide (solvent control)	10 μl/ml	100	1							1		1	
		100			2					2	0.75	2	0.75
		100								0		0	
		100								0		0	
MB46513	10	100	1		1					2	3.5	2	4.0**
		100	5		1			1		5		6	
Ethyl methanesulphonate	250	100	10		10					14	16.5***	14	16.5***
		100	7	2	14					19		19	

*** P<0.001
Otherwise P>0.01

ctb Chromatid break
cte Chromatid exchange
csb Chromosome break
cse Chromosome exchange
ctg Chromatid gap
csg Chromosome gap
others Cells with greater than 10 aberrations, pulverised cells and pulverised chromosomes

TABLE 12

Metaphase analysis data - second test, 32 hour harvest

With S-9 mix

Test agent	Concentration µg/ml	No. cells examined	Aberrations						No. of aberrant cells			
			Chromatid type		Chromosome type		Others	Gaps	Exc. gaps	Mean %	Inc. gaps	Mean %
			ctb	cte	csb	cse		ctg csg				
Dimethyl sulphoxide (solvent control)	10 µl/ml	100	2						2	2.75	2	2.75
		100	3		1				4		4	
		100	1		1				2		2	
		100	2		1				3		3	
MB46513	60	100	4		2				5	4.0	5	4.0
		100	2		1				3		3	
Cyclophosphamide	5	100	15		10				20	21.0***	20	21.0***
		100	13	3	19			1	22		22	

*** P<0.001
Otherwise P>0.01

ctb Chromatid break
cte Chromatid exchange
csb Chromosome break
cse Chromosome exchange
ctg Chromatid gap
csg Chromosome gap
others Cells with greater than 10 aberrations, pulverised cells and pulverised chromosomes

DATA EVALUATION RECORD

MB 46513-FIPRONIL METABOLITE

Study Type: 85-1; Metabolism of ^{14}C-MB 46513 in Rats

Work Assignment No. 3-23P (MRID 44262817)

Prepared for
Health Effects Division
Office of Pesticide Programs
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Date: 10/10/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

MB46513-Fipronil Metabolite Metabolism (§85-1)

EPA Reviewer: Alberto Protzel, Ph.D.
Toxicology Branch I (7509C)

11/3/97

EPA Secondary Reviewer: Marion Copley, DVM, DABT
Registration Action Branch I (2002)

11/5/97

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat
OPPTS Number: 870.7485 OPP Guideline Number: §85-1

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (Fipronil metabolite; 99.4% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole

CITATION: Totis, M. (1996) MB46513: Absorption, Distribution, Metabolism, and Excretion in the Rat: Amended (Final Report): Rhône-Poulenc Agrochimie & Rhône-Poulenc Agriculture Ltd. Lab Project Number: SA 95304, July 31, 1996. MRID 44262817. Unpublished.

SPONSOR: Rhône-Poulenc Ag Company, 2 T.W. Alexander Drive, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In a rat metabolism study (MRID 44262817), [U-^{14}C-phenyl] MB46513 (>99% a.i.) was administered to five Sprague-Dawley CD strain rats/sex/dose by gavage as a single dose at 1 or 10 mg/kg or as a single dose at 1 mg/kg following a 14-day pretreatment with unlabeled MB46513 at 1 mg/kg.

Within 168 hours of dosing, 93-101% of the administered dose was recovered from both sexes of each dose group, of which 46.4-69.5% was in the feces, 19.9-41.1% was in the tissues and carcass, and 4.4-10.8% was in the urine. In all test groups, fecal excretion was higher for males (60.1-69.5%) than for females (46.4-56.0%), and less radioactivity was retained in the carcasses and tissues of males (19.9-26.6%) than females (30.0-41.1%). Levels of urinary excretion were comparable between sexes.

Excretion of the radioactivity was increased slightly by pretreatment and at the high dose level. Within 168 hours of dosing, the single low-dose (SOLD) animals excreted 51.6-67.1% of the dose in feces and urine (including cage wash), whereas the

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repeated low-dose (ROLD) animals excreted 66.4-73.7%, and the single high-dose (SOHD) animals excreted 69.9-80.6%.

Radioactivity was excreted gradually by all dose groups, but the rate of excretion differed between dose groups. Fecal excretion peaked on Day-1 for the SOLD group, on Day-6 for the ROLD group, and on Day-5 for the SOHD group. Urinary excretion showed a similar pattern within dose groups.

Maximum concentrations of radioactivity in blood were attained within 46 to 73 hours of dosing and were similar between sexes within dose group (low dose, 0.15 ppm; high dose, 2.03-2.31 ppm). For both dose groups, elimination half-lives were 156-170 hours for males and 210-221 hours for females. The ratio of the areas under the concentration curves (AUC) for high to low-dose animals was 15.2 for males and 10.9 for females, reflecting the difference in dose levels.

The general distribution of radioactivity among tissues was the same between dose groups and sexes, although the actual levels differed between dose groups and sexes. Concentrations of radioactivity were highest in fat [fat/plasma ratios: 6.3-12.8 in males and 16.4-25.2 in females], followed usually by the adrenals and liver. Females also had high concentrations associated with the ovaries. The lowest concentrations of radioactivity were generally associated with the brain, spleen, muscle, whole blood, and stomach. With the exception of whole blood and plasma, concentrations of radioactivity in all tissues were generally higher for females than for males, e.g. radioactivity in fat was 1.6-2.8 times higher in females than in males. Among the dose groups, ^{14}C-residues were lowest in tissues from the SOLD group with the exceptions of residual carcass and skin plus fur. Pretreatment with MB46513 increased the residue levels in tissues, and residue levels in tissues from the SOHD group were 10-30 times higher than in tissues from the SOLD group.

The major radioactive component identified in excreta was unchanged MB46513 (males, 28.6-44.2%; females, 35.4-39.6%), nearly all of which was found in the feces. Unchanged MB46513 in urine accounted for <0.1% of the dose. The only other components in excreta accounting for >5% of the dose were MB46400 (males, 5.7-10.6%; females, 3.1-7.1%) and the 4-cyano-5-(N)cysteine conjugate of MB46513 (males, 7.2-14.2%; females, 3.8-9.2%). Other minor components identified in excreta included: RPA 105048; the sulfate conjugate of MB46513 (≤2.4%); a 4-cyano-5-(N) cysteine glycine conjugate of MB46513 (0.7-3.8%); and a 5-(N) cysteine conjugate of MB46513 (1.9-3.5%).

Within each dose group, the metabolite profile was the same among sexes, although metabolite levels were generally higher in males than females. The metabolic profile was also similar between

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dose groups, although there were differences in the relative levels of metabolites. Pretreatment resulted in lower levels of MB46513 and higher levels of metabolites than in SOLD animals. Levels of MB46513 were similar in excreta of SOHD and SOLD groups, but levels of metabolites were generally higher for the SOHD group.

These data indicate that fecal excretion of unchanged MB46513 is the principal pathway for elimination of MB46513 from rats. The metabolism of MB46513 in rats involves substitution of the trifluoromethyl or cyano groups on the pyrazole ring and/or sulfate, glucuronide, or glutathione conjugation at the amide on the pyrazole ring. The high levels of radioactivity in fat compared to blood and the prolonged elimination half-life indicate that there is a potential for bioaccumulation of MB46513 in fatty tissues.

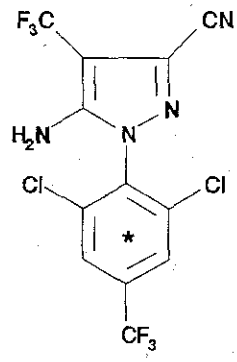
This metabolism study in the rat is classified **Acceptable (Nonguideline)** as it is not a required guideline study. It is acceptable for the purposes for which it was intended (metabolism information on MB46513, a photolytic metabolite of fipronil) as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: [U-^{14}C-phenyl]MB46513
   Radiochemical purity: ≥99.4 [determined by TLC and HPLC]
   Specific activity: 697.63-935.45 MBq/mmol for 1 mg/kg doses, and 168.15-181.79 MBq/mmol for 10 mg/kg doses.
   Lot/Batch: GHS 847
   Structure:



* denotes position of ^{14}C-label

   Non-radioactive MB46513
   Purity: 99.5% [determined by HPLC]
   Lot/Batch No.: 10DGM22
   Contaminants: Not reported

2. Vehicle control: Aqueous suspension of methyl cellulose 0.5% (w/w) and TWEEN 80 (0.01% w/v).

3. Test animals: Species: Rat
   Strain: Sprague-Dawley, Iffa Credo CD strain
   Age at study initiation: unspecified
   Weight at study initiation: 148-280 g for males and 153-217 g for females in all tests, except the tissue metabolism test which used 3 males weighing 425-485 g.
   Source: Iffa Credo, France
   Housing: For the excretion and distribution studies, animals were individually housed in metabolism units (Jencon's Metabowls Mk III) equipped with containers for collection of urine and feces, at least 1 day prior to dosing. For the blood pharmacokinetic and tissue metabolism studies, animals were housed in wire mesh bottomed cages over disposable absorbent paper.
   Diet: Type A04C, U.A.R., ad libitum
   Water: Water, ad libitum
   Environmental conditions:

Temperature: 69.8 ± 3.6 F
Humidity: Unspecified
Air Changes: Unspecified
Photoperiod: 12 hours light/12 hours dark
Acclimation period: Unspecified

B. STUDY DESIGN:

The study was designed to determine the absorption, metabolism, distribution, and excretion of [^{14}C]MB46513, a photolytic metabolite of fipronil, as a function of single and repeated oral dosing of rats. This is a special, nonguideline study.

1. Group Arrangements

Animals were randomly assigned to each test group based upon body weights (Table 1). For the elimination and distribution study, 5 animals/sex/group were used to examine excretion, distribution, and recovery of the ^{14}C-labeled test material. Rats in the single low-dose (SOLD) and single high-dose (SOHD) groups received a single oral dose of [^{14}C]MB46513 at 1 or 10 mg/kg, respectively. Rats in the repeated low-dose (ROLD) group received a single oral dose of [^{14}C]MB46513 at 1 mg/kg following 14 consecutive days of oral dosing with unlabeled MB46513 at 1 mg/kg. The basis for selecting the high dose level was not indicated; however, the report cited an acute oral toxicity study on rats indicating that the oral LD_{50} of MB46513 for male and female rats is 18 and 15 mg/kg, respectively.

For the blood pharmacokinetic study, 5 animals/sex/group were used to examine the concentration and elimination of radioactivity in the blood over time following a single oral dose of [^{14}C]MB46513 at 1 mg/kg (SOLDPK) or 10 mg/kg (SOHDPK). A group (SOLDTM) of 3 males were dosed once orally with [^{14}C]MB46513 at 1 mg/kg in order to provide tissue samples for metabolite characterization.

Table 1. Dosing groups for [^{14}C]MB46513 studies.

Test Group [a]	Nominal dose (mg/kg)	Remarks
Single Oral Low Dose (SOLD)	1	Excretion and Distribution Study: Actual dose levels were 0.94 and 0.96 mg/kg for SOLD males and females; 0.92 and 0.93 mg/kg for ROLD males and females; and 10.74 and 10.81 mg/kg for SOHD males and females. Feces, urine, cage wash and tissue/organ samples were collected. Urine and feces were used for metabolite characterization.
Repeated Oral Low Dose (ROLD)	1	
Single Oral High Dose (SOHD)	10	
Single Low Dose (SOLDPK)	1	Blood Pharmacokinetic Study: Actual dose level was 1.07 mg/kg for SOLDPK males and females, and 11.18 and 10.95 mg/kg for SOHDPK males and females. Blood was sampled over time.
Single High Dose (SOHDPK)	10	
Single Low Dose (SOLDTM)	1	Tissue Metabolism Study: Actual dose level was 1.23 mg/kg. Tissues were collected for metabolite characterization.

a Dose groups were comprised of 5 animals/sex with the exception of the SOLDTM group which consisted of 3 male rats.

2. Dosing and Sample Collection

On the day of dosing, the ^{14}C-labeled test material was prepared by dissolving in an aqueous suspension of methyl cellulose (0.5% w/w) with TWEEN 80 (0.01% w/v). Low-dose solutions were prepared using undiluted [^{14}C]MB46513, and high-dose solutions were prepared by diluting [^{14}C]MB46513 with unlabeled MB46513. Each dose solution was assayed for the concentration of active ingredient and radioactivity before, during, and after dosing. The rodent diet ration was withheld for 18 hours prior to dosing with [^{14}C]MB46513 and for 1 hour after dosing. Doses were administered orally by gavage such that rats received 1 g of dosing suspension/200 g of body wt. [^{14}C]MB46513 was administered at nominal levels of 1 or 10 mg/kg for the low and high-dose groups, respectively. Actual average dose levels are presented in Table 1.

a. Excretion and distribution studies

One day prior to dosing with [^{14}C]MB46513, animals in each dose group (SOLD, ROLD, and SOHD) were placed in metabolism cages equipped with containers for collection of urine and feces. Urine and feces were collected at 24-hour intervals post-dose, and cages were rinsed with distilled water every 24 hours. Animals were

exsanguinated under anaesthesia 168 hours after dosing and the following samples were collected for analysis: liver, kidneys, heart, lungs, thyroid, brain, spleen, muscle, abdominal fat, gonads, uterus, stomach with contents, intestinal tract with contents, bone and marrow, pancreas, whole blood, plasma, skin with fur, and residual carcass. Samples were analyzed as they were collected or stored at -20°C in the dark until analysis.

Urine, plasma, and cage washing samples were analyzed for total radioactivity directly by liquid scintillation counting (LSC). Fecal samples were extracted with methanol, and the methanol extracts were analyzed for total radioactivity using LSC. The residual insoluble fecal fractions were combusted and radioassayed by LSC. Tissue and organ samples were combusted and radioassayed by LSC. Skin with fur and the residual carcasses were solubilized in alcoholic 2M KOH at 50°C for 24 hours and aliquots were then radioassayed by LSC.

b. Blood pharmacokinetic study

Blood samples were collected from the tails of animals prior to dosing with [^{14}C]MB46513 and at 0.5, 1, 2, 4, 6, 8, and 24 hours after dosing for both dose groups (SOLDPK and SOHDPK), and at 24-hour intervals up to 360 hours post-dosing for low-dose animals and 408 hours post-dosing for high-dose animals. Thereafter, blood samples were collected at 48- to 72-hour intervals until 648 hours post-dosing. Blood samples were dried, combusted, and radioassayed by LSC.

c. Tissue metabolism study

Three males, dosed with [^{14}C]MB46513 at 1 mg/kg, were exsanguinated under anaesthesia 168 hours after dosing and abdominal fat, liver, and skin with fur samples were collected. Kidneys, heart, lungs, thyroid, brain, spleen, muscle, abdominal fat, testes, stomach with contents, intestinal tract with contents, bone and marrow, pancreas were removed and the residual carcass was retained for analysis. Samples were stored at -20°C in the dark until analysis for metabolite characterization.

3. Metabolite Characterization

For metabolite characterization and quantitation, the following samples were analyzed by reverse-phase HPLC with UV and radioactive monitoring: urine, methanol extracts of feces, and methanol/water extracts of solid fecal residues.

Urine samples from individual animals over the entire sampling period were pooled by sex and test group in 24- or 48-hour increments. The pooled urine samples were purified using a solid phase extraction cartridge (SPE RP18) eluted with methanol and were concentrated prior to HPLC analysis. In addition, aliquots of pooled Day-4 urine samples from male and female high-dose rats were subjected to enzymatic hydrolysis using bovine ß-glucuronidase and Helix pomatia sulfatase. The enzyme reactions were conducted in 0.2 M sodium acetate at pH 5 at 37 C for 16 hours, and the resulting hydrolysates were concentrated by SPE RP18 prior to HPLC analysis. Pooled Day-4 urine samples were also subjected to acid hydrolysis (1 M HCl, 4 hours at 37°C), concentrated by SPE RP18, and analyzed by HPLC.

For HPLC analysis of fecal ^{14}C-residues, the methanolic fecal extracts from individual animals over the entire sampling period were also pooled by sex/group in 24-hour increments and concentrated prior to HPLC analysis. Solid fecal residues were pooled by sex/group in 24- or 48-hour increments and extracted sequentially with methanol/water (3:1, v/v), methanol/water (1:1, v/v), and water. The resulting methanolic and aqueous extracts were combined by sample and centrifuged, the supernatants were dried and reconstituted in methanol for HPLC analysis. In addition, aliquots of pooled Day-3 methanol extracts of feces from male and female high-dose rats were concentrated and subjected to acid hydrolysis (1 M HCl, 4 hours at 37°C). The resulting hydrolysates were purified by SPE RP18, concentrated, and analyzed by HPLC.

For analysis of ^{14}C-residues in tissues, solvent extracts of liver, fat, skin/fur, and carcass were analyzed by LC/MS. Samples of liver, fat, and carcass were pooled separately by dose group and extracted by homogenization with acetonitrile (ACN). The resulting ACN extracts were partitioned against hexane, concentrated to dryness, and reconstituted in methanol prior to analysis. Pooled skin/fur samples were crushed in liquid nitrogen and extracted with methanol (all animals) and acetone (females only). The skin extracts were concentrated and redissolved in methanol prior to analysis.

To confirm the identify of ^{14}C-components isolated by HPLC, isolated components from selected samples were cochromatographed with reference standards, and/or analyzed by LC/MS, LC/MS/MS, GC/MS (with and without trimethyl-silyl derivatization), and NMR.

4. Statistics

For urine and fecal samples, radioactivity in terms of the % of the administered dose was reported for individual samples and for the total from each animal. The means of the five animals/sex/dose group were calculated by the reviewer. For tissue/blood samples, radioactivity, in terms of concentration (μg equivalents/g, i.e. ppm) and the % of administered dose, was reported for individual samples and as the mean (with ± S.D.) of five animals/sex/dose group.

For the blood kinetics study, radioactivity in whole blood was reported in terms of concentration (μg equivalents/g) for individual samples and as the mean (with ± S.D.) of five animals/sex/dose group. A computer program, TopFit V 1.1, was used to generate the following plasma kinetic parameters: elimination phase half-life ($t_{½}$), area under the concentration time curve (AUC_{0-648}), maximum concentration in the blood (C_{max}), and the time (T_{max}) required to reach C_{max}.

II. RESULTS

A. Excretion, Distribution, and Recovery

1. Excretion

As shown in Tables 2, 3 and 4, excretion of radioactivity was gradual and occurred primarily via the feces (46.4-69.5%), with urinary excretion being a secondary pathway (4.4-10.8%). For each dose group, fecal excretion of the dose was higher for males (60.1-69.5%) than for females (46.4-56.0%); whereas, levels of urinary excretion were similar for males (6.1-10.3%) and females (4.4-10.8%). Total excretion was increased slightly by pretreatment and by increasing the dose level. Within 168 hours of dosing, the SOLD animals excreted 51.6-67.1% of the dose in feces and urine (including cage wash), while the ROLD groups excreted 66.4-73.7%, and the SOHD groups excreted 69.9-80.6%.

The most notable difference in excretion between the dose groups was in the rate of excretion. For the SOLD groups (Table 2), the rate of fecal excretion was highest on Day-1 (16.5-22.7%) and declined steadily throughout the study to 3.1-3.4% on Day-7; the rate of urinary excretion peaked on Day-2 (0.9-1.2%), declining to 0.5% by Day-7. For both ROLD and SOHD groups (Tables 3 and 4, respectively), there was an initial burst of radioactivity recovered in feces (6.9-15.0%) on Day-1, but rates declined by Day-2 (3.0-6.5%). The rates of fecal excretion were highest on Day-6 (10.5-

12.7%) for ROLD animals and on Day-5 (15.8-15.9%) for SOHD animals. Urinary excretion increased steadily following dosing and peaked on Day-5 (2.1-2.4%) for ROLD animals and on Day-4 (2.3-2.9%) for SOHD animals.

a) Single low dose (SOLD): Following a single low dose of [^{14}C]MB46513, elimination of radioactivity was gradual and occurred primarily via the feces (Table 2). Fecal excretion of radioactivity was highest during the initial 24-hour post-dose period (males, 22.7%; females 16.5%) and declined steadily to 3.1-3.4% of the dose during the final 24 hours of the study (144-168 hours). Urinary excretion of radioactivity was low throughout the study, totaling 6.1 and 4.4% of the dose for males and females, respectively. As with fecal excretion, the rate of urinary excretion peaked early (24-48 hours) at 0.9-1.2% of the dose and declined steadily to 0.5% of the dose during the final 24-hour period. Cumulative fecal and urinary excretion (plus cage wash) for males and females was 67.1% and 51.6% of the administered dose, respectively.

Table 2. Recovery over time of radioactivity in excreta of rats following administration of a single oral dose of [^{14}C]MB46513 at 1 mg/kg.[a]

Sample	Percent of radioactive dose administered							
	Males							
	0-24 hr	24-48 hr	48-72 hr	72-96 hr	96-120 hr	120-144 hr	144-168 hr	Total
Urine	1.05	1.21	1.05	0.82	0.76	0.63	0.54	6.06
Feces[b]	22.65	10.62	8.77	5.79	4.84	4.03	3.38	60.08
Cage Wash	NR[c]	NR	NR	NR	NR	NR	NR	0.95
Total	23.70	11.83	9.82	6.61	5.60	4.66	3.92	67.09
Sample	Females							
	0-24 hr	24-48 hr	48-72 hr	72-96 hr	96-120 hr	120-144 hr	144-168 hr	Total
Urine	0.69	0.89	0.70	0.59	0.57	0.54	0.46	4.44
Feces[b]	16.46	8.38	6.56	4.16	4.14	3.53	3.12	46.35
Cage Wash	NR	NR	NR	NR	NR	NR	NR	0.82
Total	17.15	9.27	7.26	4.75	4.71	4.07	3.58	51.61

a Data are the mean of 5 animals/sex at each sampling interval and were calculated by the reviewer using data from Tables 8, 9 and 14 (pages 41, 42 and 47) of the study report.
b Radioactivity in fecal extracts and residual solids.
c NR = Not reported.

b) Repeated low dose (ROLD): Pretreatment with MB46513 prior to a single low dose of [^{14}C]MB46513 had a slight effect on the total amounts of radioactivity excreted in the

urine and feces, but fecal excretion remained the principal route of elimination as in the single low-dose groups (Table 3). As in the SOLD groups, the percentage of the dose excreted was higher for males (73.7%) than for females (66.4%). The total amount of radioactivity excreted in the feces was comparable for the ROLD males (61.1%) and SOLD males (60.1%), but fecal excretion of radioactivity was higher from ROLD females (53.4%) than from SOLD females (46.4%). Both ROLD males and females excreted more of the dose in urine (10.3-10.8%) than did the SOLD rats. However, the most pronounced effect from pretreatment was a change in the rates of both fecal and urinary excretion of radioactivity. In the first 24 hours following dosing, there was an initial burst in the amount of radioactivity recovered in feces (6.9-11.0%) followed by a decrease on Day-2 (3.4-6.5%). Subsequently, the rate of fecal excretion increased and peaked on Day-6 (10.5-12.7%). In urine, the rate of excretion increased steadily following dosing and peaked on Day-5 at 2.1-2.4% of the dose. As in the SOLD groups, excretion of the dose was higher for males (73.7%) than for females (66.4%).

Table 3. Recovery over time of radioactivity in excreta of rats following administration of a single oral dose of [^{14}C]MB46513 at 1 mg/kg after 14 consecutive daily doses of MB46513 at 1 mg/kg.[a]

Sample	Percent of radioactive dose administered							
	Males							
	0-24 hr	24-48 hr	48-72 hr	72-96 hr	96-120 hr	120-144 hr	144-168 hr	Total
Urine	0.39	0.92	1.46	1.89	2.37	1.84	1.42	10.29
Feces[b]	10.97	3.36	5.95	9.03	10.78	12.65	8.34	61.08
Cage Wash	NR[c]	NR	NR	NR	NR	NR	NR	2.37
Total	11.36	4.28	7.41	10.92	13.15	14.49	9.76	73.74
Sample	Females							
	0-24 hr	24-48 hr	48-72 hr	72-96 hr	96-120 hr	120-144 hr	144-168 hr	Total
Urine	0.65	1.27	1.66	1.93	2.14	1.80	1.31	10.76
Feces[b]	6.86	6.45	8.99	4.61	9.23	10.48	6.73	53.35
Cage Wash	NR	NR	NR	NR	NR	NR	NR	2.28
Total	7.51	7.72	10.65	6.54	11.37	12.28	8.04	66.39

a Data are the mean of 5 animals/sex at each sampling interval and were calculated by the reviewer using data from Tables 15, 16 and 21 (pages 48, 49 and 54) of the study report.
b Radioactivity in fecal extracts and residual solids.
c NR = Not reported.

c) Single high dose (SOHD): As with the ROLD groups, a single high dose of [^{14}C]MB46513 at 10 mg/kg had a slight effect on the total amounts of radioactivity excreted in the urine and feces. However, as in the SOLD and ROLD groups, fecal excretion remained the principal route of elimination for all animals and a higher percentage of the dose was excreted by males (80.6%) than by females (69.9%). For both SOHD males and females, The total amount of radioactivity excreted in the feces of SOHD males (69.5%) and females (56.0%) was ~10 higher than the percent dose recovered in the feces of SOLD males (60.1%) and females (46.4%). SOHD males and females also excreted more of the dose in urine (8.8-10.7%) than did the SOLD animals. The most pronounced effect from increasing the dose was a change in the rates of both fecal and urinary excretion of radioactivity. As with the ROLD groups, there was an initial burst in the amount of radioactivity recovered in feces (0-24 hours, 7.9-15.0%) followed by a decrease on Day-2 (3.0-3.8%). Subsequently, the rate of fecal excretion increased, peaking on Day-5 at 15.8-15.9% of the dose. In urine, the rate of excretion increased steadily following dosing and peaked on Day-4 at 2.3-2.9% of the dose.

Table 4. Recovery over time of radioactivity in excreta of rats following administration of a single oral dose of [^{14}C]MB46513 at 10 mg/kg.[a]

Sample	Percent of radioactive dose administered							
	Males							
	0-24 hr	24-48 hr	48-72 hr	72-96 hr	96-120 hr	120-144 hr	144-168 hr	Total
Urine	0.28	1.07	2.00	2.34	1.33	1.09	0.69	8.80
Feces[b]	15.02	2.97	8.53	11.92	15.94	9.12	6.04	69.54
Cage Wash	NR[c]	NR	NR	NR	NR	NR	NR	2.26
Total	15.30	4.04	10.53	14.26	17.27	10.21	6.73	80.60
Sample	Females							
	0-24 hr	24-48 hr	48-72 hr	72-96 hr	96-120 hr	120-144 hr	144-168 hr	Total
Urine	0.40	1.64	2.47	2.90	1.53	1.05	0.71	10.70
Feces[b]	7.92	3.82	6.19	11.50	15.75	7.28	3.58	56.04
Cage Wash	NR	NR	NR	NR	NR	NR	NR	3.20
Total	8.32	5.46	8.66	14.40	17.28	8.33	4.29	69.94

a Data are the mean of 5 animals/sex at each sampling interval and were calculated by the reviewer using data from Tables 1, 2 and 7 (pages 34, 35 and 40) of the study report.
b Radioactivity in fecal extracts and residual solids.
c NR = Not reported.

2. Tissue Distribution

Levels of radioactivity at sacrifice (168 hours post-dose) in tissue, blood, and carcass samples from male and female rats from each dose group (SOLD, ROLD, and SOHD) are summarized in Table 5, and the ratios of radioactivity in tissues/plasma are summarized in Table 6.

The general distribution of radioactivity among tissues was the same between dose groups and sexes, although the actual levels differed between dose groups and sexes. In all animals, concentrations of radioactivity were highest in fat. The ratio of radioactivity in fat/plasma was 6.3-12.8 for males and 16.4-25.2 for females. The next highest concentrations of radioactivity were generally associated with the adrenals (tissue/plasma ratio: males, 2.1-2.5; females, 3.1-4.7) and liver (tissue/plasma ratio: males, 2.0-2.5; females, 2.4-2.9). Females also had high concentrations of radioactivity associated with the ovaries (tissue/plasma ratio: 2.6-4.4). The lowest concentrations of radioactivity were generally associated with the brain, spleen, muscle, whole blood, and stomach.

The concentration of radioactivity in all tissues was generally higher for females than for males, with the exception of whole blood and plasma, in which levels of radioactivity were equivalent between sexes. Concentrations of radioactivity in fat were 1.6-2.8 times higher in females than in males.

Among the dosed groups, ^{14}C-residues were lowest in tissues from the SOLD group. With the exceptions of residual carcass and skin plus fur, pretreatment with MB46513 increased the residue levels in tissues of ROLD males and females. Levels of radioactivity were highest in tissues from the SOHD group, with residue levels generally 10-30 times higher than in tissues from the SOLD group.

a) Single low dose (SOLD): Radioactivity was detectable in all blood and tissue samples from both male and female rats sacrificed 168 hours following a single dose with [^{14}C]MB46513 at 1 mg/kg. The concentration of radioactivity in tissues was higher for females than for males, with the exception of whole blood and plasma, in which levels of radioactivity were equivalent between sexes. For both sexes, the highest residue levels were found in fat (1.54-2.73 ppm). The next highest levels of radioactivity were generally associated with the liver, adrenals, and skin with fur (males, 0.28-0.34 ppm; females 0.31-0.60 ppm). Females also had high residue levels (0.34-0.49 ppm) in ovaries, pancreas, and intestines. For both sexes, tissues with the

lowest residues (0.04-0.15 ppm) included brain, spleen, muscle, whole blood, and stomach.

b) Repeated low dose (ROLD): Pretreatment with unlabeled MB46513 for 14 days prior to a single dose of [^{14}C]MB46513 had no apparent effect on the distribution of radioactivity in tissues. As with the SOLD group, females had higher concentrations of radioactivity in tissues than males, with the exception of whole blood and plasma. For both sexes, residue levels were highest in fat (male 1.97 ppm; females, 3.15 ppm), followed by levels in the liver and adrenals (males, 0.57-0.58 ppm; females, 0.67-0.85 ppm). The lowest residues levels were associated with brain, spleen, muscle, and stomach (males, 0.07-0.12 ppm; females, 0.21-0.26 ppm). Although the pattern of distribution was the same as in the SOLD animals, radioactivity accumulated to a greater extent in all tissues of the ROLD animals, except for the residual carcass and the skin with fur.

c) Single high dose (SOHD): Increasing the dose of [^{14}C]MB46513 to 10 mg/kg increased the accumulation of radioactivity in the tissues, but had no apparent effect on the distribution of radioactivity among tissues. As with the SOLD group, females had higher concentrations of radioactivity in tissues than males, with the exception of liver, whole blood and plasma. For both sexes, residue levels were highest in fat (male, 18.31 ppm; female 50.83 ppm), followed by levels in the liver and adrenals (males, 6.43-7.02; females, 6.66-7.40 ppm). Females also had high levels of radioactivity associated with the ovaries (9.74 pm) and uterus (10.43 ppm), although these levels may have been influenced by fat associated with these samples. The lowest concentrations of radioactivity were associated with brain, spleen, muscle, whole blood, and stomach (males, 1.25-1.79 ppm; females, 1.52-2.15 ppm).

Table 5. Distribution of radioactivity in blood, tissues, organs, and carcasses of rats sacrificed 168 hours after dosing with [^{14}C]MB46513 at 1 or 10 mg/kg.[a]

Dose	PPM in [^{14}C]MB46513 Equivalents					
	Single low dose (SOLD - 1 mg/kg)		Repeated low dose (ROLD - 1 mg/kg)		Single high dose (SOHD - 10 mg/kg)	
Tissue/organ	Male	Female	Male	Female	Male	Female
Liver	0.28	0.31	0.57	0.67	7.02	6.66
Kidney	0.15	0.24	0.30	0.41	3.37	3.83
Heart	0.09	0.14	0.20	0.34	2.34	2.91
Lung	0.14	0.21	0.33	0.53	4.07	4.70
Brain	0.06	0.11	0.12	0.26	1.75	2.15
Spleen	0.05	0.08	0.12	0.22	1.65	1.87
Muscle	0.09	0.15	0.12	0.21	1.44	1.90
Fat	1.54	2.73	1.97	3.15	18.31 [b]	50.83 [b]
Gonads	0.06	0.49	0.28	0.65	2.49	9.74
Intestine & contents	0.19	0.34	0.52	0.58	4.90	4.75
Bone & Marrow	0.05	0.08	0.13	0.18	2.04	2.31
Adrenals	0.30	0.51	0.58	0.85	6.43	7.40
Residual Carcass	0.15	0.23	0.15	0.23	1.76	2.43
Blood	0.07	0.06	0.17	0.18	1.79	1.52
Plasma	0.12	0.11	0.33	0.29	2.79	2.54
Skin & Fur	0.34	0.60	0.28	0.43	2.66	5.06
Uterus	NA[c]	0.25	NA	0.42	NA	10.43
Pancreas	0.22	0.42	0.31	0.61	3.72	4.90
Thyroid	0.20	0.28	0.21	0.38	2.64	3.20
Stomach & contents	0.04	0.07	0.07	0.21	1.25	2.01

a Data are the mean of 5 animals/sex/dose group and were obtained from Tables 3, 4, 10, 11, 17 and 18 (pages 36, 37, 43, 44, 50 and 51 of the study report).
b Data are the average of 3 animals/sex.
c NA = Not applicable.

Table 6. Ratio of radioactivity in tissues/plasma of rats sacrificed 168 hours after dosing with [^{14}C]MB46513 at 1 or 10 mg/kg.[a]

Dose	Ratio of radioactivity in tissues/plasma					
	Single low dose (1 mg/kg)		Repeated low dose (1 mg/kg)		Single high dose (10 mg/kg)	
Tissue/organ	Male	Female	Male	Female	Male	Female
Liver	2.29	2.86	2.04	2.44	2.45	2.78
Kidney	1.22	2.27	1.08	1.42	1.19	1.59
Heart	0.74	1.32	0.75	1.18	0.82	1.22
Lung	1.19	1.93	1.20	1.88	1.41	1.96
Brain	0.54	1.03	0.43	0.89	0.60	0.91
Spleen	0.43	0.72	0.44	0.71	0.57	0.77
Muscle	0.79	1.47	0.41	0.78	0.56	0.79
Fat	12.77	25.22	6.25	16.43	8.16	23.22
Gonads	0.53	4.33	0.90	2.56	0.94	4.40
Intestine & contents	1.59	3.18	1.94	1.85	1.62	2.02
Bone & Marrow	0.38	0.79	0.45	0.69	0.71	0.96
Adrenals	2.51	4.72	2.11	3.06	2.38	3.19
Residual Carcass	1.21	2.16	0.53	0.90	0.65	1.04
Blood	0.55	0.60	0.52	0.62	0.64	0.60
Skin & Fur	2.85	5.64	0.96	1.87	1.02	2.23
Uterus	NA[c]	2.34	NA	1.72	NA	4.18
Pancreas	1.78	3.99	1.08	2.19	1.32	2.07
Thyroid	1.65	2.59	0.74	1.26	0.97	1.40
Stomach & contents	0.35	0.59	0.29	0.62	0.40	0.87

[a] Data are the mean of 5 animals/sex/dose group and were obtained from Tables 5, 6, 12, 13, 19 and 20 (pages 38, 39, 45, 46, 52 and 53 of the study report).

[c] Data are the average of 3 animals/sex.

[b] NA = Not applicable.

3. Recovery of Radioactivity

The recovery of radioactivity in excreta and tissues/carcass was similar for all test groups (SOLD, ROLD, and SOHD) and are presented in Table 7. Within 168 hours post-dose, 93-101% of the administered dose was recovered from all dose groups. Fecal excretion was the principle route of elimination, accounting for ~46-70% of the administered dose. Tissues and carcass (20-41% of dose) accounted for the next largest fraction, and urinary excretion accounted for 4.4-11% of the dose. In all test groups, males excreted more of the dose in feces than did females and retained less

of the dose in their carcasses and tissues than females. Levels of urinary excretion were comparable between sexes.

Based upon data from an unreported preliminary study, significant levels of radioactivity were not expected in organic volatiles.

a) Single low dose: Following a single oral dose of [^{14}C]MB465413 at 1 mg/kg, 93.7 and 92.7% of the administered dose was recovered from male and female rats, respectively, within 168 hours. The largest fraction of the dose was recovered in the feces (males, 60.1%; females 46.4%), with the carcass and tissues (males, 26.6%; females 41.1%) accounting for the next largest fraction. Radioactivity in urine accounted for 4.4-6.1% of the dose in both sexes. Males excreted more of the dose in feces than did females and retained less of the dose in the carcass and tissues.

b) Repeated low dose (ROLD): Within 168 hours post-dose, 96.2 and 98.0% of the administered dose was recovered from male and female rats, respectively. As with the single low-dose group, the largest portions of the dose were recovered in the feces (males, 61.1%; females 53.4%) and the carcass and tissues (males, 22.5%; females 31.7%). Also, males excreted more of the dose in feces than females and retained less of the dose in the carcass and tissues. However, pretreatment with MB46513 for 14 days prior to a single oral dose of [^{14}C]MB46513 increased the amount of radioactivity excreted in the urine (10.3-10.8% both sexes) and decreased the amount of radioactivity recovered in the tissues and carcass when compared to the single low-dose group.

c) Single high dose (SOHD): Following a single oral dose of [^{14}C]MB46513 at 10 mg/kg, 100.5 and 99.9% of the administered dose was recovered from male and female rats, respectively, within 168 hours. The pattern of recovery was similar to the repeated low-dose group. The majority of the dose was recovered in the feces (males 69.5%; females 56.0%), followed by the carcass and tissues (males, 19.9%; females 30.0%), and the urine (8.8-10.7% both sexes). As in both other groups, the males excreted more of the dose in feces than did females and retained less of the dose in the carcass and tissues.

Table 7. Recovery of radioactivity from rats dosed with [^{14}C]MB46513 at 1 or 10 mg/kg.[a]

Dose	Percent of radioactive dose administered					
	Single low dose (1 mg/kg)		Repeated low dose (1 mg/kg)		Single high dose (10 mg/kg)	
Sample	Male	Female	Male	Female	Male	Female
Urine	6.06	4.44	10.29	10.76	8.80	10.70
Feces	60.08	46.35	61.08	53.35	69.54	56.04
Cage Wash	0.95	0.82	2.37	2.28	2.26	3.20
Tissues	6.62	9.86	9.14	10.30	7.70	9.12
Carcass [b]	20.02	31.26	13.31	21.35	12.24	20.84
Total	93.73	92.73	96.19	98.04	100.54	99.90

a Values are the mean of 5 animals/sex/dose group and were calculated from Tables 7, 14, 21 and G1-G6 (pages 40, 47, 54, and 139-144 of the study report).
b Includes residual carcass, skin, and fur.

B. Blood Kinetics

The study report presented data on the concentration of radioactivity in whole blood from 0 to 648 hours following a single oral dose of [^{14}C]MB46513 at 1 or 10 mg/kg. These data were used to calculate the kinetic parameters for the elimination radioactivity from blood, which are presented below in Table 8.

a) Single low dose (SOLDPK): The maximum concentration of radioactivity (C_{max}) attained in blood was similar for both males (0.14 ppm) and females (0.15 ppm), but the time required for concentrations to peak in the blood was longer for females (61 hours) than for males (46 hours). The elimination half-life ($t_{½}$) was also longer for females (210 hours) than for males (156 hours), and the AUC values were higher for females (49 ppm/hr) than for males (33 ppm/hr).

b) Single high dose (SOHDPK): The calculated blood kinetic parameters were similar for both males and females from the high-dose group; although the $t_{½}$ was slighter longer and the C_{max} and AUC values were slightly higher for females than for males.

A comparison of the high and low dose data indicate that the maximum concentrations of radioactivity attained in the blood increased approximately in proportion to the 10-fold increase in the dose. The ratio of C_{max} for high-dose to low animals is 14.5 for males and 15.4 for females. In addition, the ratio of AUC values for high to low-dose animals is 15.2 for males and 10.9 for females. Dose level had only a minor affect on

$t_{½}$, slightly increasing $t_{½}$ times at the high-dose for both males and females. The high dose also increased T_{max} times for both males and females, although the increase for females was not as large as for males.

Table 8. Kinetic parameters determined for whole blood following a single dose of [^{14}C]MB46513 at 1 or 10 mg/kg body wt.[a]

Kinetic parameters	Single low dose (1 mg/kg)		Single high dose (10 mg/kg)	
	Male	Female	Male	Female
C_{max} (µg/g)	0.14 ± 0.02	0.15 ± 0.03	2.03 ± 0.47	2.31 ± 0.9
T_{max} (hr)	45.93 ± 13.63	60.65 ± 17.14	72.53 ± 9.08	70.52 ± 8.3
$t_{½}$ (hr)	156.3 ± 17.89	209.9 ± 13.75	170.1 ± 21.2	220.6 ± 55.71
$AUC_{(0-648)}$ (µg/g) x hr	33.18 ± 5.13	49.45 ± 7.33	503.4 ± 55.5	539.9 ± 79.26

a Data are the mean (±SD) of 5 animals/sex/dose and are extracted from Table 28, page 59 of the study report (MRID 44262817).

C. Metabolite characterization studies:

Quantitative HPLC analyses isolated up to 17 radioactive components from urine and up to 12 radioactive components from methanolic and methanol/water extracts of feces. The results of these analyses are summarized in Table 9 and the chemical structures appear in Figure 1.

In urine and feces, MB46513 (see Figure 1) was identified by HPLC and LC/MS analyses, and MB46400 was identified by LC/MS, GC/MS, and NMR analyses. The metabolite RPA 105048 and the sulfate conjugate of MB46513 (UMET/3 in Figure 1) were identified by LC/MS analysis and were found only in urine. Several conjugates of MB46513 were tentatively identified in urine and/or feces using LC/MS and NMR analyses; these included a 4-cyano-5-(N) cysteine conjugate (FMET/10 & UMET/15 in Figure 1), a 4-cyano-5-(N) cysteine glycine conjugate (FMET/7 in Figure 1), and a 5-(N) cysteine conjugate (FMET/9 & UMET/14 in Figure 1). Enzyme hydrolysis data and LC/MS analyses also tentatively identified two glucuronide conjugates of MB46513 in urine.

In addition, LC/MS analyses of extracts from liver, fat, carcass, and skin/fur of low-dose males (SOLDTM) isolated only one radioactive component. This component was identified as unchanged MB46513, indicating that the percent dose associated

with these tissues (15.0-34.3%) may be entirely composed of MB46513.

In excreta of all three dose groups, the majority of radioactivity was identified as unchanged MB46513 (males, 28.6-44.2%; females, 35.4-39.6%), nearly all of which was found in the feces. Unchanged MB46513 recovered in urine accounted for <0.1% of the dose. The only other components in excreta accounting for >5% of the dose were MB46400 (males, 5.7-10.6%; females, 3.1-7.1%), which was found in approximately equal amounts in urine and feces, and the 4-cyano-5-(N)cysteine conjugate of MB46513 (males, 7.2-14.2%; females, 3.8-9.2%), which was found primarily in feces (≤1.8% in urine).

Two minor components that were identified only in urine were RPA 105048 (<0.8%) and the sulfate conjugate of MB46513 (≤2.4%). Two other minor components tentatively identified in both urine and feces were a 4-cyano-5-(N) cysteine glycine conjugate of MB46513 (0.7-3.8%) and a 5-(N) cysteine conjugate of MB46513 (1.9-3.5%). The remaining unknown components isolated from urine each accounted for <1.5% of the dose, and unknown components isolated from feces each accounted for <5% of the dose.

Within each dose group, the metabolite profile was similar between sexes. The same metabolites were identified in both sexes.

The metabolite profile was qualitatively the same between dose groups and the levels of metabolites were also generally similar. However, there were differences in the relative levels of metabolites between dose groups. Levels of MB46513 were lower in ROLD rats (28.6-35.4%) than in SOLD rats (38.6-44.2%), and levels of all metabolites were higher in ROLD rats than SOLD rats. The largest relative difference was in the level of the sulfate conjugate of MB46513 (SOLD rats, ~0.2%; ROLD rats, ~2.3%). Levels of MB46513 were similar in excreta of SOHD (39.6-44.0%) and SOLD (38.6-44.2%) rats, but SOHD rats had much higher levels of all metabolites than the SOLD rats, with the exception of the sulfate conjugate.

These data indicate that fecal excretion of unchanged MB46513 is the principal pathway for elimination of MB46513 from rats. The metabolism of MB46513 in rats involves substitution of the trifluoromethyl or cyano groups on the pyrazole ring and/or sulfate, glucuronide, or glutathione conjugation at the amide on the pryazole ring. The registrant's proposed metabolic pathway for MB46513 in rats is shown in Figure 1.

a) Single low dose (SOLD): A total of 61.5 and 48.7% of the dose was identified in excreta of male and female SOLD rats,

respectively. Although levels of metabolites were higher in the excreta of males than females, the metabolic profile was similar for both sexes. The major component identified was unchanged MB46513 (38.6-44.2%), of which <0.1% was found in urine. The only other two components in excreta accounting for >5% of the dose were MB46400 (males, 5.7%; females, 3.1%) and the 4-cyano-5-(N)cysteine conjugate of MB46513 (males, 7.2%; females, 3.8%). Approximately equal amounts of MB46400 were recovered from urine and feces, but the 4-cyano-5-(N) cysteine conjugate was found primarily in feces (<0.6% in urine).

Two minor components identified only in urine were RPA 105048 (≤0.4%) and the sulfate conjugate of MB46513 (<0.3%). Two other minor components tentatively identified in both urine and feces were a 4-cyano-5-(N) cysteine glycine conjugate of MB46513 (0.7-1.4%) and a 5-(N) cysteine conjugate of MB46513 (1.9-2.3%).

In addition to the compounds identified in excreta, LC/MS analyses of extracts from liver, fat, carcass, and skin/fur of low-dose males indicate that radioactivity associated with these tissues is comprised solely of MB46513. These data indicate that unchanged MB46513 could account for up to 66.7 and 72.9% of the dose for males and females, respectively.

b) Repeated low dose (ROLD): A total of 60.6 and 58.9% of the dose was identified in excreta of male and female ROLD rats, respectively. As with the SOLD rats, the metabolic profile was similar for both sexes. The major component identified was unchanged MB46513 (28.6-35.4%), of which ≤0.02% was found in urine. The only other two components in excreta accounting for >5% of the dose were MB46400 (males, 9.8%; females, 5.6%), which was found in approximately equal amounts in urine and feces, and the 4-cyano-5-(N)cysteine conjugate of MB46513 (males, 12.2%; females, 9.2%), which was found primarily in feces (≤1.6% in urine).

Two minor components identified only in urine were RPA 105048 (≤0.8%) and the sulfate conjugate of MB46513 (≤2.4%). Two other minor components tentatively identified in both urine and feces were a 4-cyano-5-(N) cysteine glycine conjugate of MB46513 (2.4-3.4%) and a 5-(N) cysteine conjugate of MB46513 (3.1%).

Compared to the SOLD rats, levels of MB46513 in excreta of ROLD rats were lower and levels of metabolites were higher.

Based upon analyses of tissue extracts from SOLD males, radioactivity associated with the liver, fat, carcass, and skin/fur of ROLD rats is likely to be comprised solely of MB46513. In which case, unchanged MB46513 recovered from ROLD

rats would account for up to 44.8 and 59.9% of the dose for males and females, respectively.

c) Single high dose (SOHD): A total of 77.7 and 64.1% of the dose was identified in excreta of male and female SOHD rats, respectively. As with the other two dose groups, the metabolic profile was similar for both sexes. The major component identified was unchanged MB46513 (39.6-44.0%), of which ≤0.06% was found in urine. The only other two components in excreta accounting for >5% of the dose were MB46400 (males, 10.6%; females, 7.1%), which was found in approximately equal amounts in urine and feces, and the 4-cyano-5-(N)cysteine conjugate of MB46513 (males, 14.2%; females, 8.9%), which was found primarily in feces (≤1.8% in urine).

Two minor components identified only in urine were RPA 105048 (≤0.9%) and the sulfate conjugate of MB46513 (≤0.9%). Two other minor components tentatively identified in both urine and feces were a 4-cyano-5-(N) cysteine glycine conjugate of MB46513 (3.1-3.8%) and a 5-(N) cysteine conjugate of MB46513 (3.4-3.5%).

Compared to the SOLD rats, levels of MB46513 recovered in excreta of SOHD rats were the same but levels of metabolites were higher.

Based upon analyses of tissue extracts from SOLD males, radioactivity associated with the liver, fat, carcass, and skin/fur of SOHD rats is likely to be comprised solely of MB46513. In which case, unchanged MB46513 recovered from SOHD rats would account for up to 59.0 and 63.7% of the dose for males and females, respectively.

Table 9. Metabolite profile in excreta from rats dosed with [^{14}C]MB46513.[a]

Dose Group	Percent of administered dose					
	Single Low Dose 1 mg/kg		Repeated Low Dose 1 mg/kg		Single High Dose 10 mg/kg	
Compound/fraction	Male	Female	Male	Female	Male	Female
MB46513	44.19	38.60	28.55	35.39	43.97	39.61
MB46400	5.67	3.05	9.80	5.57	10.61	7.08
RPA 105048	0.35	0.09	0.76	0.41	0.62	0.47
Sulphate conjugate of MB46513 (UMET/3)	0.24	0.13	2.35	2.29	0.60	0.90
4-cyano-5-(N)Cysteine conjugate of MB46513 [b] (FMET/10 & UMET/15)	7.17	3.79	12.17	9.18	14.23	8.89
4-cyano-5-(N)cysteine glycine conjugate of MB46513 [b] (FMET7)	1.42	0.73	3.38	2.41	3.81	3.07
Glucuronide conjugates of MB46513 [b] (UMET/4 & UMET/8)	0.26	0.45	0.51	0.50	0.48	0.59
5-(N)Cysteine conjugate of MB46513 [b] (FMET/9 & UMET/14)	2.25	1.86	3.08	3.11	3.40	3.46
Total identified	**61.55**	**48.70**	**60.60**	**58.86**	**77.72**	**64.07**
Unidentified HPLC Peaks Urine, 4 to 8 peaks each <1.5% of the dose	2.84	1.47	2.03	2.25	1.58	2.20
Feces, 4 to 8 peaks each <5% of the dose	1.78	0.63	8.71	3.48	8.84	5.81
Total unidentified	**4.62**	**2.10**	**10.74**	**5.73**	**10.42**	**8.01**
Unanalyzed fractions[c] Liver, fat, carcass, skin & fur[d]	22.52	34.33	16.21	24.50	15.03	24.04
Tissues (excluding liver and fat)	4.12	6.79	6.24	7.15	4.91	5.92
Cage washes	0.95	0.82	2.37	2.28	2.26	3.20
Total Unanalyzed	**27.59**	**41.94**	**24.82**	**33.93**	**22.20**	**33.16**
Total accounted for[e]	93.76	92.74	96.16	98.52	110.34	105.24
Total recovered[f]	148.94	176.62	145.80	166.38	154.74	171.56

a Quantitative metabolite data were obtained from HPLC analyses of urine and fecal extracts and residues (Tables 33-44, pages 61-72 of study report). FMET/10 = fecal metabolite 10, UMET/15 = urinary metabolite 15 etc.
b Metabolites were tentatively identified by LC/MS and NMR analyses.
c Data were obtained from Table G1-G6, pages 139-144 of the study report.
d Based upon LC/MS analyses of tissue extracts from SOLD males, radioactivity in liver, fat, carcass, and skin/fur is likely to be comprised solely of MB46513.
e Total accounted for = (Total identified) + (Total unidentified) + (Total Unanalyzed).
f Total recovered from Table 6 of this report.

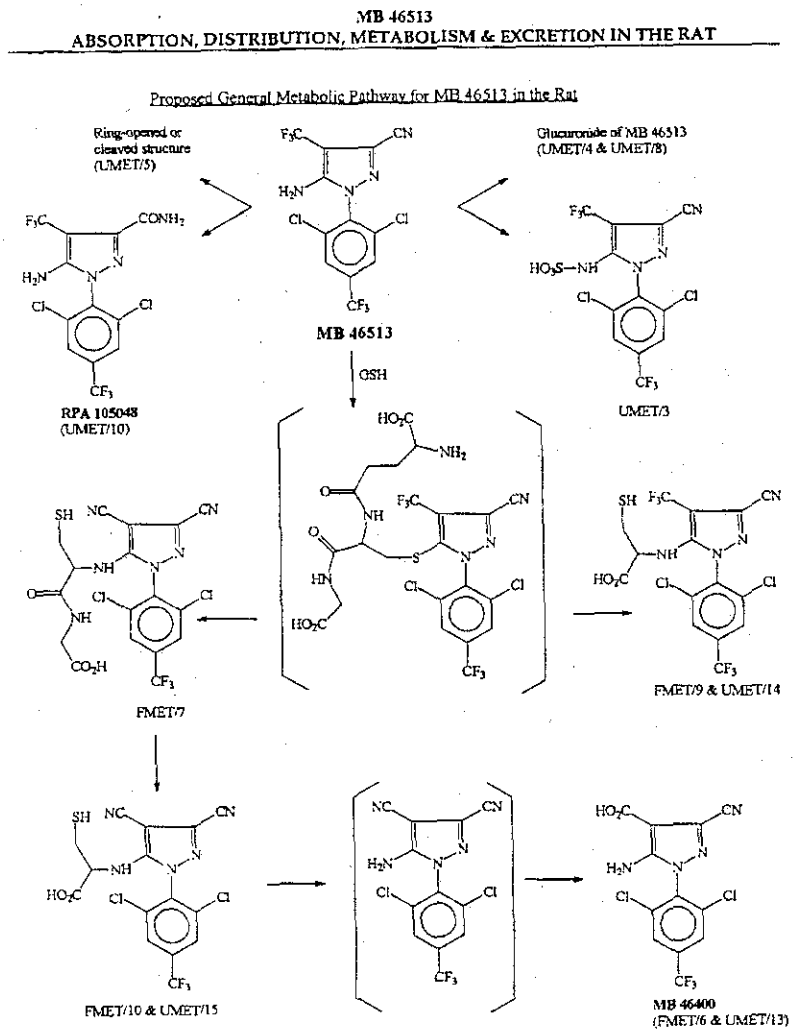


Figure 1. Proposed Metabolic Pathway for MB46513 in the Rat (Scanned from p.316 of the Study Report, MRID 44262817).

III. DISCUSSION

A. Investigator's Conclusions

Following oral dosing, [^{14}C]MB46513 was relatively well absorbed at nominal doses of 1 or 10 mg/kg in both sexes and metabolized. The elimination of [^{14}C]MB46513 was not very rapid with the majority of the administered dose being excreted in the feces rather than in the urine. Small differences were observed between the single and repeated low-dose groups in terms of elimination and distribution. Compared to the single low-dose rats, the repeated low-dose rats excreted more radioactivity indicating that predosing may increase the metabolic capacity of rats. Radioactivity was widely distributed in the tissues with a predominance in fatty tissues. A significant part of the radioactivity (8-17%) remained in the residual carcass at 168 hour post-dose. These results and the relatively long elimination half-life suggest the presence of a deep compartment such as fat.

B. Reviewer's Discussion

Within 168 hours of dosing with [^{14}C]MB46513, 93-101% of the administered dose was recovered from all test groups. Fecal excretion was the principle route of elimination, accounting for 46.4-69.5% of the administered dose. Tissues and carcasses accounted for the next largest fraction of the radioactivity (19.9-41.1%), and urinary excretion accounted for 4.4-10.8% of the dose. In all test groups, fecal excretion was higher for males (60.1-69.5%) than for females (46.4-56.0%), and less radioactivity was retained in the carcasses and tissues of males (19.9-26.6%) than females (30.0-41.1%). Levels of urinary excretion were comparable between sexes (males, 6.1-10.3%; females, 4.4-10.8%).

In the absence of biliary excretion data it is not possible determine the extent of absorption of the test material. However, based on the urinary excretion of radioactivity and on the residual radioactivity in carcass (Table 7) it is possible to estimate that at least 23.6-35.7% of the dose was absorbed by the SOLD and ROLD rats and 21.0-31.3% of the dose was absorbed in the SOHD rats. These values may increase by a few percent (< 10.0%) if radioactivity in other tissues is considered.

Excretion of the radioactivity was increased slightly by pretreatment and at the high dose level. Within 168 hours of dosing, the SOLD animals excreted 51.6-67.1% of the dose in feces and urine (including cage wash), whereas ROLD animals excreted 66.4-73.7% and SOHD animals excreted 69.9-80.6%.

Radioactivity was excreted gradually by all test groups (<35.5% of dose was excreted in feces within 48 hours), but the rates of excretion differed noticeably among groups. Fecal excretion of radioactivity was highest on Day-1 for the SOLD rats (16.5-22.7%), on Day-6 (10.5-12.7%) for ROLD rats, and on Day-5 (15.8-15.9%) for SOHD rats. Urinary excretion showed a similar pattern, being highest on Day-2 for the SOLD rats (0.9-1.2%), on Day-5 for ROLD animals (2.1-2.4%), and on Day-4 for SOHD animals (2.3-2.9%).

Maximum concentrations of radioactivity in whole blood were attained within 46-61 hours for low-dose animals and within 71-73 hours for high-dose animals. Maximum concentrations were similar between sexes within a dose group (low dose, 0.14-0.15 ppm; high dose, 2.03-2.31 ppm), and were 15 times greater in the high-dose group than the low-dose group. Elimination of radioactivity from the blood was protracted for both dose groups; elimination half-lives were 156-170 hours for males and 210-221 hours for females. In addition, the ratio of AUC values for high to low-dose animals was 15.2 for males and 10.9 for females, reflecting the difference in dose levels.

The general distribution of radioactivity among tissues was the same between dose groups and sexes, although the actual levels differed between dose groups and sexes. In all animals, concentrations of radioactivity were highest in fat (1.54-50.83 ppm). The ratio of radioactivity in fat/plasma was 6.3-12.8 for males and 16.4-25.2 for females. The next highest concentrations of radioactivity were generally associated with the adrenals (males, 0.30-6.43 ppm; females, 0.51-7.40 ppm) and liver (males, 0.28-7.02 ppm; females, 0.31-6.66 ppm). Females also had high concentrations associated with the ovaries (0.49-9.74 ppm). The lowest concentrations of radioactivity were generally associated with the brain, spleen, muscle, whole blood, and stomach.

With the exception of whole blood and plasma, concentrations of radioactivity in tissues were generally higher for females than for males, e.g. radioactivity in fat was 1.6-2.8 times higher in females than in males.

Among the dose groups, ^{14}C-residues were lowest in tissues from the SOLD group with the exceptions of residual carcass and skin plus fur. Pretreatment with MB46513 increased the residue levels in tissues. Levels of radioactivity were highest in tissues from the SOHD group, with residue levels generally 10-30 times higher than in tissues from the SOLD group.

For each dose group, the major radioactive component identified in excreta was unchanged MB46513 (males, 28.6-44.2%; females, 35.4-39.6%), nearly all of which was found in

the feces. Unchanged MB46513 recovered in urine accounted for <0.1% of the dose. The only other components in excreta accounting for >5% of the dose were MB46400 (males, 5.7-10.6%; females, 3.1-7.1%) and the 4-cyano-5-(N)cysteine conjugate of MB46513 (males, 7.2-14.2%; females, 3.8-9.2%). Other minor components identified in excreta included: RPA 105048 (<0.8%); the sulfate conjugate of MB46513 (≤2.4%); a 4-cyano-5-(N) cysteine glycine conjugate of MB46513 (0.7-3.8%); and a 5-(N) cysteine conjugate of MB46513 (1.9-3.5%).

Within each dose group, the metabolite profile was the same between sexes, although metabolite levels were generally higher in males than females. The metabolic profile was also similar between dose groups, although there were differences in the relative levels of metabolites. Pretreatment resulted in lower levels of MB46513 in excreta (ROLD groups, 28.6-35.4%; SOLD groups, 38.6-44.2%). In addition, levels of all metabolites were higher in ROLD rats than SOLD rats. Levels of MB46513 were similar in excreta of SOHD (39.6-44.0%) and SOLD (38.6-44.2%) rats, but level of metabolites were generally higher in SOHD rats than SOLD rats.

In addition, analyses of extracts from liver, fat, carcass, and skin/fur of low-dose males found only one radioactive component, unchanged MB46513, indicating that the radioactivity associated with tissues and the carcass (15.0-34.3% of dose) may be entirely composed of MB46513.

These data indicate that fecal excretion of unchanged MB46513 is the principal pathway for elimination of MB46513 from rats. The metabolism of MB46513 in rats involves substitution of the trifluoromethyl or cyano groups on the pyrazole ring and/or sulfate, glucuronide, or glutathione conjugation at the amide on the pryazole ring. High levels of radioactivity in fat compared to blood and the prolonged elimination half-life of MB46513 indicate that there is a potential for bioaccumulation of MB46513 in fatty tissues.

IV. **STUDY DEFICIENCIES**

An intravenous route of administration was not employed in this study, and no reasons were provided to justify excluding intravenous dosing. However, sufficient data are available to conclude that this metabolism study in the rat is an acceptable nonguideline study.

Reviewed by: Marion Copley, DVM, DABT Marion Copley 12/10/97
Registration Action Branch 1 (7509C)
Secondary Reviewer: Melba Morrow, DVM Melba Morrow 12/11/97
Registration Action Branch 1(7509C)

DATA EVALUATION REPORT - AMENDMENT
(Original document TXR No. 011714)

STUDY TYPE:	Subchronic Toxicity/Rats (82-1)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 435595-01
TEST MATERIAL:	M B 46513 Synonym: Fipronil Metabolite
STUDY NUMBER:	SA 93226
TESTING FACILITY:	Rhone-Poulenc-Secteur Agro Sophia Antipolis Cedex
SPONSOR:	Rhone-Poulenc Ag Company
TITLE OF REPORT:	M&B 46513: 90-Day Toxicity Study in the Rat by Dietary Administration
AUTHOR(S):	M. Dange
REPORT ISSUED:	June 17, 1994

EXECUTIVE SUMMARY: In this subchronic rat study (MRID # 43559501), M B 46513 was administered in the diet to groups of ten male and ten female CD rats at dosages of 0, 0.5, 3, 10 or 30 ppm (males: 0, 0.029, 0.177, 0.594 and 1.772 mg/kg/day; females: 0, 0.035, 0.210, 0.709, and 2.101 mg/kg/day, respectively) daily for 90 days.

There were four deaths in both sexes of the 30 ppm group during the treatment period. There was an increased incidence of clinical signs of neurotoxicity (aggressivity, irritability to touch, increased motor activity and curling up on handling) in the 10 and 30 ppm group males and females. One male in the 3 ppm group was also observed to display most of these signs. Mean body weights were statistically decreased in the 30 ppm group males and females and the 10 ppm group males at multiple weekly measurements during the study. Overall mean body weight gains for the 10 and 30 ppm group males was decreased 15.4% and 12.9% respectively. Mean weekly food consumption and food conversion efficiency for the 30 ppm group males and females were lower than the controls during the first two weeks of the study only. There were no treatment-related changes in hematology or urinalysis parameters. Alterations in clinical chemistry parameters were of no toxicological

significance. Treatment-related decreases were seen in T_4 at weeks 2 and 10 in the 30 ppm group males and in the 30 ppm group females at week 10. There was also a decrease in T_3 in the 30 ppm group males at week 10. However, there were no changes in TSH, or the thyroid gland on macroscopic or microscopic examination. Therefore, the toxicological significance of the hormone alterations is questionable. There were no treatment-related macroscopic or microscopic necropsy changes. **The study demonstrates that the metabolite is more toxic than the parent chemical (MB 46030) when administered to rats for 90 days. The Lowest Observed Effect Level (LOEL) was 3 ppm (0.177 and 0.210 mg/kg/day for males and females, respectively) based on the occurrence of agressivity, irritability to touch and increased motor activity in one male (these signs are also observed in the mouse). The No Observed Effect Level (NOEL) was 0.5 ppm (0.029 and 0.035 mg/kg/day for males and females, respectively).**

This study is classified as **Acceptable** and **satisfies** the data requirements for a subchronic rat study (82-1).

A. **JUSTIFICATION FOR LOWERING THE NOEL FOR THE SUBCHRONIC TOXICITY STUDY IN THE RAT (MRID 43559501)** using photodegradate MB 46513

The HED Hazard ID Assessment Review Committee met on December 9, 1997 to reevaluate the NOEL/LOEL for this study. They were previously set at: **(NOEL) was 3 ppm (0.177 and 0.210 mg/kg/day for males and females, respectively). The Lowest Observed Effect Level (LOEL) was 10 ppm for females (0.594 and 0.709 mg/kg/day for males and females, respectively).**

Justification for lowering these values is as follows:

Signs of toxicity at the high dose of 30 ppm include

- 4 deaths (M & F)
- clinical signs of neurotoxicity (aggressivity, irritability to touch increased motor activity and/or curling up on handling (
- decr BW, FC, FE (M & F); BWG (M)

Signs at 10 ppm

- clinical signs of neurotoxicity (aggressivity, irritability to touch increased motor activity and curling up on handling) (M & F)
- decr BW (M), BWG (M)

Signs at 3 ppm

- clinical signs of neurotoxicity (aggressivity, irritability to touch increased motor activity and curling up on handling) (1 M)

Table 1 Clinical Signs of Toxicity in Rats Treated with MB 46513 in the Diet for 90 Days[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	3.0	10.0	30.0	0	0.5	3.0	10.0	30.0
Increased motor activity	0	0	0	0	1	0	0	0	1	9
Aggressivity	0	0	1	1	4	0	0	0	0	0
Excessive vocalization	0	0	1	2	0	0	0	0	0	1
Curls up at handling	0	0	0	0	5	0	0	0	0	1
Irritability to touch	0	0	1	6	6	0	0	0	5	8

a Extracted from Table 1 (pages 41-44) of the study report.

The signs observed in the one 3 ppm male are the same signs observed in the higher doses. These signs are also seen in other rat and mouse studies with this compound.

The LOEL was 3 ppm (0.177 mg/kg/day) based on neurologic signs in one male rat (females were affected at the next higher dose). The NOEL was 0.5 ppm (0.029 mg/kg/day).

There are no other changes or modifications to the original DER TXR No. 011714.

DATA EVALUATION RECORD

MB46513-Fipronil

Study Type: §85-3; Dermal Absorption Study - Rats

Work Assignment No. 3-23Q (MRID 44262816)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

MB46513-Fipronil Metabolite Dermal Absorption - Rat (§85-3)

EPA Reviewer: Alberto Protzel, Ph.D.
Reregistration Branch 1 (4002)

11/12/97

EPA Work Assignment Manager: Marion Copley, DVM, DABT
Registration Action Branch 1 (2002)

11/12/97

DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption - Rat
OPPTS Number: 870.7600 OPP Guideline Number: §85-3

DP BARCODE: D237893 SUBMISSION CODE: S524626
P.C. CODE: 129121 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): MB46513 (Fipronil Metabolite, 99.2% a.i.)

SYNONYMS: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole

CITATION: Cheng, T. (1996) Dermal Absorption of (^{14}C)-MB 46513 in Male Rats (Preliminary and Definitive Phases): Corning Hazleton, Inc, Madison, Wisconsin. Lab Project ID. CHW 6224-230, September 27, 1996. MRID 44262816. Unpublished.

SPONSOR: Rhône-Poulenc Ag Company, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a dermal absorption study (MRID 44262816), 24 male Crl:CD BR rats/dose group were dosed dermally with [^{14}C]MB4613 (99% a.i.) as a 1% carboxymethylcellulose aqueous suspension at dose levels of 0.081, 0.881, or 7.17 mg/rat (0.006, 0.071, or 0.574 mg/cm^2). Four rats/dose were sacrificed for assessment of dermal absorption after 0.5, 1, 2, 4, 10, and 24 hours of exposure.

Dosed radioactivity was quantitatively recovered from each dose group (92.5-103%). After 24 hours of exposure, dermal absorption of MB46513 was minimal. For all dose groups, the majority of the dose was not absorbed (90.2-102.3%), and only trace amounts (0.1%) of radioactivity were excreted in the urine and feces.

2-4

For the low- and mid-dose groups, radioactivity remaining in/on the skin after washing increased with the duration of exposure, reaching maximums of 3.97% and 1.05% of the dose, respectively, by 24 hours. However, duration of exposure had no effect on accumulation of radioactivity in/on the skin for the high-dose group.

For the low-dose group, absorption (measured as amount excreted plus amount retained in the body) increased over time from <0.005% of the dose at 0.5 hours to 2.64% of the dose at 24 hours. Potential absorption (amount absorbed plus amount retained in/on skin) also increased over time from 0.74% of the dose at 0.5 hours to 6.61% of the dose at 24 hours. For the mid-dose group, absorption increased over time from <0.01% of the dose at 0.5 hours to 0.35% of the dose at 24 hours. Potential absorption increased from 0.28% of the dose at 0.5 hours to 1.40% of the dose at 24 hours. For the high-dose group, the maximum amount of absorption was 0.14% of the dose at 0.5 hours, and the maximum potential absorption was 0.66% of the dose at 4 hours. There was 2.56% adhered to the akin and absorbed at the 10 hour time point with the lowest dose applied (0.006 mg/cm^2).

SELECT DERMAL ABSORPTION VALUES
(Total % adhered to the skin and absorbed)

ave. dose	1 hour	10 hours	24 hours
0.006 mg/cm^2	<1	**2.35**	6.61
0.071 mg/cm^2	<1	<1	1.4
0.574 mg/cm^2	<1	<1	<1

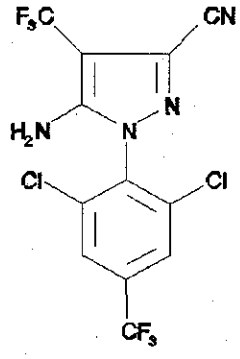
This study is classified as Acceptable (Guideline) and satisfies the requirement for a dermal absorption study (§85-3) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MB46513
   Chemical Name: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole
   Description: Yellow solid
   Lot/Batch #: 805 DAP DA 999
   Purity: 99.2% a.i.
   CAS #: 120068-37-3
   Structure:



   [^{14}C-U-phenyl]MB46513
   Description: Pale yellow cream
   Reference #: GHS 847.2
   Radiochemical purity: >99%, determined by HPLC
   Specific activity: 836 MBq/mmol

2. Vehicle: [^{14}C]MB46513 was administered as a 1% carboxymethyl cellulose aqueous suspension.

3. Test animals: Species: Rat (male)
   Strain: Crl:CD BR
   Age and weight at start of treatment: approximately 8 weeks, 162-190 g
   Source: Charles River Laboratories, Inc., Portage, MI
   Acclimation period: ≥7 days
   Diet: Rodent Diet #5002 (PMI Feed, Inc.), ad libitum
   Water: tap, ad libitum

B. STUDY DESIGN and METHODS

This study was designed to characterize the dermal absorption of [^{14}C-U-phenyl]MB46513 in male rats. A pilot study and definitive study were conducted. The pilot study consisted of two groups of 4 rats/group dosed dermally at target doses of 0.08 or 8 mg/animal. Actual average dose levels were 0.09 and 11.2 mg/animal (0.007 and 0.899 mg/cm^2). Each group was exposed for 0.5 hour.

The definitive study consisted of three groups of 24 rats/group dosed dermally at target doses of 0.08, 0.8, or 8.0 mg/animal. Actual average dose levels were 0.081, 0.881, or 7.17 mg/animal (0.006, 0.071, or 0.574 mg/cm^2). Four rats per dose group were exposed for durations of 0.5 1, 2, 3, 10 or 24 hours.

1. Dose selection

Middle and high target doses of 0.8 and 8 mg/animal were selected because these dose levels were previously used as low and middle doses in a fipronil dermal absorption study, and MB46513 is a photolytic metabolite of fipronil. The low target dose of 0.08 mg/animal was selected because MB46513 formed in the field is likely to represent only a fraction of the total amount of fipronil applied.

2. Dose preparation

For the middle and high doses, [^{14}C]MB46513 was diluted with unlabeled MB46513 to specific activities of 2.91 x 10^{-4} dpm/μg and 3.03 x 10^{-3} dpm/μg, respectively. [^{14}C]MB46513 (1.29 x10^{-5} dpm/μg) was not diluted for the low dose.

Dose suspensions were prepared by combining the required amounts of [^{14}C]MB46513, unlabeled MB46513, and the 1% carboxymethylcellulose carrier. The solutions were mixed by magnetic stirring, vortex-mixing, and sonication. Dosing suspensions were stirred constantly and were used on the day of preparation or were stored refrigerated overnight prior to dosing. Duplicate samples of each dosing solution were collected before and after dosing to determine the stability, homogeneity, and radiochemical and chemical purity of each dosing solution.

3. Animal preparation and dosing

For the pilot study, four animals were assigned to each dose group. For the definitive study, twenty-four animals were assigned to each of three dose groups. At least 16 hours before dosing, the back and shoulders of each animal were shaved and washed with water, taking care not to abrade the skin. A rectangular plastic enclosure (approximately 12.5 cm^2) was glued to the back of each rat and sealed with a silicone sealant.

A 100 μL topical application of dose suspension was pipetted onto the skin within the enclosure and spread evenly with a glass rod. The glass rod was rinsed and wiped after application and these samples were retained. The treatment site was then covered with a non-occlusive filter paper cover, and an Elizabethan collar was placed on each animal's neck. Rats were then placed in individual metabolism cages for collection of urine and feces until sacrifice.

4. Sample collection

Urine and feces were collected on dry ice located beneath each metabolism cage. For the pilot study, 4 rats/group were sacrificed at 0.5 hours post-dose, and for the definitive study, 4 rats/group were sacrificed at 0.5, 1, 2, 4, 10, and 24 hours after dosing. Rats were anesthetized with ketamine and the enclosure cover was removed and saved. The skin surface at the treatment site was then washed alternately with 2% Ivory soap solution and water using gauze pads, and dried with cotton swabs.

The rats were anesthetized with halothane and blood samples (definitive study only) were collected by cardiac puncture. Residual urine in the bladder was collected and added to the urine sample from the animal. The skin at the application site was excised and retained. The plastic enclosure and the gauze pads and cotton swabs used to wash the skin were retained for analysis. The cages were washed with 1% trisodium phosphate solution and wiped with gauze pads. The wash and gauze pads were retained for analysis.

The following samples were analyzed for radioactivity:

Enclosure cover	Plastic enclosure
Skin wash	Gauze pads and swabs from skin wash
Whole blood	Skin from treatment site
Carcass	Cage wash
Cage wipe	Urine
Feces	Spreader rinse and wipe

3. Data analysis

Means and standard deviations were calculated for samples from each dose group, and the relative standard deviation was also calculated for pre- and post-dosing samples of the dosing solution. The limit of detection (LOD) for the radioanalyses was taken as 2x background (20-84 dpm).

II. RESULTS:

1. Analysis of dose formulations

Concentrations of radioactivity (dpm/g) in dosing suspensions were determined in duplicate samples taken pre- and post-dosing and these values were used to determine the actual dose applied to each animal. The actual doses (mg/animal and mg/cm^2) applied at each time point are presented in Table 1. In the definitive study, actual doses were 99-103% of the target dose for the low-dose group, 107-114% for the mid-dose group, and 84-93% for the high-dose group. Concentrations of radioactivity in dose suspension

had relative standard deviations of 0.87-2.54%. HPLC analyses of pre- and post-dosing samples indicated that the test material was stable and that the chemical and radiochemical purity of the test substance were both >99%.

2. Dose distribution

a. Pilot study

After an exposure duration of 0.5 hours, recoveries of radioactivity from the 0.093 and 11.2 mg/animal dose groups were 94.3 and 99.4%, respectively.

b. Definitive study

The dose distributions for the definitive study are presented in Tables 1 and 2. Table 1 contains the distribution of radioactivity (percent) in the various tissues and analyzed fractions. Table 2 summarizes the data in Table 1 and contains percent of dose absorbed, not absorbed, total excreted, and total in the body. In addition, Table 2 contains the sum of percent of dose remaining in/on the skin (i.e. fraction not removable by washing) plus the percent of the dose absorbed.

The dosed radioactivity was quantitatively recovered from each dose group at all exposure intervals. Recoveries of radioactivity were 92.5-103% of the dose. For each dose at each interval, 89.8-102% of the dose was recovered in the skin wash, with skin from the test site (0.13-3.97%) accounting for the next largest portion of the dose. Only trace amounts (≤0.1%) of radioactivity were excreted in the urine and feces.

For the low- and mid-dose groups, the percent dose absorbed and associated with skin at the treatment site generally increased with the duration of exposure. However, length of exposure had no effect on absorption or the radioactivity in/on the skin for the high-dose group. Among dose groups, the percent of the dose absorbed generally decreased with increasing dose levels; although the actual amount of material in/on treated skin increased with dose level. By 24 hours, the amount of [^{14}C]MB46513 in/on treated skin was 0.278, 0.767, and 1.89 $\mu g/cm^2$ for the low-, mid-, and high-dose, respectively.

- At 0.081 mg/animal (0.006 mg/cm^2), 90.2-94.2% of the dose remained unabsorbed throughout the 24-hour period; although absorption increased over time, with absorbed radioactivity accounting for 2.64% by 24 hours. A

similar trend was observed for skin at the treatment site; radioactivity increased from 0.74% at 0.5 hours to 3.97% by 24 hours. Radioactivity in the body (2.54%) and excreted (0.1%) were also highest at 24 hours. Radioactivity absorbed and associated with the treated skin was highest at 24 hours and accounted for 6.61% of the dose.

- At 0.088 mg/animal (0.071 mg/cm^2), 93.2-101.4% of the dose remained unabsorbed throughout the 24-hour period. Absorption increased over time, although this trend was less pronounced than at the low-dose. By 24 hours, 0.35% of the dose was absorbed. A similar trend was observed for skin at the treatment site; radioactivity increased from 0.28% at 0.5 hours to 1.05% by 24 hours. Radioactivity in the body (0.31%) and excreted (0.04%) were also highest at 24 hours. Radioactivity absorbed and associated with the treated skin was highest at 24 hours, accounting for 1.40% of the dose.

- At 7.17 mg/animal (0.574 mg/cm^2), 96.1-102.3% of the dose remained unabsorbed throughout the 24-hour period. There was no correlation between the duration of exposure and the amount of radioactivity absorbed. Essentially all the radioactivity was either not absorbed or associated with the skin from the treatment site. Radioactivity associated with the treated skin was highest at 4 hours, accounting for 0.61% of the dose.

III. DISCUSSION

Investigator's Conclusion

The study author concluded that dermal absorption of MB46513 was minimal. The maximum amount of direct absorption (0.82 µg/cm^2, 0.14% of applied dose) was noted in the high-dose group at 0.5 hours of exposure. Due to the minimum amount of absorption, no correlation was noted between the amount of absorption and the duration of exposure. For the low- and mid-dose groups, there was some indication of an increasing amount of indirect absorption (amount absorbed + amount on skin) with increasing time. However, for the high-dose group, it appeared that indirect absorption had reached saturation, regardless of the duration of exposure.

Reviewer's Discussion

Male Crl:CD BR rats were dosed dermally with [^{14}C]MB4613 in 1% carboxymethylcellulose at doses of 0.08, 0.88, or 7.17 mg/rat (0.006, 0.071, or 0.574 mg/cm^2) and exposed for durations of 0.5 to 24 hours. Dosed radioactivity was quantitatively recovered from each dose group (92.5-103%).

After 24 hours of exposure, dermal absorption of MB46513 was minimal, accounting for 2.64%, 0.35%, and <0.01% of the dose in the low-, mid- and high-dose group, respectively. For all dose groups, the majority of the dose was not absorbed (90.2-102.3%), and only trace amounts (≤0.1%) of radioactivity were excreted in the urine and feces.

For the low- and mid-dose groups, radioactivity remaining in/on the skin after washing increased with the duration of exposure. However, a similar increase was not observed in the high-dose group, suggesting that the potential for uptake by the skin was saturated at the high-dose. The percent of the dose retained in/on the skin was inversely related to the dose level, although the actual amounts of material retained in/on the skin increased with dose level. The maximum amount of the dose in/on the skin was 3.97%, 1.05% and 0.61% for the low-, mid-, and high-dose groups, respectively, which are equivalent to 0.278, 0.767, and 3.61 ug/cm^2.

Amounts of radioactivity absorbed plus radioactivity in/on the skin (potential absorption) increased with the length of exposure, reaching maximums of 6.61% for the low-dose group and 1.40% for the mid-dose by 24 hours. Duration of exposure had no effect on potential absorption for the high-dose group, which reached a maximum by 4 hours of 0.66% of the dose.

IV. STUDY DEFICIENCIES

No deficiencies were noted in this dermal absorption study.

Table 1. Dose distribution of ^{14}C-MB46513 following a single dermal dose to male rats.

Average dose (mg/cm^2)	Actual dose[a]		Exposure (hrs)	Recovered radioactivity expressed as % of applied dose[b]								
	(mg/cm^2)	mg/rat		Nonocclusive cover	Enclosure	Skin wash	Skin (test site)	Blood	Carcass	Urine	Feces	Total Recovered
0.006	0.007	0.082	0.5	ND	0.08	94.1	0.74	ND	ND	<0.005	ND	94.9
	0.006	0.079	1	0.04	0.27	91.1	0.78	ND	ND	<0.005	ND	92.5
	0.006	0.080	2	0.03	0.09	92.4	1.39	ND	ND	<0.005	ND	93.9
	0.007	0.081	4	0.19	0.07	91.4	2.56	ND	ND	<0.005	ND	94.3
	0.006	0.080	10	0.06	0.19	91.4	1.68	ND	0.67	<0.005	ND	94.1
	0.007	0.082	24	0.16	0.24	89.8	3.97	ND	2.54	0.03	0.07	96.8
0.071	0.070	0.870	0.5	ND	0.17	97.1	0.28	<0.005	ND	<0.005	<0.005	97.7
	0.071	0.889	1	ND	0.26	92.9	0.37	<0.005	0.58	<0.005	<0.005	94.1
	0.069	0.864	2	1.37	0.05	97.8	0.75	ND	ND	<0.005	<0.005	100
	0.069	0.856	4	2.09	0.11	99.2	1.04	<0.005	ND	<0.005	<0.005	102
	0.071	0.894	10	2.05	0.08	98.8	0.83	<0.005	0.13	<0.005	<0.005	102
	0.073	0.915	24	0.03	0.08	98.1	1.05	ND	0.31	0.01	0.03	99.6
0.574	0.584	7.297	0.5	<0.01	0.05	102.0	0.13	ND	0.14	<0.005	<0.005	102
	0.598	7.470	1	<0.01	0.30	99.3	0.27	ND	ND	<0.005	<0.005	99.9
	0.536	6.705	2	0.18	0.08	102.0	0.41	ND	ND	<0.005	<0.005	103
	0.592	7.401	4	0.03	0.14	100.0	0.61	ND	0.05	<0.005	<0.005	101
	0.577	7.206	10	0.01	0.04	101.0	0.18	ND	ND	<0.005	ND	101
	0.556	6.949	24	0.74	0.06	95.3	0.34	ND	ND	<0.01	ND	96.5

a Data were calculated by reviewer from Tables 3-5, pages 26-28 of study report.

b Data are the mean of 4 rats/dose group and were obtained from Tables 7-9 (pages 30-38) of the study report; Data for cage washes and wipes were not reported as radioactivity was non-detectable in all of these samples.

Table 2. Summary of dose distribution values for [^{14}C]MB46513 following a single dermal dose to male rats.[a]

Average dose (mg/cm^2)	Actual dose		Exposure (hrs)	Amount of dose							
				Not Absorbed[b]	Skin at test site		In Body[c]	Excreted[d]	Absorbed[e]		Total of skin and absorbed
	(mg/cm^2)	mg/rat		(%)	(%)	$\mu g/cm^2$	(%)	(%)	(%)	$\mu g/cm^2$	(%)
0.006	0.007	0.082	0.5	94.2	0.74	0.052	ND	<0.005	<0.005	<0.001	0.74
	0.006	0.079	1	91.4	0.78	0.047	ND	<0.005	<0.005	<0.001	0.78
	0.006	0.080	2	92.5	1.39	0.083	ND	<0.005	<0.005	<0.001	1.39
	0.007	0.081	4	91.7	2.56	0.179	ND	<0.005	<0.005	<0.001	2.56
	0.006	0.080	10	91.7	1.68	0.101	0.67	<0.005	0.67	0.040	2.35
	0.007	0.082	24	90.2	3.97	0.278	2.54	0.10	2.64	0.185	6.61
0.071	0.070	0.870	0.5	97.3	0.28	0.196	<0.005	<0.01	<0.01	<0.007	0.28
	0.071	0.889	1	93.2	0.37	0.263	0.58	<0.01	0.58	0.412	0.95
	0.069	0.864	2	99.2	0.75	0.518	ND	<0.01	<0.01	<0.007	0.75
	0.069	0.856	4	101.4	1.04	0.718	<0.005	<0.01	<0.01	<0.007	1.04
	0.071	0.894	10	100.9	0.83	0.589	0.13	<0.01	0.13	0.092	0.96
	0.073	0.915	24	98.2	1.05	0.767	0.31	0.04	0.35	0.256	1.40
0.574	0.584	7.297	0.5	102.1	0.13	0.759	0.14	<0.01	0.14	0.818	0.27
	0.598	7.470	1	99.6	0.27	1.615	ND	<0.01	<0.01	<0.060	0.27
	0.536	6.705	2	102.3	0.41	2.198	ND	<0.01	<0.01	<0.054	0.41
	0.592	7.401	4	100.2	0.61	3.611	0.05	<0.01	<0.01	<0.059	0.66
	0.577	7.206	10	101.1	0.18	1.039	ND	<0.005	<0.005	<0.058	0.18
	0.556	6.949	24	96.1	0.34	1.890	ND	<0.01	<0.01	<0.056	0.34

a Data obtained from Table 1 of this report or calculated by reviewer from data in Table 1.
b Includes radioactivity in/on cover, enclosure, and skin wash.
c Includes radioactivity in carcass and blood.
d Includes radioactivity in urine and feces.
e Includes radioactivity in the body and excreted.