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DATA EVALUATION REPORT

STUDY TYPE:	Subchronic Toxicity/Dogs (82-1)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 429186-42
TEST MATERIAL:	M&B 46030 Synonym: Fipronil
STUDY NUMBER:	LSR 90/RHA310/0842
TESTING FACILITY:	Life Science Research Limited Suffolk, England
SPONSOR:	Rhone-Poulenc Ag Company
TITLE OF REPORT:	M&B 46030: Toxicity Study By Oral (Capsule) Administration to Beagle Dogs for 13 Weeks
AUTHOR(S):	P. Holmes
REPORT ISSUED:	November 21, 1991

EXECUTIVE SUMMARY: In this subchronic non-rodent study (MRID # 429186-42), M&B 46030 was administered in gelatin capsules to four male and four female beagles per group at dosages of 0, 0.5, 2.0 or 10.0 mg/kg/day for thirteen weeks.

At **10.0 mg/kg/day**, there were significant clinical signs of toxicity which were most prominent during the first three weeks of treatment in males and during the first two weeks in females. One male and three females in this group were euthanized during the second week of treatment due to their poor condition. Signs observed in these animals included inappetence, weight loss, emaciation, dehydration, hypothermia, subdued behavior, excessive salivation, hindlimb extension, convulsions, disorientation, apparent lack of vision, absent menace reaction, ataxia and blood-stained saliva around mouth. Clinical signs in the surviving animals in this group included inappetence, emaciation, underactivity, hunched posture, convulsions, head nodding and body tremors. The only clinical sign of toxicity observed in the **2.0 mg/kg/day group** was inappetence in two females. There were no treatment-related signs in the **0.5 mg/kg/day group**. 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.) One male in the **10.0 mg/kg/day group** had higher absolute and relative spleen and thymus weights and higher absolute

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adrenal weight in comparison to the controls. Another male in this group also had increased absolute and relative thymus weights. However, the group means for these organs were comparable to the controls. Follicular and parafollicular atrophy of the mesenteric lymph nodes was reported in one male and cortical atrophy of the thymus was seen in the same male and one female in the 10.0 mg/kg/day group which was euthanized during the treatment period. The findings were considered to be related to stress rather than a direct result of treatment. **The LOEL is 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). The NOEL is 2.0 mg/kg/day for males and 0.5 mg/kg/day for females.**

The study is <u>Core Guideline</u> and satisfies the guideline requirements (82-1) for a subchronic toxicity study in the dog.

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I. MATERIALS

A. Test Material

Name: M&B 46030
Synonym: Fipronil
Chemical Name: 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole
Purity: 95.4%
Batch Number: PGS963
Description: Off-white powder
Storage Conditions: Room temperature protected from artificial light

After 5 and 9 months of storage, samples were taken from the bulk container and returned to the registrant for analysis. Appendix 1 of the study report contains results of these analyses which show that the concentration of M&B 46030 remained stable.

B. Administration: gelatin capsules

C. Test Animals

Species: Purebred beagle dogs
Source: Consort Limited, Herefordshire, England
Age: 19 to 23 weeks at commencement of treatment
Weight: Males - 8.0 to 9.8 kg; Females - 7.3 to 9.5 kg at commencement of treatment
Housing: Individually in indoor kennels
Environmental Conditions: Temperature: target of 21° C
Relative humidity: target of 55%
Photoperiod: 12 hours light/dark
Air changes: 12 per hour
Food and Water: 400 g daily of a complete pelleted diet (Laboratory Diet A) and water *ad libitum*
Acclimation Period: 27 days

All dogs were subjected to a hematology screen for evidence of ill health or Factor VII deficiency shortly after they arrived at the facility.

II. METHODS

A. Dosage and Administration

Sixteen male and sixteen female dogs were randomly assigned to the following treatment groups using derived latin squares:

Dosage Level (mg/kg/day)	Number of Dogs Male	Female
0 (Control)	4	4
0.5	4	4
2.0	4	4
10.0	4	4

The test chemical was administered in gelatin capsules once daily seven days per week after feeding. Control dogs received empty capsules.

B. Experimental Design

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

physical examinations - before dosing and after 3, 7 and 11 weeks of treatment
neurological examination* - once before dosing and after 6 and 12 weeks of treatment
clinical signs of toxicity - inspected regularly throughout working day - individual daily observations recorded before and shortly after each dose
body weights - at weekly intervals during acclimation and treatment periods
food consumption - at weekly intervals during the final two weeks of acclimation and throughout the treatment period
ophthalmoscopic examinations - pretest and after 6 and 12 weeks
hematology, clinical chemistry and urinalysis - once prior to study initiation and at 6 and 12 weeks
gross necropsy - all animals
histopathology - designated organs and tissues from all animals

* The following reflexes were tested and observations performed during the neurological examination.

Cranial nerve reflexes

Pupillary light and consensual light
Palpebral - blink and corneal
Gag
General examination of the head to assess other cranial nerves

Segmental reflexes

Flexor (withdrawal) including crossed extensor
Patellar
Extensor tone

Postural reactions

Placing reactions - visual and tactile
Extensor postural thrust
Righting reactions
Tonic neck reactions
Hopping reflex

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Pathological Parameters

r hematology and clinical chemistry evaluations, blood was drawn om the jugular vein following an overnight fasting period. Urine s also collected after an overnight fast. The CHECKED (X) matology parameters were examined.

_Hematocrit (HCT)*
_Hemoglobin (HGB)*
_Leukocyte count (WBC)*
_Erythrocyte count (RBC)*
_Platelet count*
_Prothrombin Time
_Reticulocyte count
__Total plasma protein (TP)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc. (MCHC)
X Mean corpuscular volume (MCV)
X Activated thromboplastin time

EPA guideline requirement

he CHECKED (X) clinical chemistry evaluations were done.

lectrolytes:
_Calcium*
_Chloride*
_Magnesium*
_Phosphorus*
_Potassium*
_Sodium*

Other:
__Albumin*
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
__Globulins
X Glucose*
X Total Bilirubin*
X Total Protein*
__Triglycerides

nzymes:
(Alkaline phosphatase
__Cholinesterase
(Creatine phosphokinase*
__Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)*
X Serum aspartate aminotransferase (also SGOT)*
X Protein electrophoresis

* EPA guideline requirement

The CHECKED (X) urinalysis parameters were measured.

X Appearance*
X Volume*
X Specific gravity*
X pH
X Sediment (microscopic)*
X Protein*
X Glucose*
X Ketones*
X Bilirubin*
X Blood*
X Nitrate
X Total reducing substances

* EPA guideline requirement

Animals judged to be moribund during the treatment period were sacrificed. Blood samples were taken ante mortem and a physical examination was performed; urine was collected at necropsy. A

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complete necropsy was performed. At the end of the study, surviving animals were anesthetized with intravenous sodium pentobarbitone and exsanguinated. Gross examinations were done; the following CHECKED (X) tissues were preserved. The (XX) organ(s) in addition were weighed.

Digestive System	Cardiovasc./Hemat. System	Neurologic System
__Tongue	X Aorta*	XXBrain*
X Salivary glands*	XXHeart*	X Periph. nerve*
X Esophagus*	X Bone marrow*	X Spinal cord (3 levels)
X Stomach	X Lymph nodes*	XXPituitary*
X Duodenum*	XXSpleen*	X Eyes (Optic n.)*
X Jejunum*	XXThymus*	Glandular
X Ileum*	Urogenital System	XXAdrenals*
X Cecum*	XXKidneys*	__Lacrimal gland
X Colon*	X Urinary bladder*	X Mammary gland*
X Rectum*	XXTestes*	XXParathyroids*
XXLiver*	X Epididymides	XXThyroids*
X Gall bladder*	XXProstate/urethra	Other
X Pancreas*	__Seminal vesicle	X Bone*
Respiratory System	XXOvaries	X Skeletal muscle*
X Trachea*	XXUterus*	X Skin
XXLung*	X Vagina	X All gross lesions and masses

* EPA Guideline Requirement

The following samples were preserved but not examined:

bronchi
salivary gland - right submandibular (left was examined)
sciatic nerve - right (left was examined)
tongue

In addition, a costal bone marrow smear was taken, fixed and stained.

D. Statistical Analyses

The significance of inter-group differences in bodyweight change, blood composition and quantitative urinalysis were assessed by Student's t-test using a pooled error variance. For organ weight data, homogeneity of variance was tested using Bartlett's test. If this was found to be statistically significant, a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used.

E. Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality. A signed "Flagging Statements" indicates that the study neither meets nor exceeds the criteria of 40 CFR 158.34.

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III. RESULTS

A. Mortality

One male and three female dogs in the **10.0 mg/kg/day group** were euthanized during the second week of treatment. The following clinical signs were observed in the animals.

Sex & Number	Day of Euthanasia	Clinical Signs
M, 3910	10	inappetence, weight loss, emaciation, dehydration, hypothermia
F, 3865	12	inappetence, emaciation, weight loss, subdued behavior, dehydration, excessive salivation, hindlimb extension
F, 3873	8	inappetence, weight loss, convulsions, disorientation, ataxia, cardiac arrhythmia, excessive salivation, apparent lack of vision, absent menace reaction, blood-stained saliva around mouth
F, 3879	12	inappetence, weight loss, emaciation, suspected convulsion, ataxia, limb [illegible], lack of awareness, constricted pupils

The only change noted on clinical pathology examinations prior to death was an increase in the RBC parameters (HCT, HGB and RBC) and a decrease in prothrombin and activated thromboplastin time. The changes were considered to be due to the overall poor health of the animals. On necropsy, there were no treatment-related changes in appearance or organ weights.

B. Clinical Signs, Physical and Neurological Examinations

Clinical Signs

Animals in the **10.0 mg/kg/day group**, excluding those which were euthanized, were observed to have clinical signs indicative of a general toxic effect, including inappetence, emaciation, underactivity and hunched posture. The signs were more prominent in males during the first three weeks of treatment and in females during the first two weeks. Neurological signs in this group not noted under Mortality included convulsions at Week 2 and head nodding at Weeks 3 and 7 for one male, convulsions and body tremors at Week 2 and body tremors with head nodding at Week 6 for one female. There was a slow recovery in surviving animals so that by Weeks 11 and 12 only inappetence was noted in one female.

The study report states that for the **2.0 mg/kg/day group** the only sign of reaction to treatment was inappetence which was noted for two of the females (one at Week 2 and one at Weeks 1 to 4). According to Table 1A (page 42) of the study report, inappetence

was also reported in one female in this group at Week 9. Salivation was observed in both the treated and control groups.

There were no treatment-related clinical signs for the animals in the 0.5 mg/kg/day group, except for underactivity in one female at Weeks 1-3.

Physical Examinations

The findings of the physical examinations on animals in the 10.0 mg/kg/day group euthanized during the treatment period are summarized above under Mortality. Unscheduled examinations of surviving animals revealed convulsive episodes in two different males at Weeks 8 and 13. At Week 2, a female had subdued behavior, ataxia, convulsions, tremors, head nodding and facial twitching over a two-day period.

A routine examination at Week 3 revealed emaciation and occasional twitching of the whole body in one male in the 10.0 mg/kg/day group. The study report indicates that there were no other significant observations.

Neurological Examinations

One male in the 10.0 mg/kg/day group had head nodding, facial twitching and exaggerated blink and gag responses at the Week 6 examination. At the Week 12 examination, one female in this group had a depressed tactile placing response.

C. Body Weight and Body Weight Gain

On Day 7, body weight loss was observed in two males and three females in the 10.0 mg/kg/day group. Further weight loss in one of the males and the three females in this group contributed to the decision to euthanize these animals during Week 2 of the study. The other male lost weight until Day 21 but then gained so the initial loss was recovered by Day 35.

The study report states that two females in the 2.0 mg/kg/day group had weight loss during Week 2 of the study. However, on review of individual animal data (Appendix 4, page 114), one female lost 0.1 kg from Day 7 to Day 14 and another stayed at the same weight for this interval. Group means were comparable to the controls, however the mean of the 10.0 mg/kg/day group was based on one dog after Day 14.

Overall weight gain during the study was reduced by 17 and 12% in the 2.0 and 10.0 mg/kg/day groups, respectively. If the surviving animals are considered, the treated animals were comparable to the controls.

Table 1 summarizes weight changes from pretest to study termination.

Table 1
Body Weight Changes (Kg) in Dogs
Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (mg/kg/day)							
	Males				Females			
	0	0.5	2.0	10.0*	0	0.5	2.0	10.0*
Change from Day 0 to 91	2.[illegible]	2.7	2.6	2.6	2.4	..3	2.0	2.1
Percent of control value	-	113	108	108	-	96	83	88

a Extracted from Table 3 (pages 48-4[illegible]) of the study report.
* Group mean was based on three male and one female dogs beginning with Day 14.

D Food Consumption and Food Efficiency

Food Consumption

Food consumption in one male in the **10 mg/kg/day group** was not affected by the treatment. The intake of the other three males and three of the females in this group was markedly decreased beginning on the first day of treatment; the other female was moderately affected. Eating was encouraged by moistening the food on Day 4 and supplementing the diet with meat on Day 5. Those males which survived beyond Week 2 had a gradual improvement and returned to normal intakes by Weeks 3 or 5. The one surviving female continued to be affected during Weeks 5 and 6 and was not eating consistently until Week 9 (Appendix 3A, page 108).

One female in the **2.0 mg/kg/day group** had slightly lower intake during the first four weeks of treatment; another female was affected during Week 2. Consumption for the dogs in the **0.5 mg/kg/day group** was comparable to the controls. Table 2 summarizes overall food consumption on a g/dog/week basis.

Table 2
Mean Food Consumption (g/dog/week)
in Dogs Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (mg/kg/day)							
	Males				Females			
	0	0.5	2.0	10.0*	0	0.5	2.0	10.0*
Total Weeks 1-13	36.2	36.4	36.4	32.6	35.0	36.0	33.9	33.1
% Control Value	-	101	101	91	-	103	97	95

a Extracted from Table 2 (page 47) of the study report.
* Mean based on three males and one female beginning with Week 7

Food Efficiency

Food efficiency values were not determined.

E. Ophthalmoscopic Examinations

There were no treatment-related lesions.

F. Clinical Pathology

Hematology

There was no evidence of a treatment-related effect on hematology parameters. A few statistically significant differences were seen at all the sampling times but they were randomly distributed throughout the groups.

Clinical Chemistry

The study report states that higher alkaline phosphatase and lower cholesterol levels were seen in the 10.0 mg/kg/day group after 6 and 12 weeks of treatment. Higher AST values in males in the 10.0 mg/kg/day group and higher urea levels in females in the 2.0 mg/kg/day group were seen after six weeks. However, if these values are compared to those prior to treatment, there is no evidence of an effect. Table 3 summarizes the data for these parameters.

Table 3
Changes in Selected Blood Chemistry Parameters
in Dogs Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (mg/kg/day)							
	Males				Females			
	0	0.5	2.0	10.0	0	0.5	2.0	10.0
Alkaline Phosphatase								
Pre-treatment	127	112	124	123	122	133	119	132
Six weeks	99	92	105	123**	90	124	97	91
Twelve weeks	77	74	81	110***	75	101	83	86
Cholesterol								
Pre-treatment	135	136	134	124	136	144	135	117
Six weeks	133	124	132	100*	106	108	103	121
Twelve weeks	132	127	130	109	112	118	109	118
Aspartate amino-transferase (AST)								
Pre-treatment	28	30	34	34	29	24	28	27
Six weeks	27	29	32	36*	33	31	36	29
Urea								
Pre-treatment	24	23	25	20	21	23	21	24
Six weeks	30	28	28	27	24	26	29*	27

a Extracted from Table 5B (pages 56-67) of the study report.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
***Significantly different from controls, $p<0.001$

Urinalysis

There were no treatment-related changes.

G. Necropsy Findings

Gross Necropsy

There were no treatment-related changes on post-mortem macroscopic examination.

Organ Weights

The study report states that one male in the **10.0 mg/kg/day group** had higher absolute and relative spleen and thymus weights and higher absolute adrenal weight in comparison to the controls. Another male in this group also had increased absolute and relative thymus weights. However, these differences were not apparent when group means were compared.

Histopathology

Two animals in the **10.0 mg/kg/day group** which were euthanized during the treatment period had changes on histopathology. Follicular and parafollicular atrophy of the mesenteric lymph nodes was reported in one male and cortical atrophy of the thymus was seen in the same male and one female. These findings were considered the result of stress rather than a direct result of treatment.

H. Conclusion from Study Report

The study report concluded that the no-effect level was 0.5 mg/kg/day and the maximum-tolerated-dosage was between 2 and 10 mg/kg/day.

I. DISCUSSION

In this subchronic non-rodent study (MRID # 429186-42), M&B 46030 was administered in gelatin capsules to four male and four female beagles per group at dosages of 0, 0.5, 2.0 or 10.0 mg/kg/day for thirteen weeks.

At **10.0 mg/kg/day**, there were significant clinical signs of toxicity involving the central nervous system which were most prominent during the first three weeks of treatment in males and during the first two weeks in females. One male and three females in this group were euthanized during the second week of treatment due to their poor condition. The only clinical sign of toxicity observed in the **2.0 mg/kg/day group** was inappetence in two females. There were no treatment-related signs in the **0.5 mg/kg/day group.** 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.)

One male in the **10.0 mg/kg/day group** had higher absolute and

relative spleen and thymus weights and higher absolute adrenal weight in comparison to the controls. Another male in this group also had increased absolute and relative thymus weights. However, the group means for these organs were comparable to the controls. Follicular and parafollicular atrophy of the mesenteric lymph nodes was reported in one male and cortical atrophy of the thymus was seen in the same male and one female in the 10.0 mg/kg/day group which was euthanized during the treatment period. The findings were considered to be related to stress rather than a direct result of treatment.

IV. CONCLUSIONS

The LOEL is 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). The NOEL is 2.0 mg/kg/day for females and 0.5 mg/kg/day for males.

The study is **Core Guideline** and **satisfies** the guideline requirements (82-1) for a subchronic toxicity study in the dog.