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DATA EVALUATION REPORT

STUDY TYPE:	Chronic Toxicity/Dogs (83-1)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 429186-45
TEST MATERIAL:	M&B 46030 Synonym: Fipronil
STUDY NUMBER:	LSR 92/RHA311/0464
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 52 Weeks
AUTHOR(S):	P. Holmes
REPORT ISSUED:	November 16, 1992

EXECUTIVE SUMMARY: In this chronic dog study (MRID # 429186-45), M&B 46030 was administered in gelatin capsules to six male and six female beagle dogs per group at dosages of 0, 0.2, 2.0 or 5.0 mg/kg/day for 52 weeks. For the first fifteen days, the chemical was weighed directly into the capsules, but for the remainder of the study an admixture of M&B 46030 and lactose was prepared to increase the accuracy of the dose administration. Standard ante-mortem and post-mortem evaluations of toxicity were included in the study with the addition of perfusion fixation of a small number of animals in each group.

One male in the 2.0 mg/kg/day group and two in the 5.0 mg/kg/day group were sacrificed during the treatment period due to poor condition. Clinical signs of neurotoxicity observed in these animals included convulsions, vocalization, overactivity, body twitches/tremors, salivation, stiffened limbs, ataxia and incoordination. Clinical signs of neurotoxicity in the surviving animals were observed beginning in Week 2 of treatment and were similar to those described in the animals that were sacrificed prematurely. On physical examination at selected times during the study, signs of neurotoxicity observed in the 2.0 and 5.0 mg/kg/day males and females included tenseness, nervous and excitable behavior, abnormal stiffness or positioning of the hindlimbs, twitching of the facial muscles and hyperesthesia. On neurological examination at selected times, similar signs were observed in these groups with abnormal examinations in three males and two females in the 5.0 mg/kg/day group and two females in the 2.0 mg/kg/day group.

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Body weight and weight gain in the treated males were comparable to the control group. Females in the 5.0 mg/kg/day group had weight gains that were decreased in relation to the controls during the first 26 weeks (88% of the control value for weeks 0-13 and 73% for weeks 13-26) and for the overall study (84% of the control value), however the mean decrease was due to reduced gain in one female alone.

There were no other treatment-related changes observed during the study.

The No Observed Effect Level (NOEL) is 0.2 mg/kg/day in males and females.

The Lowest Observed Effect Level (LOEL) is 2.0 mg/kg/day based on clinical signs of neurotoxicity and abnormal neurological examinations.

The study is **Core Guideline** and **satisfies** the guideline requirements (83-1) for a chronic toxicity study in the dog.

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-trifluoromethylsulphinylpyrazole
Purity: 96.8%
Batch Number: PGS963
Description: Fine off-white powder
Storage Conditions: In a cool store (not exceeding 15° C) and protected from light

Six months after receipt and at six-month intervals thereafter, 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: 20 to 23 weeks at commencement of treatment
Weight: Males - Approximately 8.0 kg; Females - approximately 7.2 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)[1] and water *ad libitum*
Acclimation Period: At least four weeks

All dogs were vaccinated and treated with an anthelmintic prior to commencement of the study.

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[1] The basal diet was modified by moistening the food or adding a meat supplement to encourage intake in some of the dogs in poor condition. During Weeks 16 to 18, the daily quantity of food offered to each animal was increased to 600 g because of overactivity and weight loss in one female in the 0.2 mg/kg/day group.

II. METHODS

A. Dosage and Administration

Twenty-four (24) male and 24 female dogs were randomly assigned to the following treatment groups using "a random procedure which ensured that all groups contained populations of animals with similar initial mean and range of bodyweights":

Dosage Level (mg/kg/day)	Number of Dogs Male	Female
0 (Control)	6	6
0.2	6	6
2.0	6	6
5.0	6	6

The test chemical was weighed directly into gelatin capsules for the first 15 days. Thereafter, it was added to the capsules in the form of a 1 in 20 M&B 46030: lactose mixture. The study report states that this procedure was adopted to increase the accuracy of dose administration by enabling the addition of larger quantities of material into the capsules. Batches of the admixture were prepared during Weeks 3, 4, 6, 9, 13, 16, 19, 22, 25, 28, 31, 35, 38, 42, 45, 49 and 52. Measured amounts of lactose and the test substance were mixed using a planetary mixer to provide an admixture with a final M&B 46030 concentration of 50 mg/g. Each batch was then used to supply all treated animals until depletion of that mix, subject to the constraint of the available stability data.

Chemical analyses were done on the contents of six capsules prepared on Day 2 of treatment for animals in the 0.2 ppm group. During the first week of treatment, samples of the M&B 46030: lactose admixture were assayed for homogeneity and stability (after 3, 8, 14 and 35 days of storage). The concentration of the test chemical in the admixture was determined in Weeks 3, 4, 6, 10, 18, 26, 34, 42 and 50 of treatment.

Control dogs received empty capsules on Days 1 to 15 of treatment and thereafter were given capsules which contained lactose at a dosage of 100 mg/kg/day (equivalent to the quantity of admixture supplied to animals of the high dosage group).

B. Experimental Design

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

physical examinations - six days before dosing and after 11, 23, 35 and 47 weeks of treatment
neurological examination* - six days before dosing and after 12,

24, 38 and 50 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 and before necropsy
food consumption - for final two weeks of acclimation period and for each week throughout the treatment period
ophthalmoscopic examinations - five days before dosing and after 12, 24 and 50 weeks
hematology, clinical chemistry - once four days prior to dosing and after 12, 24 and 50 weeks of treatment
urinalysis - once six days before dosing and after 11, 23 and 48 weeks of treatment
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

Direct pupillary light
Consensual (indirect) pupillary light
Palpebral - blink
- corneal
Gag
General examination of the head to assess other cranial nerves

Segmental reflexes

Flexor (withdrawal)
Patellar
Crossed extensor

Postural reactions

Placing reactions - visual
- tactile
Extensor postural thrust
Righting - optic
- vestibular
Hopping
Tonic neck

C. Pathological Parameters

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

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X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Prothrombin Time
X 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

The CHECKED (X) clinical chemistry evaluations were done.

Electrolytes:
X Calcium*
X Chloride*
__Magnesium*
X Phosphorus*
X Potassium*
X Sodium*

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

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

* EPA guideline requirement

Plasma and serum samples were taken after 50 and 51 weeks of treatment, respectively, and frozen for possible future analysis.

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 veterinary and neurological examinations were performed. Bone marrow and urine samples were obtained and a complete necropsy was performed. At the end of the study, four male and four female animals from each group of those surviving were anesthetized with intravenous sodium pentobarbitone and exsanguinated. The remaining animals in the

groups were placed under deep sodium barbitone anesthesia and killed by perfusion fixation. Gross examinations were done on all animals. The following CHECKED (X) tissues were preserved at the routine necropsy; the (XX) organ(s) in addition were weighed (the perfused organs were not 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

For the animals subjected to a routine necropsy, approximately 5 g samples of brain from the frontal lobes and abdominal adipose tissue were taken with the minimum of delay and retained deep frozen for possible future analysis.

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 urinalysis (volume, specific gravity and pH only) were assessed by Student's t-test using a pooled error variance. For organ weights, 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. Inter-group differences in macroscopic pathology and histopathology were assessed using Fisher's Exact test.

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" indicated that the study neither meets nor exceeds the criteria of 40 CFR 158.34.

III. RESULTS

A. M&B 46030 Content in Admixture

Analyses of six samples for the concentration of M&B 46030 in the capsules on Day 2 showed that the percentage of the intended content ranged from 77% to 130%. Due to this wide variability, the chemical was then mixed with lactose to create an admixture which was placed in capsules. During the first week of treatment, the homogeneity of the 50.0 mg/g admixture was tested and found to have a coefficient of variation of 5.15%. These samples were then pooled and tested for stability. The analyses showed that the chemical was stable in the lactose admixture for at least 35 days. The concentration of the chemical in the admixture over the course of the study ranged between 93 and 106% as a percentage of the intended concentration.

B. Mortality

Three males were sacrificed during the treatment period due to poor condition. The clinical signs in these animals ante mortem are listed below.

Group & Number	Day of Euthanasia	Clinical Signs
2 - 4512	76	convulsions observed and presumed on Days 18 and 73, respectively, inappetence, vocalization, overactivity, body tremors, salivation, limbs stiffened, underactivity, ataxia, incoordination, irregular or labored respiration
4 - 4492	214	convulsions on Days 183 and 213-214, nervous behavior, salivation, ataxia, twitching of whole body, incoordination, twitching of head and pinnae muscles, prostrate, unsteady gait, inappetence, irregular respiration
4 - 4496	232	convulsions Day 232-233, inappetence, tucked-up abdomen, labored and increased respiration, muscle tremors, aggressive behavior, underactivity, distended abdomen, nervous behavior, vocalization, rales, salivation, twitching, ataxia, stiffened limbs

C. Clinical Signs, Physical and Neurological Examinations

Clinical Signs

Signs indicative of neurotoxicity were observed in the 2.0 and 5.0 mg/kg/day groups beginning in Week 2 of treatment. The signs included convulsions, localized and generalized twitching or tremors, nervous behavior, abnormalities of posture and gait, extensor rigidity of the limbs, vocalization, head nodding, aggression and resistance to dosing. One female dog (number 4473) in the 0.2 mg/kg/day group was observed to be markedly overactive during Weeks 13 to 18 of treatment, so much so that it lost weight and developed lesions on the forepads from continuous pacing. The animal's cage was modified during this period with obstacles in an effort to reduce its continuous activity. During Weeks 18 to 19, however, this animal was underactive. The study report concluded that this behavior was unlikely to have been associated with treatment since no similar changes were seen in other treated animals. However, overactivity was reported in other animals. In Appendix 3, Veterinary conditions and treatments, a female in the 5.0 mg/kg/day group was observed to have signs of anxiety with pacing in the pen. In Appendix 4, Summary of selected clinical signs during the treatment period, overactivity was observed in one male each in the 2.0 and 5.0 mg/kg/day groups, one female in the 2.0 mg/kg/day group and two females in the 5.0 mg/kg/day group; it was also reported in one control group female. Table 1 summarizes the incidences of selected clinical signs.

Table 1
Group Incidences of Selected Clinical Signs
in Dogs Treated with M&B 46030 for 52 Weeks[a]

	Dosage Levels (mg/kg/day)							
	Total Number Affected/Number in Group[b]							
	Males				Females			
Sign	0	0.2	2.0	5.0	0	0.2	2.0	5.0
Convulsion	0/6	0/6	1/5	2/5	0/6	0/6	1/6	0/6
Extensor rigidity of limbs	0/6	0/6	4/5	3/4	0/6	0/6	3/6	6/6
Nervous	1/6	0/6	2/5	3/4	0/6	0/6	2/6	1/6
Abnormal stance/gait	0/6	0/6	1/5	4/4	0/6	0/6	2/6	2/6
Tremors/twitching of muscles	0/6	0/6	2/5	3/4	0/6	0/6	2/6	2/6

a Extracted from Table 1 (page 49-52) of the study report.
b One male in the 2.0 mg/kg/day group was sacrificed during Week 11; two males in the 5.0 mg/kg/day group were sacrificed during Weeks 31 and 34.

Physical Examinations

The clinical signs observed at the physical examinations of the animals which were sacrificed during the treatment period are listed under Mortality. Of the surviving animals, the signs observed in the 2.0 and 5.0 mg/kg/day groups during the examinations included tenseness, nervous and excitable behavior, abnormal stiffness or positioning of the hindlimbs, twitching of the facial muscles and hyperesthesia.

Neurological Examinations

Abnormal neurological examinations attributable to treatment were observed in three males and five females in the 5.0 mg/kg/day group and two females in the 2.0 mg/kg/day group. Tenseness was observed in the 5.0 mg/kg/day group females from Week 12 on and in the 2.0 mg/kg/day group females at Week 25. The finding was also reported in the males of these groups but not as consistently.

Beginning with the Week 12 examination, it was observed that one male in the 5.0 mg/kg/day group had a stiff gait in the hindquarters and another had a slightly exaggerated hopping reaction in the hindquarters. At Week 24, both had an abnormal stance with their hindlegs extended behind them and their feet placed wide apart. One female in this group also had an abnormal stance. For all three, the "knuckle test" was normal and the "foot sliding test" was abnormal. By Week 38, two males and three females in this group had abnormal stances with the same results in the "knuckle test" and the "foot sliding test". At Week 50, one male and two females were observed to have this stance, however an additional two females had other moderately abnormal stances.

Three females in the 5.0 mg/kg/day group were noted to have slightly exaggerated gag reflexes, two had slightly exaggerated corneal reflexes and one had a slightly exaggerated blink reflex at Week 50.

D. Body Weight and Body Weight Gain

Body weight and weight gain in the treated males were comparable to the control group. Females in the 5.0 mg/kg/day group had weight gains during the first 26 weeks and for the overall study that were decreased in relation to the controls. The study report states that this decrease was due to reduced gain in one female alone. Table 2 summarizes weight gain for the females only.

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Table 2
Body Weight Gain in Females Treated with M&B 46030 for 52 Weeks[a]

	Dosage Levels (mg/kg/day)			
Weight gain (kg)	0	0.2	2.0	5.0
Weeks 0-13	2.4	2.3	2.2	2.1
% of control value	-	96	92	88
Weeks 13-26	1.1	1.0	1.1	0.8
% of control value	-	91	100	73
Weeks 0-52	4.5	4.1	4.4	3.8
% of control value	-	91	98	84

[a] Extracted from Table 3 (pages 61-64) of the study report; % calculated by the reviewer

E. Food Consumption

Food intake in the treated groups was comparable to the controls. As discussed previously, the basal diet was altered to enhance palatability. In addition, the amount offered was increased to 600 g per day during Weeks 16 to 18 to maintain the weight of one female in the 0.2 mg/kg/day group which became overactive at that time.

F. Ophthalmoscopic Examinations

There were no treatment-related lesions.

F. Clinical Pathology

Hematology

After 50 weeks of treatment, females in the 2.0 and 5.0 mg/kg/day groups had significantly increased HCT, HGB and RBC levels, however the differences were minor and of questionable toxicological significance.

Clinical Chemistry

The only alteration which could have been treatment-related was a statistically significant increase in ALT in the 5.0 mg/kg/day group females after 50 weeks of treatment, however there were no histopathological changes in the liver. Although there were other statistically significant differences, they were sporadic and not dose-related.

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

Terminal body weights for females in the 5.0 mg/kg/day group were significantly decreased as compared to the controls. There were few significant alterations in the absolute and relative organ weights in the treated groups and none which were consistent or dose-related.

Histopathology

There were no treatment-related changes on histopathology.

H. Conclusion from Study Report

The study report concluded that the no-effect level was 0.2 mg/kg/day.

I. DISCUSSION

In this chronic dog study (MRID # 429186-45), M&B 46030 was administered in gelatin capsules to six male and six female beagle dogs per group at dosages of 0, 0.2, 2.0 or 5.0 mg/kg/day for 52 weeks. For the first fifteen days, the chemical was weighed directly into the capsules, but for the remainder of the study an admixture of M&B 46030 and lactose was prepared to increase the accuracy of the dose administration. Standard ante-mortem and post-mortem evaluations of toxicity were included in the study with the addition of perfusion fixation of a small number of animals in each group.

One male in the 2.0 mg/kg/day group and two in the 5.0 mg/kg/day group were sacrificed during the treatment period due to poor condition. Clinical signs of neurotoxicity observed in these animals included convulsions, vocalization, overactivity, body twitches/tremors, salivation, stiffened limbs, ataxia and incoordination. Clinical signs of neurotoxicity in the surviving animals were observed beginning in Week 2 of treatment and were similar to those described in the animals that were sacrificed prematurely. One female dog (number 4473) in the 0.2 mg/kg/day group was observed to be markedly overactive during Weeks 13 to 18 of treatment, so much so that it lost weight and developed lesions

on the forepads from continuous pacing. The study report concluded that this behavior was unlikely to have been associated with treatment since no similar changes were seen in other treated animals. However, overactivity was reported in other animals. A female in the 5.0 mg/kg/day group was observed to have signs of anxiety with pacing in the pen. Overactivity was observed in one male each in the 2.0 and 5.0 mg/kg/day groups, one female in the 2.0 mg/kg/day group and two females in the 5.0 mg/kg/day group; it was also reported in one control group female. Although the extent of the hyperactivity was not seen in other animals in the higher dosage groups, the possibility that this animal was extremely sensitive to the chemical cannot be dismissed.

On physical examination at selected times during the study, signs of neurotoxicity observed in the 2.0 and 5.0 mg/kg/day males and females included tenseness, nervous and excitable behavior, abnormal stiffness or positioning of the hindlimbs, twitching of the facial muscles and hyperesthesia. On neurological examination at selected times, similar signs were observed in these groups with abnormal examinations in three males and two females in the 5.0 mg/kg/day group and two females in the 2.0 mg/kg/day group.

Body weight and weight gain in the treated males were comparable to the control group. Females in the 5.0 mg/kg/day group had weight gains that were decreased in relation to the controls during the first 26 weeks (88% of the control value for weeks 0-13 and 73% for weeks 13-26) and for the overall study (84% of the control value), however the mean decrease was due to reduced gain in one female alone.

After 50 weeks of treatment, females in the 2.0 and 5.0 mg/kg/day groups had significantly increased HCT, HGB and RBC levels, however the differences were minor and of questionable toxicological significance. The only alteration in clinical chemistry which could have been treatment-related was a statistically significant increase in ALT in the 5.0 mg/kg/day group females after 50 weeks of treatment, however there were no histopathological changes in the liver.

There were no other treatment-related changes observed during the study.

The No Observed Effect Level (NOEL) is 0.2 mg/kg/day in males and females.

The Lowest Observed Effect Level (LOEL) is 0.5 mg/kg/day based on clinical signs of neurotoxicity and abnormal neurological examinations.