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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 28 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: M&B 46030 (Fipronil) - Review of Toxicology Data Submitted by the Registrant

PC Code: 129121
DP Barcode: D197450
Case: 285247
Submission: S454829

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer Virginia A Dobozy 6/20/94
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Robert Brennis/Daphne Waldo/PM 10
Registration Division (7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C) Marcia van Gemert 6/27/94

Registrant: Rhone-Poulenc AG Company

Action Requested: Completion of review of toxicology data base for M&B 46030 (Fipronil); Toxicology Branch II previously forwarded recommendations on a request for an EUP/temporary tolerance for use of the chemical on field corn

Recommendation: Toxicology Branch II has completed review of the data base for this chemical; issues involving carcinogenicity and neurotoxicity will be presented to the appropriate peer review committees. (See RECOMMENDATIONS.)

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DATA SUMMARY

I. **ACUTE STUDIES**

Acute Neurotoxicity Study/Rat (81-8): MRID # 429186-35

Material Tested: M&B 46030 (96.7% a.i.)

A single dose of M&B 46030 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. 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 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

II. SUBCHRONIC TOXICITY TESTING

Subchronic Toxicity/Dog (82-1): MRID # 429186-42

Material Tested: M&B 46030 (95.4% a.i.)

M&B 46030 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. One male and three females in the 10 mg/kg/day group were euthanized during the second wee.. 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

Subchronic Toxicity/Rat (82-1): MRID # 429186-43

Material Tested: M&B 46030 (95.4% a.i.)

M&B 46030 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. 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 β 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: Supplementary; study may be upgraded with the submission of the data from the neurological examinations.

21-Day Dermal Toxicity Study/Rabbit (82-2): MRID # 429186-44

Material Tested: M&B 46030 (96.7% a.i.)

M&B 460430 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. 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

III. CHRONIC TOXICITY STUDIES

Chronic Toxicity/Dog (83-1): MRID # 429186-45

Material Tested: M&B 46030 (96.8% a.i.)

Male and female beagle dogs were administered M&B 46030 in capsules at dosages of 0, 0.2, 2.0 or 5.0 mg/kg/day for 52 weeks. 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 = 0.5 mg/kg/day

Classification: Guideline

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Carcinogenicity/Mouse (83-2): MRID # 429186-49

Material Tested: M&B 46030 (95.4% a.i.)

Six groups of 20 male and 20 female CD-1 mice/group were administered M&B 46030 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. 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 M&B 46030 is not carcinogenic to CD-1 mice when administered at doses of 30 ppm.

Classification: Minimum

Combined Chronic Toxicity/Carcinogenicity/Rat (83-5): MRID # 429186-48

Material Tested: M&B 46030 (95.4%)

Fifteen (15) CD rats/sex/group were administered M&B 46030 in the diet for 52 weeks to assess the chronic toxicity of the chemical. 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 (50) 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.

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.

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)

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

IV. DEVELOPMENTAL AND REPRODUCTION TOXICITY STUDIES

Developmental Toxicity/Rat [83-3(a)]: MRID # 429779-03

Material Tested: M&B 46030 (93% a.i.)

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 M&B 46030 by oral gavage from gestation days 6 through 15, inclusive. 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

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Developmental Toxicity/Rabbit [83-3(b)]: MRID # 429186-46

Material Tested: M&B 46030 (95.4% a.i.)

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 M&B 46030 by oral gavage from gestation days 6 through 19, inclusive. 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

Multigeneration Reproduction/Rat (83-4): MRID # 429186-47

Material Tested: M&B 46030 (95.4% a.i.)

Thirty CD rats/sex/group received M&B 46030 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). 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

V. MUTAGENICITY STUDIES

A. Studies Conducted with M&B 46030

Salmonella/mammalian activation gene mutation assay (84-2): MRID # 429186-52

Material Tested: M&B 46030 (90.6% a.i.)

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In two independent experiments, M&B 46030 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

In vitro gene mutation assay in mammalian cells/Chinese hamster V79 cells (84-2): MRID # 429186-51

Material Tested: M&B 46030 (97.2% a.i.)

In two independent experiments, M&B 46030 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

In vivo micronucleus assay/mouse (84-2): MRID # 429186-50

Material Tested: M&B 46030 (97.2% a.i.)

M&B 46030 was neither cytotoxic to the target organ nor caused a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow cells from CD-1 mice after oral gavage administration of doses up to 50 mg/kg. The study was unacceptable because there were observations from only two animals/sex at this dose.

Classification: Unacceptable

Cytogenic assay/human lymphocytes (84-2): MRID # 429186-53

Material Tested: M&B 46030 (90.6% a.i.)

There was no evidence of a clastogenic effect when human lymphocytes were exposed in vitro to M&B 46030 at doses of 75, 150 or 300 µg/ml with and without S9 activation.

Classification: Acceptable

B. Studies Conducted with Metabolite M&B 46136

Salmonella/mammalian activation gene mutation assay (84-2): MRID # 429186-79

Material Tested: M&B 46136 (98.7% a.i.)

In two independent experiments, M&B 46030 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

Cytogenic assay/human lymphocytes (84-2): MRID # 429186-80

Material Tested: M&B 46136 (98.7% a.i.)

There was no evidence of a clastogenic effect when human lymphocytes were exposed in vitro to M&B 46136 at doses of 75, 150 or 300 µg/ml with and without S9 activation.

Classification: Acceptable

VI. METABOLISM STUDY

Metabolism Study/Rat (85-1): MRID # 429186-55

Material Tested: M&B 46030 (^{14}-C Fipronil, >97.0% radiochemical purity; unlabelled Fipronil, >99% a.i.)

^{14}C-Fipronilwas 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). 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 metabolites in urine included two ring-opened products of the metabolite M&B 45,897, two oxidation products (M&B 46,136 and RPA200766), and parent chemical (M&B 46,030). In feces, parent M&B 46,030 was detected as a significant fraction of the sample radioactivity as well as the oxidation products M&B 46,136 and M&B 45,950. 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

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VII. SPECIAL STUDIES

Thyroid Function/Rat: MRID # 429779-04

Material Tested: M&B 46030 (95.4% a.i.)

Four groups of 27 male Crl:CD (SD) BR rats per group were administered either methylcellulose (vehicle control), 10 mg/kg/day M&B 46030, 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. Six hours later, 9 males per group received either 10 or 25 mg/kg potassium perchlorate or 0.9% saline solution. The treatment with M&B 46030 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 M&B 46030 or Noxyflex.

Classification: Supplementary

Thyroxine Clearance: MRID # 429186-54

Material Tested: 95.4%

Six groups of six male Crl:CD (SD) rats per group were administered either M&B 46030 (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. Four hours after the final dose of either test substance, each rat received [^{125}I]thyroxine at a dosage of 10 µCi/kg. M&B 46030 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 M&B 46030 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

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Toxicology Profile for M&B 46030 (Fipronil) (40 CFR 158.340)

A. Data Requirements - Technical	Required	Satisfied
81-1 Acute oral	yes	yes*
81-2 Acute dermal	yes	yes*
81-3 Acute inhalation	yes	no*
81-4 Primary eye irritation	yes	yes*
81-5 Primary dermal irritation	yes	yes*
81-6 Dermal sensitization	yes	no*
81-8 Acute neurotoxicity	no	yes
82-1 Subchronic feeding (rat)	yes	no[1]
82-1 Subchronic feeding (dog)	yes	yes
82-2 21-Day dermal	yes	yes
82-3 90-Day dermal	no	n/a
82-4 Subchronic inhalation	no	n/a
82-5 21-Day delayed neurotoxicity	no	n/a
83-1 Chronic feeding (rodent)	yes	no[1]
83-1 Chronic feeding (nonrodent)	yes	yes
83-2 Carcinogenicity (rat)	yes	no[1]
83-2 Carcinogenicity (mouse)	yes	yes
83-3 Developmental toxicity (rat)	yes	yes
83-3 Developmental toxicity (rabbit)	yes	yes
83-4 Multigeneration reproduction (rat)	yes	yes
83-5 Combined chronic toxicity/ carcinogenicity (rat)	yes	yes
84-2 Mutagenicity (gene mutation in bacterial cells)	yes	yes
84-2 Mutagenicity (gene mutation in mammalian cells)	yes	yes
84-2 Mutagenicity (in vivo assay for chromosomal effects)	yes	no
85-1 Metabolism	yes	yes
85-2 Domestic animal safety	no	n/a
85-3 Dermal absorption	no	n/a

* Reviewed in a previous memorandum
[1] Satisfied with an acceptable combined chronic toxicity/ carcinogenicity study

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B. Data Requirements - Formulation[2]	Required	Satisfied
81-1 Acute oral	yes	yes*
81-2 Acute dermal	yes	yes*
81-3 Acute inhalation	yes	yes*
81-4 Primary eye irritation	yes	yes*
81-5 Primary dermal irritation	yes	yes*
81-6 Dermal sensitization	yes	no*

[2] EXP 60655A (1.6% fipronil)
* Reviewed in a previous memorandum

B. Toxicology Issues

1. RfD

The RfD/Peer Review Committee will consider the oral RfD for M&B 46030 (Fipronil) in the near future.

2. Carcinogenicity

The chemical was carcinogenic at a dosage of 300 ppm (12.68 mg/kg/day for males and 16.75 mg/kg/day for females) in rats and will be considered by the Cancer Peer Review Committee in the near future.

3. Toxicology data gaps

Studies with technical chemical

- acute inhalation
- dermal sensitization
- mutagenicity (in vivo assay for chromosome effects)

Studies with formulation

- dermal sensitization

4. Updated, selected one-liners

Attached are updated, selected one-liners to support the data requirements.

5. Labeling Issues

The label for the formulation, FIPRONIL 1.5% Granular, was reviewed and found to adhere to the required warning and precautionary statements in 40 CFR 156.10.

C. Recommendations

Toxicology Branch II has determined that the toxicology data requirements for registration of the chemical have not been satisfied. In addition, there are concerns about the effects of the chemical on the nervous system and the thyroid gland. Neurotoxicity was a consistent finding in the three species involved in the chronic studies (rat, dog and mouse). The lowest dose at which clinical signs of neurotoxicity were observed was 1.5 ppm in the rat (0.059 and 0.078 mg/kg/day for males and females, respectively), 2.0 mg/kg/day in the dog, and 60 ppm in the mouse (≈ 8.5 mg/kg/day, the animals at this dosage in the carcinogenicity study were sacrificed prematurely due to excessive mortality). The chemical has a potent effect on thyroid function. In the combined chronic toxicity/carcinogenicity study (the only one which measured thyroid hormone levels), T_4 levels were zero after one week of treatment in both male and female rats in the 300 ppm group (12.68 and 16.75 mg/kg/day for males and females, respectively). The T_4 levels were decreased at all of the dose levels (as low as 0.5 ppm, ≈ 0.02 mg/kg/day) at some time point during the 52-week evaluation period. During the reversibility period of this study (13 weeks of no treatment), the T_4 levels in the treated males did not return to levels comparable to the control animals until 11 weeks post-treatment. Continuous thyroid stimulation, in response to the reduced T_4 levels, resulted in a high incidence of follicular cell tumors in the 300 ppm group animals. In addition, the chemical persists in the body. In the metabolism study, significant amounts of residual radioactivity were found in various organs and fat at 168 hours post-dosing for all dose groups. In summary, the data from the metabolism and chronic toxicity studies indicate that this is a persistent chemical which has the potential for nervous system and thyroid toxicity after long-term exposure at low dosages.

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Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 6/14/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. J M I 6/14/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Neurotoxicity/Rats (81-8)
EPA ID NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 429186-35
TEST MATERIAL:	M&B 46030 Synonym: Fipronil
STUDY NUMBER:	91N0099
TESTING FACILITY:	Bushy Run Research Centre Export, PA
SPONSOR:	Rhone-Poulenc Ag Company
TITLE OF REPORT:	M&B 46030: Single Exposure Peroral (Gavage) Neurotoxicity Study in Sprague Dawley® Rats
AUTHOR(S):	M. W. Gill, C. L. Wagner and C. D. Driscoll
REPORT ISSUED:	April 26, 1993

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID # 429186-35), a single dose M&B 46,030 in corn oil was administered by gavage to four groups of 15 Sprague-Dawley® rats/sex/group at dosages of either 0, 0.5, 5.0 or 50.0 mg/kg. Standard evaluations for evidence of toxicity included observations for mortality and clinical signs of toxicity and measurements of body weight. Neurobehavioral evaluations included a functional observational battery (FOB) (done at 7 hours, 7 days and 14 days post-treatment), quantitative assessment of motor activity (done 1 hour after each FOB) and post-mortem neuropathological examinations (on Day 15 of the study).

Five males and one female in the 50 mg/kg group died during the study. Clinical signs of toxicity were seen only in the 50 mg/kg group with the incidence of neurological signs more prevalent in males. On the day of dosing, males and females in this group were observed to have either clonic or tonic/clonic convulsions. Other clinical signs of toxicity included emaciation, dehydration, unkempt appearance, urine stains, cold extremities and/or pallor. Males in the 50 mg/kg group had decreased body weights in comparison to the controls at 7 and 14 days post-treatment.

Treatment-related effects on FOB parameters at seven hours post-treatment were far more pronounced in the 50 mg/kg group with the males of the group more often affected. During the open field

evaluations, 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 Day 7, the only FOB parameter which was significantly changed was an increase in the number of rears in the 50 mg/kg group males. Females in the 50 mg/kg group had a statistically significant increase in hind leg splay at the 7-day evaluation.

On Day 14, the only statistically significant changes were decreased urination in the 50 mg/kg group males, increased rectal temperature in the 0.5 and 50 mg/kg group females and increased hind leg splay in the 50 mg/kg group females.

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 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.

The study is classified as **Minimum** and **satisfies** the guideline requirements (81-8) for an acute neurotoxicity study in rats.

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: 96.7%
Lot Number: 78/GC/90
Description: White powder
Storage Conditions: In a sealed container at room temperature

The 5.0 mg/ml suspension of M&B 46030 in corn oil was prepared and then dilutions with corn oil were made to form the 0.05 mg/ml and 0.5 mg/ml solutions. All three formulations were analyzed for homogeneity and concentration; the 0.05 and 5.0 mg/ml solutions were analyzed for stability. The analyses showed that the suspensions were uniform and stable for at least 6 days. The concentrations ranged from 93.0 to 104.4% of the nominal values.

B. Test Animals

Species: Male and female Sprague Dawley® rats
Source: Harlan Sprague Dawley Inc., Indianapolis, IN
Age: 28 days when received
Weight: Males - 221.8 to 291.0 g; Females - 144.5 to 186.3 g at the time of dosing
Housing: Two rats/cage for approximately 7 days and then individually in stainless steel, wire mesh cages
Food and Water: Certified Rodent Chow® #5002 and water ad libitum
Environmental Conditions: Temperature: 66-77^{0}F
Relative humidity: 40-70%
Photoperiod: 12 hours light/dark during acclimation period; thereafter 13 hours light/12 hours dark
Acclimation Period: Three weeks

II. METHODS

Preliminary Study

In a preliminary study, 4 rats/sex/group were administered a single dose of M&B 46030 by gavage at a dosage of either 0, 50 or 80 mg/kg. An abbreviated functional observational battery (FOB) was performed every hour for 7 hours on the day of dosing and 24 hours after dosing. Gait and arousal measures were evaluated during a 1 minute observation period after the animals were removed from their cages. Piloerection, breathing pattern, body position and defecation were evaluated at each observation. Clinical

observations were recorded daily, and body weights were measured on Days 1, 5 and 8. All of the males and three of the four females in the 80 mg/kg group died during the first two days of the study. One male in the 50 mg/kg group died within 24 hours of dosing. Decreases in body weight gain were also observed in males and females of this group. The following neurological abnormalities were noted: gait alterations in both groups; clonic convulsions, alternating tonic and clonic convulsions and/or tremors in all males in the 80 mg/kg group; clonic convulsions in one male in the 50 mg/kg group; and salivation in one male and one female in the 80 mg/kg group and one male in the 50 mg/kg group.

Dosage and Administration

The animals were assigned to the following groups using a computerized randomization procedure.

Group	Number of Animals		Dosage	Concentration
	Male	Female	(mg/kg)	(mg/ml)
Control	15	15	0	0
Low	15	15	0.5	0.05
Intermediate	15	15	5.0	0.5
High	15	15	50.0	5.0

The test substance was administered once by gavage at a volume of 10 ml/kg. Control animals were given the vehicle (corn oil) at the same volume. Dosing was staggered over a four-day period to accommodate the schedule for behavioral testing.

Observations and Measurements

Standard Evaluations -

Mortality and clinical signs of toxicity - twice daily
Body weight - day prior to dosing and then weekly

Neurobehavioral Evaluations -

Functional Observational Battery (FOB) - The animals were divided into 8 testing blocks of 7 or 8 animals/sex with two blocks/sex being evaluated on each test day. Observations were performed the week prior to dosing and approximately 7 hours, 7 days and 14 days following dosing. The animals were evaluated by trained technicians who were blinded to the animals' treatment group. The endpoints included in the FOB from page 12 of the study report are attached to the DER.

Motor Activity - Motor activity evaluations were performed after the FOB testing. The testing was conducted for approximately 90 minutes using an automated recording

apparatus designed to measure activity (San Diego Instruments, Inc., San Diego, CA). Data for ambulatory activity, fine motor activity, rearing and the sum of the individual types of activity were collected. The list of motor activity parameters evaluated (from page 110 of the study report) is attached to the DER.

Neuropathology - Fifteen days following dosing, 10 animals/sex/group were anesthetized with sodium pentobarbital and perfused *in situ* by cardiac perfusion with 10% buffered formalin. The cranium and the vertebral arches covering the brain were removed and the peripheral nerves of the hindlimb exposed. These tissues were then further fixed in neutral buffered formalin. An abbreviated necropsy was performed on the thoracic and peritoneal cavities. Tissues from 6 animals in the control and high dose groups were examined microscopically. The following tissues were paraffin embedded, sectioned and stained: coronal slices of the brain, cross and longitudinal slices of the spinal cord and trigeminal nerve and Gasserian ganglia as well as numerous dorsal root ganglia and associated nerve roots. Sections of the sciatic, peroneal, sural and tibial nerves were embedded in glycol methacrylate, sectioned and stained. Animals not selected for the neuropathology examinations were sacrificed and discarded.

Data Analyses

A description of the data analyses from the study report is attached to the DER.

III. RESULTS

Mortality and Clinical Observations

Five males and one female in the 50 mg/kg group were found dead during the study, five within two days of treatment and one after five days. Diffuse brain hemorrhage was found in five of the animals which were found dead within two days of dosing but it is questionable if that was the cause of death or an agonal change. Clinical signs of toxicity were seen only in the 50 mg/kg group with the incidence of neurological signs more prevalent in males. On the day of dosing, two males and one female had clonic convulsions and two males had tonic/clonic convulsions. In addition, convulsions were observed in four males and one female at the 7-hour FOB evaluation. Other signs observed within two days of dosing in both males and females included emaciation, dehydration, unkempt appearance, urine stains, cold extremities and/or pallor.

Body Weight

Mean body weight of the 50 mg/kg group males was significantly decreased in comparison to the control group at Day 14. Table 1 presents the body weight data.

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Table 1
Body Weights in Rats Treated
with a Single Oral Dose of M&B 46030[a]

	Dosage Levels (mg/kg)							
	Males				Females			
	0	0.5	5.0	50.0	0	0.5	5.0	50.0
Pre-treatment	211.12	207.14	208.96	212.09	147.77	145.16	148.28	147.14
Seven hours	250.07	248.65	249.73	246.81	165.31	165.52	166.65	164.18
% control value	-	99.4	99.9	98.7	-	100.1	100.8	99.3
Seven days	289.59	286.92	285.95	259.58**	186.19	187.66	190.60	181.13
% control value	-	99.1	98.7	89.6	-	100.8	102.4	97.3
Fourteen days	321.63	318.44	316.88	302.51**	203.77	203.03	210.04	211.91
% control value	-	99.0	98.5	94.1	-	99.6	103.1	99.1

a Extracted from Tables 5-12 (pages 24-40) of the study report; percentages calculated by the reviewer.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

Functional Observations

Treatment-related effects on FOB parameters were more pronounced in the 50 mg/kg group at the 7-hour evaluation period with the males of the group more often affected. During the homecage observations, 5/15 males in this group were observed to have dropping eyelids. During the open field evaluations, animals showed both a stimulation and depression of the nervous system. Some of the signs of stimulation included: clonic convulsions in 4/15 males; fine tremors in 6/15 males and 6/15 females; and coarse tremors in 5/15 males and 1/15 females. Nervous system depression was evidenced by a depression of open field activity (decreased arousal and rearing activity), decreases in several reflexes (approach response, tail pinch response, air righting reflex), decreased muscle tone, altered gait and decreased pupil size. The only parameter that was considered to be affected by treatment in the 5.0 mg/kg group males and females was a decrease in mean hind leg splay. Females in the 0.5 and 5.0 mg/kg groups both had significantly decreased numbers of rears but the effect was not considered treatment-related. The study report argues that the number of open field rearing events was lower for animals in the low and mid dose groups compared to the controls at each measurement period, including pretreatment. In addition, changes in motor activity were not noted in these groups and there were no other FOB changes in these groups except for the decrease in mean hind limb splay in the 5.0 mg/kg group females. Also, there were no changes on neuropathologic examination of the central and peripheral nervous system. Table 2 presents the

incidence of the 7-hour data for those parameters with a statistically significant effect.

Table 2
Summary of Affected Functional Observations 7 Hours Post-treatment in Rats Treated with a Single Dose of M&B 46030[a]

	Dosage Levels (mg/kg)							
	Males				Females			
	0	0.5	5.0	50.0	0	0.5	5.0	50.0
Gait								
Normal	15	15	15	5**	11	14	14	7
Splayed	0	0	0	8	0	0	0	4
Hypotonic	0	0	0	2				
Walks on toes					4	1	1	4
Fine Tremors								
None	15	15	15	9*	15	15	15	9*
Whole body	0	0	0	1	0	0	0	4
Limbs	0	0	0	5	0	0	0	1
Head					0	0	0	1
Coarse Tremors								
None	15	15	15	10*	15	15	15	14
Whole body	0	0	0	5				
Limbs					0	0	0	1
Urine								
None	15	15	14	7**	13	14	13	9
Present	0	0	1	8	2	1	2	6
Rears								
Mean number	3.67	6.53	3.93	.20*	18.00	9.07**	10.53**	.60**
Approach Response[b]								
Noticeable	15	15	13	9*				
None	0	0	2	6				
Pupil Size								
Normal	14	15	15	6**	10	11	13	7
Decreased	1	0	0	9	5	4	2	8
Muscle Tone								
Normal	15	15	15	10*	14	15	14	5**
Decreased	0	0	0	5	1	0	1	10

Table 2 Continued

Rectal Temperature								
Mean	37.95	38.08	38.23**	35.21**	38.75	38.48	38.45	35.79**
Air Righting[b]								
Feet/coordinated	14	15	15	7*				
Feet/uncoordinated	1	0	0	1				
Back	0	0	0	4				
Side	0	0	0	3				
Hind Leg Splay								
Mean (cm)	7.92	7.18	7.00*	5.72**	7.54	7.64	6.55*	5.34**

a Extracted from Tables 6 (pages 26-28) and 10 (pages 35-37) of the study report.
b Females exhibited normal findings for these parameters.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

On Day 7, some males in the 50 mg/kg group appeared to have a stimulation of activity as evidenced by hyperactive arousal behavior in 3/10 animals, exaggerated startle response in 3/10 animals, exaggerated tail pinch response in 2/10 animals and an increased mean number of rears. Only the increase in rears was statistically significant. Females in the 50 mg/kg group had a statistically significant increase in hind leg splay at the 7-day evaluation.

On Day 14, the only statistically significant changes were decreased urination in the 50 mg/kg group males, increased rectal temperature in the 0.5 and 50 mg/kg group females and increased hind leg splay in the 50 mg/kg group females.

Motor Activity

Mean motor activity was decreased by 90 and 93% in the 50 mg/kg group males and females, respectively, at the 8-hour evaluation. Treatment with M&B 46030 affected the shape of the curve for total motor activity vs test session time. The changes were attributable to clear differences at 50.0 mg/kg for males and females and a tendency for differences at 5.0 mg/kg for males and females [Figures 6 (page 54) and 7 (page 59) of the study report].

At Day 7, significant increases in mean activity for the 0.5 and 5.0 mg/kg group males were observed. However, the study report indicates that the same dose-response profiles were observed at the pretreatment, 8-hour and Day 14 measurements suggesting that the Day 7 changes were the result of biological variation between groups rather than treatment with M&B 46030. To test this hypothesis, first , a nested analysis of the motor activity data was performed for the pretreatment, Day 7 and Day 14 measurements to determine if treatment affected the interaction between groups. The 8-hour data were excluded because of the treatment-related changes in the 50 mg/kg group. Significant interactions between

treatment groups and measurement periods were not observed. A second statistical analysis was performed by converting the individual animal data from Day 7 to percent of their respective preexposure data. Group comparisons were then made on the transformed data and there were no statistically significant differences. The study report concludes 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. A summary of the motor activity data are presented in Table 3.

Table 3
Mean Cumulative Test Session Counts of Motor Activity[a] in Rats Treated with a Single Oral Dose of M&B 46030

	Dosage Levels (mg/kg)							
	Males				Females			
	0	0.5	5.0	50	0	0.5	5.0	50
Pretreatment	502.9	623.6	623.2	630.7	822.3	833.1	841.7	736.5
8 Hours Post-treatment	557.4	613.9	531.2	56.0**	981.5	945.3	1008.5	68.9**
7 Days Post-treatment	584.8	752.0*	836.1*	709.2	1021.1	822.3	964.5	993.0
14 Days Post-treatment	623.4	798.5	803.4	581.1	909.0	916.7	754.7	932.3

a Extracted from Tables 13 (page 46) and 14 (page 48) of the study report.
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

Post-mortem Neuropathological Examinations

There were no treatment-related gross or microscopic changes.

IV. CONCLUSIONS FROM STUDY REPORT

The study report concluded that the No Observed Effect Level (NOEL) for this study was 0.5 mg/kg.

V. STUDY DEFICIENCIES

1. The study did not employ a positive control. The Pesticide Assessment Guidelines, Subdivision F, Addendum 10, Neurotoxicity, require that positive control data be submitted from the laboratory performing the test to demonstrate the sensitivity of the procedures being used. Historical control data may be used if all the essential aspects of the experimental protocol are the same. It can be argued that such a control is not needed for this study since the test substance did produce effects at the high dose level.

2. Tissues were examined from only the control and high dose group. The Guidelines require that at least five males and five females shall be used in each dose and control group for terminal neuropathology. However, since there were no lesions reported from the examinations of the high dose group, there is little likelihood that lesions would have been observed at the low or mid dose levels.

VI. COMPLIANCE

Signed statements of Quality Assurance and compliance with the GLP regulations were submitted by the testing facility. A signed Confidentiality Statement claiming no data confidentiality was submitted by the sponsor.

VII. DISCUSSION/CONCLUSIONS

In an acute neurotoxicity study (MRID # 429186-35), a single dose M&B 46,030 in corn oil was administered by gavage to four groups of 15 Sprague-Dawley® rats/sex/group at dosages of either 0, 0.5, 5.0 or 50.0 mg/kg. Standard evaluations for evidence of toxicity included observations for mortality and clinical signs of toxicity and measurement of body weight. Neurobehavioral evaluations included a functional observational battery (FOB) (done at 7 hours, 7 days and 14 days post-treatment), quantitative assessment of motor activity (done 1 hour after each FOB) and post-mortem neuropathological examinations (on Day 15 of the study).

Five males and one female in the 50 mg/kg group died during the study. Clinical signs of toxicity were seen only in the 50 mg/kg group with the incidence of neurological signs more prevalent in males. On the day of dosing, males and females in this group were observed to have either clonic or tonic/clonic convulsions. Other clinical signs of toxicity included emaciation, dehydration, unkempt appearance, urine stains, cold extremities and/or pallor. Males in the 50 mg/kg group had decreased body weights in comparison to the controls at 7 and 14 days post-treatment.

Treatment-related effects on FOB parameters at seven hours post-treatment were far more pronounced in the 50 mg/kg group with the males of the group more often affected. During the open field evaluations, effects of both stimulation and depression of the nervous system were seen. Those parameters for which there were statistically significant changes included gait (decreased number of males with normal gait), fine tremors (decreased number of males and females with no tremors), coarse tremors (decreased number of males with no tremors), urination (decreased number of males with none), mean number of rears (decreased in males and females), approach response (decreased number of males with a noticeable response), pupil size (decreased number of males with a normal pupil size), muscle tone (decreased number of males and females with normal muscle tone), air righting (decreased number of males with feet/coordinated) and mean hind leg splay (decreased mean measurement in males and females). 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 a decreased mean hind leg splay in males and females.

On Day 7, some males in the 50 mg/kg group appeared to have a stimulation of activity as evidenced by hyperactive arousal behavior in 3/10 animals, exaggerated startle response in 3/10 animals, exaggerated tail pinch response in 2/10 animals and an increased mean number of rears. Only the increase in rears was statistically significant. Females in the 50 mg/kg group had a statistically significant increase in hind leg splay at the 7-day evaluation.

On Day 14, the only statistically significant changes were decreased urination in the 50 mg/kg group males, increased rectal temperature in the 0.5 and 50 mg/kg group females and increased hind leg splay in the 50 mg/kg group females.

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 Lowest Observed Effect Level (LOEL) = 5.0 mg/kg for males and females based on decreased hind leg splay at the seven hour post-treatment evaluation.

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Pages 27 through 30 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 2/28/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. YMI 2/28/94
Section I, Toxicology Branch II (7509C)

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 **Core Guideline** and **satisfies** the guideline requirements (82-1) for a subchronic 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-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 21^0 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:

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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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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.

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

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

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.

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 jerks, 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	2.4	2.7	2.6	2.6	2.4	2.3	2.0	2.1
Percent of control value	-	113	108	108	-	96	83	88

a Extracted from Table 3 (pages 48-49) 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.

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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	35.0	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 2.

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	[illegible]	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.

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 3/9/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. JMI 3/10/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Subchronic Toxicity/Rats (82-1)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 429186-43
TEST MATERIAL:	M&B 46030 Synonym: Fipronil
STUDY NUMBER:	LSR 90/RHA298/0781
TESTING FACILITY:	Life Science Research Limited Suffolk, England
SPONSOR:	Rhone-Poulenc Ag Company
TITLE OF REPORT:	M&B 46030: Toxicity Study By Dietary Administration to CD Rats for 13 Weeks
AUTHOR(S):	P. Holmes
REPORT ISSUED:	April 9, 1991

EXECUTIVE SUMMARY: In this subchronic rat study (MRID # 429186-43), M&B 46030 was administered in the diet to groups of ten male and ten female CD rats at dosages of 0, 1, 5, 30 or 300 ppm (males: 0, 0.07, 0.33, 1.93, 19.87 mg/kg/day; females: 0, 0.07, 0.37, 2.28, 24.03 mg/kg/day, respectively) daily for thirteen weeks.

There were no deaths during the study. The incidence of two skin lesions, tail encrustations and abrasions, was higher in the 300 ppm group females.

Overall mean body weight gain was slightly decreased (9% lower than the control value) in the 300 ppm group females. Overall mean food consumption and food conversion ratios were comparable between the treated and control groups.

Statistically altered hematology values were seen in the treated groups, however the changes were minor and inconsistent and therefore of questionable biological significance. The 300 ppm group males and females had higher total protein concentrations than the control in association with higher values for $\alpha 1$, $\alpha 2$ and β 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. Other changes were either minor or not dose-related and were not considered of toxicological significance.

There were no treatment-related changes on macroscopic post-mortem examination. Significantly higher absolute and relative thyroid

weights were reported in the 300 ppm group males and females in comparison to the controls. Absolute weights of the thyroid were also increased in the 30 ppm group females. Absolute liver weights were increased in the 300 ppm group males and in females which received 5 ppm or above. 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. The incidence of follicular cell hyperplasia was also increased in comparison to the controls but not significantly. Liver sections stained with hematoxylin and eosin revealed a low incidence of panacinar fatty vacuolation in the 300 ppm group males and females, however when sections were stained with Oil-Red-O, the incidence and distribution of fat in the liver was significantly higher and more extensive in the 300 ppm group males. **The No Effect Level (NOEL) is 5 ppm for males (0.33 mg/kg/day) and females (0.37 mg/kg/day). The Lowest Effect Level (LOEL) is 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.**

The study is **Core Supplementary** and **does not satisfy** the guideline requirements (82-1) for a subchronic toxicity study in the rat. The study may be upgraded with the submission of the data from the neurological examinations.

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: 95.4%
Batch Number: PGS963
Description: Fine white powder
Storage Conditions: Room temperature protected from light

B. Administration: dietary

C. Test Animals

Species: CD rats
Source: Charles River (France), St Aubin-les-Elbeuf, France
Age: approximately three to four weeks upon arrival at testing facility
Weight: approximately 69 to 103 g upon arrival at testing facility
Housing: Five of one sex per cage
Environmental Conditions: Temperature: target of 21° C
Relative humidity: target of 55%
Photoperiod: 12 hours light/dark
Air changes: 20 per hour
Food and Water: Complete powdered rodent diet (Laboratory Animal Diet No. 2) and tap water *ad libitum*
Acclimation Period: 13 days

II. METHODS

A. Diet Preparation and Analysis

M&B 46030 was initially mixed with a small quantity of the basal diet to create a pre-mix which was then milled. The pre-mix was diluted with the basal diet and mixed in a Hobart mixer to prepare the 300 ppm concentration which was then serially diluted to give the other diet formulations. Batches of the diets were prepared fresh weekly.

Samples of the highest and lowest dietary concentrations taken from six positions in the mixer were taken to test for **homogeneity** of the diet formulations prior to commencement of treatment. The unused portions of the homogeneity samples were then tested for **stability** after one and two weeks of storage at room temperature. The **concentration** of the test chemical in all the diets was determined at Weeks 1 and 13 of treatment.

B. Dosage and Administration

The animals were assigned randomly to the following treatment groups using a latin square arrangement.

Group	Treatment	Dietary Concentration (ppm)	Number of Animals Males	Females
1	Control	0	10	10
2	M&B 46030	1	10	10
3	M&B 46030	5	10	10
4	M&B 46030	30	10	10
5	M&B 46030	300	10	10

The diets were administered continuously for at least thirteen weeks.

C. Experimental Design

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

physical examinations - detailed weekly examination
neurological examination* - after 12 weeks of treatment on all animals from Groups 1 and 5
clinical signs of toxicity - twice daily
body weights - on first day of dosing and then weekly throughout the treatment period
food consumption - weekly intervals during the treatment period
food conversion - calculated at weekly intervals
ophthalmoscopic examinations - all animals before treatment; Groups 1 and 5 after 12 weeks of treatment
hematology, clinical chemistry and urinalysis - after 12 weeks of treatment on all animals
gross necropsy - all animals
organ weights - designated organs from 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
Sta...
General examination of the head to assess other cranial nerves

Segmental reflexes

Flexor (withdrawal)

Postural reactions

Placing reactions - visual and tactile
Righting reactions
Grasping

General observations

Behavioral changes, e.g. aggression, sedation
Abnormalities of gait and stance
Presence of tremor or other dyskinesias

D. Pathological Parameters

For hematology and clinical chemistry evaluations, blood was drawn from the retro-orbital sinus under light ether anesthesia after an overnight fast. The CHECKED (X) hematology parameters were examined.

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

* 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)*

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

* EPA guideline requirement

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The CHECKED (X) urinalysis parameters were measured.

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

* EPA guideline requirement

At the end of the treatment period, the animals were sacrificed by carbon dioxide inhalation. Gross examinations were done over a four-day period; the following CHECKED (X) tissues were preserved. The (XX) organ(s) in addition were weighed.

Digestive System
__Tongue
XXSalivary glands*
__Esophagus*
X Stomach
X Duodenum*
X Jejunum*
X Ileum*
X Cecum*
X Colon*
X Rectum*
XXLiver*
__Gall bladder*
X Pancreas*

Respiratory System
X Trachea*
XXLung*

Cardiovasc./Hemat. System
X Aorta*
XXHeart*
__Bone marrow*
X Lymph nodes*
XXSpleen*
XXThymus*

Urogenital System
XXKidneys*
X Urinary bladder*
XXTestes*
X Epididymides
XXProstate/urethra
X Seminal vesicle
XXOvaries
XXUterus*
X Vagina

Neurologic System
XXBrain*
X Periph. nerve*
X Spinal cord
XXPituitary*
X Eyes (Optic n.)*

Glandular
XXAdrenals*
__Lacrimal gland
X Mammary gland*
XXParathyroids*
XXThyroids*

Other
X Bone*
X Skeletal muscle*
X Skin
__All gross lesions and masses

The following samples were preserved but not examined:

eye and optic nerve - right (left was examined)
harderian glands
mammary glands - cranial (caudal were examined)
salivary gland - right submandibular (left was examined)
sciatic nerve - right (left was examined)
tongue

In addition, femoral bone marrow smears were taken, fixed and stained.

Histological examinations were done on the following: 1) preserved tissues listed above from all rats in Groups 1 and 5; 2) the thyroids, parathyroids, kidneys, livers and lungs from all rats in Groups 2, 3 and 4; and 3) Oil-Red-O stained sections of liver from all animals in all groups.

E. 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 within-group error variance. Homogeneity of variance was tested using Bartlett's test for organ weights. 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. The statistical significance of the incidences of macroscopic and microscopic findings was tested using Fisher's exact probability test as a two-tailed test.

F. 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.

III. RESULTS

A. Achieved Dosages

The following actual mean dosages were received during the course of treatment (extracted from Table 4, Page 43 of the study report).

	Dosage Levels (ppm)							
	Males				Females			
	1	5	30	300	1	5	30	300
Mean Achieved Dosage	0.07	0.33	1.93	19.87	0.07	0.37	2.28	24.03

B. Diet Analyses

Analyses of the 1.0 and 300 ppm group diet formulations for **homogeneity** showed that the mean concentration of M&B 46030 in the six samples was 96 and 91% of the intended concentration, respectively (Appendix 2B, page 79). Analyses of these samples for **stability** after 7 and 14 days revealed that the chemical has an estimated 14-day shelf life (Appendix 2C, page 80). Analyses of all the diets showed that the percent of the intended M&B 46030 **concentration** in each diet ranged from 89 to 106% at Week 1 and from 91 to 104% at Week 13 (Appendix 2D, page 81).

C. Mortality

There were no deaths during the treatment period.

D. Clinical Signs

The study report states that there were no clinical signs clearly related to treatment. The report notes that the incidence of two skin lesions (tail encrustations and abrasions) was higher in the 300 ppm group females. However, individual animal data (Appendix 3, pages 82-91) indicate that the following signs were seen in the treated groups but not in the controls: salivation in one male and one female in the 30 ppm group; salivation and a clonic convulsion in one male in the 300 ppm group; and slow, deep and noisy respiration in one male in the 300 ppm group.

E. Neurological Examinations

The study report states that the results of this examination, which did not show any evidence of abnormalities, were not included with the study but are held in the archives.

F. Body Weight and Body Weight Gain

There were no statistically significant differences in body weight during the study. The study report indicates that body weight gain was slightly inferior to the control in the 30 ppm group males and statistically decreased in the 300 ppm group males and females during the first week of treatment. For the duration of the study, weight gain in the 300 ppm group males was significantly higher than the control group and that of the other treated groups was similar or superior to the controls. When the duration of the study was considered, weight gain in the 300 ppm group females was lower than the control group, although not statistically significant. Overall weight gain in the other treated females and all the treated males either exceeded or was comparable to the controls. The study report states that fluctuations during Week 13 were attributed to clinical pathology investigations at that time, although the weight gain depression was most marked in the 300 ppm group males. Table 1 summarizes weight changes at selected times during the study.

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

	Males					Females				
Body weight change	0	1	5	30	300	0	1	5	30	300
Week 0-1	65	64	63	60*	33***	31	35	35	32	19***
% of control value	-	98	97	92	51	-	113	113	103	61
Week 1-12	309	323	310	321	348**	130	147	151	152	133
Week 12-13	-4	-1	0	0	-13	-11	-15	-12	-15	-16
Week 0-13	370	386	372	381	368	150	167	173	168	136
% of control value	-	104	101	103	99	-	111	115	112	91

a Extracted from Table 2 (pages 40-41) of the study report.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
*** Significantly different from controls, $p<0.001$

G. Food Consumption and Food Conversion Ratio

Food Consumption

Weekly group mean food consumption was determined by dividing the total amount of food consumed by the group by the number of rat-days and then multiplying the result by seven. Rat-days were calculated as the total number of rats alive in the group summed for each day during the week.

Intake was markedly lower than that of the controls in the **300 ppm group males** during the first two weeks of treatment and in the **300 ppm group females** and **30 ppm group males** during the first week of treatment. In the subsequent weeks, food consumption was increased in these groups so that overall intake during the course of the study was comparable between the treated and control groups. The other treated groups were unaffected by treatment. The study report indicates that low intake during Week 13 was the result of clinical pathology procedures. Table 3 summarizes food consumption at selected times during the study.

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Table 3
Mean Food Consumption (g/rat/week)
in Rats Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (mg/kg/day)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
Week 1	187	189	183	175	138	142	147	142	147	122
% of control value	-	101	98	94	74	-	103	100	103	83
Week 2	193	195	182	183	174	140	142	144	149	144
% of control value	-	101	94	95	90	-	101	103	106	103
Weeks 1-13 (total)	2421	2438	2397	2351	2340	1742	1737	1782	1823	1787
% of control value	-	101	99	97	97	-	100	102	105	103

a Extracted from Table 1 (page 39) of the study report.

Food Conversion Ratio

Food conversion ratios were calculated by dividing the amount of food consumed by each group by the body weight gain of the group. The ratios of the **300 ppm group males and females** were higher (lower food utilization efficiency) than the control during the first week of treatment, however in subsequent weeks the ratios were lower (higher food utilization efficiency) than the controls. The values of the other treated groups were comparable to the controls. Table 4 summarizes the food conversion ratios at selected times during the study.

Table 4
Food Conversion Ratios in Rats
Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
Week 1	2.9	3.0	2.9	2.9	4.2	4.6	4.2	4.6	4.6	6.5
Week 1-13	6.5	6.3	6.4	6.2	6.4	11.6	10.4	10.3	10.8	13.1

a Extracted from Table 3 (page 42) of the study report.

E. Ophthalmoscopic Examinations

There were no treatment-related lesions.

F. Clinical Pathology

Hematology

Females in the 300 ppm group had statistically altered hematology values after 12 weeks of treatment in comparison to the controls, including lower PCV, HGB, MCV, MCH and prothrombin time and higher platelet count. Lower prothrombin times were also noted in the **30 ppm group females.** HGB values lower than those of the controls were seen in the **300 ppm group males** and in the **1, 5 and 30 ppm group females.** The study report states that the female control HGB value was high in comparison to background data for the parameter (mean of 15.5 g% with a normal range of 14.0 to 17.0 g%). The report further indicates that the change in HGB, in conjunction with the other changes in erythrocytic parameters, represent minor treatment-related effects in the 300 ppm group females. Other differences were not attributable to the test chemical. Table 5 summarizes the affected hematology parameters.

Table 5
Selected Hematology Parameters in Rats
Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
PCV (%)	46	46	46	45	45	45	44	44	44*	43***
HGB (g%)	15.9	16.0	16.2	15.9	15.3**	15.9	15.6*	15.5*	15.4**	15.3***
MCV (cμ)	52	51	52	52	52	54	53	54	53	51***
MCH (pg)	18	18	19*	18	18	19	19	19	19	18**
Platelets (1000/cmm)	852	858	911	948*	920	913	937	933	993	1028*
PT (secs)	15.0	15.8*	14.7	15.2	14.7	14.2	14.4	14.0	13.7*	13.5**

a Extracted from Table 5B (pages 45-46) of the study report.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
***Significantly different from controls, $p<0.001$

Clinical Chemistry

The **300 ppm group males and females** had higher total protein concentrations than the controls in association with higher values for α1, α2 and β globulins and lower albumin/globulin (A/G) ratios. The **5 and 30 ppm group males and females** had similar alterations in the protein values but the A/G ratios were comparable to the controls. Total protein, α2, and β globulin concentrations were also higher than the controls in the **1 ppm group females**, however the difference from the controls was not related to dosage and was not considered toxicologically significant.

Other changes included the following: 1) higher BUN values in all treated males; 2) lower AST levels in all treated females; 3) lower ALT values in **30 and 300 ppm group males and females**; and 4) higher glucose levels in females at **5 ppm or above**. Table 6 summarizes the changes in these parameters.

Table 6
Selected Clinical Chemistry Parameters
in Rats Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
ALT (iu/l)	34	31	30	28	32	30	28	27	24*	24*
AST (iu/l)	73	63	63	61*	71	74	59*	52***	40***	48***
Urea (mg%)	25	29*	30**	31***	31**	34	38	33	37	32
Glucose (mg%)	140	132	127	135	146	125	136	140*	151***	144**
Total Protein (g%)	6.8	6.9	7.1**	7.1**	7.4***	7.2	7.8**	7.6*	7.8**	7.9**
α1 globulin (g%)	1.3	1.3	1.5*	1.5**	1.7***	1.1	1.1	1.2	1.3*	1.4**
α2 globulin (g%)	0.4	0.4	0.4	0.4	0.5***	0.4	0.5**	0.4*	0.5**	0.6***
β globulin (g%)	1.7	1.6	1.8	1.6	2.0**	1.4	1.6*	1.6	1.7**	1.8***
A/G ratio	0.8	0.9	0.7	0.8	0.6**	1.1	1.0	1.1	1.0	0.9***

a Extracted from Table 6 (pages 47-48) 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

Higher absolute and relative thyroid weights were reported in the **300 ppm group males and females.** Animals in the **30 ppm group** had similar tendencies, however only the absolute weights in the **30 ppm group females** were statistically significant.

Absolute liver weights were increased in the **300 ppm group males** and in females which received **5 ppm or above.** Relative liver weights were increased in the **30 and 300 ppm group males and females.**

The absolute and relative weights of the salivary gland of treated females tended to be lower than the control. According to the study report, the differences were not statistically significant. However, Table 8A (page 52) of the study report shows that the absolute and relative weights are statistically significantly ($p<0.05$) lower in the **300 ppm group females.** The study report states that there was no dosage-relationship and histological changes in the gland were not observed so the changes were not considered to be significant. Table 7 summarizes the data for the affected organs.

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Table 7
Absolute and Relative Weights of Selected Organs from Rats Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
Thyroids										
A	0.024	0.024	0.025	0.030	0.048**	0.019	0.019	0.021	0.023*	0.032**
R	0.0044	0.0042	0.0046	0.0054	0.0091**	0.0061	0.0059	0.0063	0.0071	0.0107**
Liver										
A	19.1	21.0	19.4	21.8	27.2**	10.8	11.3	12.7*	13.4**	16.6**
R	3.54	3.72	3.59	3.99*	5.05**	3.52	3.48	3.86	4.13**	5.57**
Salivary glands										
A	0.637	0.654	0.660	0.684	0.624	0.427	0.384	0.397	0.423	0.365*
R	0.1179	0.1157	0.1225	0.1256	0.1167	0.1409	0.1184**	0.1214*	0.1298	0.1227*

a Extracted from Tables 8A and 8B (pages 50-57) of the study report.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
*** Significantly different from controls, $p<0.001$

Histopathology

There was a statistically significant increase in the incidence of hypertrophy of the follicular epithelium of the thyroid in **300 ppm group males and females.** The incidence of follicular cell hyperplasia was also increased in comparison to the controls but not significantly.

Liver sections stained with hematoxylin and eosin revealed a low incidence of panacinar fatty vacuolation in the **300 ppm group males and females.** The incidence of congestion in the liver was also increased in the **300 ppm group males and females,** although there was no dose-response relationship. In sections stained with Oil-Red-O, there was a high incidence of fat in the livers of all animals, including the control animals. However, the incidence in the **300 ppm group males** was significantly higher than the controls and the distribution was more widespread (panacinar compared with centriacinar in controls). Table 8 summarizes the findings for these organs.

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Table 8
Incidence of Histopathological Findings in Liver and Thyroids from Rats Treated with M&B 46030 for Thirteen Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
Number Examined	10	10	10	10	10	10	10	10	10	10
Liver - H&E Stain										
Panacinar hepatocytic fatty vacuolation	0	0	0	0	2	0	0	0	0	2
Congestion	4	2	3	3	6	2	0	1	0	5
Liver - Oil Red O Stain										
Panacinar hepatocytic fatty vacuolation	0	2	0	1	7**	0	0	0	0	1
Centriacinar hepatocytic fatty vacuolation	4	3	2	6	3	7	9	6	10	7
Thyroids										
Hypertrophy of follicular epithelium	3	1	0	5	8	1	0	0	0	10***
Follicular cell hyperplasia	2	0	0	1	6	0	0	1	1	2

a Extracted from Table 10 (pages 63-64) of the study report.
** Significantly different from controls, $p<0.01$
*** Significantly different from controls, $p<0.001$

H. Conclusion from Study Report

Under the DISCUSSION section of the study report, the following conclusions were made:

1) An impairment of growth performance and lowering of food intake and efficiency of food utilization was apparent for animals receiving **300 ppm** during the first week of treatment. Low food intake was also noted in the second week, however the animals became adapted to treatment after that.

2) The dosage-related higher liver weights in the rats receiving **5 ppm** and above, together with changes in plasma amino-transferase activity and protein, urea and glucose levels in treated animals, were considered to be indicative of altered liver function. The changes were minor in animals receiving up to **30 ppm** and there were no associated histopathological changes, therefore they were determined to be an adaptive rather than toxicological response.

3) The histopathological changes in the liver and thyroid of the **300 ppm group males and females** indicate a possible hypothalamic-pituitary-thyroid-liver axis. A possible mechanism would be enhanced thyroxine clearance by the liver resulting from an increased metabolic activity, leading to increased thyroid stimulating hormone (TSH) stimulation of the thyroid gland.

4) The study report concluded that the no-effect level was **1 ppm** and the maximum-tolerated-dosage was close to, but above, 300 ppm.

I. STUDY DEFICIENCY

The study report states that the neurological examinations, which were not included in this submission, did not show any abnormalities. These data should have been submitted. Although the Subdivision F Guidelines do not require these examinations, if they were done the data should be reviewed. Additionally, it was noted previously in this review that salivation and one episode of convulsions were observed in the individual animal data (Appendix 3).

IV. DISCUSSION

Ten male and ten female CD rats per group were administered M&B 46030 in the diet at dosages of 0, 1, 5, 30 or 300 ppm (males: 0, 0.07, 0.33, 1.93, 19.87 mg/kg/day; females: 0, 0.07, 0.37, 2.28, 24.03 mg/kg/day, respectively) daily for thirteen weeks.

There were no deaths during the study. A clonic convulsion was noted in one male in the 300 ppm group; salivation was observed in one male and one female in the 30 ppm group. The incidence of two skin lesions, tail encrustations and abrasions, was higher in the 300 ppm group females. The study report indicates that neurological examinations of the control and 300 ppm group rats were normal after twelve weeks of treatment, however the data have not been submitted.

Body weight gain in the 30 ppm group males was 8% lower than the controls during the first week of treatment; the difference was not statistically significant. Weight gain in the 300 ppm group males and females was 49% and 39%, respectively, lower than the control group during the same period. However, for the duration of the study, the values were increased or comparable to the control group so that the overall weight gain was only slightly decreased (9% lower than the control value) in the 300 ppm group females. Food consumption was 26% and 17% lower, respectively, in the 300 ppm group males and females during the first week of the study. Intake was 6% lower in the 30 ppm group males. During the second week of the study, males in the 300 ppm group had a 10% lower intake than the controls. However, when the overall study duration is considered, food consumption was comparable between the treated and control groups. Food conversion ratios indicated that food efficiency was lower for the 300 ppm group males and females during the first week of treatment, but overall values were comparable to the controls. The data suggest that effects on food consumption,

food conversion and body weight gain during the first one to two weeks may have been both adaptive and toxic in nature. Although the 300 ppm group males and females adapted to the level of the test chemical in the diet, the degree of decrease in body weight gain was not comparable to the decrease in food consumption. In addition, food conversion ratios indicated that food utilization efficiency was decreased.

Females in the 300 ppm group had statistically altered hematology values after 12 weeks of treatment in comparison to the controls, including lower PCV, HGB, MCV, MCH and prothrombin time and higher platelet count. Lower prothrombin times were also noted in the 30 ppm group females. HGB values lower than those of the controls were seen in the 300 ppm group males and in the 1, 5 and 30 ppm group females. The biological significance of these changes is questionable. Although the PCV, HGB and MCV were all statistically different than the control at $p<0.001$, the values were probably within the normal ranges for these parameters (no normal ranges were submitted) and were most likely due to individual variation.

The 300 ppm group males and females had higher total protein concentrations than the control in association with higher values for $\alpha 1$, $\alpha 2$ and β 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. Total protein, $\alpha 2$, and β globulins were also higher than the controls for the 1 ppm group females. The biological significance of these changes will be discussed along with the post-mortem findings. Other changes in BUN, AST, ALT and glucose were either minor or not dosage-related and were not considered of toxicological significance.

There were no treatment-related changes on macroscopic post-mortem examination. Significantly higher absolute and relative thyroid weights were reported in the 300 ppm group males and females in comparison to the controls. Absolute weights of the thyroid were also increased in the 30 ppm group females. Absolute liver weights were increased in the 300 ppm group males and in females which received 5 ppm or above. Relative liver weights were increased in the 30 and 300 ppm group males and females. The absolute weight of the salivary gland was significantly decreased in the 300 ppm group females; the relative weight was significantly decreased in the 1, 5 and 300 ppm group females. These changes in the salivary gland were not considered biologically significant since there was no dose-response relationship.

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. The incidence of follicular cell hyperplasia was also increased in comparison to the controls but not significantly. Liver sections stained with hematoxylin and eosin revealed a low incidence of panacinar fatty vacuolation in the 300 ppm group males and females. The incidence of congestion in the liver was also increased in the 300 ppm group males and females, although there was no dose-response

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relationship. In sections stained with Oil-Red-O, the incidence of and distribution of fat in the liver was significantly higher and more extensive in the 300 ppm group males. Based on the histopathological findings in the liver, the biological significance of the alterations in serum protein levels is probably meaningful in the 300 ppm group males and females.

Table 9 summarizes the findings of the study.

Table 9
Summary of Study Findings

	Dosage Levels (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
Body weight gain decreased - first week				✓ 8%	✓ 49%					✓ 39%
Food consumption decreased - first week				✓ 6%	✓ 26%					✓ 17%
Food consumption decreased - second week					✓ 10%					
Food efficiency decreased - first week					✓					✓
Total protein increased			✓	✓	✓		✓	✓	✓	✓
α1 increased			✓	✓	✓				✓	✓
α2 increased					✓		✓	✓	✓	✓
β increased					✓		✓		✓	✓
A/G ratio decreased					✓					✓
Absolute & relative thyroid weight increased					✓					✓
Absolute thyroid weight increased									✓	
Absolute liver weight increased					✓					✓
Relative liver weight increased				✓	✓				✓	✓
Incidence of hypertrophy of follicular epithelium in thyroid increased					✓					✓
Incidence of follicular cell hyperplasia in thyroid increased					✓					✓
Panacinar hepatocytic fatty vacuolation in liver increased					✓					✓

V. CONCLUSIONS

The No Effect Level (NOEL) is 5 ppm for males (0.33 mg/kg/day) and females (0.37 mg/kg/day). The Lowest Effect Level (LOEL) is 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.

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FINAL

DATA EVALUATION REPORT

M&B 46030

Study Type: 21-Day Dermal Toxicity Study - Rabbit

Prepared for:

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

Prepared by:

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Principal Reviewer Eileen Abt Date 6/1/94
Eileen Abt, M.S.

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QA Reviewer William L. McLellan Date 6/1/94
William McLellan, PH.D.

Contract Number: 68D10075
Work Assignment Number: 3-54
Clement Number: 228
Project Officer: Caroline Gordon

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M&B 46030 21 Day Dermal Toxicity 82-2

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Signature: Virginia A Dobozy
Review Section I, Toxicology Branch II (7509C) Date: 6/6/94

EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature: Y M Ioannou
Review Section I, Toxicology Branch II (7509C) Date: 6/6/94

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity Study - Rabbit (82-2)

P.C. CODE: 129121

MRID NUMBER: 429186-44

TEST MATERIAL: M&B 46030

SYNONYMS: Fipronil

STUDY NUMBER: 92N1165

SPONSOR: Rhone-Poulenc Ag Company, Research Triangle Park, NC

TESTING FACILITY: Bushy Run Research Center, Union Carbide Chemicals and Plastics Company Inc., Export, PA

TITLE OF REPORT: M&B 46030: Twenty-One Day Repeated Cutaneous Dose Toxicity Study in New Zealand White Rabbits #2

AUTHORS: S.J. Hermansky and C.L. Wagner

REPORT ISSUED: June 23, 1993

Executive Summary: In a 21-day dermal toxicity study, M&B 46030 (MRID #429186-44) (purity 96.7%) was applied in a 0.5% solution of carboxymethylcellulose in Milli-Q® filtered water to the intact skin of New Zealand White Rabbits [6/sex] at doses of 0, 0.5, 1.0, 5.0, or 10.0 mg/kg/day for 6 hours per day, for 15 doses, over a 21 day period.

Administration of 10 mg/kg/day caused decreases in mean body weight gain, and decreases in food consumption in males and females. One male and one female rabbit in the 10 mg/kg/day group exhibited signs of extreme hyperactivity that may be treatment related. No changes in clinical pathology measurements, organ weights, gross pathology, or microscopic pathology were noted. No erythema, edema, or other compound-related skin lesions were observed. **The NOEL for dermal irritation is 10.0 mg/kg/day; no LOEL could be determined. The NOEL for systemic toxicity is 5.0 mg/kg/day; the LOEL is 10 mg/kg/day.**

This study is core-guideline, and satisfies the guideline requirement [§ 82-2] for a 21-day dermal toxicity study in rabbits.

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Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test Material: M&B 46030

Description: White powder

Lot/Batch #: 78/GC/90

Purity: 96.7%

Stability of compound: The compound remained stable in 0.5% carboxymethylcellulose (CMC) at concentrations of 0.5 and 10 mg/mL for at least 14 days when stored at room temperature.

CAS #: 120068-37-3

2. Vehicle and/or positive control: Solution of 0.5% carboxymethylcellulose in Milli-Q® filtered water

3. Test animals: Rabbit

Strain: New Zealand White

Age and weight at study initiation: 4-5 months old; 3.0-3.6 kg for males, 3.1-3.8 kg for females

Source: Hazelton Research Products, Inc., Denver, PA

Housing: Individually, stainless steel, wire mesh cages

Environmental conditions:
Temperature: 61-70°F (target)
Humidity: 40-70% (target)
Air changes: Not reported
Photoperiod: 12-hour light/dark

Acclimation period: About 2 weeks

B. STUDY DESIGN

1. Animal assignment

Animals were selected for the study based on clinical signs and body weight. (Only animals with body weights within ±20% of the population mean for each sex were included.) Rabbits were assigned to the treatment and control groups in Table 1 using a computer randomization method based on body weights.

TABLE 1: 21-DAY REPEATED DOSE STUDY

Test Group	Dose Level[a] (mg/kg/day)	Male	Female
1 (Vehicle Control)	0[b]	6	6
2	0.5	6	6
3	1.0	6	6
4	5.0	6	6
5	10.0	6	6

[a] Dose levels were administered in a 0.5% solution of carboxymethylcellulose (CMC) in Milli-Q® filtered water. All animals were dosed at a constant volume of 1.0 mL/kg body weight/day.
[b] Controls were administered 0.5% carboxymethylcellulose in Milli-Q® filtered water.

2. Route, frequency, and duration of administration

Seven days prior to initial dose administration, the dorsal area of the trunk of the animals was clipped free of fur. The size of the shaved area encompassed the majority of the dorsal surface, from the scapular region to just above the rump (approximately 10-15 cm wide). The fur of the animals was also clipped before administration of the first dose and throughout the study as needed. The test material was applied directly to the clipped skin of the back. Animals were dosed once a day, for 6 hours, for 15 doses, over a 21 day period. The application site of each animal was covered with a nonsterile 4 x 4 inch gauze pad. Animals were then wrapped in a Lycra®/Spandex® jacket lined with PVC sheeting and held in place by Velcro®. Following each exposure period, the application site was washed with a dampened cloth to remove residual test material.

3. Diet

Animals were fed ACWAY® PROLAB® Animal Diet Rabbit (Agway Inc.) and tap water, *ad libitum*.

4. Statistics

The data for quantitative continuous variables for the treatment and control groups were compared using analysis of variance (ANOVA) and t-tests. Initially, Levene's test was applied to assess the equality of variances. Nonparametric data were evaluated using the Kruskal-Wallis and the Mann-Whitney U-tests. Incidence data were compared using Fisher's Exact test. $P < 0.05$ (two-tailed) was the critical level of significance used for all statistical analyses.

5. Quality assurance

The test was performed under Good Laboratory Practice standards. A quality assurance statement, signed June 21, 1993 was provided.

C. METHODS AND RESULTS

1. Observations

Animals were examined twice daily for mortality. Detailed clinical observations including skin irritation were conducted once a day. Skin irritation was scored using a modified Draize scoring system (see Appendix A for scoring system).

No mortality occurred during the study. One male and one female animal exposed to 10 mg/kg/day demonstrated signs of extreme hyperactivity on study day 21 and study day 20, respectively. Both animals recovered; however, the male animal was knocked unconscious during the period of hyperactivity and suffered a small laceration. It is possible that the hyperactivity was treatment-related since similar symptoms (i.e., spasms and delayed convulsions) were observed in rabbits exposed to this test material in an acute dermal toxicity study. No erythema or edema were observed in any animals during the study.

2. Body Weight

Animals were weighed prior to initiation of the first dose (day 1), and on days 8, 15, and 21, and immediately preceding sacrifice.

Males in the 10.0 mg/kg/day group had mean body weights that were 4 and 7% lower than controls at study days 15 and 21, respectively. However, only the decrease noted on study day 21 decrease was statistically significant (Table 2). The mean body weight gain in males in the 10.0 mg/kg/day group during the interval from day 1 to 21 was significantly different from that of the controls ($p < 0.01$) (Table 3). The statistically significant increase in body weight gain in the 5.0 mg/kg/day exposure group does not appear to be treatment-related because of the lack of a dose-response relationship (Table 3). For females in the 10.0 mg/kg/day group, mean absolute body weight did not appear to be affected (Table 2). Mean body weight gains were decreased 26%, 38%, and 51% at the day 1 to 8, day 1 to 15, and the day 1 to 21 intervals, respectively; however, they were not statistically significant (Table 3).

3. Food Consumption and Compound Intake

Food consumption was measured for all animals approximately every other day.

Males and females dosed with 10 mg/kg/day showed 44 and 21% decreases, respectively, in mean food consumption during the day 15 to 21 day measurement interval. However, only the male value was statistically significant ($p < 0.01$). Decreases in food consumption were also observed in males and females in this dose group during the 8 to 15 day interval; these decreases were not statistically significant (Table 4). Food consumption at other dose levels appears to be comparable to controls. The statistically significant increase in food consumption in the 1.0 mg/kg/day group during the 8 to 15 day interval was not considered to be treatment related since no dose-response relationship was observed.

4. Ophthalmoscopic examination

No opthalmoscopic examination was conducted.

5. Clinical pathology

Blood for hematology and clinical chemistry was collected from all rabbits by bleeding the caudal ear artery prior to sacrifice. All animals were fasted for about 24 hours prior to the bleeding procedures. The CHECKED (X) parameters were examined.

a. Hematology

X Hematocrit (HCT)
X Hemoglobin (HGB)
X Leukocyte count (WBC)
X Erythrocyte count (RBC)
X Platelet count
Blood clotting measurements
(Thromboplastin time)
(Clotting time)
(Prothrombin time)

X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpusc. HGB conc. (MCHC)
X Mean corpuscular volume (MCV)
Reticulocyte count

* Control and 10.0 mg/kg/day animals

No treatment related effects were observed. Slight variations in the mean corpuscular hemoglobin concentration for the 5.0 and 10.0 mg/kg/day groups of female rabbits were not considered to be treatment related since no dose-response relationship was observed.

b. Clinical Chemistry

Electrolytes	Other
X Calcium	X Albumin
X Chloride	X Blood creatinine
Magnesium	X Blood urea nitrogen
X Phosphorus	Cholesterol
X Potassium	X Globulins
X Sodium	X Glucose
Enzymes	X Total bilirubin
X Alkaline phosphatase	X Total serum protein
Cholinesterase	Triglycerides
X Creatine phosphokinase	Serum protein electrophores
X Lactic acid dehydrogenase	Phospholipids
X Serum alanine aminotransferase (also SGPT)	
X Serum aspartate aminotransferase (also SGOT)	
X Gamma glutamyl transferase	
Glutamate dehydrogenase	

No treatment related effects were observed. A slight increase in albumin concentration in female rabbits in the 5.0 mg/kg/day test group was noted in a single animal, and therefore was not considered to be treatment related.

6. Urinalysis

No urinalysis was performed.

7. Sacrifice and Pathology

Animals sacrificed at the end of the study were subject to gross pathological examination. The CHECKED (X) tissues were collected for histologic examination in the control and high dose groups. The CHECKED (XX) organs were also weighed.

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue	X Aorta	X Brain
X Salivary glands	X Heart	Periph. nerve
X Esophagus	Bone marrow	X Spinal cord (3 levels)
X Stomach	X Lymph nodes	X Pituitary
X Duodenum	X Spleen	X Eyes (optic n.)
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	XX Adrenal gland
X Cecum	XX Kidneys	Lacrimal gland
X Colon	X Urinary bladder	X Mammary gland
X Rectum	XX Testes	X Parathyroids
XX Liver	X Epididymides	X Thyroids
X Gall bladder	X Prostate	Other
X Pancreas	X Seminal vesicle	Bone
Respiratory	XX Ovaries	Skeletal muscle
X Trachea	X Uterus	X Skin (treated and untreated)
X Lung	X Vagina	All gross lesions and masses
Nose		
Pharynx		
Larynx		

a. Organ weight - No treatment related effects on absolute or relative organ weights in males or females were observed. Statistically significant increases in the absolute and relative weights of the adrenal glands of male rabbits at 0.5 and 1.0 mg/kg/day were observed, but were not considered to be treatment related since no dose-response relationship was seen.

b. Gross pathology - No treatment related effects were observed.

c. Microscopic pathology - No treatment related effects were observed.

E. DISCUSSION

Review of the data suggests that the conduct of the study was adequate and the reporting of the results was accurate.

No changes in clinical pathology measurements, organ weights, gross pathology, or microscopic pathology were noted. In the 10 mg/kg/day dose group, the mean absolute body weight of male rabbits and the mean body weight gain of males and females was decreased throughout the study. Male and female rabbits dosed with 10 mg/kg/day had decreased mean food consumption from day 8 to 21 of the study.

One male and one female rabbit exposed to 10 mg/kg/day exhibited signs of extreme hyperactivity near the end of the study. These effects may be treatment related since similar effects were observed in rabbits

exposed to this test material in an acute dermal toxicity study. The NOEL for dermal irritation is 10.0 mg/kg/day; no LOEL could be determined. The NOEL for systemic toxicity is 5.0 mg/kg/day; the LOEL is 10 mg/kg/day.

This study satisfies the guideline requirements for a 21-day dermal toxicity study in rabbits and is classified as Core Guideline.

Appendix A

Dermal Irritation Scoring System

Erythema Formation	Value
No erythema	0
Barely Perceptible Erythema	1
Well-defined Erythema	2
Moderate to Severe Erythema	3
Severe Erythema	4

Edema Formation	
No edema	0
Barely Perceptible Edema	1
Well-defined Edema	2
Moderate to Severe Edema	3
Severe Edema	4

TABLE 2. Mean Body Weights (g ± S.E.) for Rabbits Dermally Exposed to M&B 46030[a]

Dose Level (mg/kg/day)	Mean Body Weight (g ± S.E.) at Day: 1	21	Body Weight Gain (g) (day 1 - 21)	% of Control
		Males		
0	3313.7 ± 136.47	3539.0 ± 188.78	225.3	
0.5	3370.8 ± 96.85	3640.4 ± 172.78	269.6	119.7
1.0	3328.1 ± 209.45	3585.2 ± 280.43	257.1	114.1
5.0	3337.3 ± 209.27	3648.9 ± 208.48	311.6	138.3
10.0	3323.1 ± 136.79	3275.7 ± 183.31*	-47.4	-21.0
		Females		
0	3391.8 ± 239.91	3644.0 ± 311.75	252.2	
0.5	3410.7 ± 245.07	3728.6 ± 265.02	317.9	126.1
1.0	3409.0 ± 180.37	3768.7 ± 258.80	359.7	142.6
5.0	3422.8 ± 185.37	3769.4 ± 172.20	346.6	137.4
10.0	3451.0 ± 183.82	3575.7 ± 286.83	124.7	49.4

[a] Data extracted from Tables 2 and 4, pages 18 and 20

* Significantly different from control group ($p < 0.05$)

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TABLE 3. Mean Body Weight Gain (g ± S.E.) for Rabbits Dermally Exposed to M&B 46030[a]

Dose Level (mg/kg/day)	Mean Body Weight Gain (g ± S.E.) from: Day 1 to 8	Day 1 to 15	Day 1 to 21
		Males	
0.0	68.7 ± 58.28	100.7 ± 50.08	225.2 ± 110.66
0.5	90.9 ± 48.55	176.0 ± 104.69	269.6 ± 112.04
1.0	91.0 ± 59.42	163.4 ± 163.82	257.1 ± 122.06
5.0	151.8 ± 65.56*	221.2 ± 80.84	311.6 ± 107.90
10.0	28.9 ± 65.19	-35.9 ± 183.51	-47.4 ± 207.09**
		Females	
0.0	99.0 ± 66.56	156.3 ± 126.47	252.2 ± 117.85
0.5	102.0 ± 56.24	195.8 ± 72.88	317.9 ± 99.14
1.0	118.5 ± 58.61	243.0 ± 89.79	359.7 ± 142.09
5.0	117.1 ± 55.81	212.7 ± 126.58	346.6 ± 125.73
10.0	73.9 ± 53.66	97.2 ± 130.68	124.8 ± 163.93

[a] Data extracted from Tables 3 and 5, pages 19 and 21

* Significantly different from control group ($p < 0.05$)
** Significantly different from control group ($p < 0.01$)

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TABLE 4. Mean Food Consumption (g/animal/day ± S.E.) for Rabbits Dermally Exposed to M&B 46030[a,b]

Dose Level (mg/kg/day)	Mean Food Consumption (g/animal/day ± S.E.) from: Day 1 to 8	Day 8 to 15	Day 15 to 21
		Males	
0.0	193.1 ± 37.84	176.9 ± 40.59	206.7 ± 44.18
0.5	209.8 ± 26.71	202.2 ± 26.07	200.5 ± 23.22
1.0	213.7 ± 35.84	197.1 ± 59.29	214.8 ± 37.79
5.0	222.5 ± 31.60	200.8 ± 33.95	204.7 ± 59.70
10.0	186.1 ± 44.11	146.7 ± 63.32 (17%)	119.8 ± 29.99** (44 %)
		Females	
0.0	212.4 ± 47.82	190.9 ± 31.44	202.0 ± 45.09
0.5	218.4 ± 45.16	224.7 ± 37.71	227.7 ± 33.36
1.0	226.2 ± 29.08	233.8 ±34.97*	243.0 ± 19.07
5.0	233.9 ± 17.04	229.5 ± 29.44	237.0 ± 35.36
10.0	223.0 ± 31.80	175.4 ± 35.39 (8%)	158.6 ± 48.36 (21 %)

[a] Data extracted from Tables 6 and 7, page 22 and 23
[b] Values in parentheses represent percent decrease compared to control

* Significantly different from control group, $p < 0.05$
** Significantly different from control group, $p < 0.01$

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Section I, Toxicology Branch II (7509C)

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.

[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.

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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.

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	X Ovaries	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	0/5	0/5	1/6	0/6
Extensor rigidity of limbs	0/6	0/6	4/5	3/4	0/5	0/5	3/6	6/6
Nervous	1/6	0/6	2/5	3/4	0/5	0/5	2/6	1/6
Abnormal stance/gait	0/6	0/6	1/5	4/4	0/5	0/5	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.

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DATA EVALUATION REPORT

STUDY TYPE: Carcinogenicity/Mice (83-2)

EPA I.D. NUMBERS: P. C. CODE: 129121
MRID NUMBER: 429186-49

TEST MATERIAL: M&B 46030
Synonym: Fipronil

STUDY NUMBER: LSR 92/RHA313/0971

TESTING FACILITY: Life Science Research Limited
Suffolk, England

SPONSOR: Rhone-Poulenc Ag Company

TITLE OF REPORT: M&B 46030: Oncogenicity study by dietary administration to CD-1 mice for 78 weeks

AUTHOR(S): A. Broadmeadow

REPORT ISSUED: March 9, 1993

EXECUTIVE SUMMARY: In this carcinogenicity study (MRID # 429186-49), six groups of 20 male and 20 female CD-1 mice per group were treated with M&B 46030 in the diet at dosages of either 0, 0.1, 0.5, 10, 30 or 60 ppm for 52 weeks to measure the chronic toxicity of the chemical. An additional six groups of 52 male and female mice were treated at the same dosages for 78 weeks to test the carcinogenic potential of the chemical. The standard measures of *ante-* and *post-mortem* toxicity were evaluated.

Due to excessive mortality, males and females in the 60 ppm groups were sacrificed during Week 10 of the study. Survival in the other groups was comparable or exceeded the control group. Systemic 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 (percentage of the control value ranged from 74-86% in males and 81-86% in females); values for the 10 ppm group were also decreased but less consistently; 2) decreased food consumption in the 30 ppm group females (less than 90% of the control value); 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 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.

The Lowest Observed Effect Level (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

The No Observed Effect Level (NOEL) = 0.5 ppm (0.055 mg/kg/day for males and 0.063 mg/kg/day for females)

The study demonstrated that M&B 46030 is not carcinogenic when administered at doses of 30 ppm or greater to CD-1 mice. The chemical was tested at doses sufficient to measure its carcinogenic potential. At 30 ppm, signs of toxicity included decreased body weight gain, decreased food consumption (females), decreased food efficiency, altered WBC differential counts, macroscopic and microscopic post-mortem changes.

The study is **core minimum** and **satisfies** the guideline requirements (83-2) for a carcinogenicity study in mice.

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: 95.4%
Batch Number: PGS 963
Description: Off-white powder
Storage Conditions: In the dark at room temperature

Samples of the test material were returned to the sponsor at six-month intervals to test for the integrity of the test material. Result of the analyses (Appendix 1) show that there was satisfactory quality throughout the study.

B. Administration: dietary

C. Test Animals

Species: CD-1 mice
Source: Charles River (UK) Limited, Kent, England
Age: 21 tc 28 days on arrival
Weight: 16 to 21 g on arrival
Housing: Four of one sex per cage
Environmental Conditions: Temperature: target of 21^0 C
Relative humidity: target of 55%
Photoperiod: 12 hours light/dark
Air changes: 15 per hour
Food and Water: Powdered rodent diet (Laboratory Animal Diet No. 2) and tap water *ad libitum*
Acclimation Period: 13 days

II. METHODS

A. Diet Preparation and Analysis

M&B 46030 was initially mixed with a small quantity of the basal diet to create a pre-mix which was milled in an ultracentrifugal mill. The pre-mix was then diluted with further quantities of the diet to produce the 60 ppm concentration which was then serially diluted to prepare the other concentrations. Batches of the diets were prepared fresh weekly.

Samples of the lowest and highest dietary concentrations from six positions in the mixer were taken to test for **homogeneity** of the diet formulations prior to commencement of treatment. The unused portions of the homogeneity samples containing 0.1 ppm of the test chemical were then tested for **stability** after one and two weeks of

storage at room temperature. The study report states that the stability of 60 ppm in the diet was demonstrated in a previous study (LSR Report No. 90/RHA299/0325). The concentration of the test chemical in all the diets was determined at Weeks 1, 2, 3, 4 and at eight-week intervals thereafter during the treatment period and in Week 78.

B. Dosage and Administration

The animals were assigned randomly to the following treatment groups using computer-generated random numbers.

			Number of Animals			
			Toxicity		Carcinogenicity	
Group	Treatment	Concentration (ppm)	Males	Females	Males	Females
1	Control	0	20	20	52	52
2	M&B 46030	0.1	20	20	52	52
3	M&B 46030	0.5	20	20	52	52
4	M&B 46030	10	20	20	52	52
5	M&B 46030	30	20	20	52	52
6	M&B 46030	60	20	20	52	52

The diets were administered continuously for 52 weeks and 78 weeks to animals in the toxicity and carcinogenicity phases, respectively. An additional eight male and eight female mice served as veterinary controls to monitor disease outbreaks.

The dosages selected for this study were based on the results of a preliminary study (LSR Report No. 90/RHA299/0325) in which 110 ppm of the chemical in the diet of mice was associated with high mortality, overactivity/irritability, convulsions, low food intake, poor weight performance, inferior food conversion efficiency and high liver weights. At 40 ppm, 2/24 animals died and at 15 and 40 ppm there was decreased food intake, weight gain, food conversion efficiency and increased liver weights.

C. Experimental Design

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

detailed examinations[1] - weekly

[1] Examinations included palpation for swellings. The location, size, consistency, time of first observation and subsequent history were recorded.

clinical signs of toxicity - twice daily
body weights - during acclimation period, on first day of dosing, weekly for the first 14 weeks of treatment and then every two weeks and before necropsy
food consumption - weekly
food conversion ratios - calculated weekly for the first 14 weeks
hematology - differential WBC counts on control and 30 ppm group animals after 50 and 76 weeks of treatment
gross necropsy - all animals
organ weights (absolute and relative) - designated organs from all animals
histopathology - designated organs and tissues from certain animals (See description under Pathological Parameters)

D. Pathological Parameters

Hematology

Blood was drawn from the tail vein of surviving carcinogenicity phase animals without anesthesia after 50 and 76 weeks of treatment. Differential leukocyte counts were done on animals in the control and 30 ppm groups.

Post-mortem Pathology

Gross necropsies were done on animals which died or were sacrificed during the treatment period and on animals euthanized at the terminal sacrifices after either 53 or 78 weeks of treatment. The following CHECKED (X) tissues were preserved; the (XX) organ(s) in addition were weighed.

Digestive System
__Tongue
X_Salivary glands*
__Esophagus*
X_Stomach
X_Duodenum*
X_Jejunum*
X_Ileum*
X_Cecum*
X_Colon*
X_Rectum*
XXLiver*
X_Gall bladder*
X_Pancreas*

Respiratory System
X_Trachea*
XXLung*

Cardiovasc./Hemat. System
X_Aorta*
XXHeart*
X_Bone marrow*
X_Lymph nodes*
XXSpleen*
X_Thymus*

Urogenital System
XXKidneys*
X_Urinary bladder*
XXTestes*
X_Epididymides
X_Prostate/urethra
X_Seminal vesicle
XXOvaries
XXUterus*
X_Vagina

Neurologic System
XXBrain*
X_Periph. nerve*
X_Spinal cord
X_Pituitary*
X_Eyes (Optic n.)*

Glandular
XXAdrenals*
__Lacrimal gland
X_Mammary gland*
X_Parathyroids*
X_Thyroids*

Other
X_Bone*
X_Skeletal muscle*
X_Skin
__All gross lesions and masses

* EPA guideline requirement

The following samples were preserved but not examined:

eye and optic nerve - right (left was examined)
harderian glands
mammary glands - cranial (caudal were examined)
salivary gland - right submandibular (left was examined)
sciatic nerve - right (left was examined)
tongue

In addition, bone marrow smears were taken from all mice, fixed and stained.

Microscopic examinations were done on the following tissues: 1) the kidneys, liver, lungs and thyroid with parathyroids from mice in Groups 1 and 5 of the toxicity phase; 2) the tissues specified above from Group 1 and 5 mice of the carcinogenicity phase; 3) the kidneys, liver and lungs from mice not included in 1 and 2; 4) tissues specified above from all mice killed or dying during the treatment period; 5) tissues found to be abnormal on macroscopic examination.

E. Statistical Analyses

The description of the statistical methods used to analyze data from the study is attached to the DER.

F. 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.

III. RESULTS

A. Diet Analyses

Analyses of the 0.1 and 60 ppm group diet formulations for **homogeneity** showed that the mean concentration of M&B 46030 in the six samples was 94.9 and 85.3% of the intended concentration, respectively (Appendix 2B, page 233) The coefficient of variation for the 0.1 and 60 ppm samples was 8.9% and 2.6%, respectively. Analyses of the 0.1 ppm sample for **stability** after 7 and 14 days revealed that the chemical has an estimated 14-day shelf life in the diet (Appendix 2C, page 234). Although the study report (page 22) indicates that satisfactory stability for the 60 ppm concentration was demonstrated in a previous study, results in Appendix 2C are for a 800 ppm concentration. Analyses of all the diets showed that the percent of the intended M&B 46030 **concentration** in each diet averaged 100±11%, 92±11%, 88±7% and

90±6% for the 0.1, 0.5, 10 and 30 ppm concentrations, respectively (Appendix 2E, page 241).

B. Mortality

Fourteen males and seven females in the 60 ppm groups died during the first nine weeks of treatment. One male had a convulsion but no significant clinical signs were seen *ante mortem* in the other mice which died; necropsy examinations did not determine the cause of death. As a result of these treatment-related mortalities, all surviving animals in this group were sacrificed during Week 10. Statistical analyses showed that there was a negative trend in mortality (when humane sacrifices were censored) in the remaining groups of females. There was no treatment-related effect on mortality in males. The number of deaths/sacrifices during each phase of the study is presented below.

	Dosage Levels (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Toxicity Phase	6 (30)[1]	5 (25)	1 (5)	4 (20)	2 (10)	2 (10)	1 (5)	5 (25)	3 (15)	7 (35)
Carcinogenicity Phase	28 (54)	21 (40)	26 (50)	26 (50)	26 (50)	20 (38)	20 (38)	26 (50)	15 (29)	14 (27)

[1] Numbers in parentheses indicate percent mortality.

C. Clinical Signs

The study report states that treatment-related clinical signs observed during the study were limited to convulsions in three males in the 60 ppm group during Week 2. However, the report does not include either a table or individual observations of clinical signs.

There was no evidence of a treatment-related effect on the incidence, location, multiplicity or mean time of onset of palpable swellings.

D. Body Weight and Body Weight Gain

In the study report, mean body weights and body weight gains were tabulated for all 72 animals combined rather than reporting each phase individually. Weight gains were decreased in the 60 ppm group males and females (69% and 76%, respectively) during the first nine weeks of the study. The body weight gain results for the other groups were analyzed using the following intervals: 0-13, 13-26, 26-52, 52-78, 0-26, 0-52 and 0-78. Statistically significant decreases in weight gain were seen in the following groups: 1) 10 and 30 ppm group males and females during the 0-13 week period; 2)

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30 ppm group males and females during the 0-26 week period; 3) 30 ppm group males and females during the 0-52 week period; and 4) 30 ppm group females during the 0-78 week period. There was an increase in weight gain in the 30 ppm group males at the 26-52 week time period. Values were comparable to the controls for all the other treatment groups. Table 1 summarizes the data.

Table 1
Body Weight Changes (G) in Mice Treated with M&B 46030 in the Diet for up to 78 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
Interval	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Weeks 0-13	15.9	14.7	15.1	14.0*	11.7**	10.9	10.5	10.7	9.1*	9.1*
Percent of control	-	92	95	88	74	-	96	98	83	83
Weeks 0-26	21.1	19.8	20.5	20.0	16.1**	17.6	16.9	18.1	14.9	14.7*
Percent of control	-	94	97	95	76	-	96	103	85	84
Weeks 0-52	23.4	23.0	23.6	22.0	19.7**	22.2	21.0	23.7	21.1	19.1*
Percent of control	-	98	98	94	84	-	95	107	95	86
Weeks 0-78	23.1	21.9	23.7	21.3	19.9*	25.4	21.7	25.8	22.1	20.6*
Percent of control	-	95	103	92	86	-	85	102	87	81

a Extracted from Table 3B (pages 63-65) of the study report
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$

E. Food Consumption and Food Conversion Ratio

Food Consumption

Mean weekly food consumption per mouse was calculated for each cage from the weight of food supplied, that remaining and an estimate of spillage. For the 60 ppm group, food intake was decreased in males for the first two weeks and in females for the first nine weeks. Results of total food consumption for the other groups were analyzed using the following intervals: 1-13, 14-26, 27-52, 53-78, 1-26, 1-52 and 1-78. There were no statistically significant changes, although the values for the 30 ppm group females were consistently less than 90% of the control value throughout the study. Table 2 summarizes food consumption at selected times during the study.

Table 2
Group Mean Total Food Consumption (g/mouse) in Mice Treated with M&B 46030 in the Diet for up to 78 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Weeks 1-13	465	453	450	444	433	472	447	456	442	422
Percent of control	-	97	97	95	93	-	95	97	94	89
Weeks 1-26	950	928	918	905	878	958	903	932	886	841
Percent of control	-	98	97	96	92	-	94	97	92	88
Weeks 1-52	1951	1885	1879	1892	1804	1864	1726	1813	1712	1621
Percent of control	-	97	96	97	92	-	93	97	92	87
Weeks 1-78	2971	2848	2870	2915	2753	2774	2556	2722	2517	2391
Percent of control	-	96	97	98	93	-	92	98	91	86

a Extracted from Table 4B (pages 82-83) of the study report

Food Conversion Ratio

Food conversion efficiency in the 60 ppm group males and females was decreased in relation to the control group during the first nine weeks of the study (0.4 in the 60 ppm group males vs 8.3 in the control males and 2.9 ppm in the 60 ppm group females vs 5.6 in the control females). Overall, values in males receiving 10 or 30 ppm were lower than the controls; females in the 30 ppm group were affected for the first two weeks only. Table 3 summarizes the food conversion ratios at selected times during the study.

Table 3
Food Conversion Ratios in Mice Treated with M&B 46030 in the Diet for up to 78 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Week 1	8.3	7.8	6.5	6.4	3.8	5.6	5.0	6.4	5.2	4.3
Mean of Weeks 1-14	3.3	3.1	3.1	2.9	2.3	2.4	2.3	2.5	2.0	2.2

a Extracted from Table 5 (pages 84-85) of the study report.

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F. Achieved Dosages

Group mean dosages (mg/kg/day) were calculated for the overall study period (weeks 1-78); those values (Table 6, page 88) were as follows.

	Dosage Levels (ppm)							
	Males				Females			
	0.1	0.5	10	30	0.1	0.5	10	30
Mean Achieved Dosage (mg/kg/day)	0.011	0.055	1.181	3.430	0.012	0.063	1.230	3.616

G. Hematology

The differential leukocyte counts were comparable between the treated and control groups after 50 weeks of treatment and in the male animals after 76 weeks. The 30 ppm group females had a slightly lower percentage of neutrophils and slightly higher percentage of lymphocytes after 76 weeks of treatment; these findings are summarized in Table 4.

Table 4
Differential Leukocyte Counts in Female Mice
After 76 Weeks of Treatment with M&B 46030 in the Diet[a]

	WBC %				
Group	Neutrophil	Lymphocyte	Eosinophil	Basophil	Monocyte
Control	40	58	2	0	0
30 ppm	33*	64*	2	0	0

a Extracted from Table 7B (page 90) of the study report.
* Significantly different from controls, $p<0.05$

H. Necropsy Findings

Gross Necropsy

There were no treatment-related findings on gross examination of animals sacrificed at the toxicity phase necropsy or in animals which died or were sacrificed during the treatment period.

Considering all the animals in the carcinogenicity phase (terminal sacrifice animals plus those which died or were sacrificed during the treatment period), the incidences of liver enlargement and of surface changes on the liver surface were higher in the 30 ppm group males. Other statistically significant changes were of doubtful toxicological significance. Table 5 summarizes the hepatic

changes observed at gross necropsy.

Table 5
Incidence of Macroscopic Hepatic Changes in Mice
Treated with M&B 46030 in the Diet for up to 78 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Animals sacrificed or dying during treatment										
Number	28	21	26	26	26	20	20	26	15	14
Areas of Change	0	0	1	1	3	0	0	0	0	1
Appears Large	1	1	1	3	4	1	0	1	0	1
Animals sacrificed after 78 weeks of treatment										
Number	24	31	26	26	26	32	32	26	37	38
Areas of Change	0	1	2	2	5	0	0	1	0	0
Appears Large	1	1	0	1	3	0	0	0	0	0

a Extracted from Tables 9C (page 111) and 9D (page 121) of the study report

Organ Weights

The study report states that the relative weight of the livers of animals in the 60 ppm groups which died prematurely were increased in comparison to the control groups. (These data have not been tabulated.) The absolute weights of the liver were higher in the 30 ppm group males at both the toxicity and carcinogenicity phase necropsies and in the 10 and 30 ppm group females at the toxicity phase necropsy. The relative weights were increased in the 10 and 30 ppm group males and in the 30 ppm group females at both of the necropsies and in the 0.5 ppm group males at the toxicity phase necropsy. According to the study report, in animals which died or were sacrificed during treatment, liver weights were increased in the 30 ppm group. (These data were not tabulated.) Table 6 summarizes the liver weights at selected time periods.

Table 6
Absolute and Relative Liver Weights from Mice
Treated with M&B 46030 in the Diet for up to 78 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Toxicity Phase										
Body weight (g)	51.2	46.8	47.1	49.1	44.1**	38.9	40.9	41.9	40.0	38.0
Absolute weight (g)	2.61	2.64	2.70	3.02	3.37**	1.67	1.77	1.86	1.84*	2.00**
Relative weight (%)	5.079	5.663	5.719*	6.132*	7.675**	4.363	4.417	4.497	4.666	5.392**
Carcinogenicity Phase										
Body weight (g)	49.0	48.5	49.2	46.9	46.6	45.3	43.3	47.3	43.0	40.8
Absolute weight (g)	2.77	2.78	2.92	3.30	3.81**	1.99	1.93	2.06	2.02	2.13
Relative weight (%)	5.634	5.744	5.977	7.008*	8.261**	4.535	4.575	4.451	4.799	5.294**

a Extracted from Tables 8A-D (pages 91-98) of the study report.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$

Histopathology

Non-neoplastic Findings -

There was a statistically significant increase in the incidence of periacinar microvesicular vacuolation in the liver of males in the 10 and 30 ppm groups at the toxicity phase necropsy. The change was also seen in females in the 0.5 and 30 ppm groups but was not statistically significant.

During the carcinogenicity phase, the 30 ppm group males which died or were sacrificed during the study had a statistically significant increased incidence of hepatocellular hyperplasia and chronic degenerative change in the livers. The degenerative changes included necrosis and apoptosis, increased ploidy, hypertrophy and degeneration of periacinar hepatocytes, chronic inflammation and bile stasis. Non-neoplastic changes in other organs which were statistically significant were of doubtful toxicological significance.

At the carcinogenicity phase necropsy, there was a statistically significant increase in the incidence of microvesicular periacinar vacuolation of hepatocytes in the 10 and 30 ppm group males. The incidence was also increased in females receiving 0.5 ppm or greater (but not dose-related), however there was a lower incidence

of periacinar fatty vacuolation in the 0.5 and 10 ppm group females.

Neoplastic Findings -

The number of animals with tumors was comparable between the treated and control groups in animals which were sacrificed at the toxicity phase necropsy and in those which died or were sacrificed during the treatment period of the carcinogenicity phase. At the carcinogenicity phase necropsy, there was a higher incidence of malignant hepatocellular tumors in males in the 30 ppm group as compared to the controls. The study report states that the zero incidence of this tumor in the control group was below the range observed in recent studies, 2.9 to 25.0%. In addition, the difference in incidence from controls was not statistically significant and when the benign and malignant hepatocellular tumors are considered together, the incidences were similar.

The significant non-neoplastic and neoplastic findings in the study are summarized in Tables 7 and 8, respectively.

Table 7
Incidence of Non-neoplastic Findings in Mice Treated with M&B 46030 in the Diet for up to 78 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Toxicity Phase Necropsy										
Number Examined	14	15	19	16	18	18	19	15	17	13
Microvesicular periacinar vacuolation	0	2	2	7**	12***	1	1	4	1	4
Carcinogenicity Phase										
Deaths or Sacrifices During Carcinogenicity Period										
Number Examined	28	21	26	26	26	20	20	26	15	14
Hepatocellular hyperplasia	0	2	1	0	4*	0	0	0	0	0
Chronic degenerative change	3	5	3	5	11*	6	4	5	4	2
Terminal Sacrifice										
Number Examined	24	31	26	26	26	32	32	26	37	38
Microvesicular periacinar vacuolation	5	7	7	13*	13*	0	0	4*	3	7*
Periacinar hepatocyte fatty vacuolation	3	9	5	2	0	7	1	1	1*	6

a Extracted from Tables 10B (pages 145-148), 10D (pages 151-160) and 10F (pages 163-170) of the study report
* Significantly different from controls, $p < 0.05$
** Significantly different from controls, $p < 0.01$
*** Significantly different from controls, $p < 0.001$

Table 8
Incidence of Hepatocellular Neoplasm in Mice
Treated with M&B 46030 in the Diet for up to 78 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
Toxicity Phase Necropsy										
Number examined	14	15	19	16	18	18	19	15	17	13
Hepatocellular carcinoma	0	0	0	0	1	0	0	0	0	1
Carcinogenicity Phase										
Animals sacrificed or dying during treatment period										
Number examined	28	21	26	26	26	20	20	26	15	14
Hepatocellular carcinoma	1	0	0	0	2	0	0	0	0	1
Hepatocellular adenoma	3	1	2	0	1	0	0	0	0	1
Terminal Necropsy										
Number examined	24	31	26	26	26	32	32	26	37	38
Hepatocellular carcinoma	0	1	2	1	3	0	0	0	0	1
Hepatocellular adenoma	7	2	0	6	5	0	0	0	0	1
Carcinogenicity Phase - All Animals										
Number examined	52	52	52	52	52	52	52	52	52	52
Hepatocellular carcinoma	1	1	2	1	5	0	0	0	0	1
Hepatocellular adenoma	10	3	2	6	6	0	0	0	0	1

a Extracted from Tables 10B (pages 145-148), 10C (pages 149-150), 10E (page 161-162) and 10G (page 171) of the study report

I. Conclusion from Study Report

The study report concluded that M&B 46030 showed no carcinogenic potential when administered to CD-1 mice at levels of up to 30 ppm for 78 weeks. The target organ of M&B 46030 was the liver. The No Observed Effect Level (NOEL) was 0.5 ppm.

IV. STUDY DEFICIENCIES

1. Individual data on clinical observations during the study should have been included with the study report.

2. The study report states that the incidence of malignant hepatocellular tumors in mice in recent studies at this laboratory was 2.9 to 25.0%, however no data have been included to substantiate this statement.

3. The study report states that the stability of the chemical in the diet at 60 ppm was established in a previous study, however data are included in Appendix 2C for a 800 ppm diet formulation rather than a 60 ppm one.

IV. DISCUSSION/CONCLUSIONS

In this carcinogenicity study (MRID # 429186-49), six groups of 20 male and 20 female CD-1 mice per group were treated with M&B 46030 in the diet at dosages of either 0, 0.1, 0.5, 10, 30 or 60 ppm for 52 weeks to measure the chronic toxicity of the chemical. An additional six groups of 52 male and female mice were treated at the same dosages for 78 weeks to test the carcinogenic potential of the chemical. The mean achieved dosages for the 0.1, 0.5, 10 and 30 ppm groups were 0.011, 0.055, 1.181 and 3.430 for males and 0.012, 0.063, 1.230 and 3.616 for females. The standard measures of *ante* and *post-mortem* toxicity were evaluated.

Fourteen (14) males and 7 females in the 60 ppm group died during the first nine weeks of treatment. Convulsions were observed in one male but no significant clinical signs were seen *ante mortem* in the other mice which died. Necropsy examinations did not reveal the cause of death in the animals. As a result of these treatment-related mortalities, all surviving animals were euthanized during Week 10. Statistical analyses showed that there was a negative trend in mortality (when humane sacrifices were censored) in the remaining groups of females. There was no treatment-related effect in males.

The only treatment-related clinical sign of toxicity was convulsions in 3 males in the 60 ppm group during Week 2 of the study.

Body weight gains were decreased in the 60 ppm group males and females (69% and 76% of the control value, respectively) during the first nine weeks of the study. The body weight gain results for the other groups were analyzed using the following intervals: 0-13, 13-26, 26-52, 52-78, 0-26, 0-52 and 0-78. Statistically significant decreases in weight gain were seen in the following groups: 1) 10 and 30 ppm group males and females during the 0-13 week period (88% and 74 % of the control value for the 10 and 30 ppm group males,

respectively; 83% for both the 10 and 30 ppm group females; 2) 30 ppm group males and females during the 0-26 week period (76% and 84% of the control value for males and females, respectively); 3) 30 ppm group males and females during the 0-52 week period (84% and 86% of the control value for the males and females, respectively); and 4) 30 ppm group females during the 0-78 week period (81% of the control value). There was an increase in weight gain in the 30 ppm group males at the 26-52 week time period. Values were comparable to the controls for all the other treatment groups.

Mean weekly food consumption for the 60 ppm group was decreased in males for the first two weeks and in females for the first nine weeks. Results of total food consumption for the other groups were analyzed using the following intervals: 1-13, 14-26, 27-52, 53-78, 1-26, 1-52 and 1-78. There were no statistically significant changes, although the values for the 30 ppm group females were consistently less than 90% of the control value throughout the study.

Food conversion efficiency in the 60 ppm group males and females was decreased in relation to the control group during the first nine weeks of the study (0.4 in the 60 ppm group males vs 8.3 in the control group males and 2.9 ppm in the 60 ppm group females vs 5.6 in the control group females). Overall, values in males receiving 10 or 30 ppm were lower than the controls; females in the 30 ppm group were affected for the first two weeks only.

Differential white blood cell counts (WBC) showed that the 30 ppm group females had a slightly lower percentage of neutrophils and slightly higher percentage of lymphocytes after 76 weeks of treatment.

There were no treatment-related findings on gross examination of animals sacrificed at the toxicity phase necropsy or in animals which died or were sacrificed during the treatment period. Considering all the animals in the carcinogenicity phase (terminal sacrifice animals plus those which died or were sacrificed during the treatment period), the incidences of liver enlargement and of surface changes on the liver surface were higher in the 30 ppm group males. Other statistically significant changes were of doubtful toxicological significance.

The relative weight of the livers of animals in the 60 ppm groups which died prematurely were increased in comparison to the control groups. The absolute weights of the liver were increased in the 30 ppm group males at both the toxicity and carcinogenicity phase necropsies and in the 10 and 30 ppm group females at the toxicity phase necropsy. The relative weights were increased in the 10 and 30 ppm group males and in the 30 ppm group females at both of the necropsies and in the 0.5 ppm group males at the toxicity phase necropsy. In animals which died or were sacrificed during treatment, liver weights were increased in the 30 ppm group.

There was a statistically significant increase in the incidence of periacinar microvesicular vacuolation in the liver of males in the 10 and 30 ppm groups at the toxicity phase necropsy. The change was also seen in females in the 0.5 and 30 ppm groups but was not statistically significant. During the carcinogenicity phase, the 30 ppm group males which died or were sacrificed during the study had a statistically significant increased incidence of hepatocellular hyperplasia and chronic degenerative change in the livers. The degenerative changes included necrosis and apoptosis, increased ploidy, hypertrophy and degeneration of periacinar hepatocytes, chronic inflammation and bile stasis. Non-neoplastic changes in other organs which were statistically significant were of doubtful toxicological significance. At the carcinogenicity phase necropsy, there was a statistically significant increase in the incidence of microvesicular periacinar vacuolation of hepatocytes in the 10 and 30 ppm group males. The incidence was also increased in females receiving 0.5 ppm or greater (but not dose-related), however there was a lower incidence of periacinar fatty vacuolation in the 0.5 and 10 ppm group females.

The number of animals with tumors was comparable between the treated and control groups in animals which were sacrificed at the toxicity phase necropsy and in those which died or were sacrificed during the treatment period of the carcinogenicity phase. At the carcinogenicity phase necropsy, there was a higher incidence of malignant hepatocellular tumors in males in the 30 ppm group as compared to the controls. However, the zero incidence of this tumor in the control group was below the range observed in recent studies, 2.9 to 25.0% (according to the author). In addition, the difference in incidence from controls was not statistically significant and when the benign and malignant hepatocellular tumors are considered together, the incidences were similar.

The study demonstrated that M&B 46030 is not carcinogenic when administered at doses of 30 ppm or greater to CD-1 mice. The chemical was tested at doses sufficient to measure its carcinogenic potential. At 30 ppm, signs of toxicity included decreased body weight gain, decreased food consumption (females), decreased food efficiency, altered WBC differential counts, macroscopic and microscopic post-mortem changes.

The Lowest Observed Effect Level (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

The No Observed Effect Level (NOEL) = 0.5 ppm (0.055 mg/kg/day for males and 0.063 mg/kg/day for females)

Page 107 is not included in this copy.

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Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Combined Chronic Toxicity/Carcinogenicity/Rats (83-5)

EPA I.D. NUMBERS: P. C. C[illegible] 129121
MRID NUMBER: 429186-48

TEST MATERIAL: M&B 46030
Synonym: Fipronil

STUDY NUMBER: LSR 93/RHA432/0166

TESTING FACILITY: Pharmaco-LSR Ltd.
Suffolk, England

SPONSOR: Rhone-Poulenc Ag Company

TITLE OF REPORT: M&B 46030: Combined oncogenicity and toxicity study by dietary administration to CD rats for 104 weeks including a 13 week reversibility period on completion of 52 weeks of treatment

AUTHOR(S): P. Aughton

REPORT ISSUED: June 11, 1993

EXECUTIVE SUMMARY: In this combined chronic toxicity/carcinogenicity study in CD rats (MRID # 429186-48), 15 rats/sex/group were administered technical M&B 46030 in the diet for 52 weeks to assess the chronic toxicity of the chemical. 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 (50) 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 (males: 0, 0.019, 0.059, 1.27 and 12.68 mg/kg/day; females: 0, 0.025, 0.078, 1.61 and 16.75 mg/kg/day). Standard pre- and post-mortem evaluations of toxicity were included in the study along with measures of thyroid function.

The carcinogenicity phase of the study was terminated early (after 89 and 91 weeks of treatment 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 systemic 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

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group males and females and the 30 ppm group females (overall, 82%, 75% and 77% of the control value, respectively); 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 pm group males and females.

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). The chemical was administered at dosages sufficient to test its carcinogenic potential. At 300 ppm, there were alterations in most of the parameters measured including clinical signs of toxicity, body weight gain, food consumption, food conversion efficiency, clinical and post-mortem pathology.

The No Observed Effect Level (NOEL) = 0.5 ppm for males (0.019 mg/kg/day) and females (0.025 mg/kg/day)

The Lowest Observed Effect Level (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.

The study is **core minimum** and **satisfies** the guideline requirements (83-5) for a combined chronic toxicity/carcinogenicity study in rats.

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: 95.4%
Batch Number: PGS963
Description: Fine off-white or slightly yellow powder
Storage Conditions: In the dark in a cool store (not exceeding 15^0 C)

Samples of the test material were returned to the sponsor at six-month intervals to test for the integrity of the test material. Result of the analyses (Appendix 1) show that there was satisfactory quality throughout the study.

B. Administration: dietary

C. Test Animals

Species: CD rats
Source: Charles River (UK) Limited, Kent, England
Age: 35 to 42 days at commencement of treatment
Weight: 100 to 152 g eight days after arrival
Housing: Five of one sex per cage
Environmental Conditions: Temperature: target of 21^0 C
Relative humidity: target of 55%
Photoperiod: 12 hours light/dark
Air changes: 15 per hour
Food and Water: Powdered rodent diet (Laboratory Animal Diet No. 2) and tap water *ad libitum*
Acclimation Period: 14 days

II. METHODS

A. Diet Preparation and Analysis

M&B 46030 was initially mixed with a small quantity of the basal diet to create a pre-mix which was milled in an ultracentrifugal mill. The pre-mix was then diluted with further quantities of the diet to produce the desired concentrations and mixed in an electrically grounded (earthed) mixer. Batches of the diets were prepared fresh weekly.

Samples of the two lowest dietary concentrations taken from six positions in the mixer were taken to test for **homogeneity** of the diet formulations prior to commencement of treatment. The unused portions of the homogeneity samples were then tested for **stability**

after one and two weeks of storage at room temperature. The concentration of the test chemical in all the diets was determined at Weeks 1, 2, 3, 4, 12, 20, 28, 36, 44, 52, 60, 68, 76, 84 and 92 of treatment.

B. Dosage and Administration

The animals were assigned randomly to the following treatment groups using computer-generated random numbers.

			Number of Animals					
			Toxicity		Reversibility		Carcinogenicity	
Group	Treatment	Concentration (ppm)	Males	Females	Males	Females	Males	Females
1	Control	0	15	15	15	15	50	50
2	M&B 46030	0.5	15	15	15	15	50	50
3	M&B 46030	1.5	15	15	15	15	50	50
4	M&B 46030	30	15	15	15	15	50	50
5	M&B 46030	300	15	15	15	15	50	50

The diets were administered continuously for at least 52 weeks to the animals in the toxicity and reversibility phases. The animals in the latter phase were fed the basal diet for an additional 13 weeks after the treatment phase. The rats in the carcinogenicity phase were supposed to be fed for 104 weeks, however decreased survival (see later discussion) forced the study to be terminated prematurely.

An additional ten male and ten female animals served as veterinary controls to monitor disease outbreaks. Another ten male and ten female rats were used for pre-treatment clinical pathology testing.

The selected dosages were based on a subchronic toxicity study in rats (MRID # 429186-43) in which the NOEL and LOEL were 5 and 30 ppm, respectively. At 30 ppm, there were alterations in serum protein values and increased weight of the liver and thyroid.

C. Experimental Design

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

detailed examinations[1] - weekly
clinical signs of toxicity - twice daily
body weights - during acclimation period, on first day of dosing,

[1] Examinations included palpation for swellings. The location, size, consistency, time of first observation and subsequent history were recorded.

weekly for the first 14 weeks of treatment and then every two weeks
food consumption - weekly
food conversion ratios - calculated weekly for the first 14 weeks
ophthalmoscopic examinations - all animals before treatment; Groups 1 and 5 of the toxicity and reversibility phases after 50 weeks; Groups 1 and 5 of the carcinogenicity phase after 87 and 90 weeks of treatment for males and females, respectively
hematology, clinical chemistry and urinalysis - see the Pathological Parameters section of the DER for an explanation of the timing of these evaluations
thyroid hormone levels - see the Pathological Parameters section of the DER for an explanation of the timing of these evaluations
gross necropsy - all animals
organ weights - designated organs from all animals
histopathology - designated organs and tissues from all animals

D. Pathological Parameters

Clinical Pathology

HEMATOLOGY AND CLINICAL CHEMISTRY - Hematology and clinical chemistry evaluations were done after 24 and 50 weeks of treatment (toxicity phase animals) and after 76, 88 (males only) and 90 (females only) weeks of treatment (carcinogenicity phase) in ten male and ten female rats from each group. During the reversibility phase, samples were collected from the ten male and ten female rats from each group after 12 weeks of no treatment. Blood was drawn from the retro-orbital sinus under light ether anesthesia after an overnight fast.

The CHECKED (X) hematology parameters were examined; those marked with a cross (+) were examined at the reversibility phase evaluations.

X Hematocrit (HCT)*+	__Total plasma protein (TP)
X Hemoglobin (HGB)*+	X Leukocyte differential count[a]
X Leukocyte count (WBC)*	X Mean corpuscular HGB (MCH)+
X Erythrocyte count (RBC)*+	X Mean corpuscular HGB conc. (MCHC)+
X Platelet count*-	X Mean corpuscular volume (MCV)+
X Prothrombin Time+	X Reticulocyte count

* EPA guideline requirement

[a] Differential leukocyte counts were done on blood smears prepared from tail vein blood for Group 1 and 5 animals only after 50, 76, 88 (males only) and 91 (females only) weeks of treatment.

The CHECKED (X) clinical chemistry evaluations were done; those marked with a cross (+) were examined at the reversibility phase evaluations.

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)*

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

* EPA guideline requirement

URINALYSIS - Urine was collected in a metabolism cage from ten male and ten female animals per group deprived of water for approximately 12 hours. Sampling was done after 23 and 49 weeks of treatment (toxicity phase animals) and after 75, 87 (males only) and 90 (females only) weeks of treatment (carcinogenicity phase animals). During the reversibility phase, evaluations were done in ten male and ten female animals per group after 6 and 11 weeks. The CHECKED (X) urinalysis parameters were measured; those marked with a cross (+) were examined at the reversibility phase evaluations.

X Appearance*+
X Volume*+
X Specific gravity*
X pH+
X Sediment (microscopic)*
X Protein*+
X Urobilinogen

X Glucose*
X Ketones*
X Bilirubin*
X Blood*
X Nitrite
X Total reducing substances

* EPA guideline requirement

THYROID HORMONES - Thyroid function was evaluated in ten males and ten females per group after 1, 4, 12, 24 and 50 weeks of treatment and after 2, 4, 7 and 11 weeks of the reversibility period. Blood was drawn from the retro-orbital sinus under ether anesthesia after an overnight fast. The following levels were measured:

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Triiodothyronine concentration (T_3)
Thyroxine concentration (T_4)
Thyroid stimulating hormone (TSH)

Post-mortem Pathology

Sacrifices were done at the following times: after 52 weeks of treatment (toxicity phase); after 13 weeks of no treatment following 52 weeks of treatment (reversibility phase); after 89 weeks and five days (males in the carcinogenicity phase); and after 91 weeks of treatment (females in the carcinogenicity phase). The animals were sacrificed by carbon dioxide inhalation. Gross examinations were done on all animals. 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*
__Esophagus*	X Bone marrow*	X Spinal cord
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*
__Gall bladder*	XXProstate/urethra	Other
X Pancreas*	X Seminal vesicle	X Bone*
Respiratory System	XXOvaries	X Skeletal muscle*
X Trachea*	XXUterus*	X Skin
XXLung*	X Vagina	__All gross lesions and masses

* EPA guideline requirement

The following samples were preserved but not examined:

eye and optic nerve - right (left was examined)
harderian glands
mammary glands - cranial (caudal were examined)
salivary gland - right submandibular (left was examined)
sciatic nerve - right (left was examined)
tongue

In addition, bone marrow smears were taken, fixed and stained.

Microscopic examination of the preserved tissues listed above were examined in: 1) all rats in Groups 1 and 5 of the toxicity and carcinogenicity phases; 2) the kidneys, liver, lungs and thyroids from all rats in Groups 2, 3 and 4 of the toxicity and carcinogenicity phases and from all rats sacrificed at the end of the reversibility period; and 3) tissues found to be abnormal on macroscopic examination.

E. Statistical Analyses

The description of the statistical methods used to analyze data from the study is attached to the DER.

F. 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 meets or exceeds the criteria numbered 1 and 2 in 40 CFR 158.34.

III. RESULTS

A. Achieved Dosages

Group mean dosages were calculated for the overall study period (weeks 1-90) only; those values (Table 7, page 117) were as follows.

	Dosage Levels (ppm)							
	Males				Females			
	0.5	1.5	30	300	0.5	1.5	30	300
Mean Achieved Dosage (mg/kg/day)	0.019	0.059	1.27	12.68	0.025	0.078	1.61	16.75

B. Diet Analyses

Analyses of the 1.0 and 1.5 ppm group diet formulations for homogeneity showed that the mean concentration of M&B 46030 in the six samples was 98.8 and 107% of the intended concentration, respectively (Appendix 2B, page 401). The coefficient of variation for the 0.5 and 1.5 ppm samples was 6.40% and 4.51%, respectively. Analyses of these samples for stability after 7 and 14 days revealed that the chemical has an estimated 14-day shelf life (Appendix 2C, page 403). Analyses of all the diets showed that the percent of the intended M&B 46030 concentration in each diet averaged 104±12.0%, 102±13.5%, 95.7±4.2% and 98.4±3.4% for the 0.5, 1.5, 30 and 300 ppm concentrations, respectively (Appendix 2E, page 410).

C. Mortality

The study was terminated prior to 104 weeks due to excessive mortality and to ensure that a sufficient number of animals would

be available for the terminal sacrifices. Males were sacrificed after 89 weeks of treatment when the number of surviving animals in the 300 ppm group was 25% of the original number. Females were sacrificed after 91 weeks when survival in the 30 ppm group had fallen to 25%. Survival at the end of 78 weeks was above 50% in all the groups.

According to the study report, the number of animals which died or were killed for humane reasons was slightly higher in the 300 ppm groups as compared to the controls. The increased mortality was most likely due to deaths associated with convulsive episodes during the first few weeks in this group. In the second half of the study, the number of deaths among the 30 ppm group females was greater than the controls. There were no statistically significant differences in mortality among males. There was a significant difference between the 30 ppm group females and the controls when the humane sacrifice were discounted, but no differences were apparent when these sacrifices were included.

Table 1 summarizes the cumulative mortality data.

Table 1
Cumulative Morality in Rats Treated with
M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
Week Number	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Toxicity Phase - 52 Weeks of Treatment- 15 Rats/group										
1-14	0	0	0	0	1	0	0	0	0	0
29-32	1	0	1	0	1	0	0	0	0	0
53	3	1	1	0	3	1	1	1	1	2
Reversibility Phase - 52 Weeks of Treatment Followed by 13 Weeks of No Treatment - 15 Rats/Group										
2-43	0	0	0	0	2	0	0	0	0	1
48-54	0	0	0	1	2	1	2	0	0	1
65	2	0	2	2	5	2	2	4	0	5
Carcinogenicity Phase - Up to 91 Weeks of Treatment - 50 Rats/group										
9-22	0	0	0	0	2	0	0	0	0	1
38-41	0	2	1	3	3	0	1	0	0	4
43	2	2	2	3	4	0	1	0	1	4
53	3	6	2	6	6	2	1	0	5	4
78	19	20	13	22	20	16	12	12	23	15
90	29	36	28*	30	37*	26	27	28	34	15
91	30	36	28*	30	38*	27	29	29	37	18

a Extracted from Table 3A (pages 91-95) of the study report
* Includes animals killed or dying during the terminal sacrifice

D. Clinical Signs

Seizures, lasting up to 25 minutes, were observed in eight males and twelve females in the 300 ppm group, one male and three females in the 30 ppm group and three males in the 1.5 ppm group. The seizures were associated with death in four males and three females in the 300 ppm group, one female in the 30 ppm group and one male in the 1.5 ppm group.

Other signs of neurotoxicity, including irritability, overactivity, vocalization, salivation, aggressive behavior and grinding of the teeth were observed throughout the treatment period in females in the 1.5, 30 and 300 ppm groups. The study report states that these neurological signs were not evident during the reversibility

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period. The number of males receiving 300 ppm which were noted to be thin was increased as compared to the control group. Clinical signs data are summarized in Table 2.

Table 2
Incidence of Selected Clinical Signs in Rats
Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Toxicity Phase - 52 Weeks of Treatment - 15 Rats/group										
Aggression	1	0	0	0	1	0	0	1	0	1
Irritability	0	1	0	0	1	0	0	2	2	5
Vocalization	2	0	0	1	1	0	0	2	3	5
Convulsion	0	0	0	0	1	0	0	0	0	0
Reversibility Phase - 52 Weeks of Treatment Followed by 13 Weeks of No Treatment - 15 Rats/group										
Aggression	0	0	0	1	1	0	0	0	0	4
Irritability	0	0	1	1	0	0	0	0	0	5
Overactivity	0	0	0	0	1	0	0	0	2	3
Vocalization	0	0	0	1	0	0	0	1	1	5
Salivation	0	0	0	0	0	0	0	0	1	0
Carcinogenicity Phase - Up to 91 Weeks of Treatment - 50 Rats/group										
Thin	14	8	12	14	22	9	11	13	18	9
Aggression	0	3	1	2	4	0	0	4	2	9
Irritability	3	4	3	4	6	2	2	5	6	18
Overactivity	0	0	0	0	1	0	0	1	0	3
Vocalization	4	9	3	6	10	4	2	11	7	19
Salivation	0	0	1	0	1	0	0	0	2	8
Convulsion	0	0	3	1	5	0	0	0	2	11

a Extracted from Tables 1A-1D (pages 70-89) of the study report

The study report states that there was no adverse effect of treatment on the group distribution of animals with swellings or on the location, multiplicity or mean time of onset of the palpable swellings (Table 2, page 90).

E. Body Weight and Body Weight Gain

In the study report, mean body weights are tabulated for all 80 animals combined rather than reporting each phase individually. Body weight gains in the 300 ppm group males and females were only 42% and 46%, respectively, of the control values during the first week of treatment. After 52 weeks, the values were 85% and 82%, respectively. Body weight gains in the 30 ppm group males and females were significantly decreased during the first week but were comparable to the control groups throughout the study except for the 0-90 week measure which was reduced in the females. Weight gain was comparable to the controls for all the other treatment groups. Table 3 summarizes the data.

Table 3
Body Weight Changes (G) in Rats Treated
with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
Body weight change (g)	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Week 0-1	62	62	61	58**	26**	28	29	27	25*	13**
Percent of control	-	100	98	94	42	-	104	96	89	46
Week 0-13	415	405	397	402	368**	175	181	172	175	162
Percent of control	-	98	96	97	89	-	103	98	100	93
Week 0-52	712	728	707	670	603**	338	350	339	330	278**
Percent of control	-	102	99	94	85	-	104	100	98	82
Week 0-88	699	771	781	652	576**					
Percent of control	-	100	112	93	82					
Week 0-90						451	420	438	346*	339**
Percent of control	-					-	93	97	77	75

a Extracted from Table 4A (page 101) of the study report; some calculations performed by reviewer from Table 4A (pages 95-100)
* Significantly different from controls, $p < 0.05$
** Significantly different from controls, $p < 0.01$

During the reversibility period, weight gain in the 30 and 300 ppm groups was still decreased in comparison to the controls (Table 4B, page 102).

F. Food Consumption and Food Conversion Ratio

Food Consumption

Mean weekly food consumption per rat was calculated for each cage from the weight of food supplied, that remaining and an estimate of spillage. Values were decreased in relation to the controls for the

300 ppm group during the first week for females and for the first two weeks for males. However, total intake was comparable to the controls for all groups. Table 4 summarizes food consumption at selected times during the study.

Table 4
Mean Food Consumption (g/rat/week) in Rats
Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (mg/kg/day)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Week 1	196	198	195	193	146	152	152	154	149	119
Percent of control	-	101	99	98	74	-	100	101	98	78
Week 2	208	205	200	203	186	154	155	155	156	149
Percent of control	-	99	96	98	89	-	101	101	101	97
Week 13	194	193	188	189	188	135	141	137	137	138
Percent of control	-	99	97	97	97	-	104	101	101	102
Week 52	207	210	205	213	205	155	161	157	157	149
Percent of control	-	101	99	103	99	-	104	101	101	96
Weeks 1-89	18098	18000	17742	18677	17614					
Percent of control	-	99	98	103	97					
Weeks 1-90						13931	14027	14043	14020	13700
Percent of control						-	101	101	101	98

a Extracted from Table 5A (pages 103-111) of the study report; some of the percent of control calculations were done by the reviewer.

During the reversibility period, food consumption between the treated and control groups was comparable.

Food Conversion Ratio

To calculate the mean food conversion ratios, weekly cage values were first calculated from the body weight gain of the animals alive at the end of the week and the total weight of food consumed in the cage. The food efficiency in the 300 ppm group males and females was less than the control group during the first week of the study but similar during the remainder of the 14 weeks for which the values were calculated. Table 5 summarizes the food conversion ratios at selected times during the study.

Table 5
Food Conversion Ratios in Rats Treated
with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Week 1	31.4	31.3	31.1	30.0	17.5	18.5	19.0	17.6	16.7	10.4
Mean of Weeks 1-14	15.2	14.9	15.0	15.1	14.7	8.9	9.1	8.8	9.0	8.6

[a] Extracted from Table 6 (page 114) of the study report.

G. Ophthalmoscopic Examinations

There were no treatment-related lesions.

H. Clinical Pathology

Hematology

Males and females in the 300 ppm group had significantly lower PCV, Hb and RBC values than the controls at most of the evaluation periods. Animals in the 30 ppm and 1.5 ppm groups were affected similarly at some of the evaluation periods. Prothrombin times and platelet counts were also occasionally affected in either one or both sexes in the 30 and 300 ppm groups. However, many of the values which were significantly different from the controls were similar to those found in the pre-treatment evaluations. Table 6 summarizes the hematology data for the treated animals; Table 7 lists the pre-treatment values for the affected parameters.

Table 6
Selected Hematology Parameters in Rats Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
After 24 Weeks of Treatment										
PCV (%)	46	45	45	45	43**	44	45	45	44	41**
Hb (g%)	16.1	16.0	16.1	15.7	15.0***	15.5	15.9	15.8	15.3	14.7**
RBC (mil/cmm)	9.37	9.23	9.01*	8.95*	8.90*	8.11	8.41	8.37	8.14	8.11
MCV (cμ)	49	49	51	50	48	54	53	54	54	51***
MCH (pg)	17	17	18	18	17	19	19	19	19	18***
PT (secs)	14.1	14.6*	14.0	14.1	13.6*	13.4	13.8*	13.5	13.0*	12.7**
After 50 Weeks of Treatment										
PCV (%)	47	47	47	46	43***	46	45	44*	43***	41***
Hb (g%)	16.1	16.1	16.1	15.7	14.9***	15.7	15.7	15.2	15.1*	14.2***
RBC (mil/cmm)	9.26	9.10	8.97	8.82	8.76*	8.15	8.16	8.01	7.90	7.87
MCV (cμ)	51	52	53	52	50	57	56	55*	55*	52***
MCH (pg)	18	18	18	18	17	20	19	19	19	18***
PT (secs)	15.2	16.0	15.0	15.5	14.7	14.3	14.9	14.1	13.9	12.9**
After 76 Weeks of Treatment										
PCV (%)	47	46	42*	41*	42*	43	42	44	42	39*
Hb (g%)	16.2	15.1	14.6	14.0*	14.4*	15.0	14.8	15.5	14.4	13.6*
Platelets 1000/cmm	1042	1048*	1121	1296*	1246***	852	1058*	958	1021*	1193***
PT (secs)	13.5	14.1	13.7	13.3	13.0	13.4	12.3***	12.7*	12.2***	12.0***
After 88 Weeks of Treatment										
PCV (%)	46	46	46	42	41*					
Hb (g%)	15.6	15.3	15.1	13.9*	13.7*					
Platelets (1000/cmm)	918	917	1050	1185*	1338***					
After 90 Weeks of Treatment										
MCHC (%)						34	35	35	34	33*

a Extracted from Tables 9B-G (pages 123-132) of the study report
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
*** Significantly different from controls, $p<0.002$

Table 7
Selected Pre-Treatment Hematology Values in Rats[a]

	PCV (%)	Hb (g%)	RBC (mil/cmm)	MCV (cμ)	MCH (pg)	Platelets (1000/cmm)	PT (secs)
Males	40±1	13.0±0.3	6.18±0.26	65±2	21±1	1072±123	15.4±0.8
Females	41	13.4	6.36	64	21	996	14.8

a Extracted from Table 9A (page 122) of the study report.

After the reversibility period, the hematology values for the treated and control groups were comparable with the exception of the prothrombin time which was significantly lower than the control in the 30 and 300 ppm group females (Table 9D, page 127).

Clinical Chemistry

The clinical chemistry parameters which were thought to be affected by the treatment (i.e., were consistent and dose-related) are as follows:

Cholesterol - increased in the 30 and 300 ppm group males and females
Calcium - increased in the 300 ppm group males and females
Protein alterations[2] - 30 and 300 ppm group males and females; 1.5 ppm group males after 76 and 88 weeks; and after 76 weeks in the 0.5 ppm group males

Table 8 summarizes the changes in these parameters.

[2] High total protein, low albumin, high alpha and beta globulins and low albumin to globulin ratio

Table 8
Selected Clinical Chemistry Parameters in Rats
Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
After 26 Weeks of Treatment										
Cholesterol (mg %)	56	67	65	69	82*	76	66	63	79	135***
Total Protein (g %)	6.8	6.9	6.8	7.1*	7.4***	7.3	6.9	7.3	7.6	7.8*
Albumin (g %)	2.9	2.9	2.9	2.9	2.5***	3.9	3.6	3.7	3.8	3.6
α 1 globulin (g %)	1.4	1.6	1.5	1.8**	1.9***	1.2	1.2	1.3	1.4	1.7***
α 2 globulin (g %)	0.5	0.5	0.5	0.5	0.6***	0.4	0.4	0.5	0.5*	0.5***
ß globulin (g %)	1.8	1.7	1.7	1.7	2.2***	1.5	1.4	1.5	1.6	1.7*
A/G ratio (-:1)	0.8	0.7	0.7	0.7*	0.5***	1.1	1.1	1.0	1.0*	0.9***
Calcium (mmol/l)	2.5	2.5	2.5	2.6**	2.6**	2.5	2.6	2.5	2.6**	2.7***
After 58 Weeks of Treatment										
Cholesterol (mg %)	88	82	103	102	117	113	101	114	137	229***
Total protein (g %)	6.9	6.8	6.8	6.9	7.3***	7.4	7.4	7.7	8.0***	8.2***
Albumin (g %)	2.8	3.0	2.7	2.8	2.7	3.7	3.9	3.8	3.8	3.5
α 1 globulin (g %)	1.6	1.5	1.7	1.8	2.0**	1.4	1.3	1.5	1.9**	2.1***
α 2 globulin (g %)	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.8***
ß globulin (g %)	1.8	1.7	1.8	1.7	2.0*	1.5	1.5	1.6	1.6	1.6
A/G ratio (-:1)	0.7	0.8	0.6	0.7	0.6**	1.0	1.1	1.0	0.9	0.8***
Calcium (mmol/l)	2.7	2.6	2.7	2.7	2.8*	2.7	2.7	2.7	2.7	2.8***

Table 8 Continued

	0	0.5	1.5	30	300	0	0.5	1.5	30	300
After 76 Weeks of Treatment										
Cholesterol (mg%)	104	125	140	135	149*	95	169*	128	169*	228***
Total protein (g%)	6.7	6.7	6.7	6.9	6.9	7.2	7.5	7.3	7.5	8.1***
Albumin (g%)	2.9	2.5*	2.5*	2.5**	2.4**	3.5	3.2	3.3	3.1	3.0*
α 1 globulin (g%)	1.4	1.7*	1.7*	1.9**	1.9**	1.3	1.7	1.5	1.7	2.0**
α 2 globulin (g%)	0.4	0.4	0.4	0.5**	0.6***	0.5	0.6	0.6	0.6**	0.7***
ß globulin (g%)	1.8	1.9	1.9	1.9	1.8	1.6	1.8	1.7	1.8	2.0*
A/G ratio (-:1)	0.7	0.6*	0.6*	0.6**	0.5***	0.9	0.8	0.9	0.8	0.5***
Calcium (mmol/l)	2.6	2.7	2.7	2.8*	2.7	2.7	2.7	2.7	2.7	2.9**
Prior to terminal necropsy[b]										
Cholesterol (mg%)	134	127	135	174	170	143	170	178	231*	230*
Total protein (g%)	7.5	7.1	7.2	7.3	7.5	8.0	8.2	8.1	8.2	8.5*
Albumin (g%)	3.1	2.9	2.7**	2.4***	2.3***	3.8	3.5	3.4	3.0**	3.0**
α 1 globulin (g%)	1.7	1.6	1.8	2.1*	2.3**	1.4	1.8	1.9*	2.3**	2.1**
α 2 globulin (g%)	0.5	0.5	0.5	0.6	0.6*	0.7	0.7	0.7	0.8*	0.9***
ß globulin (g%)	2.0	2.0	2.1	2.1	2.1	1.9	1.9	1.8	1.9	2.2***
A/G ratio (-:1)	0.7	0.7	0.6*	0.5***	0.5***	0.9	0.8	0.7*	0.6**	0.6***
Calcium (mmol/l)	2.7	2.6	2.6	2.7	2.8	2.8	2.8	2.8	2.7	2.9*

a Extracted from Tables 11B-G (pages 138-156) of the study report
b After 88 weeks of treatment in the males and 90 weeks of treatment in the females
* Significantly different from controls, $p < 0.05$
** Significantly different from controls, $p < 0.01$
*** Significantly different from controls, $p < 0.001$

Other statistically significant clinical chemistry changes were either inconsistent, not dose-related or not of biological significance. Those which were consistent but considered not of biological significance included decreases in the liver enzymes (AP, AST and ALT), mostly in the 30 and 300 ppm group but with

occasional effects in the 1.5 ppm group.

The pre-treatment clinical chemistry values for the affected parameters are summarized in Table 9.

Table 9
Selected Pre-treatment Clinical Chemistry Parameters in Rats[a]

	Cholesterol	Total protein	Albumin	α 1 globulin	α 2 globulin	ß globulin	A/G ratio	Calcium
Males	78±11	5.5±0.1	3.1±0.1	0.8±0.1	0.4±0.0	1.1±0.0	1.3±0.1	2.6±0.1
Females	84±7	5.4±0.1	2.9±0.3	0.8±0.1	0.4±0.0	1.1±0.1	1.2±0.2	2.6±0.1

a Extracted from Table 11 A (pages 136-137) of the study report

At the end of the reversibility period, females in the 300 ppm group still had elevated cholesterol and calcium concentrations along with high total protein, alpha and beta globulins and low albumin to globulin ratios (Table 11D, pages 146-147).

Thyroid Hormones

Thyroid hormones (TSH, T_4 and T_3) were measured after 1, 4, 12, 24 and 50 weeks of treatment and after 2, 4, 7 and 11 weeks of the reversibility period. The TSH and T_4 were the most consistently affected parameters. The TSH levels were significantly elevated in the 30 ppm group males and the 300 ppm group males and females at most of the time points during the study. The T_4 levels were significantly decreased in the 1.5, 30 and 300 ppm group males and females at most of the time points. After one week of treatment, the value was zero for both the males and females in the 300 ppm groups. Occasionally, all of the treated groups were affected in a definite dose-responsive relationship. There were only occasional significant differences in the T_3 values of the treated animals. Table 10 summarizes the TSH and T_4 values during treatment.

Table 10
Thyroid Hormone Parameters in Rats Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
After 1 Week of Treatment										
TSH (ng/ml)	4.7	7.1	6.2	11.8***	20.3***	3.5	3.5	3.2	3.6	7.6***
T_4 (µg/dl)	2.93	3.02	2.23*	1.16***	0.00***	2.32	1.86	2.58	1.26**	0.00***
After 4 Weeks of Treatment										
TSH	5.2	8.0	6.5	11.2**	22.9***	3.8	3.9	3.3	3.9	7.5***
T_4	3.14	2.70*	2.56**	1.84***	0.39***	3.03	2.48*	2.36*	1.46***	0.79***
After 12 Weeks of Treatment										
TSH	5.7	7.2 (6.0)[b]	5.8	6.1	18.4***	3.4	3.4	2.9	3.5	8.7***
T_4	5.18	4.74 (4.38*)	3.96**	3.50***	1.22***	3.62	2.85**	2.87*	2.05***	1.10***
After 24 Weeks of Treatment										
TSH	7.2	10.0	6.9	8.6	21.0***	3.2	3.7	3.2	3.9	6.6***
T_4	4.58	3.81*	3.35***	2.43***	0.76***	2.85	3.09	3.49**	2.98	1.46***
After 50 Weeks of Treatment										
TSH	13.0	17.1	12.4	26.6*	57.3***	6.2	8.0	5.5	6.1	13.5***
T_4	5.95	5.51	4.83**	3.90***	2.07***	3.31	3.46	3.00	2.06***	1.38***

a Extracted from Tables 12A-E (pages 156-160) of the study report.
b Values in parenthesis calculated after exclusion of one outlier
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
*** Significantly different from controls, $p<0.001$

During the reversibility period, the TSH and T_4 levels in the females were comparable to the controls; the T_3 levels were significantly elevated in the 30 and 300 ppm groups at some of the time points. The TSH levels in the 300 ppm group males remained significantly elevated through the reversibility period, although the values decreased at each subsequent time point. The T_4 levels in the treated males were not comparable to the control group until after 11 weeks of reversibility period. The T_3 levels in the treated males were essentially comparable to the controls (Table 12F, pages 161-164).

Urinalysis

Urine pH values tended to be lower in the treated animals with some

statistically significant differences in the 30 and 300 ppm groups at some time points. Protein values tended to be elevated in the higher dose groups with significant differences in the 300 ppm group females after 49 and 90 weeks of treatment. After 87 weeks of treatment, males in the 30 and 300 ppm groups also had significantly elevated urine volumes with decreased specific gravities.

During the reversibility period, the pH and protein changes were still observed.

G. Necropsy Findings

Gross Necropsy

The study report states that in the animals which died or were sacrificed during the study (toxicity or carcinogenicity phase), the incidences of large and/or pale kidneys and large livers, adrenals and thyroids were increased in the 300 ppm group males and females as compared to the respective controls. It is also noted that the incidence of large parathyroids was increased in the 300 ppm group males.

At the interim necropsy, the incidences of large livers and thyroids were increased in the 30 and 300 ppm groups.

At the necropsy following the reversibility phase, large kidneys and livers were observed in males in the 30 and 300 ppm groups and 300 ppm group, respectively.

At the terminal sacrifice, the incidences of large and pale kidneys were increased in male and females in the 30 and 300 ppm groups. There were also increases in the incidences of granular kidneys, large livers and large thyroids in males in the 30 ppm group and males and females in the 300 ppm group. The findings are summarized in Tables 11 and 12.

Table 11
Incidence of Macroscopic Findings in Rats Killed/ Died During Treatment with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Toxicity Phase[b]										
Number Examined	3	1	1	0	3	1	1	1	1	2
Large Adrenals	0	0	0	0	1	0	0	0	0	0
Pale Kidneys	0	0	0	0	3	0	0	1	0	0
Large Kidneys	0	0	0	0	2	0	0	1	0	0
Large Parathyroids	0	0	0	0	2	0	0	1	0	0
Reversibility Phase[b,c]										
Number Examined	2	0	2	2	5	2	2	4	0	5
Large Adrenals	0	0	0	0	0	0	1	2	0	0
Pale Kidneys	0	0	0	0	1	0	0	0	0	0
Large Parathyroids	0	0	0	0	0	0	0	1	0	0
Carcinogenicity Phase										
Number Examined	30	36	28	30	38	27	29	29	37	28
Large Adrenals	5	8	8	8	20**	14	16	13	15	18
Pale Kidneys	8	7	4	7	20*	3	2	5	11	4
Large Kidneys	14	16	9	16	28*	2	4	4	12*	4
Large Liver	4	9	5	11	16*	2	3	3	1	7
Large Parathyroids	6	7	6	11	15	2	1	3	8	3
Large Thyroids	2	3	1	0	11*	1	0	0	1	1

a Extracted from Tables 15A-D(pages 198-223) of the study report
b Result of the examination of the thyroids do not appear for this phase.
c The results of findings during and after the treatment portion of the reversibility phase (Tables 15C and 15D) have been combined
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$

Table 12
Incidence of Macroscopic Findings at Scheduled Necropsies in Rats Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Interim Sacrifice - After 52 Weeks of Treatment										
Number Examined	12	14	14	15	12	14	14	14	14	13
Large Liver	0	0	0	0	4	0	0	0	0	1
Large Thyroids	0	0	0	1	1	0	0	0	0	0
After 13 Weeks of Reversibility										
Number Examined	13	15	13	13	10	13	13	11	15	10
Large Kidneys	0	0	1	3	2	0	0	0	0	0
Large Liver	0	1	0	0	2	0	0	0	0	0
Terminal Sacrifice[b]										
Number Examined	20	14	22	20	12	23	21	21	13	22
Pale Kidneys	1	1	3	8*	7**	0	2	0	4*	4*
Granular Kidneys	2	1	4	8	8**	0	1	0	4	4
Large Kidneys	3	2	6	11*	10***	0	2	0	5**	2
Large Liver	1	0	0	3	6**	1	1	0	0	3
Large Thyroids	0	0	1	3	5**	1	0	0	1	3

a Extracted from Tables 15E-H (pages 224-249) of the study report
b Males and females were sacrificed after 89 and 91 weeks of treatment, respectively
* Significantly different from controls, $p < 0.05$
** Significantly different from controls, $p < 0.01$

Organ Weights

Interim Necropsy

The absolute weights of the liver and thyroids were increased in the 300 ppm group males and females.

The following increases in relative organ weights were recorded: liver and thyroids in the 30 and 300 ppm group males and females; heart in the 300 ppm group females and uterus and cervix in the 300 ppm group males.

Necropsy Following Reversibility Period

The terminal body weight of the 300 ppm group males was decreased.

The absolute weights of the brain and lungs were decreased in the 300 ppm group females and males, respectively.[3]

The following increases in relative organ weights were recorded: adrenals in the 300 ppm group females; heart in the 300 ppm group males and females; kidneys in the 300 ppm group females; liver in the 30 ppm group females and the 300 ppm group males and females; lungs in the 300 ppm group females; testes in the 300 ppm group males; and thyroids in the 300 ppm group males and females.

Terminal Necropsy (After 89 weeks in males and 91 weeks in females)

The terminal body weight of the 30 ppm group females and the 300 ppm group males and females was decreased as compared to the controls; the 0.5 ppm group males was increased.

The absolute weight of the following organs was increased: adrenals in the 300 ppm group males; kidneys in the 30 and 300 ppm group males; liver in the 300 ppm group males and females; spleen in the 0.5, 1.5 and 300 ppm group males; thyroids in the 300 ppm group females and all the male groups; uterus and cervix in the 300 ppm group females. The absolute weight of the thymus was decreased in the 300 ppm group females.

The relative weight of the following organs was increased: brain in the 0.5 and 300 ppm group males and the 30 and 300 ppm group females; adrenals in the 30 and 300 ppm group males and females; heart in the 30 and 300 ppm group males and females; kidneys in the 0.5 group females and in the 30 and 300 ppm group males and females; liver in the 30 and 300 ppm group males and females; lungs in the 0.5 ppm group males, 30 ppm group females and the 300 ppm group males and females; spleen in the 0.5 and 30 ppm group females and 300 ppm group males and females; thyroid in the 30 and 300 ppm group males and females; and uterus and cervix in the 300 ppm group females.

The study report states that organ weights of animals killed or dying during the treatment period indicated that the weights of the livers and thyroids of males and females in the 300 ppm group and the kidneys of males in the 300 ppm group tended to be higher than those of the controls which died or were sacrificed prematurely. However, these data are not tabulated.

Table 13 summarizes the data for the affected organs.

[3] The mean absolute weight of the thyroids was calculated excluding animals with thyroid tumors.

Table 13
Absolute and Relative Weights of Selected Organs
from Rats Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Interim Necropsy (After 52 Weeks of Treatment)										
Body weight (g)	843.9	868.7	855.2	797.9	769.8	461.8	478.5	460.0	442.3	427.7
Liver										
A (g)	29.5	30.0	29.1	34.3	40.5 **	16.4	17.3	17.1	19.1	23.4 **
R (%)	3.53	3.44	3.40	4.32 **	5.26 **	3.56	3.62	3.70	4.31 **	5.49 **
Thyroids										
A (g)	0.039	0.035	0.042	0.047	0.056 **	0.027	0.031	0.030	0.032 *	0.045 **
R (%)	0.0047	0.0040	0.0050	0.0058 *	0.0073 **	0.0059	0.0064	0.0065	0.0073 *	0.0107 **
Heart										
A (g)	1.99	2.06	2.06	1.97	1.96	1.36	1.41	1.37	1.37	1.42
R (%)	0.239	0.241	0.244	0.252	0.255	0.298	0.300	0.299	0.313	0.336 *
Uterus and cervix										
A (g)						0.80	0.81	0.89	0.91	0.96
R (%)						0.176	0.177	0.194	0.207	0.225 *
Reversibility Necropsy (After 52 Weeks of Treatment and 13 Weeks of No Treatment)										
Body Weight (g)	887.2	840.6	954.5	840.4	687.3 *	541.7	535.3	498.9	546.4	425.0
Brain										
A (g)	2.34	2.35	2.31	2.35	2.24	2.10	2.11	2.01	2.08	2.01 *
R (%)	0.283	0.293	0.247	0.284	0.335	0.408	0.411	0.414	0.395	0.481
Lungs										
A (g)	2.56	2.54	2.56	2.85	2.36 *	1.90	1.94	1.77	1.92	1.88
R (%)	0.308	0.314	0.273	0.347	0.355	0.363	0.374	0.363	0.360	0.446 **
Adrenals										
A (g)	0.065	0.063	0.063	0.068	0.065	0.092	0.191	0.097	0.092	0.097
R (%)	0.0077	0.0078	0.0066	0.0082	0.0098	0.0177	0.0316	0.0199	0.0173	0.0233 *

Table 13 continued

	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Heart										
A (g)	2.11	2.04	2.17	2.32	1.96	1.49	1.45	1.45	1.53	1.43
R (%)	0.247	0.250	0.230	0.279	0.292 *	0.283	0.279	0.293	0.286	0.339 **
Kidneys										
A (g)	6.03	6.01	6.80	8.22	6.20	3.92	3.72	3.71	4.16	4.32
R (%)	0.719	0.745	0.716	0.994	0.940	0.742	0.701	0.744	0.774	1.038 *
Liver										
A (g)	30.3	30.3	32.9	30.9	30.6	18.5	19.4	17.8	21.0	19.7
R (%)	3.50	3.70	3.44	3.69	4.55 *	3.42	3.60	3.54	3.81 *	4.68 **
Testes										
A (g)	3.57	3.80	3.80	3.81	3.63					
R (%)	0.423	0.471	0.407	0.461	0.548 *					
Thyroids[b]										
A (g)	0.038	0.039	0.043	0.045	0.045	0.031	0.031	0.030	0.034	0.035
R (%)	0.0045	0.0047	0.0045	0.0054	0.0067 *	0.0059	0.0058	0.0061	0.0063	0.0080 **
Terminal Necropsy (After 89 Weeks of Treatment in Males and 91 Weeks in Females)										
Body Weight (g)	863.0	1006.6 *	949.4	809.7	732.2 **	596.1	556.2	566.4	496.4 *	463.6 **
Brain										
A (g)	2.37	2.35	2.39	2.33	2.35	2.10	2.11	2.11	2.10	2.13
R (%)	0.284	0.238 *	0.260	0.310	0.325 *	0.363	0.391	0.386	0.445 *	0.476 **
Adrenals										
A (g)	0.074	0.088	0.086	0.092	0.109 **	0.108	0.138	0.123	0.125	0.128
R (%)	0.0086	0.0088	0.0094	0.0124 *	0.0155 *	0.0188	0.0256	0.0222	0.0262 *	0.0299 *
Heart										
A (g)	2.19	2.53 *	2.36	2.34	2.30	1.64	1.65	1.65	1.64	1.63
R (%)	0.256	0.255	0.253	0.304 *	0.316 **	0.276	0.302	0.330	0.342 **	0.369 **
Kidneys										
A (g)	6.32	7.29	7.24	8.56 **	9.86 *	4.23	4.51	4.15	5.75	4.89
R (%)	0.737	0.741	0.791	1.144 **	1.354 **	0.716	0.829 *	0.751	1.207 **	1.119 **

Table 13 continued

	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Liver										
A (g)	28.3	32.4	32.1	33.9	39.4 **	23.0	22.0	21.5	25.0	27.9 *
R (%)	3.30	3.24	3.48	4.40 **	5.41 **	3.88	3.98	3.82	5.12 **	6.14 **
Lungs										
A (g)	2.56	2.63	2.63	2.59	2.62	1.89	2.01	1.91	1.91	1.93
R (%)	0.303	0.265 *	0.284	0.342	0.360 *	0.326	0.371	0.351	0.401 *	0.433 **
Spleen										
A (g)	1.224	1.939 *	1.540 *	1.790	1.558 *	0.858	0.987	0.822	0.918	0.876
R (%)	0.1416	0.1963	0.1633	0.2378	0.2122 **	0.1453	0.1802 *	0.1468	0.1900 **	0.1939 **
Thyroids										
A (g)	0.042	0.051 *	0.053 *	0.063 **	0.094 **	0.036	0.038	0.036	0.044	0.072 *
R (%)	0.0049	0.0052	0.0056	0.0082 **	0.0129* *	0.0060	0.0070	0.0065	0.0090 **	0.0156 *
Uterus and cervix										
A (g)						0.73	2.02	0.91	1.43	0.91 *
R (%)						0.130	0.394	0.167	0.306	0.207 **

a Extracted from Tables 14A-H (pages 174-197) of the study report.
A = absolute weight; R = relative weight
b Group mean calculated excluding animals with thyroid tumors.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
*** Significantly different from controls, $p<0.001$

Histopathology

Non-neoplastic Findings

The incidence and severity of progressive senile nephropathy was increased in the 300 ppm group males and females during the toxicity phase and in the 30 and 300 ppm group males and females during the carcinogenicity phase. This change was still observed at the reversibility necropsy. The incidence of nephropathy was higher in the 30 and 300 ppm group females and the severity was greater in all the treated animals which received 1.5 ppm or more of the chemical. Table 14 summarizes the data.

Table 14
Incidence and Severity of Progressive Senile Nephropathy in Rats Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Toxicity Phase										
Number Examined	15	15	15	15	15	15	15	15	15	15
Number Affected	6	3	5	7	11	4	6	4	6	8
Number Graded Minimal or Slight	4	2	1	3	7	3	5	2	2	5
Number Graded Moderate to Severe	2	1	4	4	4	1	1	2	4	3
Reversibility Phase										
Number Examined	15	15	15	15	15	15	15	15	15	15
Number Affected	8	7	9	8	9	5	4	7	11	13**
Number Scored Minimal or Slight	8	6	5	2	5	4	4	5	7	8
Number Scored Moderate to Severe	0	1	4	6	4	1	0	2	4	5
Oncogenicity Phase (Includes animals which died during treatment)										
Number Examined	50	50	50	50	50	50	50	50	50	50
Number Affected	26	28	32	42**	44***	14	21	17	31**	24
Number Graded Minimal or Slight	12	10	13	12	10	7	11	6	7	12
Number Graded Moderate to Severe	14	18	19	30	34	7	10	11	24	12

a Extracted from Tables K-M (pages 322-324) of the study report; some calculations performed by the reviewer.
* Significantly different from controls, $p<0.05$
** Significantly different from controls, $p<0.01$
*** Significantly different from controls, $p<0.001$

The study report states that other findings were common to this strain and age of rat and this laboratory. It is noted in Table 16J (page 293) that in the carcinogenicity phase (includes all animals) there is a dramatic increase in the incidence of cortical hemorrhagic degeneration of the adrenals in female rats as compared to the male rats. Of 50 animals necropsied, the finding was present in 6, 0, 3, 0, 3 males in the 0, 0.5, 1.5, 30 and 300 ppm groups, respectively, whereas the number of females affected was 33, 31, 34, 29 and 28, respectively. The difference was not observed in the other phases.

There were a wide variety of histological changes in the liver in all the phases. The incidence of the findings were essentially comparable to the controls and do not offer an explanation for the consistent increase in organ weights in the 30 and 300 ppm groups. Also noted is the increase in hyperplasia of the parathyroids in the 300 ppm group (especially males) during the carcinogenicity phase (Table 16J, page 298); the majority of the animals with the finding died or were sacrificed during the treatment period (Table 16A, page 254).

Neoplastic Findings

Neoplastic changes were observed in the thyroid gland. Considering all the animals in the carcinogenicity phase, the incidence of benign follicular cell adenomas was higher in the 300 ppm group males and females and the incidence of follicular cell carcinomas was higher in the 300 ppm group males. The incidence of follicular cell tumors was higher in the 1.5 and 30 ppm group males, however the study report states that the incidences were within the historical range for this laboratory.

The study report states that there were no treatment-related tumors in the toxicity phase. (One parafollicular cell adenoma was observed in a control female and a 300 ppm group male; a follicular cell adenoma was seen in a 30 ppm group male.) In the reversibility phase, six treated animals had thyroid tumors. A follicular cell carcinoma was observed in one male each in the 30 and 300 ppm groups; follicular cell adenomas were found in one male in the 300 ppm group, one female in the 1.5 ppm group and two females in the 300 ppm group. Table 15 summarizes the data on thyroid tumors; Table 16 totals the number of tumors for each group.

Table 15
Incidence of Benign and Malignant Thyroid Tumors
in Rats Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Dosage Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Toxicity Phase										
Number Examined	15	15	15	15	15	15	15	15	15	15
Follicular Cell Adenoma	0	0	0	1	0	0	0	0	0	0
Reversibility Phase										
Deaths or Sacrifices During Reversibility Period										
Number Examined	2	0	2	1	2	1	0	4	0	4
Follicular Cell Adenoma	0	0	0	0	0	0	0	0	0	1
Terminal Sacrifice										
Number Examined	13	15	13	13	10	13	13	11	15	10
Follicular Cell Carcinoma	0	0	0	1	1	0	0	0	0	0
Follicular Cell Adenoma	0	0	0	0	1	0	0	1	0	1
Carcinogenicity Phase										
Deaths or Sacrifices During Treatment										
Number Examined	29	34	28	30	38	27	29	29	37	28
Follicular Cell Carcinoma	0	0	0	0	3	0	0	0	1	-
Follicular Cell Adenoma	0	0	4	0	8**	0	0	0	0	6*
Terminal Sacrifice										
Number Examined	20	14	22	20	12	23	21	21	13	22
Follicular Cell Carcinoma	0	0	0	0	2	0	1	0	0	1
Follicular Cell Adenoma	0	1	1	3	4*	0	0	0	0	2

a Extracted from Tables 17A-G (pages 306-318) of the study report.
* Significantly different from controls, $p < 0.05$
** Significantly different from controls, $p < 0.01$
*** Significantly different from controls, $p < 0.001$

Table 16
Total Number of Thyroid Tumors in Rats
Treated with M&B 46030 in the Diet for up to 91 Weeks[a]

	Follicular Cell Adenoma	Follicular Cell Carcinoma	Total
Males[b]			
0	0	0	0
0.5	1	0	1 (1.3%)
1.5	5	0	5 (6.3%)
30	4	1	5 (6.3%)
300	13	6	19 (23.8%)
Females[b]			
0	0	0	0
0.5	0	1	1 (1.3%)
1.5	1	0	1 (1.3%)
30	0	1	1 (1.3%)
300	10	2	12 (15.0%)

a Differs from Text-table 1 (page 53) of the study report due to inclusion of tumors reported in the toxicity and reversibility phases

b Eighty animals per sex per group were examined in all the phases.

The historical incidences of the these tumors in this laboratory are as follows:

	Male	Female
Number Examined	359	365
Follicular Cell Carcinoma	4 (1.1%)	6 (1.6%)
Follicular Cell Adenoma	22 (6.1%)	5 (1.4%)
Total Follicular Cell Tumors	26 (7.2%)	10 (2.7%)

J. Conclusion from Study Report

The study report concluded that functional and morphological changes were seen in the liver, thyroid and kidneys and functional effects, only, were noted for the nervous system. The highest level of treatment produced thyroid follicular cell tumors, although they

were clearly the result of hormonal effects in a species known to be more sensitive than man to thyroid changes. The study report also concluded that the No Adverse Effect Level (NOEL) level was 0.5 ppm.

IV. STUDY DEFICIENCY

There was excessive mortality during the latter part of the study and treatment was terminated after 89 and 91 weeks in males and females, respectively, in order that there be a sufficient number of animals for the terminal necropsy. The change does not affect the validity of the study for several reasons. First, the premature termination occurred near the end of the study. Second, the registrant has cited literature references indicating that in general, the longevity of the CD rat has been decreasing and therefore, a shortened life span was not unique to this study. Third, the study was long enough to have tumors develop in the treated groups.

IV. DISCUSSION/CONCLUSIONS

In this combined chronic toxicity/carcinogenicity study in CD rats, 15 rats/sex/group were administered technical M&B 46030 in the diet for 52 weeks to assess the chronic toxicity of the chemical. 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 (50) 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 (males: 0, 0.019, 0.059, 1.27 and 12.68 mg/kg/day; females: 0, 0.025, 0.078, 1.61 and 16.75 mg/kg/day). Standard pre- and post-mortem evaluations of toxicity were included in the study along with measures of thyroid function.

The carcinogenicity phase of the study was terminated early (after 89 and 91 weeks of treatment in males and females, respectively) due to excessive mortality and to ensure that a sufficient number of animals were available for the terminal sacrifices. The number of animals which died or were killed for humane reasons was slightly higher in the 300 ppm group males and females during the first few weeks of the study, most likely due to convulsive episodes in these animals. No statistically significant differences in mortality between the groups were observed in the male animals. No significant differences were observed in females when humane sacrifices were taken as uncensored. However, when treated as censored observations, the 30 ppm and control groups were significantly different.

Seizures (sometimes causing death) were observed in the 30 and 300 ppm group males and females and in the 1.5 ppm group males. Other signs of neurotoxicity, including irritability, overactivity, vocalization, salivation, aggressive behavior and grinding of the teeth were observed throughout the treatment periods of all phases in females in the 1.5, 30 and 300 ppm groups. The incidence of

thinness in males in the 300 ppm group was increased as compared to the controls. The neurological signs were not observed during the reversibility period.

Body weight gain was affected in the 30 ppm group males and the 300 ppm group males and females. (This parameter was tabulated for all of the animals in the study rather than reporting each phase individually.) During the first week of treatment, mean body weight gain was 42% and 46% of the control value in the 300 ppm group males and females, respectively. Weight gain for weeks 0-13 was 89% and 93% of the control value for the 300 ppm males and females, respectively. After 52 weeks of treatment, the values were 85% and 82%, respectively; overall weight gain was 82% and 75% of the control value. For the 30 ppm group males and females, the values were 94% and 89% of the control value, respectively, after one week of treatment, 94% and 98% after 52 weeks and 93% and 77% overall. During the reversibility period, weight differences remained between the 30 and 300 ppm group males and females and the controls.

Mean food consumption was decreased during the first week and the first two weeks in the 300 ppm group females and males, respectively, but was comparable to the controls for the remainder of the study. Food conversion efficiency was decreased in the 300 ppm group males and females but then was comparable to the controls for the remainder of the study.

Decreased hematology parameters (hemoglobin, hematocrit and RBC counts) were seen in the 300 ppm males and females at most of the evaluation periods and in the 1.5 and 30 ppm groups at some of the periods. Although these changes were statistically significant, there is a question of their biological significance since the values were comparable to or sometimes exceeded the pre-treatment values for the parameters. The prothrombin times were occasionally decreased and the platelet counts increased in either one or both sexes in the 30 and 300 ppm groups. Again, the biological significance of these changes is questionable. At the end of the reversibility period, the parameters were comparable between the treated and control groups.

Consistent treatment-related changes were seen in some clinical chemistry parameters. Increases in cholesterol were seen in the 30 and 300 ppm group males and females at all of the evaluation periods, however not all of the differences were statistically significant. Calcium levels were significantly increased in the 300 ppm group males and females. Serum protein alterations, including high total protein, low albumin, high alpha and beta globulins and low albumin to globulin ratio, were seen in the 30 and 300 ppm group males and females throughout the study, in the 1.5 ppm group males after 76 and 81 weeks of treatment and in the 0.5 ppm group males after 76 weeks of treatment. At the end of the reversibility period, females in the 300 ppm group still had elevated cholesterol and calcium concentrations along with high total protein, alpha and beta globulins and low albumin to globulin ratios.

Dramatic alterations in thyroid function were seen as the result of treatment. TSH levels were increased in the 30 ppm group males and in the 300 ppm group males and females. After one week of treatment, the TSH levels in the 300 ppm group males and females were 4.3X and 2.2X the control levels, respectively; the level for the 30 ppm group males was 2.5X the control value. T_4 levels were dramatically reduced in the 300 ppm group males and females with both values being zero after one week of treatment. Statistically significant decreases in T_4 values were seen in all the treated groups at some time points during the study. During the reversibility period, the TSH and T_4 levels in the females were comparable to the controls. The TSH levels in the 300 ppm group males remained significantly elevated throughout the reversibility period, although the values decreased at each successive time point. The T_4 levels in the treated males were not comparable to the control group until 11 weeks of the reversibility period.

Changes in urinalysis parameters included lower pH values in the treated animals with some statistically significant differences in the 30 and 300 ppm groups at some time points. Protein values tended to be elevated in the higher dose groups with significant differences in the 300 ppm group females after 49 and 90 weeks of treatment. After 87 weeks of treatment, males in the 30 and 300 ppm groups also had significantly elevated urine volumes with decreased specific gravities. During the reversibility period, the pH and protein changes were still observed.

On gross necropsy of animals which died or were sacrificed during the treatment period, the incidence of large and/or pale kidneys and large livers, adrenals and thyroids were increased in the 300 ppm group males and females. The incidence of large parathyroids was also increased in the 300 ppm group males. At the scheduled interim necropsy (after 52 weeks of treatment), the incidences of large livers and thyroids were increased in the 30 ppm group males and in the 300 ppm group males and females. At the necropsy following the reversibility period, large kidneys and livers were observed in the males in the 30 and 300 ppm groups and the 300 ppm group, respectively. At the terminal sacrifice, the incidences of large and pale kidneys were increased in males and females in the 30 and 300 ppm groups. There were also increases in the incidences of granular kidneys, large livers and large thyroids in males in the 30 ppm group and males and females in the 300 ppm group.

Changes in organ weights were relatively consistent regardless of the time at which the necropsy was conducted. At the interim necropsy, absolute and relative weights of the liver and thyroids were increased in the 30 and 300 ppm group males and females. At the necropsy following the reversibility period, the relative weights of a variety of organs were increased, mostly in the 300 ppm group males and/or females including the adrenals, heart kidneys, lungs, testes and thyroids; the relative weight of the liver was also increased in the 30 ppm group females. Some of the changes may have been treatment-related, however others may have resulted from the decreased terminal body weight in the 300 ppm

group males (statistically significant) and females. At the terminal necropsy, the absolute weight of a variety of organs was increased in the 300 ppm group males and females mostly; the weight of the thyroid was increased in the 300 ppm group females and all the treated males. The relative weights of a larger variety of organs was increased in the 30 and 300 group males and/or females, mostly. Again, the significance of some of the changes is questionable due to the decreased terminal body weights of the 30 ppm group females and the 300 ppm group males and females.

On histopathology, the incidence and severity of progressive senile nephropathy was increased in the 300 ppm group males and females during the toxicity phase and in the 30 and 300 ppm group males and females during the carcinogenicity phase. At the necropsy after the reversibility period, the incidence was higher in the 30 and 300 ppm group females and the severity was greater in all the treated animals. There were a wide variety of histological changes in the liver in all phases of the study. The incidences of the findings were essentially comparable to the controls and do not offer an explanation for the consistent increase in liver weight in the 30 and 300 ppm groups. Other non-neoplastic changes were seen with comparable frequency in the treated and control groups.

Benign and malignant 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).

The No Observed Effect Level (NOEL) = 0.5 ppm for males (0.019 mg/kg/day) and females (0.025 mg/kg/day)

The Lowest Observed Effect Level (LOEL) = 1.5 ppm for males (0.059 mg/kg/day) and females (0.78 mg/kg/day) based on an increased incidence of clinical signs and alterations in clinical chemistry and thyroid parameters.

The Maximum Tolerated Dose (MTD) = 300 ppm for males (12.68 mg/kg/day) and females (16.75 mg/kg/day) based on an increased incidence of clinical signs, decreased body weight gain, decreased food consumption, altered clinical chemistry and thyroid parameters and necropsy findings.

D11086

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DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat Guideline: §83-3 a

EPA ID No.s: EPA MRID # 42977903
EPA DP Barcode D197450
EPA Submission No. S454829
EPA Pesticide Chemical Code 129121
Toxicology Chemical Code [new chemical]

Test Material: M&B 46,030 (93% a.i., with 7% M&B 46136, Lot No.: IGB444, Batch No. JJW 2070)

Synonyms: Fipronil, 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole, $C_{12}H_4C_{12}F_6N_4OS$, Molecular wgt 437

Sponsor: Rhone-Poulenc Ltd.
Rainham Road South, DAGENHAM, Essex, RM10 7XS, UK

Report Title: THE EFFECT OF M&B 46,030 ON PREGNANCY OF THE RAT

Testing Facility: Huntingdon Research Centre Ltd.
P.O. Box 2, HUNTINGDON, Cambridgeshire, PE18 6ES, UK

Study Number(s): M&B 335+326/90582

Author(s): Amanda J. Brooker, David M. John

Report Issued: August 13, 1991

Executive Summary: In a developmental toxicity (teratology) study (MRID# 42977903), 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 M&B 46,030 (93% a.i.) by oral gavage from gestation days 6 through 15, inclusive.

Maternal toxicity was noted at the high dose (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 pe[illegible]including the dosing plus post dosing period (90.1% of control, gestation days 6 through 20) and for the entire gestation period (91.8% of control, gestation day 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 (gestation 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. **The LOEL for maternal toxicity is 20 mg/kg/day with a NOEL for maternal toxicity of 4 mg/kg/day** based on reduced body weight gain, increased water consumption, reduced food consumption and reduced food efficiency.

No effects were noted in developmental toxicity parameters. **The LOEL for developmental toxicity is greater than 20 mg/kg/day with a NOEL for developmental toxicity of 20 mg/kg/day or higher.**

The study is classified as **Core Minimum Data (Acceptable)** and satisfies the guideline requirement (§ 83-3a) for a developmental toxicity (teratology study) in rats.

A. Materials and Methods: A copy of the "materials and methods" section from the investigators report is appended.

Test Compound:
Purity: 93% a.i.
Density: not provided
Description: A white powder
Lot No.: IGB444, Batch No. JJW 2070
Receipt date: May 16, 1989
Other provided information: stored at room temperature in the dark
Contaminants: [redacted] M&B 46136

Vehicle(s): 0.5% methylcellulose

Test Animal(s):
Species: Specific Pathogen Free female rats
Strain: Crl: CD^{R} (SD) BR VAF/Plus
Source: Charles River, St. Aubin les Elbeuf, France
Age: not provided
Body Weight: 165-262 g on arrival
Males: same strain
Animals were received in 2 batches on different days.
ANIMALS WERE RECEIVED TIMED PREGNANT

B. Study Design

This study was designed to assess the developmental toxicity potential of M&B 46,030 when administered by oral gavage to pregnant rats on gestation days 6 through 15, inclusive.

Mating Procedure

ANIMALS WERE RECEIVED TIMED PREGNANT
From the vendor: natural mating was used with the day of mating judged by the appearance of sperm in the vaginal smear or the presence of a vaginal plug. This was considered Gestation Day 0.

Animal Husbandry

Animals were kept under standard animal care conditions. The animals were not acclimatized to the laboratory as they were received timed pregnant. They received Labsure Laboratory Animal Diet No. 1 and tap water *ad libitum* (food and water were analyzed for contaminants).

Group Arrangement:

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	0.5% MC	25
Low Dose	1	25
Mid Dose	4	25
High Dose	20	25

Dose Administration:

All doses were administered in a volume of 1.0 ml/100g of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration but not stability. Dosing was based on gestation day 6, 8, 10, 12, and 14 body weight. Doses were based on a range finding study (provided), A PRELIMINARY STUDY OF THE EFFECT OF M&B 46,030 ON THE PREGNANCY OF THE RAT, Study No. M&B/326 where groups of 10 animals per dose received either 0, 5, 10, or 20 mg/kg/day M&B 46,030 by oral gavage from gestation day 6 through 15, inclusive. There was a dose related decrease in body weight gain and food and water consumption at dose levels of 10 mg/kg/day and above, no other treatment related observations were noted. It was concluded from this study that *a suitable high dosage for an embryotoxicity study in the rat could be 20 mg/kg/day.*

Observations

The animals were checked daily for mortality or abnormal condition. *Food consumption was measured from weighday to weighday.* Water consumption was measured from Day 2. The animals were weighed initially (≈Day 1 or 2) and then on Day 2 (second receipt batch of animal), 4, 6, 8, 10, 12, 14, 16, 18, and 20 of gestation. Dams were sacrificed on day 20 of gestation. Examinations at sacrifice consisted of a dissection and examination *for congenital abnormalities and macroscopic pathological changes in maternal organs.* The ovaries and uteri were examined for the *number of corpora lutea,* the *number and distribution of live young,* the *number and distribution of embryofetal deaths* (divided into early and late), the *individual fetal weight from which the litter weight was calculated,* and *fetal abnormalities. Uteri or individual uterine horns without visible implantation were immersed in a 10% solution of ammonium sulphide to reveal evidence of embryonic death at very early stages of implantation.*

The fetuses were examined in the following manner: *Live young were examined externally and weighed. Half the fetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique); the remainder were fixed in 74 OP industrial spirit for subsequent*

macroscopic examination, evisceration, clearing and alizarin staining (modified Dawson technique) for skeletal examination. Young showing suspected abnormalities were processed by the more appropriate technique for clarification of initial observations. All fetuses were sexed by gonadal inspection following preservation. References for techniques were provided.

Historical control data **were not** provided to allow comparison with concurrent controls.

Statistical analysis

The following statistical analysis methods were employed (from the investigators report):

> **Statistical analyses were routinely performed on litter data. Significance tests were normally two-tailed.**
>
> **Litter data**
>
> **The basic sample unit was the litter, and due to the preponderance of non-normal distributions, non-parametric analyses have generally proved the most consistent.**
>
> **Mean values of litter size, pre- and post implantation loss, litter weight, mean pup weight and the incidence of anomalous offspring were analysed by the Kruskal-Wallis test[1]. Intergroup comparisons were made by the non-parametric equivalent of the Williams' test[2] following a significant H statistic.**
>
> **Where 75% of the values for a given variable consisted of one value, a Fisher's exact test[3] was used.**

Compliance

A signed and dated STATEMENT OF NO CONFIDENTIALITY CLAIM was provided.

A signed and dated COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS was provided.

A signed and dated FLAGGING STATEMENT for 40 CFR 158.34 was provided. According to the investigators, the study neither meets nor exceeds the applicable criteria.

A signed and dated DEPARTMENT OF QUALITY ASSURANCE REPORT AUDIT STATEMENT was provided.

B. Results

Analysis of Test Compound:

According to the investigators: *Analysis of M&B 46,030 in liquid formulation was within 15% of nominal on all occasions sampled* (mean 107%, range 98-115%). The provided data supported this statement.

Maternal Toxicity:

Mortality

No animals were reported to have died.

Clinical Observations

No clinical signs attributable to treatment were noted in the provided data.

Body Weight

The investigators supplied group mean, graphical depiction and individual animal data, the following table presents body weight gain data:

Table I: Body Weight Gains (grams)[a]

Gestation Days:	2-6	6-16	16-20[1]	6-20	2-20[1]
Control	42.6	93.9	75.9	169.8	212.4
LDT	40.9	94.2	73.8	167.9 (98.9)	208.9 (98.3)
MDT	42.8	88.8 (94.6)[2]	65.9	154.6 (91.0)	197.4 (92.9)
HDT	41.9	77.6 (82.6)	75.4	153.0 (90.1)	194.9 (91.8)

[1] = calculated by reviewer from means, [2] = percent of control
[a] = Data extracted from M&B/335 Table 3.

The above data indicate that the high dose group gained less weight than the control during the dosing period (gestation days 6-16) and to a lesser extent for the period including the dosing plus post dosing period (gestation days 6 through 20) and for the entire gestation period (gestation day 2 through 20).

Food and Water Consumption

The investigators supplied group mean, graphical depiction and individual animal data; the following table from the investigators report presents food and water consumption data:

Table II

Food and water consumption - group mean values

Group:	1	2	3	4
Compound:	Control		M&B 46,030	
Dose (mg/kg/day):	-	1	4	20

Group	Number of animals	Consumption (g/rat/day) during days of gestation								6-15
		4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	
Water consumption										
1	25	37	35	37	40	39	41	46	46	38
2	25	36	35	36	39	38	42	44	45	38
3	25	37	35	38	40	40	43	47	47	39
4	25	41	36	43	48	50	50	55	55	45
Food consumption										
1	25	29	28	29	31	31	32	35	34	30
2	25	29	28	29	30	30	32	33	34	30
3	25	29	28	28	30	31	31	33	33	30
4	25	29	24	22	28	30	32	35	36	27

Treatment period Days 6 to 15 of pregnancy inclusive

From the provided data, there appears to be an increase in water consumption in the high dose group during the dosing period, although prior to dosing there seemed to be increased consumption. The percents of control were 110.8, 102.9, 116.2, 120.0, 128.2, 122.0, 119.6, and 119.6% for the above time periods (118% of control for gestation days 6-15). Food consumption was slightly decreased in the high dose group at the beginning of the dosing period (gestation days 6-11; 85.7, 75,9, 90.3 and 96.8% for the 6-7, 8-9, 10-11, and 12-13 time periods; 90% of control for gestation days 6-15), after which no treatment related effect was noted. A calculation of food efficiency indicates that 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.

Gross Pathological Observations

The investigators supplied individual animal data, no treatment related effects were noted.

Cesarean section Observations

Table III: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	25	25	25	25
#Animals Mated/Inseminated	25	25	25	25
#Animals Pregnant	24	24	25	25
Pregnancy Rate (%)	96	96	100	100
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	1	1	0	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea[1]	387	379	398	392
Corpora Lutea/dam	16.1	15.8	15.9	15.7
Total Implantations[1]	338	340	324	332
Implantations/Dam	14.0	14.0	13.0	13.3
Total Live Fetuses[1]	320	323	298	316
Live Fetuses/Dam	13.3	13.5	11.9	12.6
Total Resorptions[1]	15	14	26	16
Early[1]	14	14	24	16
Late[1]	1	0	2	0
Resorptions/Dam	0.6	0.6	1.0	0.6
Mean Fetal Weight (gm)	3.96	3.98	3.82	3.96
Preimplantation Loss(%)	14.5	11.6	19.7	15.0
Postimplantation Loss(%)	3.9	5.8	7.4	5.0
Sex Ratio (% Male)	43.7	51.9	52.2	50.4

[1] = calculated by reviewer from individual animal data

[a] = Data extracted from M&B/335 Tables 4, 5 and Appendix 5.

No treatment related effects were noted in the above data.

Developmental Toxicity

No treatment related effects were noted in external, visceral or skeletal examination data. The investigators provided group summary and individual animal data; however, they did not provide litter incidence (except in individual data) for the skeletal *variant* data, but based on those observation for fetal incidence alone and inspection of the individual animal data, no treatment related effect was noted. Tables 6 through 10 from the investigators report are appended.

C. Discussion/Conclusions

a. Maternal Toxicity:

Maternal toxicity was noted at the high dose (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 through 20) and for the entire gestation period (91.8% of control, gestation day 3 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 hig dose group for gestation days 6-15. Food consumption was slightly decreased in the high dose group at the beginning of the dosing period (gestation days 6-11) with an overall reduction of 9% 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, 27.3, and 25.3% for the control, low, mid and high dose groups respectively.

b. Developmental Toxicity:

i. Deaths/Resorptions:

No treatment related effects were noted.

ii. Altered Growth:

No treatment related effects were noted.

iii. Developmental Anomalies:

No treatment related effects were noted.

iv. Malformations:

No treatment related effects were noted.

D. Study Deficiencies:

No specific study deficiencies.

E. Core Classification: Core Minimum Data (Acceptable).

Maternal Toxicity NOEL = 4 mg/kg/day
Maternal Toxicity LOEL = 20 mg/kg/day

Developmental Toxicity NOEL => 20 mg/kg/day
Developmental Toxicity LOEL > 20 mg/kg/day

Page_____ is not included in this copy.

Pages 153 through 163 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

011086

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 3/15/94
Senior Pharmacologist, Review Section I, TB II/HED (7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. YMI 3/15/94
Section Head, Review Section I, TB II/HED (7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit Guideline: §83-3 b

EPA ID No.s: EPA MRID # 42918646
EPA DP Barcode D197450
EPA Submission No. S454829
EPA Pesticide Chemical Code 129121
Toxicology Chemical Code [new chemical]

Test Material: M&B 46030 (95.4% w/w, with [redacted] M&B 46136, [redacted] M&B 45,950 and [redacted] of 3 unidentified, Batch No. PGS 963)

Synonyms: Fipronil, 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethyl sulphinylpyrazole, $C_{12}H_4C_{12}F_6N_4OS$, Molecular weight 437, Class: phenylpyrazole

Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet, Boite Postale 9163, F-69263 LYON CEDEX 09, FRANCE

Title of Report: M&B 46030: TERATOLOGY STUDY IN THE RABBIT

Testing Facility: Life Science Research Limited
Eye, Suffolk, IP23 7PX, England

Study Number(s): LSR Report No: 90/RHA321/0722
LSR Schedule No: RHA/321/46030

Author(s): V.C. King

Report Issued: November 29, 1990

Executive Summary: In a developmental toxicity (teratology) study (MRID# 42918646), 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 M&B 46030 (95.4% w/w) by oral gavage from gestation days 6 through 19, inclusive.

Maternal toxicity was noted at all dose levels tested in the form of reduced body weight gain noted at all gestation day periods determined. Body weight gains for the treatment period (gestation days 6-20) were 0.30, 0.22, 0.22, 0.15, and 0.09 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively, (73, 73, 50, and 30% of control, respectively). For gestation days 20-

28, the body weight gains were 0.12, 0.16, 0.14, 0.17, and 0.16 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups respectively. For gestation days 0-28, the body weight gains were 0.57, 0.50, 0.49, 0.46, and 0.38 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups respectively (this is 88, 86, 81, and 67% of control for this time period). All treated groups consumed less food than that of the control group during the dosing period (gestation days 6-20), 2748, 2444, 2494, 2438, and 2158 gm for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively the 2 highest dose groups achieving statistical significance for gestation days 6-12 and 13-19). Food Efficiency was 10.9, 9.0, 8.8, 6.2 and 4.2% for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively. No other parameters showed a treatment related effect. **The LOEL for maternal toxicity is equal to or less than 0.1 mg/kg/day with a NOEL for maternal toxicity of less than 0.1 mg/kg/day** based on reduced body weight gain, reduced food consumption and efficiency.

No effects were noted in developmental toxicity parameters. **The LOEL for developmental toxicity is greater than 1.0 mg/kg/day with a NOEL for developmental toxicity of equal to or greater than 1.0 mg/kg/day.**

The study is classified as **Core Minimum Data (Acceptable)** and satisfies the guideline requirement (§ 83-3b for a developmental toxicity (teratology) study in rabbits.

011056

A. Materials and Methods: A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: **M&B 46030**
Purity: 95.4% w/w
Density: not provided
Description: Fine off white powder, also identified as a creamy-yellow crystalline powder
Batch No. PGS963
Receipt date: January 24, 1990
other provided information: stored at room temperature in the dark
Contaminants: [redacted] M&B 46136, [redacted] M&B 45,950 and [redacted] of 3 unidentified manufacturing impurities

Vehicle(s): 1.5% w v aqueous methylcellulose mucilage and 0.5% w v Tween 80

Test Animal(s): Species: sexually mature virgin female rabbits
Strain: New Zealand White
Source: Ranch Rabbits, Crawley Down, Sussex, England
Age: 16-24 weeks
Body Weight: 3.31-4.82 kg at start of study
Males: same strain

B. Study Design

This study was designed to assess the developmental toxicity potential of M&B 46030 when administered by oral gavage to pregnant rabbits on gestation days 6 through 19, inclusive.

Mating Procedure

Artificial insemination was employed using pooled semen from New Zealand White bucks of established fertility. According to the investigators, following insemination, each female was injected intravenously with 25 I.u. of luteinizing hormone (Profasi, Serono) to ensure successful ovulation. The day of insemination was considered as Gestation Day 0. It should be noted that according to the investigators, 3 weeks prior to the expected date of insemination, the rabbits were injected with 25 I.u. of the luteinizing hormone to synchronize ovulation.

Animal Husbandry

Animals were kept under standard animal care conditions and acclimatized to the laboratory conditions for a minimum of 1 week. They received S.D.S. Standard Rabbit Diet (Special Diet Services Limited, Witham, Essex, England and tap water (by an automatic system) ad libitum (food & water were analyzed for contaminants).

Group Arrangement:

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	0.5% MC	22
Low Dose	0.1	22
Low Mid Dose	0.2	22
High Mid Dose	0.5	22
High Dose	1.0	22

Dose Administration:

All doses were administered in a volume of 5 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration, homogeneity and stability. Dosing was based on the most recent gestation day body weight. Dose selection was based on a range finding study LSR Report No. 90/RHA354/0541, which was **not** provided.

Observations

The animals were checked daily for mortality or abnormal condition. The animals were weighed daily. Food consumption was determined for the following *phases*: gestation days 1-5, 6-12, 13-19, 20-23, 24-28 (all inclusive). Dams were sacrificed on day 29 of gestation. Examinations at sacrifice consisted of a macroscopic examination *for evidence of disease or adverse reaction to treatment.... The reproductive tract, complete with ovaries was dissected out and the following recorded: the number of corpora lutea in each ovary, number of implantation sites,* the *number of resorption sites* (divided into early and late), the *number and distribution of live and dead fetuses in each uterine hor*. *In apparently non-pregnant animals, presence of implantation sites was checked using a staining technique* (reference citation provided).

The fetuses were examined in the following manner: *The following were recorded:* the *weight of individual fetuses,* the *weight of individual placentae, external abnormalities of individual fetuses and placentae. All fetuses were killed by subcutaneous injection of pentobarbitone sodium. The neck and the thoracic and abdominal cavities of all fetuses from each litter were dissected, the contents examined and sex recorded. Following examination, the fetuses were eviscerated and one-third of the fetuses in each litter were decapitated and the heads fixed in Bouin's fluid for subsequent examination following free-hand serial sectioning. Torsos and the remaining intact fetuses were fixed in industrial methylated spirit (74° o.p.). The eviscerated fetuses were processed using a modification of the Dawson Alizarin staining technique* (reference citation provided) *and the skeletons were examined.*

Historical control data for some parameters were provided to allow comparison with concurrent controls.

Statistical analysis

The following statistical analysis methods were employed (from the investigators report):

> The significance of suggestive inter-group differences was tested using appropriate statistical tests, each of which has been specified where significance was found.
>
> The following tests were used:
>
> | | |
> |---|---|
> | T-test | Bodyweight change |
> | Pearson's (product moment) correlation coefficient | Day 6 bodyweight versus bodyweight change |
> | Pearson's (product moment) correlation coefficient followed by analysis of co-variance | Day 6 bodyweight versus absolute bodyweight |
> | Mann-Whitney U-test | Food consumption |
>
> Evaluation of the tests performed on bodyweight data revealed essentially similar results and only the results of the t-test have been presented in the report.

Compliance

A signed and dated statement of no confidentiality claims was provided.

A signed and dated compliance with good laboratory practice standards was provided.

A signed and dated FLAGGING STATEMENT for 40 CFR 158.34 was provided. According to the investigators, the study neither meets nor exceeds the applicable criteria.

A signed and dated QUALITY ASSURANCE INSPECTIONS statement was provided.

B. Results

Analysis of Test Compound:

According to the investigators the compound was stable in the dosing solutions for at least 6 hours, the maximum period of time used for dosing, although they tested concentrations up to 48 hours with little loss (according to the data provided). Concentration analysis found that during the first week the solutions were 121, 124, 102, and 82.6% for the 0.5, 1.0, 2.5, and 5.0 mg target concentrations, respectively; and for the last week the solutions were 111.6, 87.5, 97,2 and 100.2% for the 0.5, 1.0, 2.5, and 5.0 mg target concentrations, respectively.

Maternal Toxicity:

Mortality

One control animal was *sacrificed in extremis* due to clinical condition, with the necropsy report showing evidence of an intra-peritoneal infection.

Clinical Observations

No clinical signs attributable to treatment were noted in the data provided.

Body Weight

The investigators supplied group mean, graphical depiction and individual animal data; the following graphical depiction and table from the investigators report presents body weight gain data. As noted on the following table, the body weight gains for the treatment period (gestation days 6-20) were 0.30, 0.22, 0.22, 0.15, and 0.09 for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day, respectively, which is 73, 73, 50, and 30% of control, respectively. Calculated by the reviewer from means, for gestation days 20-28, the body weight gains were 0.12, 0.16, 0.14, 0.17, and 0.16 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively. For gestation days 0-28, the body weight gains were 0.57, 0.50, 0.49, 0.46, and 0.38 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups respectively (this is 88, 86, 81, and 67% as compared to control for this time period). It is apparent that all treated groups gained less weight than that of the control group.

FIGURE 1

Bodyweight change of females during gestation

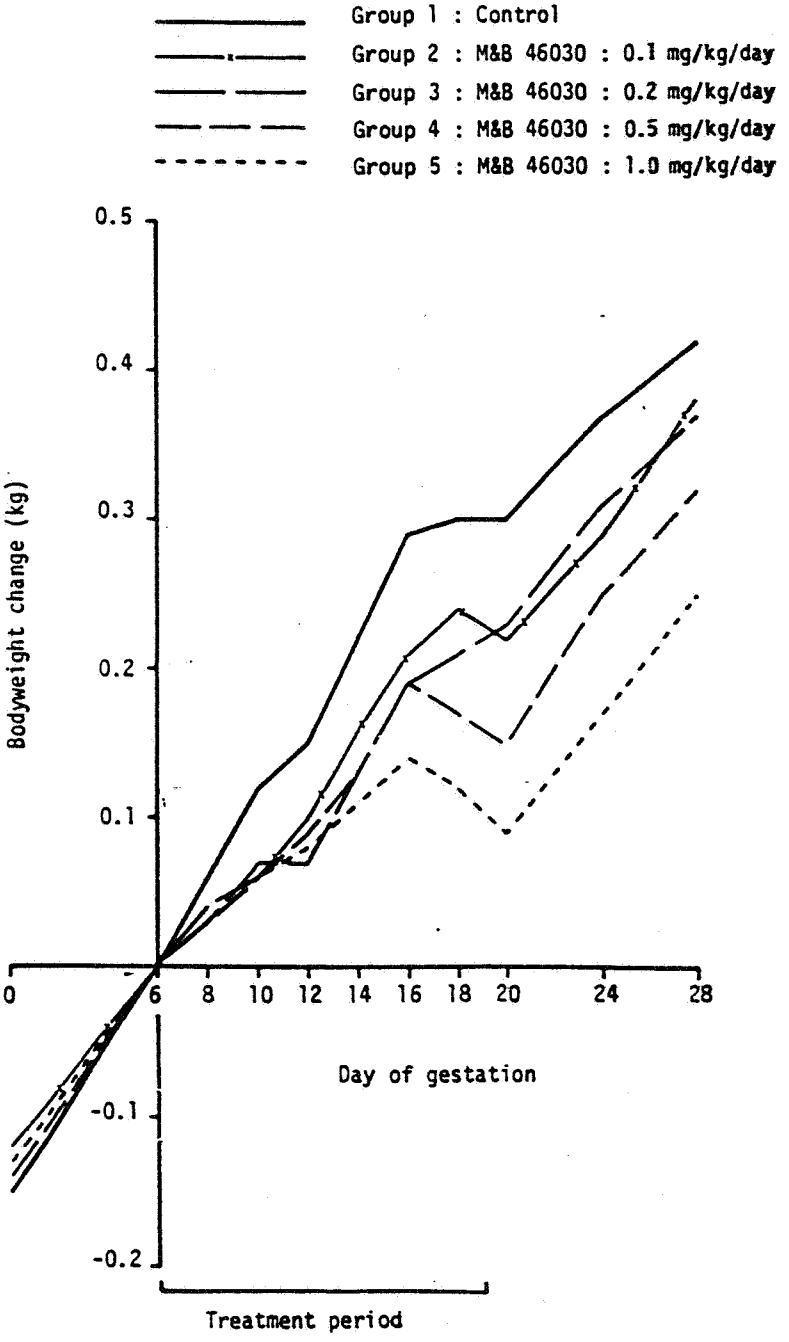

TABLE 3

Group mean bodyweight change (kg) of females during gestation

Group	:	1	2	3	4	5
Compound	:	Control	---------	M&B 46030	---------	
Dosage (mg/kg/day)	:	0	0.1	0.2	0.5	1.0

Group	Number of animals		Day of gestation									
			0-6	6-8	6-10	6-12	6-14	6-16	6-18	6-20	6-24	6-28
1	19	Mean	0.15	0.06	0.11	0.15	0.22	0.29	0.30	0.30	0.37	0.42
		SD	0.08	0.04	0.05	0.06	0.08	0.09	0.07	0.10	0.09	0.12
2	19	Mean	0.12	0.04(67)[1]	0.05***(46)	0.10(67)	0.16(73)	0.21(72)	0.24(80)	0.22(73)	0.29(78)	0.38(91)
		SD	0.10	0.04	0.04	0.07	0.09	0.12	0.10	0.12	0.13	0.12
3	21	Mean	0.12	0.03*(50)	0.07*(64)	0.07*(47)	0.13*(59)	0.19*(64)	0.21*(70)	0.22(73)	0.31(84)	0.37(88)
		SD	0.07	0.04	0.06	0.15	0.19	0.21	0.19	0.23	0.19	0.21
4	18	Mean	0.14	0.03**(50)	0.06***(55)	0.09*(60)	0.13*(59)	0.19*(64)	0.17*(57)	0.15**(50)	0.25*(68)	0.32(76)
		SD	0.06	0.04	0.05	0.07	0.10	0.13	0.14	0.15	0.15	0.18
5	18	Mean	0.13	0.03*(50)	0.06**(54)	0.08*(53)	0.11**(50)	0.14**(48)	0.12***(40)	0.09***(30)	0.17***(46)	0.25**(60)
		SD	0.05	0.03	0.05	0.06	0.09	0.12	0.15	0.17	0.12	0.16

1 = % of Control

SD Standard deviation.
* Bodyweight change with respect to Day 6 of gestation significantly different from Controls, P<0.05 (t-test).
** Bodyweight change with respect to Day 6 of gestation significantly different from Controls, P<0.01 (t-test).
*** Bodyweight change with respect to Day 6 of gestation significantly different from Controls, P<0.001 (t-test).

011086

Food Consumption

The investigators supplied group mean, and individual animal data, the following table from the investigators report presents food consumption data:

Food intake - group mean values (g/rabbit/day)

Group	1	2	3	4	5
Compound	Control	---	M&B 46030	---	
Dosage (mg/kg/day)	0	0.1	0.2	0.5	1.0

Group		Days of gestation 1-5	6-12	13-19	20-23	24-28	6-20[1]
1	Mean	185	187	184	151	132	2748
	SD	31	26	26	30	32	
	n	19	18	19	18	18	
2	Mean	169	171	157	148	133	2444
	SD	39	33	48	32	25	
	n	19	18	18	19	19	
3	Mean	181	168	166	156	131	2494
	SD	27	41	46	32	38	
	n	21	21	21	21	21	
4	Mean	186	180	146*	156	131	2438
	SD	25	27	51	22	36	
	n	18	18	17	16	17	
5	Mean	178	165*	124**	135	130	2158
	SD	21	21	50	35	28	
	n	18	18	17	17	18	

1 = calculated by reviewer from means
SD Standard deviation.
n Number of animals.
* Food intake with respect to Days 1-5 significantly different from Controls, P<0.05 (Mann Whitney U-test).
** Food intake with respect to Days 1-5 significantly different from Controls, P<0.01 (Mann Whitney U-test).

From the provided data, all treated groups consumed less food than that of the control group during the dosing period (gestation days 6-20), 2748, 2444, 2494, 2438, and 2158 gm for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively (the 2 highest dose groups achieving statistical significance for gestation days 6-12 and 13-19). Food Efficiency during the dosing period was 10.9, 9.0, 8.8, 6.2 and 4.2% for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively; note the considerable decrease in the 2 highest dose groups.

Gross Pathological Observations

The investigators supplied individual animal data, no treatment related effects were noted.

Cesarean Section Observations

Table III: Cesarean Section Observations[a]

Dose:	Cont.	LDT	LMDT	HMDT	HDT	HC
#Animals Assigned	22	22	22	22	22	
#Animals Mated/Insem.	22	22	22	22	22	
#Animals Pregnant	20	21	21	18	19	
Pregnancy Rate (%)	90.9	95.5	95.5	81.8	86.4	
Maternal Wastage						
#Died	1	0	0	0	0	
#Non pregnant	1	1	1	4	3	
#Aborted	0	1	0	0	0	
#Total lit. loss	1	1	0	0	0	
Total litters	19	19	21	18	18	
Total Corpora Lutea[1]	204	211	219	188	195	
Corpora Lutea/dam	11.4	11.1	10.4	10.4	10.8	11.0
Total Implantations[1]	203	175	181	162	175	
Implantations/Dam	10.7	9.2	9.0	9.0	9.7	9.1
Total Live Fetuses[1]	169	157	171	146	155	
Live Fetuses/Dam	8.9	8.3	8.1	8.1	8.6	8.3
Total Resorptions[1]	34	18	18	16	20	
Early[1]	9	10	9	12	12	
Late[1]	25	8	9	4	8	
Resorptions/Dam	1.8	0.9	0.9	0.9	1.1	0.8
Mean Fetal Weight (gm)	40.2	41.7	41.0	40.8	39.5	40.8
Preimplantation Loss(%)	6.5	17.1	14.9	14.7	10.3	17.3
Postimplantation Loss(%)	16.7	10.3	9.5	9.9	11.4	9.3
Sex Ratio (Male/female)	4.7/4.2	4.1/4.1	4.4/3.8	4.1/4.1	4.5/4.1	4.2/4.2

[1] = calculated by reviewer from individual animal data; HC = historical control for 47 studies, mean and low and high data provided.
[a] = Data extracted from M&B/335 Tables 1, 5 and Appendix 5.

No treatment related effects were noted in the above data.

Developmental Toxicity

No treatment related effects were noted in external, visceral or skeletal examination data. The investigators provided group summary and individual animal data. Tables 6 through 8 from the investigators report are appended.

C. Discussion/Conclusions

a. Maternal Toxicity:

Maternal toxicity was noted at all dose levels tested in the form of reduced body weight gain noted at all gestation period determined. Body weight gains for the treatment period (gestation days 6-20) were 0.30, 0.22, 0.22, 0.15, and 0.09 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively, (73, 73, 50, and 30% of control, respectively). For gestation days 20-28, the body weight gains were 0.12, 0.16, 0.14, 0.17, and 0.16 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively. For gestation days 0-28, the body weight gains were 0.57, 0.50, 0.49, 0.46, and 0.38 kg for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively (this is 88, 86, 81, and 67% of control for this time period). All treated groups consumed less food than that of the control group during the dosing period (gestation days 6-20), 2748, 2444, 2494, 2438, and 2158 gm for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively (the 2 highest dose groups achieving statistical significance for gestation days 6-12 and 13-19). Food Efficiency was 10.9, 9.0, 8.8, 6.2 and 4.2% for the 0, 0.1, 0.2, 0.5, and 1.0 mg/kg/day dose groups, respectively. No other parameters showed a treatment related effect.

b. Developmental Toxicity:

i. Deaths/Resorptions:

No treatment related effects were noted.

ii. Altered Growth:

No treatment related effects were noted.

iii. Developmental Anomalies:

No treatment related effects were noted.

iv. Malformations:

No treatment related effects were noted.

D. Study Deficiencies:

No specific study deficiencies.

E. Core Classification: Core Minimum Data (Acceptable).

Maternal Toxicity NOEL < 0.1 mg/kg/day
Maternal Toxicity LOEL =< 0.1 mg/kg/day

Developmental Toxicity NOEL => 1.0 mg/kg/day
Developmental Toxicity LOEL > 1.0 mg/kg/day

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Pages 176 through 192 are not included.

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011086

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 6/20/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 6/20/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Multigeneration Reproduction/Rats (83-4)
EPA I.D. NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 429186-47
TEST MATERIAL:	M&B 46030
SYNONYM:	Fipronil
STUDY NUMBER:	LSR 92/RHA425/0309
TESTING FACILITY:	Life Science Research Ltd
SPONSOR:	Rhone-Poulenc
TITLE OF REPORT:	M&B 46030: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations
AUTHOR(S):	V.C. King
REPORT ISSUED:	June 26, 1992

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID # 429186-47), 30 males and 30 females per group of CD rats received M&B 46030 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). The protocol was a standard reproduction study with litters culled to 4 animals per sex on post partum Day 4 and with two matings of the F_0 generation. In addition, physical development was assessed on a litter basis by recording the day of onset and completion of pinna unfolding, hair growth, tooth eruption and eye opening.

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) the absolute and relative weights of the thyroid glands and the liver were increased in the 30 and 300 ppm group males and females of the F_0 and F_1 generations; the absolute and relative weights of the ovaries were decreased in the 300 ppm group females in the F_0 generation; the absolute weight of the pituitary gland was decreased in the 30 and 300 ppm group females and the relative

weight was decreased in all the treated female groups in the F_1 parental animals; the absolute and relative weights of the testes in the 300 ppm group males were decreased 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).

The study is classified as **Core Minimum** and **satisfies** the guideline requirements (83-4) for a multigeneration reproduction study in rats.

011086

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Name: M&B 46030
Synonym: Fipronil
Chemical Name: 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyphenyl)-4-trifluoromethylsu¹phinyl-pyrazole
Purity: 95.4%
Lot Number: PGS 963
Description: Fine off-white powder
Storage Conditions: 13°C in the dark

Samples of the test material were returned to the sponsor every six months for re-analysis throughout the study. Analyses (Appendix 50) showed that the purity of the chemical remained consistent.

2. Administration: dietary

3. Test Animals

Species: CD strain (of Sprague-Dawley origin)
Source: Charles River U.K. Limited, Kent, England
Age: 5 to 6 weeks at commencement of study
Weight: males: 115 to 168 g; females: 99 to 140 g at commencement of study
Acclimation Period: Two weeks
Housing: 1 male and 1 female per cage during mating; up to 5 per sex per cage at other times of the study
Environmental conditions: Temperature: target of 21° C
Humidity: target of 55%
Air changes: 15 per hour
Photoperiod: 12 hours light/dark
Food and water: available *ad libitum*

4. Diet Preparation and Analysis

The diet was prepared fresh weekly by mixing appropriate amounts of the test substance with SDS Laboratory Animal Diet No. 2 and was stored at room temperature. Homogeneity of the test material in the diets was determined pre-treatment for the 3 and 300 ppm formulations. The study report states that stability was determined as part of a preliminary study (LSR Report No. 90/RHA319/0544). During the study, samples of treated food were analyzed for concentration at the commencement of treatment (for the treatment levels not assessed for homogeneity), for the first four weeks of the study and then at 4-weekly intervals until termination of the

study.

B. PROCEDURES AND STUDY DESIGN

1. Mating Procedure: Females were paired with males of the same treatment group in the same cage, avoiding the pairing of siblings. Observations were made daily for evidence of ejected copulation plugs and vaginal smears were prepared to examine for the presence of sperm. The day on which evidence of mating was observed was designated as Day 0 of gestation; the pairs were then separated. Pairs were allowed a maximum of three weeks to mate. For this study, F_0 females were paired for a second mating ten days after the weaning of the F_1A litters to produce F_1B litters.

Pregnant females were placed in individual stainless steel cages from Day 16-18 post coitum to Day 14-18 post partum and then in polypropylene cages from Day 14-18 post partum until weaning.

2. Mating Schedule: The F_0 parental animals were given test diets for 71 days before they were mated. Treatment was continued throughout the mating phase for the 300 ppm group animals. Treatment for the 0, 3 and 30 ppm groups was continued through two matings and until they were sacrificed after the F_1B litters were weaned. The 300 ppm group animals were not mated but were maintained on the treated diet. Selection of one male and one female from each litter for the F_1 parental generation was made when the F_1A litter was weaned using random numbers after grossly atypical animals had been excluded. If there were not enough litters to supply 30 males and 30 females, additional offspring were randomly selected from each group. These animals were treated for 10 weeks prior to mating; treatment was continued until weaning of the F_2 litter.

3. Dose Selection Rational: Dosages were based on a preliminary study (LSR Report No. 90/RHA319/0544); the results of this study are not discussed in the present study report.

4. Animal Assignment: F_0 animals were randomly assigned to test groups using a random replicate selection procedure which ensured that not more than one offspring of each sex from each litter was present in each group. Table 1 presents the dosage groups.

Table 1: Animal Assignment

Test Groups		Dose	Animals per group	
Number	Designation	(ppm)	Males	Females
1	Control	0	30	30
2	M&B 46030	3	30	30
3	M&B 46030	30	30	30
4	M&B 46030	300	30	30

C. METHODS

1. Observation Schedule

a. Parental animals: Observations and the schedule for those observations are summarized in Table 2.

Table 2: Observation Schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality and clinical signs of toxicity	All	Twice daily throughout the study
Body weight	Males	Weekly
	Females	Weekly until mating was detected, on Days 0, 6, 13 and 20 post coitum and on Days 1, 4, 7, 14, 21 and 25 post partum
Food intake	All	Weekly for each generation until animals were paired for mating

b. Reproductive performance: The following indices were calculated:

$$\text{Percentage mating} = \frac{\text{Animals mated}}{\text{Animals paired}} \times 100$$

$$\text{Conception rate} = \frac{\text{Animals that achieved a pregnancy}}{\text{Animals mated}} \times 100$$

$$\text{Fertility index} = \frac{\text{Animals that achieved a pregnancy}}{\text{Animals paired}} \times 100$$

c. Litter observations: The following litter observations were

made (see Table 3). (Note: On day 4 post partum, litters containing more than 8 offspring were reduced to 8 by random culling, leaving 4 males and 4 females wherever possible.)

Table 3: F_1/F_2 Litter Observations

Observation	Time of Observation (Day *post partum*)
Number born (live and dead)	1
Mortality and litter size	Daily
Observations of general health	Daily
Sex ratio	Days 1, 4 (before/after culling), 14 and 25
Body weights	Days 1, 4 (before culling), 7, 14, 21 and 25
Physical development	Time of pinna unfolding, hair growth, tooth eruption and eye opening
Observations on individual pups	1

Dead pups were examined grossly for external and internal abnormalities and a possible cause of death was determined for pups born or found dead. The following F_1 and F_2 indices were calculated.

Gestation Length - number of gestation days up to and including the day on which offspring were first observed, with day of mating = Day 1

$$\text{Gestation index} = \frac{\text{Number of live litters born}}{\text{Number pregnant}} \times 100$$

$$\text{Group mean litter size} = \frac{\text{Number of live offspring}}{\text{Number of litters on day of examination}}$$

Post-implantation survival index (for F_2 generation only) =

$$\frac{\text{Number of offspring on Day 1 post partum}}{\text{Number of uterine implantation sites}} \times 100$$

Live birth index =

$$\frac{\text{Number of live offspring on Day 1 post partum}}{\text{Total number of offspring on Day 1 post partum}} \times 100$$

Viability index =

$$\frac{\text{Number of live offspring on Day 4 before culling}}{\text{Number of live offspring on Day 1 post partum}} \times 100$$

Lactation index (Days 7, 14, 21 and 25 post partum) =

$$\frac{\text{Number of live offspring on day of examination}}{\text{Number of live offspring on Day 4 after culling}} \times 100$$

d. Necropsy

1) Parental animals: F_0 parental animals that littered were sacrificed after the F_1B litters were weaned. F_1 males and females that produced litters were sacrificed after the F_2 litters were weaned. Females that did not mate, mated but did not give birth or whose litters died

before weaning were sacrificed with the majority of the females. Complete gross necropsies were performed on all adult animals.

2) Offspring: Pups culled at Day 4, any found dead, F_1 weanling pups not selected for continuation on the study, F_1B and F_2 weanling pups were examined externally and internally for macroscopic evidence of abnormalities.

3) Necropsy observations: Gross necropsies consisted of external and internal examinations including the cervical, thoracic and abdominal viscera. The following tissues (X) were prepared for microscopic examination* and weighed (XX).

XX Ovaries	XX Epididymides
XX Uterus/cervix	XX Prostate
X Vagina	XX Seminal vesicles
XX Lesions	XX Testes

Additional tissues weighed and examined microscopically included the liver, pituitary and thyroid (with parathyroids). The thyroid and liver of all adult animals were examined microscopically. The caudal mammary glands were examined microscopically in adult females with total litter losses.

* Microscopic examinations were performed on the above tissues from all parental rats from Groups 1 and 4, all adult rats that died or were sacrificed during the study and all animals that exhibited gross pathological change.

D. Statistical Analysis: The study report states only that the significance of suggestive inter-group differences was tested using appropriate statistical tests, each of which was specified where used. Differences with an associated probability of $p<0.05$ were considered to be statistically significant.

E. Compliance: Signed and dated GLP and Quality Assurance statements were provided. A signed Flagging Statements was submitted indicating that the study neither meets nor exceeds any of the applicable criteria of 40 CFR 158.34.

II. RESULTS

A. Analyses of Test Diets

Analyses of the 3 and 300 ppm diets for homogeneity showed that the diets were homogeneous with coefficients of variation of 5.77% and 3.70%, respectively. Stability analyses for concentrations of 1 and 500 ppm (from study RHA/319) showed that the chemical was stable for at least 31 days in the basal diets. Analyses of the concentration

of the chemical in the three diets over 36 weeks showed that the mean percent of the intended concentration ranged from 92.2% to 97.9%.

B. Systemic (Parental) Toxicity

1. Mortality and clinical signs: Seven animals in the F_0 300 ppm group died or were sacrificed during the study including: 1) one rat/sex died during the first week of the study; these animals were replaced; 2) male died during Week 24 with blood-stained discharge around the head; 3) one female died and another was sacrificed after convulsions, excessive salivation, pallor, abnormal respiration and limited use of hind limbs on Day 14 post partum; 4) two females were found dead on Day 17 post coitum and Day 17 post partum. Two additional animals were sacrificed, one male in the 30 ppm had decreased muscular control and a female in the control group had a marked deterioration in condition. Necropsy examinations did not reveal any treatment-related findings, however several of the animals were severely cannibalized.

Two additional females in the 300 ppm group were observed to have convulsions during the study but survived to termination.

In the F_1 generation, one female each in the 30 and 300 ppm groups was sacrificed during parturition due to prolonged vaginal bleeding. One female in the 300 ppm group was found dead on Day 16 post partum and another was sacrificed with convulsions, edema of the head and mutilation of the digits. One control group female was sacrificed after trauma to the buccal cavity.

2. Body weight, body weight gain, food consumption and food conversion efficiency: In the F_0 generation, body weight gain was statistically decreased in the 30 and 300 ppm group males as compared to the control groups at Week 1 and in the 300 ppm group males between Weeks 10 and 19. (Weight was recorded for 26 weeks.) Weight gain was decreased in all the treated female groups at Week 1 and in the 300 ppm group at Week 10 (time of mating). Food consumption (measured until pairing) was decreased in the 30 ppm group females at Week 1 and in the 300 ppm group males and females throughout the evaluation period. In general, food conversion efficiency was comparable between the treated and control groups. In the F_1 generation, body weight was lower in the 300 ppm group males at the time of selection and subsequent weight gain was low. Body weight in the 300 ppm group females was also low at selection; weight gain to pairing was unaffected. The data for these parameters are summarized in Table 4.

Table 4: Pre-mating Body Weight, Body Weight Gain, Food Consumption and Food Efficiency[a]

	Dosage Levels (ppm)			
	0	3	30	300
F_0 Generation Males - Entire Study				
Mean body weight (g)				
Week 0	138	137	135	140
1	205	203	197	172
10	548	536	530	512
19	668	655	651	622
26	740	721	705	687
Mean body weight gain (g)				
Week 0-1	67	66	62***	32***
0-10	410	399	395	372**
0-19	530	518	516	482**
0-26	602	584	570	547
Mean food consumption (g/rat/week)				
Week 1	182	183	174	124‡‡‡
10	208	203	205	199***
Mean food efficiency				
Week 1	37.0	35.7	35.7	25.0
10	6.7	7.4	8.2	9.3
F_0 Generation Females - Premating				
Mean body weight (g)				
Week 0	121	121	118	117
1	161	158	155	140
10	312	303	304	290
Mean body weight gain (g)				
Week 0-1	40	37**	37**	23***
0-10	191	182	186	173***
Mean food consumption (g/rat/week)				
Week 1	154	152	145‡	109‡‡‡
10	145	141	141	139**

Table 4 continued

Mean food efficiency				
Week 1	26.3	24.4	25.6	21.3
10	2.1	2.0	3.0	4.3
F1 Generation Males - Entire Study				
Mean body weight (g)				
Week 0	146	144	139	114‡‡‡
1	215	213	207	172
10	585	571	572	504
19	704	686	685	605
Mean body weight gain (g)				
0-10	439	427	433	390***
0-19	558	542	546	491***
Mean food consumption (g/rat/week)				
Week 1	165	168	167	152‡
10	224	211	223	205‡
Mean food efficiency				
Week 1	41.7	40.0	40.0	38.5
10	7.9	7.2	6.8	6.8

Table 4 continued

F1 Generation Females - Premating				
Mean body weight (g)				
Week 0	128	133	122	106‡‡‡
1	170	178	165	146
10	314	327	319	303
Mean body weight gain (g)				
Week 0-1	42	45**	43**	40
0-20	186	194	197	197
Mean food consumption (g/rat/week)				
Week 1	133	143	136	132
10	149	153	153	161
Mean food efficiency				
Week 1	32.3	31.3	31.3	30.3
10	6.3	5.5	6.7	5.3

a Body weight gain was calculated by the reviewer from Tables 2-3 (pages 83-85) and 44-45 (pages 135-137); food intake data was extracted from Tables 4-5 (pages 86-87) and 46-47 (pages 138-139) of the study report. Food consumption and food efficiency data were extracted from Tables 4-6 (pages 86-88) and 46-48 (pages 138-140)

* Change from Day 1 significantly different from control ($p<0.05$)
** Change from Day 1 significantly different from control ($p<0.01$)
*** Change from Day 1 significantly different from control ($p<0.001$)
‡ Significantly different from control ($p<0.05$)
‡‡ Significantly different from control ($p<0.01$)
‡‡‡ Significantly different from control ($p<0.001$)

During the F_0-F_1A gestation, body weight was significantly lower in the 300 ppm group females at Day 0 of gestation. The change in body weight from Day 0 to Day 20 was also significantly lower. During lactation, body weight in the 300 ppm group females was significantly lower. During the F_0-F_1B gestation period, only the 0, 3 and 30 ppm dosages were administered. While the body weights were lower in the treated groups during the gestation and lactation periods, the differences were not significant. During the F_1-F_2 gestation period, mean body weight for the 300 ppm group females was significantly decreased in comparison to the controls at Days 1 and 20. (Weight change was not statistically analyzed.) During lactation, body weights for the 300 ppm group females was significantly decreased in comparison to the controls at Days 1 and 25. Data for these parameters are summarized in Table 5.

Table 5: Body Weights During Gestation and Lactation[a]

	Dosage Levels (ppm)			
	0	3	30	300
F_0 Generation - Litter A				
Mean Body Weight (g)				
Day 0 of gestation	307	306	304	290‡
Day 20 of gestation	4[illegible]	458	458	418***
Day 1 post partum	352	357	354	323‡‡
Day 25 post partum	362	356	353	329‡‡
Mean body weight change				
Days 1-20 of gestation	156	152	154	128***
Days 1-25 post partum	10	-1	-1	5
F_0 Generation - Litter B				
Mean Body Weight (g)				
Day 1 of gestation	369	361	362	
Day 20 of gestation	528	513	516	
Day 1 post partum	406	399	40[illegible]	
Day 25 post partum	411	404	400	
Mean Body Weight Change				
Days 1-20 of gestation	159	152	154	
Days 1-25 post partum	5	5	-1	

Table 5 continued

F_1 Generation				
Mean Body Weight (g)				
Day 1 of gestation	322	332	321	299‡
Day 20 of gestation	474	482	478	441‡
Day 1 post partum	378	390	373	322‡‡
Day 25 post partum	377	375	361	327‡‡‡
Mean Body Weight Change				
Days 1-20 of gestation	152	150	157	142
Days 1-25 post partum	-1	-15	-12	-5

a Body weights were extracted from Tables 11 (page 93), 13 (page 95), 25 (page 109), 27 (page 111), 53 (page 145) and 55 (page 147) of the study report; body weight gains were calculated by the reviewer.
* Change from Day 0 or 1 significantly different from control ($p<0.05$)
** Change from Day 0 or 1 significantly different from control ($p<0.01$)
*** Change from Day 0 or 1 significantly different from control ($p<0.001$)
‡ Significantly different from control ($p<0.05$)
‡‡ Significantly different from control ($p<0.01$)
‡‡‡ Significantly different from control ($p<0.001$)

3. Test Substance Intake: Based on food consumption, body weight and dietary concentrations, the mg/kg/day doses at the beginning and end of the pre-mating period are presented in Table 6.

Table 6: Test Substance Intake[a]

	Dosage Levels (ppm)					
	Males			Females		
	3	30	300	3	30	300
F_0 Generation						
Week 1	0.46	4.48	33.96	0.47	4.56	36.39
Week 10	0.16	1.68	16.97	0.20	2.00	20.76
Mean of 10 weeks	0.25	2.54	24.73	0.28	2.77	27.51
F_1 Generation						
Week 1	0.40	4.14	45.60	0.39	4.06	44.94
Week 10	0.16	1.69	17.72	0.20	2.09	23.04
Mean of 10 weeks	0.24	2.54	27.32	0.26	2.71	29.28
Mean of two generations	0.25	2.54	26.03	0.27	2.74	28.40

a Extracted from Tables 7 (page 89) and 49 (page 141) of the study report; means calculated by the reviewer.

4. Reproductive Performance: Mating and fertility, as well as gestation lengths and gestation indices, in the F_0 generation were unaffected by the treatment. However, there was evidence of a treatment-related effect on the F_0-A offspring. Thirteen pups from nine litters in the 300 ppm group had convulsions between Days 14 and 20 post partum. The size of the litters in the 300 ppm group was significantly lower on Days 1 and 4 post partum and both the live birth and viability indices were reduced. The body weights of the pups before culling and weight gain to weaning were significantly lower in the 300 ppm group.

There was no evidence of a treatment-related effect on any of these parameters in the F_0-B generation in which the highest dosage group was 30 ppm.

In the F_1 generation, the percentage of animals mating was reduced in the 300 ppm group with a reduction in the fertility index. There was no effect on gestation length, gestation index or parturition. Four pups in three litters in the 300 ppm group had convulsions on Day 15 post partum and one on Day 18 post partum. Post-implantation survival and offspring postnatal viability to Day 4 post partum were significantly reduced in the 300 ppm group with reductions in postnatal litter size. Post-implantation survival was also reduced in the 3 ppm (significantly) and 30 ppm groups, but postnatal

viability was not affected. Mean pup weight for the 300 ppm group was reduced at Days 1 and 4 (precull) and weight gain to Day 26 was reduced. Results for the parental animals and the offspring are summarized in Table 7.

Table 7: Reproductive Performance[a]

	Dosage Levels (ppm)			
	0	3	30	300
F_0 Generation - Litter A				
Median precoital interval				
1-4 Days: Number (%)	27 (96)	29 (97)	30 (100)	29 (97)
Males				
Number paired	30	30	30	30[b]
Number mating	30	30	30	30
Percentage mating	100	100	100	100
Number pregnant	27	30	29	30
Conception rate (%)	90	100	97	100
Fertility index (%)	90	100	97	100
Females				
Number paired	30	30	30	30[b]
Number mating	30	30	30	30
Percentage mating	100	100	100	100
Number pregnant	27	30	29	30
Conception rate (%)	90	100	97	100
Fertility index (%)	90	100	97	100
Gestation Interval - Number (%)				
22 days	8 (33)	11 (37)	9 (31)	4 (14)[c]
22½ days	7 (29)	13 (43)	15 (52)	17 (59)
23 days	9 (38)	6 (20)	5 (17)	8 (28)

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Table 7 continued

Number of live litters born	26	30	29	29
Gestation index (%)	96	100	100	100
Mean Litter Size - Lactation Day				
1 (total)	14.8	14.0	14.1	12.1‡‡‡
4 (precull)	14.0	13.5	13.7	9.6‡‡‡
4 (postcull)	7.8	8.0	8.0	7.4
25	7.8	7.9	7.9	7.2
Live birth index (%)	98	98	100	83‡‡
Viability index (%)	97	99	97	89‡
Lactation index (%)				
Day 7	100	99	98	100
Day 25	100	99	98	97
Sex Ratio (M:F) - Day 1	1:1.02	1:1.00	1:0.90	1:0.96
Male Mean Pup Weight (g) - Lactation Day				
1	6.7	6.8	6.5	6.3‡
4 (precull)	9.9	10.2	9.7	8.8‡‡
4 (postcull)	10.0	10.2	9.8	8.9
25	90.1	90.2	86.7	69.9‡‡‡ ***
Female Mean Pup Weight (g) - Lactation Day				
1	6.3	6.5	6.1	5.9‡
4 (precull)	9.3	9.7	9.2	8.3‡‡‡ ***
4 (postcull)	9.3	9.8	9.3	8.4
25	84.4	85.1	81.9	66.3‡‡‡ ***

Table 7 continued

F_0 Generation - Litter B				
Median Precoital Interval				
1-4 Days: Number (%)	28 (97)	28 (93)	30 (100)	
Males				
Number paired	30	30	30	
Number mating	29	30	30	
Percentage mating	97	100	100	
Number pregnant	29	30	30	
Conception rate (%)	100	100	100	
Fertility index (%)	97	100	100	
Females				
Number paired	30	30	30	
Number mating	29	30	30	
Percentage mating	97	100	100	
Number pregnant	29	30	30	
Conception rate (%)	100	100	100	
Fertility index (%)	97	100	100	
Gestation Interval - Number (%)				
22 days	7 (24)	9 (30)	7 (23)	
22½ days	12 (41)	9 (30)	13 (43)	
23 days	6 (21)	7 (23)	5 (17)	
23½ days	4 (14)	4 (13)	4 (13)	
24 days	0	1 (3)	1 (3)	
Number of live litters born	29	30	30	
Gestation index (%)	100	100	100	

Table 7 continued

Mean Litter Size - Lactation Day				
1 (total)	15.8	15.1	14.9	
4 (precull)	14.0	14.7	14.6	
4 (postcull)	8.0	7.9	8.0	
25	7.9	7.8	8.0	
Live birth index (%)	99	99	99	
Viability index (%)	98	99	99	
Lactation index (%)				
Day 7	100	96	100	
Day 25	99	95	100	
Sex Ratio (M:F) - Day 1	1:0.97	1:1.02	1:1.01	
Male Mean Pup Weight (g) - Lactation Day				
1	6.6	6.8	6.7	
4 (precull)	9.6	9.82	9.6	
4 (postcull)	9.9	10.0	9.8	
25	90.1	90.4	88.8	
Female Mean Pup Weight (g) - Lactation Day				
1	6.2	6.4	6.3	
4 (precull)	8.8	9.3	8.9	
4 (postcull)	9.3	9.5	9.2	
25	84.2	85.1	82.6	
F1 Generation				
Median Precoital Interval				
1-4 Days: Number (%)	25 (83)	27 (93)	28 (93)	21 (84)
5-8 Days: Number (%)	3 (10)	1 (3)	1 (3)	4 (16)
Males				
Number paired	30	30	30	30

Table 7 continued

Number mating	30	29	29	25
Percentage mating	100	97	97	83*
Number pregnant	27	29	29	25
Conception rate (%)	90	100	93	96
Fertility index (%)	90	97	90	80
Females				
Number paired	30	30	30	30
Number mating	30	29	30	25
Percentage mating	100	97	100	83*
Number pregnant	27	29	27	24
Conception rate (%)	90	100	93	96
Fertility index (%)	90	97	93	80
Gestation Interval - Number (%)[d]				
22 days	9 (35)	3 (10)	3 (11)	3 (13)
22½ days	9 (35)	13 (45)	15 (56)	12 (50)
23 days	6 (23)	7 (24)	7 (26)	5 (21)
23½ days	2 (8)	3 (10)	2 (7)	4 (17)
24 - 25½ days	0	3 (9)	0	0
Number of live litters born	26	28	27	23
Gestation index (%)	100	97	96	96
Mean Litter Size - Lactation Day				
1 (total)	13.6	11.9	13.4	11.8
4 (precull)	13.3	12.3	13.0	10.7
4 (postcull)	7.7	7.7	7.9	7.3
25	7.7	7.4	7.8	7.2
Mean Number Implantation Sites[e]	14.9	14.1	15.1	14.6

Table 7 continued

Post-implantation survival index	90	84‡	85	81‡
Live birth index (%)	100	98	99	78‡‡‡
Viability index (%)	98	99	98	73‡‡‡
Lactation index (%)				
Day 7	100	98	98	98
Day 25	100	97	98	92
Sex Ratio (M:F) - Day 1	1:0.99	1:0.97	1:1.10	1:0.93
Male Mean Pup Weight (g) - Lactation Day				
1	6.5	6.7	6.5	6.0‡‡
4 (precull)	9.8	10.3	9.9	8.6‡
4 (postcull)	9.8	10.2	9.9	9.0
25	84.3	88.1	84.7	66.7‡‡‡ ***
Female Mean Pup Weight (g) - Lactation Day				
1	6.1	6.3	6.1	5.5‡‡‡
4 (precull)	9.1	9.7	9.2	7.8‡
4 (postcull)	9.1	9.8	9.2	7.9
25	79.3	83.2	79.6	62.0‡‡‡ ***

a Extracted from Tables 9-10 (pages 91-92), 12 (page 94), 14-18 (pages 96-100), 23-24 (pages 107-108), 26 (page 110), 28-32 (page 112-116), 51-52 (pages 143-144), 54 (page 146) and 56-60 (pages 148-152)
b One male and one female were found dead during Week 1; they were replaced
c This group excludes one animal found dead Day 17 post coitus
d Excludes female sacrificed Day 18 post coitum in control group and female in the 30 ppm group sacrificed on Day 22 post coitum
e Calculated for F_1 generation only
‡ Significantly different from control (<0.05)
‡‡ Significantly different from control (<0.01)
‡‡‡ Significantly different from control (p<0.001)
* Change from Day 1 significantly different from control (p<0.05)
** Change from Day 1 significantly different from control (p<0.01)
*** Change from Day 1 significantly different from control (p<0.001)

5. Offspring Development

There was a statistically significant delay in the onset of tooth eruption in the F_1-A offspring of the 300 ppm group. Development in the F_1-B offspring of the treated groups was comparable to the controls. In the F_2 generation, pinna unfolding was slightly (but not significantly) delayed in the

300 ppm group. The data for these two parameters in the affected generations are presented in Table 8.

Table 8: Selected Developmental Parameters[a]

	Dosage Levels (ppm)			
	0	3	30	300
F_1A Generation				
Pinna Unfolding				
Day of onset	2.2	2.2	2.1	2.3
Day of completion	3.4	3.3	3.4	3.5
Tooth Eruption				
Day of onset	9.7	9.4	9.7	10.4*
Day of completion	11.7	11.6	11.6	12.0
F_2 Generation				
Pinna Unfolding				
Day of onset	2.7	2.5	2.7	3.0
Day of completion	3.3	3.3	3.5	3.8
Tooth Eruption				
Day of onset	9.3	9.1	9.6	9.3
Day of completion	11.4	10.6	11.1	11.3

a Extracted from Tables 19 (page 101) and 61 (page 153) of the study report
* Significantly different from control ($p<0.05$)

6. Necropsy Results

Gross Necropsy - There were no treatment-related effects at any of the gross necropsy examinations of parental animals or offspring except the incidence of small pups in the 300 ppm group was increased at the necropsy of F_2 offspring culled at Day 4 [7/51 (14%) in the 300 ppm group vs 1/145 (0.7%) in the control group].

Organ Weights of Parental Animals - The absolute and relative weights of the liver and thyroid were significantly increased in the 30 and 300 ppm group males and females in the F_0 and F_1 parental animals (30 ppm males in F_0 generation were not significantly increased). The absolute and relative weights of the ovaries were decreased in the 300 ppm group females in the

F_0 generation. In the F_1 generation, the absolute weight of the pituitary gland was decreased in the 30 and 300 ppm group females and the relative weight was decreased in all the treated female groups. The absolute weight of the epididymides and ovaries were increased in the 300 ppm group males and females, respectively; the relative weights for these organs were not statistically different from the control group. The absolute and relative weights of the testes in the 300 ppm group males were decreased. The organ weight data from the study report are presented in Table 9.

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Table 9: Absolute and Relative Weight of Selected Organs[a]

		Dosage Levels (ppm)							
		Males				Females			
		0	3	30	300	0	3	30	300
F_0 Generation									
Terminal body weight		736.4	716.1	698.1	677.7*	392.1	383.8	389.8	352.0**
Pituitary	A	0.013	0.011	0.011	0.012	0.016	0.015	0.016	0.013*
	R	0.0018	0.0016	0.0016	0.0018	0.0042	0.0039	0.0040	0.0038
Liver	A	24.8	24.3	25.5	34.0**	16.4	15.6	17.8*	19.1**
	R	3.36	3.38	3.66*	5.00**	4.19	4.07	4.57**	5.45**
Thyroids	A	0.029	0.029	0.033*	0.044**	0.026	0.023	0.030	0.036**
	R	0.0040	0.0040	0.0048*	0.0064**	0.0066	0.0062	0.0078	0.0103**
Ovaries	A					0.126	0.132	0.131	0.090**
	R					0.0324	0.0345	0.0335	0.0255**
F_1 Generation									
Terminal body weight		694.1	674.8	674.7	594.8**	369.8	385.1	367.8	358.3
Pituitary	A	0.012	0.011	0.011	0.010	0.016	0.015	0.013**	0.012**
	R	0.0017	0.0017	0.0016	0.0018	0.0045	0.0039*	0.0036**	0.0035**
Liver	A	24.0	23.1	27.1**	28.8**	14.0	14.4	15.5**	19.8**
	R	3.46	3.42	4.01**	4.82**	3.79	3.74	4.23**	5.52**
Thyroids	A	0.030	0.030	0.038**	0.044**	0.023	0.024	0.027*	0.033**
	R	0.0044	0.0044	0.0057**	0.0074**	0.0064	0.0062	0.0075*	0.0094**
Ovaries	A					0.134	0.128	0.131	0.115*
	R					0.0364	0.0333	0.0359	0.0330
Testes	A	3.96	3.89	3.99	3.70**				
	R	0.576	0.579	0.598	0.628*				
Epididymides	A	1.576	1.504	1.507	1.445*				
	R	0.2287	0.2243	0.2266	0.2451				

a Extracted from Tables 38-41 (pages 124-127) and 66-69 (pages 162-165) of the study report.
* Significantly different from control (<0.05)
** Significantly different from control (<0.01)
*** Significantly different from control ($p<0.001$)

Histopathology - There was a significantly increased incidence of centriacinar fatty vacuolation in the livers of the 300 ppm group females in both the F_0 and F_1 generations. In the F_0 generation, there was an increased incidence of follicular

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epithelial hypertrophy of the thyroid glands in the 30 ppm group males (not statistically significant) and in the 300 ppm group males and females. In the F_1 generation, the incidence of this finding was increased in all the treated males (significantly increased in the 300 ppm group only) and in the 30 and 300 ppm group females (significantly increased). The study report states that the incidence was low in the 3 ppm group and could be considered within the range that might be expected in an organ which is very sensitive to stimulus in the rat. The incidences of these histopathological changes are presented in Table 9.

Table 9: Incidence of Histopathological Changes in the Liver and Thyroid

	Dosage Levels (ppm)							
	Males				Females			
	0	3	30	300	0	3	30	300
F_0 Generation								
Number Examined	30	30	29	29	29	30	30	26
Liver								
Centriacinar fatty vacuolation	0	0	0	0	0	0	0	9***
Thyroids								
Follicular epithelial hypertrophy	0	0	2	10***	0	0	0	6**
F1 Generation								
Number Examined	30	30	30	30	29	30	29	27
Liver								
Centriacinar fatty vacuolation	0	0	0	0	1	1	2	6*
Thyroids								
Follicular epithelial hypertrophy	0	2	3	9***	0	0	7*	15***

a Extracted from Tables 42 (pages 128-130) and 70 (pages 166-167) of the study report.
* Significantly different from control (<0.05)
** Significantly different from control (<0.01)
*** Significantly different from control (p<0.001)

7. Conclusion from Study Report

The study report concluded that the No Observed Effect Level was 3 ppm.

III. DISCUSSION/CONCLUSIONS

In a two-generation reproduction study, 30 males and 30 females per group of CD rats received M&B 46030 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). The protocol was a standard reproduction study with litters culled to 4 animals per sex on post partum Day 4 and with two matings of the F_0 generation. In addition, physical development was assessed on a litter basis by recording the day of onset and completion of pinna unfolding, hair growth, tooth eruption and eye opening.

In the parental animals (F_0 and F_1 generations) of the 300 ppm group, there was increased mortality and clinical signs of toxicity. Seven animals in the F_0 generation died or were sacrificed during the study with evidence of convulsions pre-mortem; two which survived were also observed to have convulsions. Three animals in the 300 ppm group of the F_1 generation died or were sacrificed during the study. One animal each of the 30 ppm group in both the F_0 and F_1 generations were sacrificed during the study.

In the F_0 generation, body weight gain was statistically decreased in the 30 and 300 ppm group males as compared to the control groups at Week 1 and in the 300 ppm group males between Weeks 10 and 19. Weight gain was decreased in all the treated female groups at Week 1 and in the 300 ppm group at Week 10. Food consumption was decreased in the 30 ppm group females at Week 1 and in the 300 ppm group males and females throughout the evaluation period. In general, food conversion efficiency was comparable between the treated and control groups. In the F_1 generation, body weight was lower in the 300 ppm group males at the time of selection and subsequent weight gain was low. Body weight in the 300 ppm group females was also low at selection; weight gain to pairing was unaffected.

During the F_0-F_1A gestation, body weight was significantly lower in the 300 ppm group females at Day 0 of gestation. The change in body weight from Day 0 to Day 20 was also significantly lower. During lactation, body weight in the 300 ppm group females was significantly lower. During the F_0-F_1B gestation period, there were no significant differences. During the F_1-F_2 gestation period, mean body weight for the 300 ppm group females was significantly decreased in comparison to the controls at Days 1 and 20. During lactation, body weights for the 300 ppm group females was significantly decreased in comparison to the controls at Days 1 and

25.

Mating and fertility, as well as gestation lengths and gestation indices, in the F_0 generation were unaffected by the treatment. However, there was evidence of a treatment-related effect on the F_0-A offspring. Thirteen pups from nine litters in the 300 ppm group had convulsions between Days 14 and 20 post partum. The size of the litters in the 300 ppm group was significantly lower on Days 1 and 4 post partum and both the live birth and viability indices were reduced. The body weights of the pups before culling and weight gain to weaning were significantly lower in the 300 ppm group. There was no evidence of a treatment-related effect on any of these parameters in the F_0-B generation. In the F_1 generation, the percentage of animals mating was reduced in the 300 ppm group with a reduction in the fertility index. There was no effect on gestation length, gestation index or parturition. Four pups in three litters in the 300 ppm group had convulsions on Day 15 post partum and one on Day 18 post partum. Post-implantation survival and offspring postnatal viability to Day 4 post partum were significantly reduced in the 300 ppm group with reductions in postnatal litter size. Post-implantation survival was also reduced in the 3 ppm (significantly) and 30 ppm groups, but postnatal viability was not affected. Mean pup weight for the 300 ppm group was reduced at Days 1 and 4 (precull) and weight gain to Day 26 was reduced.

There was a statistically significant delay in the onset of tooth eruption in the F_1-A offspring of the 300 ppm group. Development in the F_1-B offspring of the treated groups was comparable to the controls. In the F_2 generation, pinna unfolding was slightly (but not significantly) delayed in the 300 ppm group.

There were no treatment-related effects at any of the gross necropsy examinations of parental animals or offspring except the incidence of small pups in the 300 ppm group was increased at the necropsy of F_2 offspring culled at Day 4 [7/51 (14%) in the 300 ppm group vs 1/145 (0.7%) in the control group].

The absolute and relative weights of the liver and thyroid were significantly increased in the 30 and 300 ppm group males and females in the F^0 and F_1 parental animals (30 ppm males in F_0 generation were not significantly increased). The absolute and relative weights of the ovaries were decreased in the 300 ppm group females in the F_0 generation. In the F_1 generation, the absolute weight of the pituitary gland was decreased in the 30 and 300 ppm group females and the relative weight was decreased in all the treated female groups. The absolute weight of the epididymides and ovaries were increased in the 300 ppm group males and females, respectively; the relative weights for these organs were not statistically different from the control group. The absolute and relative weights of the testes in the 300 ppm group males were

decreased.

There was a significantly increased incidence of centriacinar fatty vacuolation in the livers of the 300 ppm group females in both the F_0 and F_1 generations. In the F_0 generation, there was an increased incidence of follicular epithelial hypertrophy of the thyroid glands in the 30 ppm group males (not statistically significant) and in the 300 ppm group males and females. In the F_1 generation, the incidence of this finding was increased in all the treated males (significantly increased only in the 300 ppm group) and in the 30 and 300 ppm group females (significantly increased).

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 development in the F_1 and F_2 litters. The NOEL for reproductive toxicity was 30 ppm (2.54 mg/kg/day for males and 2.74 mg/kg/day for females).

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FINAL

DATA EVALUATION REPORT

M&B 46030 (FIPRONIL)

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jennifer E Alexander Date 5/16/94
Jennifer E. Alexander, B.S.

Independent Reviewer Nancy E. McCarroll Date 5/16/94
Nancy E. McCarroll, B.S.

QA Manager William L. McLellan Date 5/16/94
William L. McLellan, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-54
Clement Number: 231
Project Officer: Caroline Gordon

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Salmonella

Fipronil

MUTAGENICITY STUDIES

EPA Reviewer: Virginia Dobozy, V.M.D., M.P.H. Signature: Virginia Dobozy
Review Section I, Toxicology Branch II Date: 5/17/94
Health Effects Division (H7509C)
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T Backus
Review Section II, Toxicology Branch II Date: 5/17/94
Health Effects Division (H7509C)

DATA EVALUATION REPORT

TEST MATERIAL: M&B 46030 (Fipronil)

TOX. CHEM. NO.: Not provided

P.C. CODE: 129121

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

MRID Number: 429186-52

SYNONYM(S): Fipronil; 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethylsulphinylpyrazole

SPONSOR: Rhone-Poulenc Ltd, Essex, United Kingdom

TESTING FACILITY: Microtest Research Limited, York, United Kingdom

TITLE OF REPORT: Study to Determine the Ability of M&B 46030 to Induce Mutation in Four Histidine-Requiring Strains of Salmonella typhimurium

AUTHOR(S): C.B. Clare

STUDY NUMBER: MAB 20/S

REPORT ISSUED: October 5, 1988

EXECUTIVE SUMMARY: In two independently performed Salmonella typhimurium/mammalian microsome reverse gene mutation assays, strains TA1535, TA1537, TA98, or TA100 were exposed to 0.8, 4, 20, 100, or 500 µg/plate M&B 46030 (initial trial) or 25, 50, 100, 200, or 400 µg/plate (confirmatory trial) both in the presence and absence of S-9 activation. The S9 fraction was derived from Aroclor 1254-induced Wistar rat livers and the test substance was delivered to the test system in dimethyl sulfoxide. These doses were derived based on a range-finding study in which M&B 46030 caused substantial toxicity at 1000 and 5000 µg/plate.

Cytotoxicity was seen in the majority of strains at 500 µg/plate +/- S9 in the initial assay; therefore, the high dose was lowered in the confirmatory assay to 400 µg/plate +/- S9. **M&B 46030 was assayed up to a sufficiently high level**

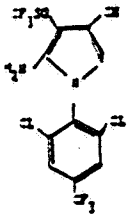
and there was no evidence of mutagenicity in any strain. Results with the positive controls were adequate to demonstrate assay sensitivity.

This study is classified as **Acceptable** and satisfies the guideline requirement for a gene mutation study [84-2].

A. MATERIALS:

1. Test Material: M&B 46030

Description: White crystalline solid
Identification numbers: Lot number IGB 438
Purity: 90.6% (see Addendum to the Study Report, p. 39)
Structure:



Stability: Not reported
CAS number: Not reported
Receipt date: April 18, 1988
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored in the dark at room temperature; dosing solutions were prepared immediately prior to use; analytical determinations were not performed on the dosing solutions.

2. Control Materials:

Solvent/final concentration: DMSO/0.1 mL/plate

Positive:

Nonactivation:

Sodium azide	2	µg/plate TA1535, TA100
9-Aminoacridine	50	µg/plate TA1537
2-Nitrofluorene	50	µg/plate TA98

Activation:

2-Aminoanthracene	5	µg/plate TA98, TA100 only

3. Activation: S9 derived from male Wistar (150-300g)

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

The rat liver S9 homogenate was prepared by the performing laboratory. The S9 mix was prepared as follows:

Component:	Volume/100 mL:
500 mM Sodium phosphate buffer (pH 7.4)	57.56
60 mg/mL Glucose-6-phosphate	10.00
25 mg/mL NADP	10.00
250 mM $MgCl_2$	8.00
1 mg/mL L-histidine HCl	2.00
1 mg/mL D-biotin	2.44
S-9	10.00 (10%)

Note: For the nonactivated tests, the above cofactor solution was prepared with 30 mL of sterile distilled water substituted for glucose-6-phosphate, NADP, and the S9 homogenate.

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 _____ TA1538
list any others:

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (histidine dependence, rfa mutation, and ampicillin resistance)? Yes.

5. Test Compound Concentrations Used:

Mutation assay:

(a) Preliminary Cytotoxicity Assay: Five doses (8, 40, 200, 1000, and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Three plates were used per dose, per condition for the test substance, and five plates were used per condition for the negative and positive controls.

(b) Mutation assay:

- Initial Trial: Five doses (0.8, 4, 20, 100, and 500 µg/plate) were tested with and without S9 activation using all strains. Three plates were used per strain, per dose, per condition for the test substance, and five plates were used with and without S9 activation for the negative and positive controls.

- Confirmatory Trial: Five doses (25, 50, 100, 200, and 400 µg/plate) were evaluated as described for the initial trial.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: x Standard plate test
___ Pre-incubation (_____) minutes
___ "Prival" modification
___ Spot test

2. Mutation Assays: A mixture containing 0.1 mL of the appropriate concentration of the test material, solvent or positive control, 0.1 mL of an overnight bacterial culture of the appropriate tester strain, and 1.0 mL of the S9-cofactor mix or cofactor solution was added to 2.5 mL of unsupplemented top agar. The contents of the tubes were mixed and poured onto minimal Davis agar; plates were incubated at 37°C for at least two days. Following incubation, revertant colonies were counted, and means and standard deviations were determined. In addition, the background lawn growth was evaluated for signs of cytotoxicity.

3. Evaluation Criteria:

(a) Valid assay: The assay was considered valid if: (1) the mean number of revertant colonies in the solvent control fell within the provided expected spontaneous range for each tester strain; (2) the positive controls induced a positive response; and (3) no more than 5% of the plates were lost due to contamination or other unexpected events.

(b) Positive response: The test material was considered positive if it induced a reproducible, dose-related increase in mutant colonies of strains TA98 or TA100 that was at least ≥ 2-fold higher than the solvent control or ≥ 3-fold higher than the solvent control for strains TA1535 or TA1537. The increases must also be accompanied "by significant F-statistics"; the p-value was not reported.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: No data were reported from the preliminary cytotoxicity assessment conducted with 8 to 5000 µg/plate +/– S9 M&B 46030. The study author indicated that the two highest dose levels with or without S9 activation (1000 and 5000 µg/plate) were cytotoxic. Accordingly, a dose range of 0.8 to 500 µg/plate was selected for the nonactivated and S9-activated mutation assay.

2. Mutation Assay:

(a) Initial Trial: Cytotoxicity, as indicated by a reduction in the background lawn of growth, was observed at the highest nonactivated dose (500 µg/plate) in all strains and at 500 µg/plate + S9 in all nonactivated strains but TA100 (Table 1). However, no appreciable increase in the number of revertants was seen at any concentration. By contrast to the uniformly negative results with the test

Table 1: Representative Results of the Initial Salmonella typhimurium/Mammalian Microsome Mutation Assay with M&B 46030

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	0.1 mL	-	20±2	11±4	23±6	72±12
	0.1 mL	+	19±10	10±1	36±3	131±8
Positive Controls						
Sodium azide	2 µg	-	521±12	--	--	639±115
9-Aminoacridine	50 µg	-	--	865±62	--	--
2-Nitrofluorene	50 µg	+	--	--	928±140	--
2-Aminoanthracene	5 µg	+	--	--	413±27	806±83
Test Material						
M&B 46030	100 µg[b]	-	27±2	11±3	16±3	80±6
	500 µg[c]	-	17±2	14±5	20±3	60±7
	100 µg[b]	+	9±4	17±5	40±2	137±12
	500 µg[c]	+	9±1	15±6	49±13	141±27

[a]Means and standard deviations of the counts from triplicate plates for the test material or quintuplicate plates for the solvent and positive controls.
[b]Findings for lower doses (0.8, 4, or 20 µg/plate +/- S9) did not suggest a mutagenic effect.
[c]The highest dose tested; thinning of the background lawn of growth reported for all strains at 500 µg/plate -S9 and for all strains except TA100 at 500 µg/plate +S9.

Note: Data were extracted from the study report, p. 20-27.

material, the positive controls produced marked increases in histidine revertants in the corresponding tester strains.

(b) Confirmatory trial: Representative results from the confimatory assay are presented in Table 2. Due to cytotoxicity at 500 µg/plate +/- S9 in the initial trial, the high dose selected for the confirmatory assay was lowered to 400 µg/plate +/- S9. As shown, M&B 46030 was neither cytotoxic nor mutagenic at any level. Allstrains responded to the mutagenic action of the appropriate nonactivated or S9-activated positive control.

From the overall results, the study author concluded that M&B 46030 was not mutagenic in this bacterial assay system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. M&B 46030 was evaluated over a concentration range that included a cytotoxic level (500 µg/plate +/- S9), but failed to induce a mutagenic response in two independently performed trials. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the responses induced by the nonactivated positive controls. Although the S9-activated positive control (5 µg/plate 2AA) was not tested with strains TA1535 or TA1537, the results with strains TA98 and TA100 clearly indicated that the S9 fraction was biologically active. We, therefore, assess that the lack of testing strains TA1535 or TA1537 with a promutagen did not affect the outcome of the study. The study provided acceptable evidence that M&B 46030 is not mutagenic in this bacterial test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated October 7, 1988.)

F. APPENDIX ATTACHED? No.

Table 2: Representative Results of the Confirmatory Salmonella typhimurium/Mammalian Microsome Mutation Assay with M&B 46030

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	0.1 mL	-	16±3	8±3	18±2	76±7
	0.1 mL	+	23±4	11±4	31±8	107±8
Positive Controls						
Sodium azide	2 µg	-	314±20	--	--	731±45
9-Aminoacridine	50 µg	-	--	524±70	--	--
2-Nitrofluorene	50 µg	-	--	--	996±50	--
2-Aminoanthracene	5 µg	+	--	--	330±58	411±32
Test Material						
M&B 46030	400 µg[b]	-	20±6	9±4	18±3	59±6
	400 µg[b]	+	19±6	13±4	36±2	123±5

[a]Means and standard deviations of the counts from triplicate plates for the test material or quintuplicate plates for the positive and solvent controls.
[b]The highest dose tested. Findings for lower levels (25, 50, 100, or 200 µg/plate +/- S9) did not suggest a mutagenic effect.

Note: Data were extracted from the study report, p. 29-36.

C11086

FINAL

DATA EVALUATION REPORT

M&B 46030
(Fipronil)

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster V79 Cells (HGPRT)

Prepared for:

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

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S. Date 5/11/94

Independent Reviewer Laura P. Schuler, B.A. Date 5/11/94

QA Manager William L. McLellan, Ph.D. Date 5/11/94

Contract Number: 68D10075
Work Assignment Number: 3-54
Clement Number: 230
Project Officer: Caroline Gordon

011086

FIPRONIL

GUIDELINE SERIES 84-2: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

EPA Reviewer: Virginia Dobozy, V.M.D., M.P.H. Signature: Virginia Dobozy
Review Section I,
Toxicology Branch II/HED (7509C) Date: 5/17/94
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T Backus
Review Section II,
Toxicology Branch II/HED (7509C) Date: 5/17/94

DATA EVALUATION REPORT

TEST MATERIAL: M&B 46030 (Fipronil)

TOX. CHEM. NO.: Not provided

PC CODE: 129121

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster V79 cells (HGPRT)

MRID NUMBER: 429186-51

SYNONYM/CAS NUMBER: Fipronil; 5-amino-1-(2,6-dichloro-4-trifluoromethyl-phenyl)-3-cyano-4-trifluoromethylsulphinylpyrazole

SPONSOR: Rhone-Poulenc Agrochimie, Lyon, France

TESTING FACILITY: Pharmaco-LSR Ltd., Suffolk, England

TITLE OF REPORT: M&B 46030: Investigation Of Mutagenic Activity At The HGPRT Locus In A Chinese Hamster V79 Cell Mutation System

AUTHOR: J.M. Lloyd

STUDY NUMBER(S): Authorized Final Report: LSR Report No. 90/RHA304/0418; Amended Final Report No. 93/RHA304/0566

REPORT ISSUED: Authorized Final Report: December 5, 1990; Amended Final Report: June 29, 1993.

EXECUTIVE SUMMARY: -In two independent Chinese hamster V79 cell HGPRT forward gene mutation assays, M&B 46030 was assayed at intended concentrations of 0.8, 4, 20, 100, or 500 µg/mL +/- S9; actual concentrations based on analytical concentrations ranged from 1.13 to 385.65 µg/mL in Trial 1 and 1.62 to 317.95 µg/mL in Trial 2. The S9 was derived from Arochlor 1254-induced CD rat livers, and M&B 46030 was delivered to the test system in dimethyl sulfoxide.

M&B 46030 was neither cytotoxic nor mutagenic in the presence or absence of S9 up to insoluble levels (≥100µg/mL +/- S9). Findings with the positive controls confirmed the sensitivity of the test system to detect mutagenesis.

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FIPRONIL

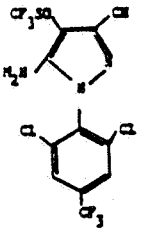
MAMMALIAN CELLS IN CULTURE GENE MUTATION

The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian cell forward gene mutation study (84-2).

A. MATERIALS:

1. Test Material: M&B 46030

   Description: Off-white powder
   Identification No.: Batch No. JJW2092/1
   Purity: 97.2%
   Receipt date: July 13, 1989
   Stability: Not determined
   Structure:



   CAS No.: Not provided
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: The sub-sample of the test material used in this assay was stored at room temperature in the dark. Dosing solutions were prepared fresh for each phase of testing; achieved concentrations were determined analytically.

2. Medium: Dulbecco's modified Eagle's minimal essential medium with 10% fetal calf serum and antibiotics

3. Control Materials:

   Solvent/final concentration: DMSO/1%

   Positive:

   Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in sterile distilled water to yield a final concentration of 1000 µg/mL.

   Activation (concentrations, solvent): Dimethylbenzanthracene (DMBA) was prepared in an DMSO to yield a final concentration of 10 µg/mL.

   Note: DMBA was also included in the nonactivated phase of testing.

4. Activation: S9 derived from young male CD (≈200 g body weight; Charles River Breeding Laboratory, Kent, England)

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 liver homogenate was prepared by the performing laboratory, stored at -196°C and used within 6 months.

The S9 mix contained the following components:

Component	Quantity
0.4 M $MgCl_2$/1.65 M KCl	0.2 mL
0.1 M Glucose-6-phosphate (sodium salt)	0.5 mL
0.1 M NADP (sodium salt)	0.4 mL
S9 homogenate	3 mL
0.1 M phosphate buffer (pH 7.4)	to a final volume of 10 mL

5. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
_____ Chinese hamster ovary cells
__x__ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Not reported.

6. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) 10 µg/mL 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

7. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Five concentrations (0.8, 4, 20, 100 and 500 µg/mL) were evaluated with and without S9 activation. Duplicate cultures were assayed per dose, per condition.

(b) Mutation assays: Two independent assays were performed with five concentrations (0.8, 4, 20, 100, and 500 µg/ml +/- S9) of the test material.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, solvent or positive controls for: 3 hours (nonactivated) 3 hours (activated)

(b) After washing, cells were cultured for 7 days (expression period) before cell selection.

(c) After expression, 10^5 cells/dish (3 dishes) were added to each of triplicate dishes and were cultured for 6 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes) were cultured for 6 days without selection medium to determine cloning efficiency (CE).

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria: No criteria were provided to evaluate assay validity, a positive response, or the biological significance of the findings.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The preliminary cytotoxicity assay was performed with five test material doses ranging from 0.3 to 500 µg/mL +/-S9. The report stated that the two highest doses (100 and 500 µg/mL) with and without S9 activation formed homogenous suspensions with the culture medium. There was, however, no indication of cytotoxicity at any nonactivated or S9-activated concentration. Based on these findings, comparable doses were selected for the mutation assays.

2. Mutation Assays:

(a) Nonactivated conditions: Representative data from the two nonactivated trials are presented in Table 1. Our reviewers noted the borderline acceptable absolute posttreatment survival (-49%) for the solvent controls in both trials but concluded that the outcome of the study was not adversely affected. As further shown, M&B 46030 was neither cytotoxic nor mutagenic in the absence of S9 activation. Although sporadic increases in the mutation frequency (MF) were seen, they were not dose-related or consistent. By contrast, the positive control (1000 µg/mL EMS) induced marked increases in total mutant colonies and the MF in both trials.

TABLE 1. Representative Results from the Chinese Hamster V79 Cell Forward Gene Mutation Assays with M & B 46030 in the Absence of S9 Activation

Substance	Dose	Average Percent Relative Survival (posttreatment)[a]	Average Total Mutant Colonies[a]	Average Percent Absolute Cloning Efficiency[a]	Average Mutation Frequency/ 10^5 survivors[b]
Solvent Control					
Dimethyl sulfoxide	1%	100.0[d] (49.8)[c,d]	0.3	106.9	0.3
		100.0[e] (49.1)[d,e]	0.0	89.5	0.0
Positive Control[f]					
Ethyl methane sulfonate	1000 µg/mL	60.9[c]	93.7	83.6	112.1
		45.0[e]	85.2	73.1	116.5
Test Material[g]					
M&B 46030	20/23.3 µg/mL[h]	96.4[c]	1.5	126.9	1.2
	500/385.7 µg/mL	>100	2.7	96.1	2.8
	20/23.9 µg/mL[h]	86.4[e]	0.0	62.9	0.0
	500/317.9 µg/mL	100.9	0.5	91.9	0.5

[a]Average values from six plates subcultured from duplicate flasks per group; calculated by our reviewers.

[b]Mutation Frequency = $\frac{\text{Average Total Mutant Colonies}}{\text{No. of Cells Plated } (1\text{X}10^5) \text{ X Absolute Cloning Efficiency}} \text{X}10^5$; calculated by our reviewers.

[c]Results from Trial 1

[d]Values in () = Absolute percent survival.

[e]Results from Trial 2

[f]Cultures were also exposed to 10 µg/mL dimethylbenzanthracene; these findings were largely negative.

[g]Presented values are the intended/actual concentrations from analytical determinations.

[h]Based on information from the preliminary study and the analytical report, our reviewers assumed that this level was the highest soluble dose, and that higher levels (100 or 500 µg/mL) formed homogeneous suspensions with the culture medium. Findings from an intermediate concentration (100 µg/mL) and lower doses (0.8 or 4 µg/mL) in both trials did not suggest a mutagenic effect.

Note: Data were extracted from the study report, pp. [illegible], 29, and 30

(b) S9-activated conditions: In agreement with the nonactivated results, there was no reproducible evidence that M&B 46030 was cytotoxic or that exposure of V79 cells to the test material increased the frequency of forward mutations at the HGPRT locus (Table 2). Marked increases in the MF were, however, observed in cultures treated with the positive control (10 µg/mL DMBA).

3. Analytical Determinations: The analysis of dosing solutions prepared for the two mutation assays indicated that lower levels (0.8, 4.0, and 20 µg/mL) contained appreciably higher test material concentrations than intended (~141, 134, and 116% of the target for Trial 1 and ~203, 144, and 119% of the target for Trial 2, respectively). Achieved levels for the 100 µg/mL dosing suspensions were within 10% of the target; however, the high dosing suspensions (500 µg/mL) contained appreciably less test material than intended (385.65 µg/mL -- 77% in Trial 1 and 317.95 µg/mL -- 64% in Trial 2). The study author attributed the differences between intended and actual concentrations to compound insolubility. We assess that since M&B 46030 was assayed to insoluble levels with no effect, the discrepancies between actual and intended concentrations did not compromise the study.

Based on the overall findings, the study author concluded that M&B 46030 was not mutagenic in this mammalian cell gene mutation assay.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the study was properly conducted and we agree with the study author's conclusions: M&B 46030 was assayed up to insoluble levels (≥~100 µg/mL +/-S9) but failed to induced a cytotoxic or mutagenic response in Chinese hamster V79 lung fibroblasts. Additionally, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the nonactivated (1000 µg/mL EMS) and S9-activated (10 µg/mL DMBA) positive controls. We concluded, therefore, that the study provided acceptable evidence that M&B 46030 is not mutagenic in this in vitro mammalian cell gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A signed quality assurance statement was dated June 29, 1993.)

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TABLE 2. Representative Results from the Chinese Hamster V79 Cell Forward Gene Mutation Assays with M & B 46030 in the Presence of S9 Activation

Substance	Dose	Average % Relative Survival (posttreatment)[a]	Average Total Mutant Colonies[a]	Average % Absolute Cloning Efficiency[a]	Average Mutation Frequency/ 10^5 survivors[b]
Solvent Control					
Dimethyl sulfoxide	1%	100 (95.0)[c,d,e] 100 (67.3)[f]	5.3[e] 0.3	97.7[e] 64.5	5.4[e] 0.5
Positive Control					
Dimethylbenz-anthracene	10 µg/mL	89.5[c] 87.3[f]	52.2 47.8	54.1 96.1	96.5 49.7
Test Material[g]					
M&B 46030	20/23.3 µg/mL[h] 500/385.7 µg/mL	>100[c] >100	1.5 0.0	75.9 92.9	2.0 0.0
	20/23.9 µg/mL[h] 500/317.9 µg/mL	94.3[f] 62.7	1.1 0.0	107.1 85.6	1.3 0 0

[a]Average values from six plates subcultured from duplicate flasks per group; calculated by our reviewers.

[b]Mutation Frequency = $\frac{\text{Average Total Mutant Colonies}}{\text{No. of Cells Plated } (1X10^5) \text{ X Absolute Cloning Efficiency}} X10^5$

[c]Results from Trial 1

[d]Value in () = Absolute percent survival

[e]Average value from three plates; one of the two replicate cultures died.

[f]Results from Trial 2

[g]Presented values are the intended/actual concentrations from analytical determinations.

[h]Based on information from the preliminary study and the analytical report, our reviewers assumed that this level was the highest soluble dose, and that higher levels (100 or 500 µg/mL) formed homogeneous suspensions with the culture medium. Findings from an intermediate concentration (100 µg/mL) and lower doses (0.8 or 4 µg/mL) in both trials did not suggest a mutagenic effect.

Note: Data were extracted from the study report, pp. 26, 28, 29, and, 31.

011086

FINAL

DATA EVALUATION REPORT

M&B 46030 (FIPRONIL)

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

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

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jennifer E Alexander Date 5/16/94
Jennifer E. Alexander, B.S.

Independent Reviewer Nancy E. McCarroll Date 5/16/94
Nancy E. McCarroll, B.S.

QA/QC Manager William L. McLellan Date 5/16/94
William L. McLellan, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-54
Clement Number: 229
Project Officer: Caroline Gordon

011086

EPA Reviewer: Virginia Dobozy, V.M.D., M.P.H. Signature: _____
Review Section I, Date: Virginia Dobozy 5/17/94
Toxicology Branch II/HED (H7509C)
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Review Section II, Date: 5/17/94
Toxicology Branch II/HED (H7509C)

DATA EVALUATION REPORT

TEST MATERIAL: M&B 46030 (Fipronil)

TOX. CHEM. NUMBER: Not provided

PC CODE: 129121

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

MRID NUMBER: 429186-50

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

SPONSOR: Rhone-Poulenc Ag Company, Lyon, France

STUDY NUMBER: 90/RHA305/1377

TESTING FACILITY: Pharmaco-LSR Ltd., Suffolk, England

TITLE OF REPORT: M&B 46030: Assessment of Clastogenic Action on Bone Marrow Erythrocytes in the Micronucleus Test

AUTHOR: C. Nicholas Edwards

REPORT ISSUED: Final Report: March 3, 1991; Amended Final Report: June 29, 1993

EXECUTIVE SUMMARY: In an in vivo micronucleus assay, groups of five male and five female CD-1 mice receiving single oral gavage administrations of 25 mg/kg M&B 46030 were sacrificed 24, 48, or 72 hours posttreatment. Similar groups were administered either 1 or 5 mg/kg, and bone marrow cells were harvested 24 hours postexposure. The test material was delivered to the animals as a suspension prepared in 0.5% methylcellulose. Dose selection for the main assay was based on the study author's claim that depression of bone marrow proliferation occurred in males and females (2/sex) administered 50 mg/kg in the dose range-finding study (cell harvest was 72 hours postexposure).

No evidence of overt toxicity, cytotoxicity to the target organ, or increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) was observed in males or females from the high-dose group. Based on the lack of an effect, slides prepared from two males and two females administered 50 mg/kg in the preliminary test were re-evaluated; these findings provided no convincing evidence of cytotoxicity and no appreciable increase in the MPE

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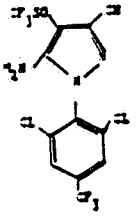
frequency. We assess that while no indication of a genotoxic effect was seen, observations from only two males and two females administered 50 mg/kg and sacrificed at 72 hours are insufficient to confirm that a genotoxic effect was not observed. Additional animals should have be tested at this dose level (or perhaps a slightly higher dose level).

This study is classified as Not Acceptable; it does not satisfy the guideline requirement for an in vivo micronucleus assay (84-2).

A. MATERIALS:

1. Test Material: M&B 46030 (Fipronil)

Description: Off-white powder
Identification no.: Batch number JJW2092/1
Purity: 97.2%
Structure:



Receipt date: July 13, 1989
Stability: Not provided
Contaminants: M&B 45,950; M&B 46,136 (see Study Appendix, p. 47)
Solvent used: 0.5% Methylcellulose
Other provided information: Homogeneity and actual concentrations were determined for dosing suspensions prepared prior to study initiation and for the preliminary toxicity and micronucleus assays. All suspensions were prepared fresh on the day of use; dosing suspensions were not adjusted for purity.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: 0.5% methylcellulose (dosing volume of 10 mL/kg) was administered by oral gavage.

Positive/final concentration/route of administration: Chlorambucil (CB), prepared in aqueous 10% ethanol, was administered by oral gavage at 30 mg/kg.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

- Preliminary toxicity test: 25, 50, 100, and 200 mg/kg
- Micronucleus assay: 1, 5, and 25 mg/kg

4. Test Animals:

(a) Species: mouse Strain: CD-1 Age (at receipt): 4-5 weeks
Weight range: preliminary toxicity test: 23.1-26.8 g (males), 21.5-23.4 g (females); micronucleus assay: 23.9-30.4 g (males), 19.7-24.2 g (females)

Source: Charles River Breeding Laboratories (UK), Kent, England

(b) Number of animals used per test dose:

- Preliminary toxicity test: 2 males; 2 females, per group
- Micronucleus assay: 15 males; 15 females, per vehicle and high-dose groups

5 males; 5 females per low- and mid-dose and positive control groups (sacrificed at 24 hours)

Note: Dosing was based on individual body weights.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
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 (high-dose group; only a 24-hour sampling was performed on the low- and mid-dose animals)

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr x 48 hr
x 72 hr

(c) Positive control:
Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): __x__ 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

__x__ bone marrow _____ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal:
at least 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: at least 1000

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the high test dose or the vehicle, the appropriate groups of animals were sacrificed by CO_2 asphyxiation and cervical dislocation. Sacrifice time for the low- and mid-dose and positive control groups was 24 hours. Animals from the preliminary toxicity test that survived to 72 hours postdosing were similarly sacrificed. Bone marrow cells were flushed from both femurs with fetal calf serum and centrifuged. Supernatants were discarded; pellets were resuspended in the remaining supernatant and spread onto slides. Prepared slides were fixed in methanol, stained with May-Grunwald and Giemsa solutions, coverslipped, coded and scored.

4. Statistical Methods: The results were evaluated for statistical significance using the Mann-Whitney test at p-values of 0.05 and 0.01.

5. Evaluation Criteria: No criteria were provided to evaluate assay validity, a positive response, or the biological significance of findings.

C. REPORTED RESULTS:

1. Preliminary Toxicity Test:

a. Analytical determination: Results from the analytical determinations indicated that the dosing suspensions were uniformly dispersed and that, with the exception of the low dosing suspension (88% of intended), all suspensions were within +/- 6% of the target concentrations.

b. Animal observation: The report indicated that the highest dose selected for the range-finding study was based on an oral LD_{50} of 97 mg/kg for rats which was provided by the sponsor. Accordingly, groups of 2 male and 2 female mice received single oral gavage administrations of 25, 50, 100, or 200 mg/kg M&B 46030. Animals were observed daily for mortality and clinical signs; body weights were recorded immediately prior to dosing and daily thereafter for 72 hours. Bone marrow cells were collected from

animals surviving to the scheduled sacrifice and examined for cytotoxic effects (PCE:NCE ratios). One mid-dose and one high-dose male were found dead approximately 18 hours after dosing. The remaining males and females in the mid- and high-dose groups experienced convulsions and increased motor activity after dosing. These animals were sacrificed in extremis 18 hours after dosing. Depression of bone marrow proliferation was reported in the low- and mid-dose animals compared to historical controls (PCE:NCE ratio was 0.5 for both the 25 and 50 mg/kg group compared to 0.9 for historical controls). Based on these findings, 25 mg/kg of the test material was selected as the high dose for the micronucleus assay.

2. Micronucleus Assay:

a. Analytical determination: Results from the analytical determinations for the micronucleus assay indicate that dosing suspensions were uniformly dispersed and that the theoretical concentrations (0.1, 0.5, and 2.5 mg/mL) differed from the actual concentrations by 20.0%, 8.0%, and 2.8%, respectively.

b. Animal observations: Groups of 15 male and 15 female mice were given a single oral gavage dose of 25 mg/kg M&B 46030. Groups of five male and five female mice received a single oral gavage dose of 1 or 5 mg/kg of the test material. No deaths occurred during the study period. No overt signs of toxicity were noted at any dose or sacrifice interval.

c. Micronucleus assay: Representative findings from the micronucleus assay are shown in Table 1. M&B 46030 was neither cytotoxic to the target organ nor caused a significant increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) in bone marrow cells harvested from male or female mice 24, 48, or 72 hours postexposure to the high dose (25 mg/kg). Similar results were obtained for low- (1 mg/kg) and mid- (5 mg/kg) dose animals at the 24-hour sacrifice time. Because no signs of toxicity or increases in the MPE frequency were seen at 25 mg/kg, the study author scored the slides prepared from two male and two female animals treated with 50 mg/kg (the maximum tolerated dose in the preliminary study. No increase in the frequency of MPEs was noted in bone marrow cells collected 72 hours post-exposure to 50 mg/kg. By contrast, the positive control (30 mg/kg CB) induced significant ($p<0.01$) genotoxic effects in both sexes.

From the overall results, the study author concluded that M&B 46030 was not genotoxic in this in vivo assay.

TABLE 1. Representative Results of the Micronucleus Assay In Mice Treated with M&B 46030.

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean MPEs/1000 PCEs ±S.D.	Mean PCE/NCE Ratio
Vehicle Control								
0.5% Methylcellulose	10 ml	24	M	5	5064	1	0.2±0.4	0.9
			F	5	5235	2 (3)[b]	0.4±0.5 (0.3)	0.9
		48	M	5	5132	5	1.0±1.0	0.8
			F	5	5210	5 (10)	1.0±1.2 (1.0)	1.0
		72	M	5	5260	4	0.8±0.8	0.8
			F	5	5173	2 (6)	0.4±0.5 (0.6)	0.8
Positive Control								
Chlorambucil	30 mg	24	M	5	4402	302	69.8±24.3*	0.4
			F	5	4882	325 (627)	65.4±24.0* (67.6)	0.5
Test Material								
M&B 46030	25 mg[c]	24	M	5	5173	4	0.8±0.8	0.9
			F	5	5100	1 (5)	0.2±0.4 (0.5)	0.8
		48	M	5	5313	2	0.4±0.5	0.9
			F	5	5191	4 (6)	0.8±1.0 (0.6)	0.9
		72	M	5	5290	6	1.1±0.8	0.9
			F	5	5188	5 (11)	1.0±0.7 (1.1)	0.8
	50 mg[d]	72	M	2	1714	2	1.2	0.5
			F	2	2056	0 (2)	0.0 (0.6)	0.7

[a]Time after compound administration by oral gavage
[b]Values in () are the combined results for both sexes.
[c]Results for the low- and mid-dose groups sacrificed 24 hours postexposure to 1 or 5 mg/kg, respectively, did not suggest a positive effect.
[d]Results from the scoring of slides made for the preliminary toxicity test.

*Significantly different ($p \leq 0.01$) than the corresponding vehicle control by the Mann-Whitney U test.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes

Note: Data were extracted from the study report, pp. 25-28 and 31.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: While no indication of a genotoxic effect using M&B 46030 in this in vivo assay exists, we have reservations about this conclusion because data are only provided for four animals dosed at 50 mg/kg. Similarly, the study author's claim that 50 mg/kg caused bone marrow suppression is based on the findings of a clear decrease in the PCE:NCE ratio for only 1 of 4 animals (see Study Report, pp. 30 and 31). It was noteworthy that a comparable PCE:NCE reduction was seen for 1 of 4 animals treated with 25 mg/kg in the preliminary test; this effect was not reproduced in the micronucleus assay. Therefore, the evidence of test material interaction with the target cell at 50 mg/kg is not convincing. Based on this consideration, we assess that the study is inconclusive.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated June 29, 1993.)

F. APPENDIX: No.

C11086

FINAL

DATA EVALUATION REPORT

M&B 46030
(Fipronil)

Study Type: Mutagenicity: Mammalian Cells in Culture Cytogenetic Assay in Human Lymphocytes

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date 5/11/94
Nancy E. McCarroll, B.S.

Independent Reviewer _____ Date 5/11/94
Laura Schuler, B.A.

QA Manager _____ Date 5/11/94
William L. McLellan, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-54
Clement Number: 232
Project Officer: Caroline Gordon

011086

FIPRONIL

GUIDELINE SERIES 84-2: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

EPA Reviewer: Virginia Dobozy, V.M.D., M.P.H. Signature: Virginia Dobozy
Review Section I, Toxicology Branch II Date: 5/17/94
Health Effects Division (7509C)
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T Backus
Review Section II, Toxicology Branch II Date: 5/17/94
Health Effects Division (7509C)

DATA EVALUATION REPORT

TEST MATERIAL: M&B 46030 (Fipronil)

TOX. CHEMICAL NO.: None provided

PC CODE: 129121

STUDY TYPE: Mammalian cells in culture cytogenetic assay in human lymphocytes

MRID NUMBER: 429186-53

SYNONYM(S): Fipronil; 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethylsulphinylpyrazole

SPONSOR: Rhone-Poulenc Ltd., Dagenham, Essex, United Kingdom

TESTING FACILITY: Microtest Research Limited, Heslington, York, United Kingdom

TITLE OF REPORT: Study To Evaluate The Chromosome Damaging Potential Of M&B 46030 By Its Effects On Cultured Human Lymphocytes Using An *In Vitro* Cytogenetics Assay

AUTHOR(S): R.R. Marshall

STUDY NUMBER: MAB 20/HLC

REPORT ISSUED: July 20, 1988

CONCLUSIONS-EXECUTIVE SUMMARY: In an *in vitro* cytogenetic assay, human lymphocytes derived from one male and one female healthy human donors were exposed to M&B 46030 doses of 75, 150, or 300 µg/mL with or without S9 activation. The S9 fraction was derived from Aroclor 1254 induced Wistar male rat livers and M&B 46030 was delivered to the test system in dimethyl sulfoxide.

Reduced mitotic indices were seen in cultures treated with 300 µg/mL +/-S9; this level was also reported to be near the solubility limit of the test material in this assay system. **There was, however, no indication of a clastogenic effect at any dose with or without S9 activation.** Findings with the positive controls confirmed the sensitivity of test system to detect clastogenesis.

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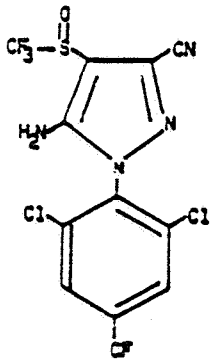
FIPRONIL MAMMALIAN CELLS IN CULTURE CYTOGENETICS

The study is classified as Acceptable and satisfies the guideline requirements for an in vitro mammalian cell cytogenetic assay (84-2).

A. MATERIALS:

1. Test Material: M&B 46030

Description: White crystalline powder
Identification number: Lot No. IGB 438
Purity: 90.6% (See Addendum to the Study Report p. 25).
Chemical structure:



CAS No.: Not provided
Receipt date: April 18, 1988
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions used in the assay were prepared immediately prior to use. Representative dosing solutions were not analyzed for actual concentrations.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Methyl methanesulfonate (MMS) was prepared in DMSO to yield final concentrations of 50, 75, and 100 µg/mL. Cells exposed to 100 µg/mL were selected for metaphase analysis.

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in DMSO to yield final concentrations of 12.5, 25, and 50 µg/mL. Cultures treated with 25 µg/mL were evaluated for structural aberrations.

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3. Activation: S9 derived from male Wistar (150-300 g)

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The rat liver homogenate was prepared by the performing laboratory. The S9 mix contained the following components:

Component	Volume
KCl (150 mM)	1.0 mL
NADP (25 mg/mL)	1.0 mL
Glucose-6-phosphate (180 mg/mL)	1.0 mL
S9	2.0 mL

Note: The final S9 concentration in culture medium was 2% (0.5 mL of the above S9-cofactor mix plus 9.5 mL of culture medium).

4. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Not performed.

(b) Cytogenetic assays: Seven doses (4.69, 9.38, 18.75, 37.5, 75, 150, and 300 µg/mL +/- S9) were initially evaluated. Cells treated with 75, 150, or 300 µg/mL were scored for chromosome aberrations.

5. Test Cells: Human lymphocytes were obtained from the blood of two healthy donors, one male and one female. Lymphocytes were initiated at 37°C in Hepes-buffered RPMI medium containing 20% fetal calf serum, 0.1 mL phytohemagglutinin, and antibiotics.

Properly maintained? Yes.
Cell line or strain periodically checked for mycoplasma contamination? Not applicable.
Cell line or strain periodically check for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

Cells exposed to test compound, solvent, or positive controls for: 3 hours (nonactivated); 3 hours (activated)

2. Cytogenetic Assay:

(a) Treatment: Approximately 44 hours after initiation, duplicate cultures were exposed to the selected test material dose, solvent or positive control in both the presence and absence of S9 activation. Lymphocytes were treated for 3 hours, washed twice,

refed fresh culture medium, and reincubated for 25 hours. Colchicine (final concentration, 1 µg/mL) was added 1 hour before all cultures were harvested. Cultures were centrifuged, swollen in 0.075 M KCl at 37°C and fixed in ice-cold methanol: glacial acetic acid (3:1). Lymphocytes were stored at least 18 hours at 4°C before slide preparation. Slides were prepared, stained in 4% Gurr's Giemsa R66, rinsed, dried, and coverslipped.

(b) Metaphase analysis: The mitotic index (MI) was determined by examining 1000 cells per culture. Slides were coded prior to metaphase analysis, but the MI was determined from uncoded slides. Two hundred metaphase spreads (100 cells/culture) from each selected dose group and the solvent control were scored for chromosome aberrations; gaps were recorded and aberration frequencies were presented with and without gaps. At least 25 cells were scored for the positive control groups.

4. Statistical Methods: The data from the experimental groups were evaluated for statistical significance ($p<0.05$) by χ^2 test.

5. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if (1) the incidence of aberrations ("in particular structural") in the solvent control cultures fell within the provided historical control range; (2) at least 160 out of 200 cells were available for analysis at each treatment level, and (3) the positive control induced statistically significant increases in incidence of aberrations ("particularly structural").

(b) Positive response: The test material was considered positive if a statistically significant increase in the incidence of chromosome aberrations was seen at one or more test material concentration and the incidence exceeded the historical control range. The study report also stated that any positive response are more likely to lead to a confident conclusion if the effect was dose-related, occurred at non-toxic doses, and was reproduced in independent experiments.

C. REPORTED RESULTS:-

1. Solubility Determination: The test material was soluble in DMSO at 31.25 mg/mL. When this stock solution was added to culture medium at a final concentration of 312.5 µg/mL, limited precipitation was observed that disappeared with shaking. From these observations, the study author concluded that 300 µg/mL was near the solubility limit of the test material; hence, 300 µg/mL was selected as the maximum dose for the cytogenetic assay.

2. Cytogenetic Assays: Representative results from the nonactivated and S9-activated cytogenetic assay with M&B 46030 are presented in Table 1. As shown, the high dose with or without S9 activation reduced the MI in lymphocyte cultures derived from male and female donors. Accordingly, cells treated with 75, 150, or 300 µg/mL +/-S9 were scored for chromosome aberrations. No appreciable increase in the incidence of structural or numerical aberrations was noted at any dose either with or without S9 activation. By contrast, lymphocytes from both donors responded to the clastogenic action of the nonactivated (100 µg/mL MMS) or S9-activated (25 µg/mL CP) positive controls.

From the overall results, the study author concluded that M&B 46030 was negative in this in vitro cytogenetic assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and we agree with the study author's interpretation of the results. M&B 46030 was tested in both the presence and absence of S9 activation to a high dose that was near the solubility limit and caused a marked reduction in the MI but failed to induce a clastogenic effect in human lymphocytes derived from a male and female donor. Additionally, the sensitivity of the test system to detect clastogenesis was adequately demonstrated by the results obtained with the positive control. We conclude, therefore, that the study provided acceptable evidence that M&B 46030 was negative in this test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated July 21, 1988.)

F. STUDY APPENDIX: No.

TABLE 1. Representative Results from the Human Lymphocyte *in vitro* Cytogenetic Assay with M&B 46030

Substance	Dose	S9 Activation	Donor	Mitotic Index (%)[a]	No. of Cells Scored	Total Number of Structural Aberrations[b]	Percent Cells with Structural Aberrations[b]	Biologically Significant Aberrations (No/Type)[c]
Solvent Control								
Dimethyl sulfoxide	1%	-	M	3.9	100	1	1	1TD
		-	F	2.9	100	1 (2)[d]	1 (1.0)[d]	1SD
	1%	+	M	2.6	100	2	1	1TD; 1SE
		+	F	4.1	100	2 (4)	2 (1.5)	1TD; 1SD
Positive Control								
Methyl methanesulfonate	100 µg/mL	-	M	ND[c]	50	14	20.0	8TD; 3TE; 3SD
		-	F	ND	25	10 (24)*	28.0 (23.0)	2TD; 5TE; 3SD
Cyclophosphamide	25 µg/mL	+	M	ND	42	7	14.0	2TD; 3TE; 2SD
		+	F	ND	25	38 (45)*	56.0 (30.0)	26TD; 4TE; 8SD
Test Material								
M&B 46030	300 µg/mL[e]	-	M	1.1	83	2	2.4	1TD; 1SD
			F	0.9	100	1 (3)	1.0 (1.6)	1SD
	300 µg/mL[e]	+	M	1.5	100	0	0.0	--
		+	F	0.8	62	0	0.0	--

[a]Number of metaphases per 1000 cells scored.
[b]Gaps excluded
[c]Abbreviations used:

TD = Chromatid deletion TE = Chromatid exchange ND = Not done
SD = Chromosome deletion

[d]Values in () are the combined results for cells derived from both sexes.
[e]Results for lower treatment groups (75 or 150 µg/mL +/-S9) did not suggest a clastogenic effect.

*Significantly higher ($p<0.001$) than the solvent control by χ^2 test.

Note: Data were extracted from the study report, pp. 19, 20, 23, and 24.

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FINAL

DATA EVALUATION REPORT

M&B 46136 (FIPRONIL METABOLITE)

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jennifer E. Alexander, B.S. Date 5/16/94

Independent Reviewer Nancy E. McCarroll, B.S. Date 5/16/94

QA Manager William L. McLellan, Ph.D. Date 5/16/94

Contract Number: 68D10075
Work Assignment Number: 3-54
Clement Number: 233
Project Officer: Caroline Gordon

Fipronil *Salmonella*

MUTAGENICITY STUDIES

EPA Reviewer: Virginia Dobozy, V.M.D., M.P.H. Signature: Virginia Dobozy
Review Section I, Toxicology Branch II Date: 5/17/94
Health Effects Division (H7509C)
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T Backus
Review Section II, Toxicology Branch II Date: 5/17/94
Health Effects Division (H7509C)

DATA EVALUATION REPORT

TEST MATERIAL: M&B 46136 (Fipronil Metabolite)

TOX. CHEM. NO.: Not provided

P.C. CODE: 129121

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

MRID Number: 429186-79

SYNONYM(S): Fipronil (Metabolite)

SPONSOR: Rhone-Poulenc Ltd, Essex, United Kingdom

TESTING FACILITY: Microtest Research Limited, York, United Kingdom

TITLE OF REPORT: Study to Determine the Ability of M&B 46136 to Induce Mutation in Four Histidine-Requiring Strains of Salmonella Typhimurium

AUTHOR(S): C.B. Clare

STUDY NUMBER: MAB 21/S

REPORT ISSUED: October 5, 1988

EXECUTIVE SUMMARY: In two independently performed Salmonella typhimurium/ mammalian microsome reverse gene mutation assays, strains TA1535, TA1537, TA98, or TA100 were exposed to 0.32, 1.6, 8, 40, or 200 µg/plate M&B 46136 without S9 activation or 0.8, 4, 20, 100, or 500 µg/plate M&B 46136 with S9 activation in the initial mutation assay. In the confirmatory assay, doses of 12.5, 25, 50, 100, or 150 µg/plate -S9 or 25, 50, 100, 200, or 400 µg/plate +S9 were evaluated. The S9 fraction was derived from Aroclor 1254-induced Wistar rat livers and the test substance was delivered to the test system in dimethyl sulfoxide.

Cytotoxicity was seen at 200 µg/plate -S9 and at 500 µg/plate +S9 in the initial assay; therefore, the high dose was lowered to 150 µg/plate -S9 and to 400 µg/plate + S9 for the confirmatory assay. M&B 46136 was assayed up to

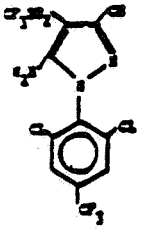
sufficiently high levels and there was no evidence of mutagenicity in any strain. Results with the positive controls were adequate to demonstrate assay sensitivity.

This study is classified as **Acceptable** and satisfies the guideline requirement for a gene mutation study [84-2].

A. MATERIALS:

1. Test Material: M&B 46136

   Description: White crystalline solid
   Identification numbers: Lot number WAB 202/1A
   Purity: 98.7%
   Structure:



   Stability: Not reported
   CAS number: Not reported
   Receipt date: April 18, 1988
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: The test material was stored in the dark at room temperature; dosing solutions were prepared immediately prior to use; analytical determinations were not performed on the dosing solutions.

2. Control Materials:

   Solvent/final concentration: DMSO/0.1 mL/plate

   Positive:

   Nonactivation:

Sodium azide	2 µg/plate TA1535, TA100
9-Aminoacridine	50 µg/plate TA1537
2-Nitrofluorene	50 µg/plate TA98

   Activation:

2-Aminoanthracene	5 µg/plate TA98, TA100 only

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

The rat liver S9 homogenate was prepared by the performing laboratory. The S9 mix was prepared as follows:

Component:	Volume/100mL:
500 mM Sodium phosphate buffer (pH 7.4)	57.56
60 mg/mL Glucose-6-phosphate	10 00
25 mg/mL NADP	10.00
250 mM $MgCl_2$	8.00
1 mg/mL L-histidine HCl	2.00
1 mg/mL D-biotin	2.44
S9	10.00 (10%)

Note: For the nonactivated tests, the above cofactor solution was prepared with 30 mL of sterile distilled water substituted for glucose-6-phosphate, NADP, and the S9 homogenate.

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 _____ TA1538
list any others:

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (histidine dependence, rfa mutation, and ampicillin resistance)? Yes.

5. Test Compound Concentrations Used:

(a) Preliminary Cytotoxicity Assay: Five doses (8, 40, 200, 1000, and 5000 µg/plate) were evaluated with and without S9 activation using only strain TA100. Three plates were used per dose, per condition for the test substance, and five plates were used per condition for the negative and positive controls.

(b) Mutation assay:

- Initial Trial: Five doses (0.32, 1.6, 8, 40, and 200 µg/plate -S9; and 0.8, 4, 20, 100, and 500 µg/plate +S9) were tested using all strains. Three plates were used per strain, per dose, per condition for the test substance, and five plates were used with and without S9 activation for the negative and positive controls.

- Confirmatory Trial: Five nonactivated doses (12.5, 25, 50, 100, and 150 µg/plate and five S9-activated doses (25, 50, 100, 200, and 400 µg/plate) were evaluated as described for the initial trial.

Fipronil *Salmonella*

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: _x_ Standard plate test
___ Pre-incubation (_____) minutes
___ "Prival" modification
___ Spot test

2. Mutation Assays: A mixture containing 0.1 mL of the appropriate concentration of the test material, solvent or positive control, 0.1 mL of an overnight bacterial culture of the appropriate tester strain, and 1.0 mL of the S9-cofactor mix or cofactor solution was added to 2.5 mL of unsupplemented top agar. The contents of the tubes were mixed and poured onto minimal Davis agar; plates were incubated at 37°C for at least two days. Following incubation, revertant colonies were counted, and means and standard deviations were determined. In addition, the background lawn growth was evaluated for signs of cytotoxicity.

3. Evaluation Criteria:

(a) Valid assay: The assay was considered valid if: (1) the mean number of revertant colonies in the solvent control fell within the provided expected spontaneous range for each tester strain; (2) the positive controls induced a positive response; and (3) no more than 5% of the plates were lost due to contamination or other unexpected events.

(b) Positive response: The test material was considered positive if it induced a reproducible, dose-related increase in mutant colonies of strains TA98 or TA100 that was at least ≥ 2-fold higher than the solvent control or ≥ 3-fold higher than the solvent control for strains TA1535 and TA1537. The increases must also be accompanied "by significant F-statistics"; the p-value was not reported.

C. REPORTED RESULTS:

1. Preliminary Cytotoxic Assay: No data were provided for the preliminary cytotoxicity assay conducted with 8 to 5000 µg/plate +/- S9 of the test material. The study author stated, however, that the three highest nonactivated levels (200, 1000, and 5000 µg/plate) and the two highest S9-activated concentrations were cytotoxic. Based on these findings, the initial mutation assay was performed with a nonactivated dose range of 0.32 to 200 µg/plate and with an S9-activated dose range of 0.8 to 500 µg/plate.

2. Mutation Assay:

(a) Initial Trial: Cytotoxicity was observed at the highest non-activated dose (200 µg/plate) in all strains and in all but strain TA100 at the highest S9-activated dose (500 µg/plate). However, no increase in the number of revertants was seen at any level. By contrast to the uniformly negative results with the test material

the positive controls produced marked increases in histidine revertants in the corresponding tester strains. The representative data from this assay are presented in Table 1.

(b) Confirmatory Trial: Due to cytotoxicity at the highest doses (200 µg/plate -S9 and 500 µg/plate +S9) in the initial trial, the high doses selected for the confirmatory assay were 150 µg/plate -S9 and 400 µg/plate +S9. Neither cytotoxicity nor mutagenicity were observed in this assay using concentration ranges of 12.5-150 µg/plate in the absence of S9 activation or 25-400 µg/plate in the presence of S9 activation. Representative data for the confimatory assay are presented in Table 2.

From the overall results, the study author concluded that M&B 46136 was not mutagenic in this bacterial assay system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. M&B 46136 was evaluated over a concentration range that included a cytotoxic level (200 µg/plate -S9 and 500 µg/plate +S9), but failed to induce a mutagenic response in two independently performed trials. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the responses induced by the nonactivated positive controls. Although the S9-activated positive control (5 µg/plate 2AA) was not tested with strains TA1535 or TA1537, the results with strains TA98 and TA100 clearly indicated that the S9 fraction was biologically active. We, therefore, assess that the lack of testing strains TA1535 or TA1537 with a promutagen did not affect the outcome of the study. The study provided acceptable evidence that M&B 46136 is not mutagenic in this bacterial test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated October 7, 1988.)

F. APPENDIX ATTACHED? No.

Table 1: Representative Results of the Initial Salmonella typhimurium/Mammalian Microsome Mutation Assay with M&B 46136

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	0.1 mL	-	23±9	11±4	23±6	72±12
	0.1 mL	+	19±10	10±1	36±3	131±8
Positive Controls						
Sodium azide	2 µg	-	543±133	--	--	639±115
9-Aminoacridine	50 µg	-	--	865±62	--	--
2-Nitrofluorene	50 µg	-	--	--	928±140	--
2-Aminoanthracene	5 µg	+	--	--	413±27	806±83
Test Material						
M&B 46136	40 µg[b]	-	24±7	9±1	21±3	97±14
	200 µg[c]	-	29±1	8±2	19±4	67[d]
	100 µg[b]	+	20±5	14±1	26±12	131±12
	500 µg[c]	+	20±8	14±1	22±2	144±2

[a]Means and standard deviations of the counts from triplicate plates for test material and quintuplicate plates for the solvent and positive control groups.
[b]Findings for lower doses (0.32, 1.6, or 8 µg/plate -S9; 0.8, 4, or 20 µg/plate +S9) did not suggest a mutagenic effect.
[c]The highest dose tested; thinning of the background lawn of growth reported for all strains at 200 µg/plate -S9 and for all strains except TA100 at 500 µg/plate +S9.
[d]One of the plates was listed as wet and not counted.

Note: Data were extracted from the study report, p. 22-29.

Table 2: Representative Results of the Confirmatory Salmonella typhimurium/Mammalian Microsome Mutation Assay with M&B 46136

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	0.1 mL	-	18±3	10±5	18±2	76±7
	0.1 mL	+	23±4	11±4	31±8	107±8
Positive Controls						
Sodium azide	2 µg	-	245±20	--	--	731±45
9-Aminoacridine	50 µg	-	--	432±50	--	--
2-Nitrofluorene	50 µg	-	--	--	996±50	--
2-Aminoanthracene	5 µg	+	--	--	330±58	411±32
Test Material						
M&B 46136	150 µg[b]	-	16±5	12±8	27±4	68±5
	400 µg[b]	+	16±4	9±3	32±8	96±14

[a]Means and standard deviations of the counts from triplicate plates for test material; positive and solvent control group means and standard deviations are based on counts from 5 replicate plates per strain, per condition.
[b]The highest dose tested. Findings for lower doses (12.5, 25, 50, or 100 µg/plate - S9; 25, 50, 100, or 200 µg/plate + S9) did not suggest a mutagenic effect.

Note: Data were extracted from the study report, p. 31-38.

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FINAL

DATA EVALUATION REPORT

M&B 46136
(Fipronil Metabolite)

Study Type: Mutagenicity: Mammalian Cells in Culture Cytogenetic Assay in Human Lymphocytes

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer	Nancy E. McCarroll, B.S.	Date 5/11/94
Independent Reviewer	Laura P. Schuler, B.A.	Date 5/11/94
QA Manager	William L. McLellan, Ph.D.	Date 5/11/94

Contract Number: 68D10075
Work Assignment Number: 3-54
Clement Number: 234
Project Officer: Caroline Gordon

011086

FIPRONIL

GUIDELINE SERIES 84-2: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

EPA Reviewer: Virginia Dobozy, V.M.D., M.P.H. Signature: Virginia Dobozy 5/17/94
Review Section I, Toxicology Branch II Date:
Health Effects Division (7509C)
EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D. Signature: Byron T Backus
Review Section II, Toxicology Branch II Date: 5/17/94
Health Effects Division (7509C)

DATA EVALUATION REPORT

TEST MATERIAL: M&B 46136

TOX. CHEMICAL NO.: None provided

PC CODE: 129121

STUDY TYPE: Mammalian cells in culture cytogenetic assay in human lymphocytes

MRID NUMBER: 429186-80

SYNONYM(S): Fipronil (metabolite)

SPONSOR: Rhone-Poulenc Ltd., Dagenham, Essex, United Kingdom

TESTING FACILITY: Microtest Research Limited, Heslington, York, United Kingdom

TITLE OF REPORT: Study To Evaluate The Chromosome Damaging Potential Of M&B 46136 By Its Effects On Cultured Human Lymphocytes Using An *In Vitro* Cytogenetics Assay

AUTHOR(S): R.R. Marshall

STUDY NUMBER: MAB 21/HLC

REPORT ISSUED: November 15, 1989

CONCLUSIONS-EXECUTIVE SUMMARY: In an *in vitro* cytogenetic assay, human lymphocytes derived from one male and one female healthy human donors were exposed to M&B 46136 doses of 75, 150, or 300 µg/mL with or without S9 activation. The S9-activated phase of testing was repeated using the high dose only. The S9 fraction was derived from Aroclor 1254 induced Wistar male rat livers and M&B 46136 was delivered to the test system in dimethyl sulfoxide.

A marked reduction in the mitotic index was seen in cultures treated with 300 µg/mL +/-S9; this level was also reported to be near the solubility limit of the test material in this assay system. **There was, however, no indication of a clastogenic effect at any dose with or without S9 activation.** Findings with the positive controls confirmed the sensitivity of test system to detect clastogenesis.

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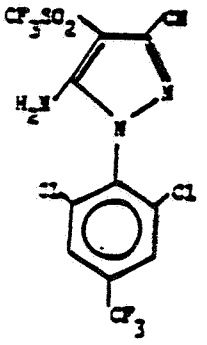
FIPRONIL MAMMALIAN CELLS IN CULTURE CYTOGENETICS

This study is classified as Acceptable and satisfies the guideline requirements for an in vitro mammalian cell cytogenetic assay (84-2).

A. MATERIALS:

1. Test Material: M&B 46136

Description: White crystalline powder
Identification number: Lot No. MAB 202/1A
Purity: Reported as 98.7%
Chemical structure:



CAS No.: Not provided
Receipt date: April 18, 1988
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions used in the assay were prepared immediately prior to use. Representative dosing solutions were not analyzed for actual concentrations

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Methyl methanesulfonate (MMS) was prepared in DMSO to yield final concentrations of 50, 75, and 100 µg/mL. Cells exposed to 100 µg/mL were selected for metaphase analysis.

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in DMSO to yield final concentrations of 12.5, 25, and 50 µg/mL. Cultures treated with 25 µg/mL were evaluated for structural aberrations.

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3. Activation: S9 derived from male Wistar (150-300 g)

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat liver homogenate was prepared by the performing laboratory. The S9 mix contained the following components:

Component	Volume
KCl (150 mM)	1.0 mL
NADP (25 mg/mL)	1.0 mL
Glucose-6-phosphate (180 mg/mL)	1.0 mL
S9	2.0 mL

Note: The final S9 concentration in culture medium was 2% (0.5 mL of the above S9-cofactor mix plus 9.5 mL of culture medium).

4. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Not performed.

(b) Cytogenetic assays:

(1) Initial assay: Seven doses (4.69, 9.38, 18.75, 37.5, 75, 150, and 300 µg/mL +/- S9) were initially evaluated. Cells treated with 75, 150, or 300 µg/mL were scored for chromosome aberrations.

(2) Repeat Assay: 250 and 300 µg/mL +S9; only cells exposed to 300 µg/mL +S9 were scored.

5. Test Cells: Human lymphocytes were obtained from the blood of two healthy donors, one male and one female. Lymphocytes were initiated at 37°C in Hepes-buffered RPMI medium containing 20% fetal calf serum, 0.1 mL phytohemagglutinin, and antibiotics.

Properly maintained? Yes.
Cell line or strain periodically checked for mycoplasma contamination? Not applicable.
Cell line or strain periodically check for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

Cells exposed to test compound, solvent, or positive controls for: 3 hours (nonactivated); 3 hours (activated)

2. Cytogenetic Assay:

(a) Treatment: Approximately 44 hours after initiation, duplicate cultures were exposed to the selected test material dose, solvent or positive control in both the presence and absence of S9 activation. Lymphocytes were treated for 3 hours, washed twice, refed fresh culture medium, and reincubated for 25 hours. Colchicine (final concentration, 1 µg/mL) was added 1 hour before all cultures were harvested. Cultures were centrifuged, swollen in 0.075 M KCl at 37°C and fixed in ice-cold methanol: glacial acetic acid (3:1). Lymphocytes were stored at least 18 hours at 4°C before slide preparation. Slides were prepared, stained in 4% Gurr's Giemsa R66, rinsed, dried and coverslipped.

(b) Metaphase analysis: The mitotic index (MI) was determined by examining 1000 cells per culture. Slides were coded prior to metaphase analysis, but the MI was determined from uncoded slides. Two hundred metaphase spreads (100 cells/culture) from each selected dose group and the solvent control were scored for chromosome aberrations; gaps were recorded and aberration frequencies were presented with and without gaps. At least 25 cells were scored for the positive control groups.

4. Statistical Methods: The data from the experimental groups were evaluated for statistical significance ($p<0.05$) by χ^2 test.

5. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if (1) the incidence of aberrations ("in particular structural") in the solvent control cultures fell within the provided historical control range; (2) at least 160 out of 200 cells were available for analysis at each treatment level, and (3) the positive control-induced statistically significant increases in incidence of aberrations ("particularly structural").

(b) Positive response: The test material was considered positive if a statistically significant increase in the incidence of chromosome aberrations was seen at one or more test material concentration and the incidence exceeded the historical control range. The study report also stated that any positive response are more likely to lead to a confident conclusion if the effect was dose-related, occurred at non-toxic doses, and was reproduced in independent experiments.

C. REPORTED RESULTS:

1. Solubility Determination: The test material was soluble in DMSO at 31.25 mg/mL. When this stock solution was added to culture medium at a final concentration of 312.5 µg/mL, limited precipitation was observed that disappeared with shaking. From these observations, the study authors concluded that 300 µg/mL was near the solubility limit of the test material; hence, 300 µg/mL was selected as the maximum dose for the cytogenetic assay.

2. Cytogenetic Assays:

(a) Nonactivated conditions: Marked reductions in the MI were recorded for cultures from both donors exposed to the two highest doses (150 and 300 µg/mL). Based on this finding, cells exposed to 75, 150, or 300 µg/mL were scored for chromosome aberrations. As shown in Table 1, there was no evidence of a clastogenic effect at any dose. Similarly, there was no appreciable increase in the frequency of numerical aberrations. Results with the positive control (100 µg/mL MMS) did, however, show a significant ($p<0.01$) increase in structural aberration in cells derived from both the male and female donors.

(b) S9-activated conditions: In agreement with the nonactivated findings, exposure to 150 or 300 µg/mL M&B 46136 resulted in mitotic suppression (data combined for both sexes were $-\geq 40\%$ lower than control). Cultures treated with the three highest doses (75, 150, or 300 µg/mL) were, therefore, selected for the cytogenetic evaluation. No significant increase in the incidence of structural aberration were noted at any dose (Table 2), and there was no effect on the frequency of numerical aberrations. Although a slight increase in structural aberrations occurred in the low-dose culture of male donor cells, the increase was limited to this culture and, therefore, not considered of biological relevance. However, the trial was repeated with the high dose because the study author stated that an insufficient number of metaphase spreads was examined at this level in the initial trial. Findings from the repeat trial were also not indicative of a clastogenic response. In both S9-activated trials, cells harvested from the male and female donors responded to the genotoxic action of the positive control (25 µg/mL CP).

From the overall results, the study author concluded that M&B 46136 was negative in this in vitro cytogenetic assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and we agree with the study author's interpretation of the results. M&B 46136 was tested in both the presence and absence of S9 activation to a high dose that was near the solubility limit and caused a marked reduction in the MI but failed to induce a clastogenic effect in human lymphocytes derived from a male and female donor. Additionally, the

TABLE 1. Summarized Results from the Nonactivated Human Lymphocyte *in vitro* Cytogenetic Assay with M&B 46136

Substance	Dose	Donor	Mitotic Index (%)[a]	No. of Cells Scored	Total Number of Structural Aberrations[b]	Percent Cells with Structural Aberrations[b]	Biologically Significant Aberrations (No/Type)[c]
Solvent Control							
Dimethyl sulfoxide	1%	M	3.9	100	1	1	1TD
	1%	F	2.9	100	1 (2)[d]	1 (1.0)[d]	1SD
Positive Control							
Methyl methanesulfonate	100 µg/mL	M	ND[c]	50	14	20	8TD; 3TE; 3SD
		F	ND	25	10 (24)[*]	28 (23)	2TD; 5TE; 3SD
Test Material							
M&B 46136	75 µg/mL	M	2.4	100	0	0	--
		F	3.0	100	1 (1)	1 (0.5)	1SE
	150 µg/mL	M	0.6	100	3	3	3TD
		F	1.2	100	1 (4)	1 (2.0)	1TD
	300 µg/mL	M	0.8	67	0	0	--
		F	1.3	100	1 (1)	1 (0.6)	1TD

[a]Number of metaphases per 1000 cells scored.
[b]Gaps excluded
[c]Abbreviations used:

TD = Chromatid deletion TE = Chromatid exchange ND = Not done
SD = Chromosome deletion SE = Chromosome exchange

[d]Values in () are the combined results for cells derived from both sexes.
[*]Significantly higher ($p<0.001$) than the solvent control by χ^2 test.

Note: Data were extracted from the study report, pp. 23, 28, and 29.

TABLE 2. Summarized Results from the S9-Activated Human Lymphocyte in vitro Cytogenetic Assay with M&B 46136

Substance	Dose	Donor	Mitotic Index (%)[a]	No. of Cells Scored	Total Number of Structural Aberrations[b]	Percent Cells with Structural Aberrations[b]	Biologically Significant Aberrations (No/Type)[c]
Solvent Control							
Dimethyl sulfoxide	1%	M	2.6[d]	100	2	1	1TD; 1SE
	1%	F	4.1	100	2 (4)[e]	2 (1.5)[e]	1TD; 1SD
	1%	M	4.9[f]	100	1	1	1TD
	1%	F	4.2	100	0 (1)	0 (0.5)	--
Positive Control							
Cyclophosphamide	25 µg/mL	M	ND[d]	42	7	14	2TD; 3TE; 2SD
		F	ND	25	38 (45)[e]	56 (30.0)[e]	26TD; 4TE; 8SD
	25 µg/mL	M	ND[f]	25	29	60	17TD; 3TE; 7SD; 2M
		F	ND	25	30 (59)[e]	60 (60.0)	12TD; 5TE; 10SD; 3M
Test Material							
M&B 46136	75 µg/mL	M	2.0[d]	100	8	5	5TD; 2SD; 1SE
		F	3.8	100	1 (9)	1 (3.0)	1SD
	150 µg/mL	M	1.0	100	2	2	2TD
		F	3.2	100	2 (4)	2 (2.0)	1TD; 1SD
	300 µg/mL	M	0.6	73	0	0	--
		F	0.7	42	0	0 (0.0)	--
	300 µg/mL	M	1.4[f]	100	2	2	2TD
		F	1.3	100	3 (5)	3 (2.5)	2TD; 1SD

[a]Number of metaphases per 1000 cells scored.
[b]Gaps excluded
[c]Abbreviations used:

TD = Chromatid deletion
SD = Chromosome deletion
TE = Chromatid exchange
SE = Chromosome exchange
M = Multiple aberrations (cell with <7 aberrations or cell with pulverized chromosomes); counted as one aberration.
ND = Not done

[d]Results from the initial trial
[e]Values in () are the combined results for cells derived from both sexes.
[f]Results from the repeat trial

*Significantly higher ($p<0.001$) than the solvent control by χ^2 test.

Note: Data were extracted from the study report, pp. 24, 25, 28, and 29.

sensitivity of the test system to detect clastogenesis was adequately demonstrated by the results obtained with the positive control. We conclude, therefore, that the study provided acceptable evidence that M&B 46136 was negative in this test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated November 15, 1989.)

F. STUDY APPENDIX: No.

Reviewed by: Timothy F. McMahon, Ph.D. 5/9/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 5/10/94
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Metabolism (85-1)

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

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

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

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

Testing Facilities: Hazleton UK, North Yorkshire, England.

Sponsor: Rhone Poulenc Agriculture, Ongar, Essex CM5 OHW

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

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

Report issued: June 26, 1992

Executive Summary:

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

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

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

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

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

Core Classification: minimum

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

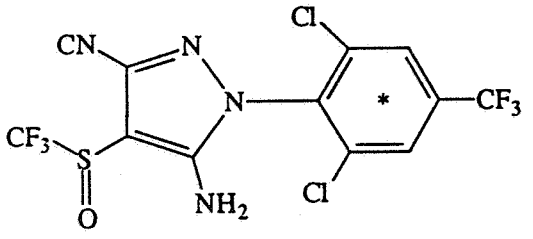
MATERIALS

A. Test Materials

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

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

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



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

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

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

II. METHODS

A. Study Design

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

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

2) Metabolite Characterization and Identification Studies

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

Urine

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

Feces

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

Tissues

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

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

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

C. Experimental

a. Animal Husbandry

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

b. Dosing

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

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

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

5/16/96

7:21

c. Sample Collection and Analysis

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

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

d. Radioassay Preparation:

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

e. Metabolite Characterization and Identification

a. Sample Analysis:

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

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

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

D. Compliance

A signed statement of No Data Confidentiality claims was provided.

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

A signed statement of quality assurance was provided.

III. RESULTS

1. Absorption

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

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

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

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

2. Distribution

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

Table 1a

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

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

Table 1b

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

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

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

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

3. Excretion

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

Table 2

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

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

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

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

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

d. Plasma Levels of 14-C Fipronil Derived Radioactivity

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

Table 3
Pharmacokinetic Parameters in Fipronil Treated Rats

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

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

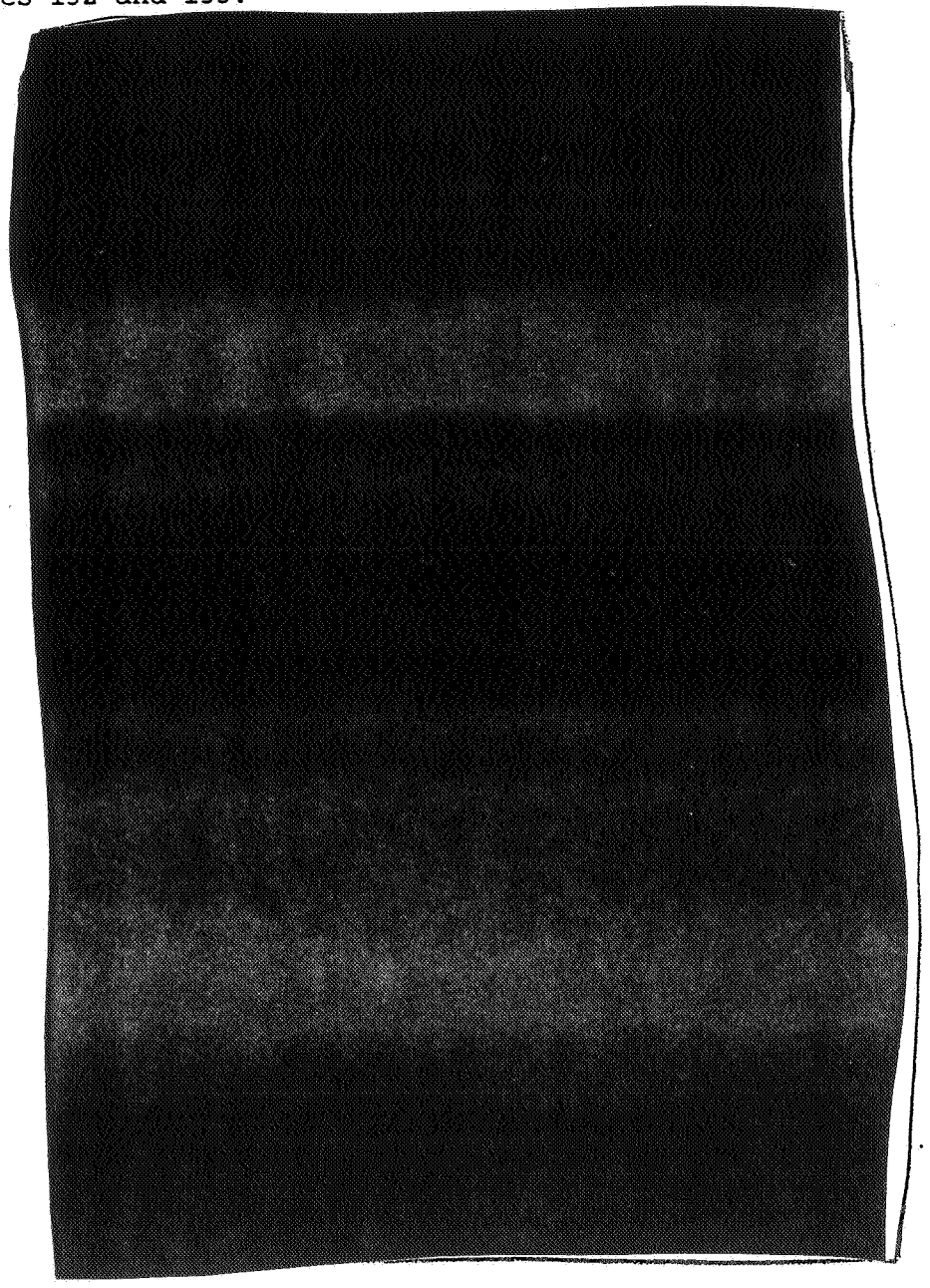
At the single high dose, pharmacokinetic parameters were again similar between male and female rats but differed from that of the low dose. At the high dose, blood concentrations of radioactivity increased slowly, with a Tmax of between 48-72 hours for male and female rats. Thereafter, blood concentration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

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

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

2) Metabolite Characterization and Identification

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



EPA Registration Data

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

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

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

a) **Urinary Metabolites**

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

Table 4

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

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

[a]represents 72 hour urine sample.

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

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

Single Low Dose

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

Repeated Low Dose

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

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

Single High Dose

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

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

b) **Fecal Metabolites**

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

Table 5

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

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

Single Low Dose

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

Repeated Low Dose

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

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

Single High Dose

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

c. Tissue Extracts

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

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

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

IV. DISCUSSION

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

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

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

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

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

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

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

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

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

Core Classification: minimum

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

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011086

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 5/31/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I. 6/3/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Special Study - Thyroid Function
EPA ID NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 429779-04
TEST MATERIAL:	M&B 46030 Synonym: Fipronil
STUDY NUMBER:	M&B 353/90920
TESTING FACILITY:	Huntingdon Research Centre, Ltd. Cambridgeshire, England
SPONSOR:	Rhone-Poulenc Ag Company
TITLE OF REPORT:	M&B 46030 - An Investigation into the Potential Effects on Thyroid Function in Male Rats Using the 'Perchlorate Discharge Test'
AUTHOR(S):	David H. Peters *et al*
REPORT ISSUED:	April 12, 1991

EXECUTIVE SUMMARY: In this special study (MRID # 429779-04), the effect of M&B 46030 on thyroid function was compared to propylthiouracil (PTU), a known inhibitor of thyroid organification, and Noxyflex, a thiourea compound known to lower thyroxine levels in rats. Four groups of 27 male Crl:CD (SD) BR rats per group were administered either methylcellulose (vehicle control), 10 mg/kg/day M&B 46030, 200 mg/kg/day 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. Six hours later, 9 males per group received either 10 or 25 mg/kg potassium perchlorate or 0.9% saline solution. Blood was immediately drawn to measure radioactivity levels. The animals were sacrificed, and the thyroids were weighed and analyzed for radioactivity.

The treatment with M&B 46030 or Noxyflex appeared to result in stimulation of the thyroid glands as evidenced by increased accumulation of ^{125}I by 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 propylthiouracil 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

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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 M&B 46030 or Noxyflex.

The study is classified as **Core Supplementary** as it is not a required guideline study.

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I. MATERIALS

A. Test Materials

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

M&B 46030 was suspended in 0.5% methylcellulose for administration. The formulation was analyzed for stability, homogeneity and accuracy of preparation on Days 1 and 14. Results in Appendix 5 of the study report show that the formulations were homogeneous, stable for at least one day and within acceptable range of the nominal concentrations.

Other materials used: Propylthiouracil
Noxyflex (noxythiolin)
Potassium perchlorate
^{125}I as sodium iodide in dilute sodium hydroxide solution

B. Test Animals

Species: Crl:CD (SD) BR male rats
Source: Charles River, St. Aubin-Les-Albeuf, France
Age: 42 ± 1 day old when received
Weight: 21 g when received
Housing: Three rats per cage
Environmental Conditions: Temperature: 21 ± 2^0 C
Relative Humidity: 55 ± 10%
Photoperiod: 12 hours light/dark
Food and Water: SDS Rat and Mouse No. 1 modified maintenance diet and water *ad libitum*
Acclimation Period: Five days

II. METHODS

Study Objective

The objective of the study was to compare the effects of M&B 46030 on thyroid function to that of propylthiouracil, a known inhibitor of thyroid organification (interference with iodination of thyroglobulin) and Noxyflex, a thiourea compound known to lower thyroxine levels in rats. Thyroid function was evaluated by measuring the effects of these chemicals on iodide incorporation and follicular stimulation. (Perchlorate was administered to release any free iodide present in the thyroid glands.)

Dosage Groups and Administration

The animals were assigned to the following groups.

Group	Treatment	Number of Animals
1	Control (0.5% methylcellulose)	27
2	M&B 46030 (10 mg/kg/day)	27
3	Propylthiouracil (PTU) (200 mg/kg/day)	27
4	Noxyflex (50 mg/kg/day)	27

M&B 46030 and PTU, both in suspension with 0.5% methylcellulose, were administered by gavage at a volume of 5 ml/kg using a syringe and a rubber catheter. Control animals received the vehicle alone at this volume. Noxyflex was administered intraperitoneally as a solution in 0.9% saline at a dosage volume of 1 ml/kg. Treatment was continuous for 14 days. On Day 15, each animal received an intraperitoneal injection of $Na^{125}I$ at a dose level of 1 μCi ^{125}I in 0.5 ml 0.9% (w/v) saline. Six hours later, 9 males from each group received intraperitoneal injections of either potassium perchlorate at a dosage of 10 mg/kg, potassium perchlorate at a dosage of 25 mg/kg or 0.9% saline solution.

Observations

The following observations were recorded at the indicated times.

Mortality - twice daily
Clinical signs of toxicity - once daily
Body weight - at time of allocation of animals to groups, on commencement of treatment and once a week thereafter
Food consumption - weekly
Food efficiency - weekly

Terminal Studies

On Day 15, each rat received an injection of a neuroleptanalgesic immediately after the intraperitoneal dose of potassium perchlorate

or saline. Blood was drawn by cardiac puncture and was analyzed for radioactivity in a gamma counter. The animals were then sacrificed and the thyroid glands were removed, weighed and analyzed for radioactivity.

Statistical Analysis

A description of the statistical procedures from the study report is attached to the DER.

III. RESULTS

Mortality and Clinical Signs

One male treated with Noxyflex was found dead on Day 2 of the study; the cause of death was not apparent. No clinical signs of toxicity were observed with the Noxyflex or M&B 46030 treatments. All rats treated with PTU salivated for up to 1 hour after dosing. Peri-oral staining immediately after dosing and matted fur up to ½ hour after dosing were also noted.

Body Weight Gain

Group mean body weight gain was significantly reduced in all the treated groups in comparison to the control group. The reduction was most severe in the PTU-treated animals (37% of the control value for weeks 0-2). The reductions for the M&B 46030- and Noxyflex-treated groups were comparable (85% and 75% of the control value, respectively).

Food Consumption

There was a significant decrease in food consumption over the two-week period in all treated groups as compared to the control group. The effect was most severe in the PTU-treated animals (73% of the control value) with the Noxyflex- and M&B 46030-treated animals being marginally affected (86% and 92% of the control value, respectively).

Food Efficiency

Food efficiency, as measured by food conversion ratios, in the Noxyflex- and M&B 46030-treated animals was similar to the control group (5.3 and 5.0, respectively vs. 4.6 for the controls). The ratio for the PTU-treated group was elevated (9.0) indicating lower food efficiency utilization.

Terminal Studies

Whole Blood Radioactivity -

Increases in the radioactivity of the whole blood were seen in the

PTU- and Noxyflex-treated groups when data from the control group rats receiving a post-treatment saline injection were compared to those for saline-injected animals in other groups. In animals injected with 10 mg/kg potassium perchlorate, the PTU-treated group had an increase in radioactivity, whereas the Noxyflex-treated group had a decrease in radioactivity when compared to saline-treated animals of the same treatment group. The changes were similar but smaller when 25 mg/kg of potassium perchlorate was administered. When the overall treatment group means were compared, the PTU-treated animals had significantly more radioactivity in their blood than the control animals. A summary of these results from Table 4 of the study report is attached to the DER.

Thyroid Gland Radioactivity -

M&B 46030 and Noxyflex produced significant increases in the radioactivity levels in the thyroids as compared to the controls in the animals that received post-treatment saline, whereas the PTU appeared to inhibit the ^{125}I uptake. The same effects were seen with both doses of perchlorate. A summary of these results from Table 5 of the study report is attached to the DER.

Thyroid Weights -

The groups were combined since there was no evidence that either perchlorate injection had an effect on thyroid weight. All of the treatments caused a significant increase in the weights with PTU inducing the most marked effect.

Ratio of blood radioactivity: thyroid weight -

The PTU-treated animals had a significant decrease in the ratio when those animals receiving saline were compared to the control group animals receiving saline. There were no significant changes when the saline-treated rats receiving the other treatments were compared to the controls. PTU-treated animals receiving either dose of perchlorate had similar decreases. The rats treated with M&B 46030 or Noxyflex and injected with 25 mg/kg of perchlorate also had significant decreases in the ratios.

Ratio of blood radioactivity: thyroid radioactivity -

M&B 46030 and Noxyflex with post-treatment saline injections both caused increases in the ratios of approximately 60%. PTU induced a decrease of approximately 90%. The same effect was seen with both levels of perchlorate.

IV. COMPLIANCE

The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in

accordance with GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality.

V. CONCLUSIONS

The treatment with M&B 46030 or Noxyflex appeared to result in stimulation of the thyroid glands as evidenced by increased accumulation of ^{125}I by 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 propylthiouracil 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 M&B 46030 or Noxyflex.

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011086

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A. Dobozy 5/6/94
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Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou 6/3/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Special Study - Thyroxine Clearance
EPA ID NUMBERS:	P. C. CODE: 129121 MRID NUMBER: 429186-54
TEST MATERIAL:	M&B 46030 Synonym: Fipronil
STUDY NUMBER:	M&B 352/90958
TESTING FACILITY:	Huntingdon Research Centre, Ltd. Cambridgeshire, England
SPONSOR:	Rhone-Poulenc Ag Company
TITLE OF REPORT:	M&B 46030 - An Investigation into the Potential Effects on Thyroid Function in Male Rats by Studying Thyroxine Clearance
AUTHOR(S):	David H. Peters *et al*
REPORT ISSUED:	April 12, 1991

EXECUTIVE SUMMARY: In a special study to measure the effect of M&B 46030 on thyroxine clearance (MRID # 429186-54), six groups of six male Crl:CD (SD) rats per group were administered either M&B 46030 (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. Four hours after the final dose of either test substance, each rat received [^{125}I] thyroxine at a dosage of 10 µCi/kg. Levels of ^{125}I in whole blood were measured for up to 30 hours following thyroxine administration and were used to calculate thyroxine terminal half-life, clearance and volume of distribution. M&B 46030 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 M&B 46030 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.

The study is classified as **Core Supplementary** as it is not a required guideline study.

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I. MATERIALS

A. Test Materials

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

M&B 46030 was suspended in 0.5% methylcellulose for administration. The formulation was analyzed for stability, homogeneity and accuracy of preparation on Days 1-2 and 14-15. Results in Appendix 5 of the study report show that the formulations were homogeneous, stable for at least one day and within acceptable range of the nominal concentrations.

Other materials used: phenobarbital - positive control
[^{125}I] thyroxine (T_4)
sodium iodide

B. Test Animals

Species: Crl:CD (SD) BR male rats
Source: Charles River, St. Aubin-Les-Albeuf, France
Age: 49 days old when received
Weight: 16 g when received
Housing: Three rats per cage
Environmental Conditions: Temperature: 21 ± 2^0 C
Relative Humidity: 55 ± 10%
Photoperiod: 12 hours light/dark
Food and Water: SDS Rat and Mouse No. 1 modified maintenance diet and water *ad libitum*
Acclimation Period: Four days

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II. METHODS

Dosage Groups and Administration

The animals were assigned to the following groups.

Group	Treatment	Subset	Number of Animals
1	Control (0.5% methylcellulose)	(a)	6
		(b)	6
2	M&B 46030 (10 mg/kg/day)	(a)	6
		(b)	6
3	Phenobarbital (80 mg/kg/day)	(a)	6
		(b)	6

The test material in a suspension with 0.5% methylcellulose was administered by gavage at a volume of 5 ml/kg using a syringe and a rubber catheter. Control animals received the vehicle alone at this volume. Phenobarbital was administered intraperitoneally as a suspension in 0.5% methylcellulose at a volume of 2 ml/kg. All of the test materials were prepared fresh daily. Subset (a) of each group was treated on Day 1 only; subset (b) was treated for a total of 14 days. On Day 1 for subset (a) and Day 14 for subset (b), the animals in each group received 1 mg NaI in 0.9% saline solution intraperitoneally at two times, 5 minutes and 10 hours 5 minutes after the test compound administration. On Day 1 four hours after dosing for subset (a), the animals also received 10 μCi/kg of [^{125}I] thyroxine in 0.9% saline solution using an IV infusion set at a constant dosage volume of 2 ml/kg. The animals in subset (b) received the identical treatment on Day 14.

Observations

The following observations were recorded at the indicated times.

Mortality - twice daily
Clinical signs of toxicity - once daily
Body weight - at time of allocation of animals to groups, Day -1, on commencement of treatment, pre-terminally for subset (a) and once a week thereafter for subset (b), including Day 13
Food consumption - Day -2 to Day 3 for subset (a) and from Day -2 to Day 7, Day 14 and termination for subset (b)
Food efficiency - calculated for subset (b) only

Thyroxine Clearance

Blood was drawn at the following times after the administration of [^{125}I] thyroxine on Day 1 for subset (a) animals and on Day 14 for subset (b) animals: 0.5, 1, 2, 4, 8, 12, 20, 24 and 30 hours. Each sample was divided into three aliquots. Two of the aliquots were used to determine total radioactivity by gamma counting and the other was used to determine protein-bound radioactivity after precipitation with trichloroacetic acid.

Statistical Analysis

A description of the statistical procedures from the study report is attached to the DER.

III. RESULTS

Mortality and Clinical Signs

None of the rats died during the study. There were no treatment-related clinical signs of toxicity in the animals treated with M&B 46030. Collapsed posture, lethargy and shallow respiration were observed on Day 1 in the rats treated with phenobarbital.

Body Weight, Food Consumption and Food Efficiency

Body weights, food consumption and food efficiency values of the M&B 46030- and phenobarbital-treated animals were comparable to the control group.

Thyroxine Clearance

Graphs of thyroxine concentration in whole blood (pg/ml) plotted against time after thyroxine administration on Days 1 and 14 (Figures 3 and 4 of the study report) showed a biphasic distribution with an absorption phase followed by a excretion/metabolism phase. Analyses of the aliquot treated with trichloroacetic acid (to precipitate out the proteins) showed that the [^{125}I] thyroxine was stable in whole blood and consequently, the levels of radioactivity measured could be related directly to the amounts of thyroxine.

Pharmacokinetic parameters, including terminal half-life, clearance and volume of distribution of thyroxine in whole blood, were calculated. A summary of these parameters found in Table 4 from the study report is attached to the DER. Statistical analyses were done to determine if there was any effect on the parameters due to the duration of treatment with the dosing material and the treatment with the dosing material *per se*. There was a significant increase in the terminal half-life and the volume of distribution for the control animals that had been on the study for 14 days as compared

to those who had been maintained for only one day. Therefore, statistical comparisons between Day 1 and Day 14 values for the treated groups were not reported since it was unclear how much age and the length of treatment contributed to the changes.

There was no evidence of an effect of M&B 46030 on terminal half-life, clearance and volume of distribution after one day of treatment. However, after 14 days, there was a significant decrease in the terminal half-life (52% of control level) along with significant increases in the clearance (261% of control level) and volume of distribution (137% of control level). Animals treated with phenobarbital had similar effects on Day 14 with a significant decrease in terminal half-life (69% of control level) and increases in clearance (184% of control level) and volume of distribution (125% of control level). There was also evidence of an effect of the phenobarbital treatment at Day 1 on all three parameters.

IV. COMPLIANCE

The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in accordance with GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality.

V. CONCLUSIONS

M&B 46030 induced increased thyroxine clearance from whole blood when administered to rats at a dosage of 10 mg/kg/day for 14 days. There was no evidence of increased clearance after one day of treatment. The quantitative effect on Day 14 was greater after treatment with M&B 46030 than with phenobarbital, although the latter chemical also had an effect at Day 1.

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