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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 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 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

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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.

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I. MATERIALS

A. Test Material

Name: M&B 46030
Synonym: Fipronil
Chemical Name: 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-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.

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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
Startle
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.

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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	4.0	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.

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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*	926	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, $p < 0.001$

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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*	53**	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	3.9•	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.

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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 thyrcid 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

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					✓					✓
Penacinar 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.