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DATA EVALUATION REPORT

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

EPA I.D. NUMBERS: P. C. CODE: 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. Auchton

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 incl[illegible] 1) neurotoxicity (including seizures which resulted in death) [illegible]ne 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.

2

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'
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

' 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.

5

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:

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	26
91	30	36	28*	30	38*	27	29	29	37	28

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

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		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	95
Week 0-52	712	726	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	17739	18677	17614					
Percent of control	-	99	98	103	97					
Weeks 1-90						13931	14027	14043	14020	13760
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.

13

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	1.7	8.9	9.1	8.8	9.0	8.6

a Extracted fr a 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 PC , 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 o 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 as 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.

91065

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	44	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*	975	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, $p<0.002$

122 15

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	14.3

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 24 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 50 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	0.6***
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 86 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	β 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•	2.55••	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	2.49••	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.

131

[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	0.032	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)	897.2	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	0.362	0.374	0.353	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..
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	8.12	6.20	3.92	3.72	3.71	4.15	4.32
R (%)	0.719	0.745	0.716	0.994	0.940	0.742	0.701	0.744	0.774	0.918
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.[illegible]	.81	4.68 **
Testes										
A (g)	3.57	3.80	3.80	3.81	3.63					
R (%)	0.425	0.471	0.407	0.461 *	0.[illegible]					
Thyroids[b]										
A (g)	0.038	0.039	0.043	0.045	0.045	0.031	0.[illegible]31	0.030	0.034	0.035
R (%)	0.0045	0.0047	0.0045	0.0056	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	6[illegible]5.2	596.4	[illegible] *	[illegible]3.6 [illegible]
Brain										
A (g)	2.37	2.35	2.39	2.33	2.35	2.10	2.11	2.11	2.13	2.13
R (%)	0.284	0.238 *	0.260	0.310 *	0.325 *	0.363	0.391	0.384	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 *	0.0259 *
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	0.300	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										
(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 1-A-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.

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Table 14
Incidence and Severity of Progressive Senile Nephropathy in Rats Treated with M&B 16030 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	4	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, $p<0.001$

28

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	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

139

32

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.