Miscfile

010608

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

SEP 30 93

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Amitraz
RfD & Developmental Toxicity Assessment

Barcode D191893
Chem. No. 106201

FROM: Ray Landolt 9/29/93
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

TO: Dennis H. Edwards, Jr., PM 19
Insecticide-Rodenticide Branch
Registration Division (H7505C)

THRU: Mike Ioannou, Section Head J.M. Ioannou 9/29/93
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Branch Chief Marcia van Gemert 9/29/92
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Nor-Am Chemical Company

Action Requested: 1. The Data Evaluation Reports (DER) on Amitraz were not adequate for a RfD evaluation. All of the chronic, reproductive and developmemtal toxicity studies conducted during 1973 and the mouse carcinogenicity study conducted in 1983 have been reevaluated. DER's and corrected one-liners for these studies are attached.

2. The Health Effects Division RfD/Peer Review Committee met on September 2, 1993 to discuss and evaluate the existing chronic, developmental and reproductive toxicity studies in support of re-registration and to reassess the Reference Dose (RfD) of Amitraz. The RfD/Peer Review Committee recommendations are presented.

Data Request: The developmemtal (83-3a) and reproductive (83-4) toxicity studies in rats are supplementary and do not permit a reliable assessment of the developmental and reproductive toxicity of amitraz. The RfD Committee requested a study addressing the developmental neurotoxicity/reproductive toxicity potential of amitraz in the rat to be submitted. The Agency should be consulted on the study protocol before the study is initiated.

1 OF 80

Background Information:

The existing RfD for this chemical was established by the Health Effects Division RfD Committee in July 1987 and verified by the Agency RfD Work Group October 1987. The RfD is based on a no observable effect level (NOEL) of 0.25 mg/kg/day for changes in blood chemistry (increased blood glucose) and hypothermia observed at the 1.0 mg/kg/day level in the two year dog feeding study. An uncertainty factor (UF) of 100 was used to account for the inter-species extrapolation and inter-species variability. On this basis the RfD was calculated to be 0.0025 mg/kg/day.

The following studies were initially reviewed in 1975 (DER 001124) and were not subjected to current acceptance criteria for guideline data (158.135) requirements. This data base was evaluated by HED in consort with the California Department of Food and Agriculture March 1989 (DER 007190) and concluded that these studies were acceptable. A data classification was not assigned to these studies.

Studies Evaluated with this Review:	MRID	Classification
83-1a Two Year Feeding/Carcinogenicity Study in Rats conducted at 0, 15, 50 or 200 ppm.	0044585	Minimum
NOEL=15 ppm (males 0.77 and females 0.97 mg/kg/day)		
LOEL=50 ppm (males 2.5 and females 3.1 mg/kg/day) with excitable and aggressive behavior.		
MTD=200 ppm (males 10.2 and females 12.6 mg/kg/day) with clinical signs and decreased b.wt. gain.		
Negative for Carcinogenicity		
RfD Committee Recommended: Change the NOEL/LOEL from 50/200 ppm to 15/50 ppm based on clinical signs at the 50 ppm level in female rats.		
83-1b Two Year Oral (capsule) Toxicity Study in Dogs conducted at 0, 0.1, 0.25 or 1.0 mg/kg/day.	0044586	Minimum
NOEL = 0.25 mg/kg/day		
LOEL = 1.0 mg/kg/day with CNS depression, hypothermia and increased blood glucose values.		
RfD Committee Recommendation: This study supports the existing RfD.		

Studies Evaluated with this Review:	MIRD	Classification
83-3a Developmental Toxicity in Rats and a One-Generation Reproduction Study in Rats conducted at 0, 1, 3 and 12 mg/kg/day were considered together in an evaluation of the developmental potential of amitraz in rats.	00029959 00029960	Supplementary Supplementary

Maternal NOEL = 3 mg/kg/day
LOEL = 12 mg/kg/day with a decrease in b.wt. gain.

Developmental NOEL = 3 mg/kg/day
LOEL = 12 mg/kg/day with decreased litter size at birth and day-4.

RfD Committee Recommended: The NOEL/LOEL for developmental toxicity of 3 and 12 mg/kg/day.

83-3b Developmental Toxicity in Rabbits conducted at 0, 1, 5 and 25 mg/kg/day	00029961	Supplementary

Maternal NOEL = 5 mg/kg/day
LOEL = 25 mg/kg/day with decreased b.wt. gain and increased abortions.

Develomenntal NOEL = 5 mg/kg/day
LOEL = 25 mg/kg/day with decreased litter size, increased pre- and post-implantation loss.

RfD Committee Recommended: While this study was deficient it was adequate for risk assessment.

83-4 Multigeneration Reproduction in Rats conducted at 0, 15, 50 and 200 ppm	00029962	Supplementary

Systemic NOEL = 50 ppm (males 4.84 and females 5.22 mg/kg/day)
LOEL = 200 ppm (males 16.41 and females 20.06 mg/kg/day) with decreased b.wt. gain and food intake.

Reproductive NOEL = 15 ppm (males 1.47 and females 1.58 mg/kg/day)
LOEL = 50 ppm (males 4.84 and females 5.22 mg/kg/day) with reduced litter size and pup survival.

RfD Committee Recommended: Concurrence with the reviewer.

Studies Evaluated with this Review, but Not Considered by the RfD Committee.	MRID	Classification
82-1a 90-day Oral (gavage) Toxicity in Rats conducted at 0, 3, 12 and 50 mg/kg/day. NOEL = 3 mg/kg/day LOEL - 12 mg/kg/day with excitable and irritable behavior, decreased b.wt. gain, relative weight of adrenals increased and relative weight of liver decreased.	0051784	Supplementary
82-1b 90-day Oral (capsule) Toxicity in Dogs conducted at 0, 0.25, 1.0 and 4.0 mg/kg/day. NOEL = 0.25 mg/kg/day LOEL = 1.0 mg/kg/day with CNS depression, hypothermia, decreased pulse, increased glucose values and histopathological findings of the liver.	0040345	Supplementary

83-2b Carcinogenicity

The carcinogenicity issue was addressed by HED Carcinogenicity Peer Review Committee January 3, 1991 (DER 008336). The carcinogenicity studies in the mouse and rat (83-2) were not discussed by the RfD/Peer Review Committee. Amitraz was classified as a "Group C" possible human carcinogen. Quantification of potential human risk using a low-dose extrapolation model (Q^*_1) was recommended.

The purpose of this review is to upgrade DER 004252 of May 23, 1984 with the information considered by the January 3, 1991 Peer Review Committee in evaluating the carcinogenicity of amitraz and supplement this review with tables from the original report.

Two Year Mouse Feeding/Carcinogenicity Study (83-2b) was conducted at 0, 25, 100, and 400 ppm (MRID 252098 to 252102).

Classification of Data - Minimum

Systemic NOEL = < 25 ppm
LOEL = 25 ppm (males 2.3 and females 2.6 mg/kg/day) with a dose-related increase in the incidence of hyperplastic nodules, basophilic and telangiectatic foci in the liver of females, accompanied by stomach hyperkeratosis and spleen hematopoiesis in males

Carcinogenicity - Positive in females at 400 ppm (50.1 mg/kg/day) with an increased incidence of hepatocellular adenomas (18%), carcinomas (21%) and combined adenomas/carcinomas (38%), accompanied by increased incidence of lung adenomas (25%) in males at the 400 ppm (44.7 mg/kg/day) level.

010608

Reviewed By: Ray Landolt 6/28/93
Section I, Toxicology Branch II - (H7509C)
Secondary Reviewer: Mike Ioannou 6/28/93
Section I, Toxicology Branch II - (H7509C)

DATA EVALUATION REPORT

Study Type: Feeding/Carcinogenicity - Mouse (83-2b)

Test Material: N'-(2,4-Dimethylphenyl)-N-[[(2,4-dimethylphenyl) imino]methyl]-N-methylmethanimidamide

Common Name: Amitraz (BTS 27419)

Classification: Insecticide/Acaricide

Barcode: 191893
Tox.Chem.No.106201
MRID 252098, 252099 252100, 252101 and 252102

Title of Study: 104 Week Tumorigenicity Study in Mice

Study Number: T233, Tox/83/179-93

Study Date: December 20, 1983

Sponsor: The Upjohn Company and Boots Company, LTD (Nor-Am Chemical Co.)

Testing Facility: FBC Limited, Huntingdon Research Center, England

Author: John Colley

Quality Assurance: Kenneth W.G. Shillam

This is to upgrade Toxicology review of May 23, 1984 (DER 004252) with the information considered by the January 3, 1991 (DER 008336) Peer Review Committee in evaluating the carcinogenic potential of amitraz. In addition, tables from the original report have been inserted in support of these findings.

Conclusion: Three groups of 75 B6C3F1 mice/sex/group were fed dietary levels of 25, 100, or 400 ppm with 100 mice/sex in the control group for 104 weeks.

Classification of Data - Minimum

Deficiency - A systemic NOEL was not demonstrated.

This study is acceptable and satisfies the guideline data requirement (83-2b) for a carcinocenicity study in mice.

Systemic NOEL = < 25 ppm (males 2.3 and females 2.6 mg/kg/day) with a dose-related increase in the incidence of hyperplastic nodules, basophilic and telangiectatic foci in the liver of females, accompanied by an increased incidence of stomach hyperkeratosis and spleen hematopoiesis in males.

Carcinogenicity - Positive in females at the 400 ppm level (50.1 mg/kg/day) with a significant increase in the incidence of hepatocellular adenomas (18%), carcinomas (21%), and combined adenomas/carcinomas (38%), accompanied by a significant increase in the incidence of lung adenomas (25%) in male mice at the 400 ppm level (44.7 mg/kg/day).

A. Materials

1. Test Material - Amitraz technical, a pale yellow lumpy powder, of batch numbers 34732Y (weeks 1-4), 52221 (weeks 5-54) and 56105 (weeks 55-107) was used in this study. The purity of the test material ranged from 88.2 to 100.8%.

2. Animals - Male and female, 33 (+ 2 days) day old, B6C3F1 mice from Charles River were used in this study. The mice were within a 5 g weight range for each sex.

B. Study Design:

1. Allocation of Animals:

Test Group	Dose Level(ppm)	Number of Mice Male	Female
1 Control	0	100	100
2 Low	25	75	75
3 Mid	100	75	75
4 High	400	75	75

Mice were not allocated to treatment groups for an interim sacrifice.

Animals were housed 5/cage and identified with individual ear marks within each cage. Temperature (22°C), humidity (50%) and a 12 hour light/dark cycle were controlled to provide a uniform environment.

A pretest macroscopic examination of 10 male and 10 female mice was negative.

Dietary levels were prepared weekly from powdered Spratt's Laboratory Diet No. 2. A premix was prepared for dilution to the required dietary concentrations. Prepared diets and tap water were available ad libitum.

Dietary levels were analyzed every 13-weeks by high pressure liquid chromatography to verify the targeted concentration.

Targeted Concentration (ppm) for	Nominal Concentration (ppm) 25	100	400
Weeks 1 to 52	24-31	92-117	348-440
Weeks 65 to 104	24-27	91-106	392-407

Statistical analysis of dietary intake was calculated from the weekly cage means for each group. Body weight gain was calculated as the mean values of body weight gain of individual animals. Analysis of variance was used to assess the significance of intergroup differences in food and body weight data. Intergroup comparisons were calculated using Student's 't' test. Bartlett's test was applied to test for heterogeneity of variance between treatments.

6

C. Methods and Results:

1. Observations - Animals were housed with 5 mice/sex/cage. All animals were observed individually for clinical signs of toxicity daily during the first four weeks and then weekly thereafter.

   Hyperactivity and aggressive behavior were observed for males fed the 400 ppm and to a lesser degree in males fed the 100 ppm level during the first 12 weeks of the study. Cutaneous lesions, as evidence of fighting, accompanied by inflammation and swelling of the perigenital and perianal areas were also observed in male mice fed the 100 and 400 ppm levels. In addition, piloerection and hunched posture were observed for male mice at the 400 ppm level during weeks 2-4. These gross affects were not observed in the females fed dietary levels of amitraz.

2. Food consumption of each cage was recorded initially and then weekly thereafter.

   Food consumption decreased significantly ($p<0.05$) at the 100 and 400 ppm levels during weeks 1-12 for males by 6% and during weeks 1-19 for females by 5 to 11%. Dietary intake of males at the 100 ppm level decreased significantly ($p<0.01$) during weeks 13-25 by 6% being comparable to the controls for the remainder of the study. An increase in food consumption was recorded at the 400 ppm level by 9 to 13% being significant for males during weeks 26-104 and for females at weeks 31-58 and 93-104. Table for food consumption, from this report is attached.

3. Food utilisation was assessed over 4-weekly periods during the first 24-weeks of the study.

   Food utilisation during the initial 24-weeks of the study was lower for males and females at the 400 ppm level as compared to the controls or the 25 and 100 ppm levels. Table for food conversion ratios, from this report is attached.

4. Mean Compound Consumed (mg/kg/day) is summarized in the following table.

	25 ppm		100 ppm		400 ppm	
Week	Male	Female	Male	Female	Male	Female
13	2.82	3.54	10.66	14.03	54.02	57.18
26	2.35	2.70	9.52	11.77	45.24	54.75
52	2.21	2.32	9.40	9.39	45.88	50.93
104	1.93	2.28	7.90	9.18	43.52	51.49
1 to 104	2.31	2.63	9.61	10.77	44.65	50.13

5. Body weight was recorded initially and then weekly thereafter

Group mean body weight gain by week 13 was reduced at the 400 ppm level in males and females by 25 and 14%, respectively accompanied by a reduced body weight gain in males at the 100 ppm level by 13%. as compared to control values.

Group mean body weight gains are summarized in the following table for week 13 (from Table 4, page 34) of this study.

Group Mean Body Weight Gain (g) for Week 13

	Control	25ppm	100ppm	400ppm
Males	8	8(0)*	-7(13)	-6(25)
Females	7	+8(14)	7(0)	-6(14)

* Percent (%) change compared to control.

As shown on the attached table (Table 5, page 38 from this report) body weight gain at the 400 ppm level was reduced by week 18 for males and females by 31%. By week 52, at the 400 ppm level, body weight gain was reduced for males and females by 29 and 55%, respectively as compared to control values.

At the 100 ppm level body weight gain was reduced significantly ($p<0.01$) by week 52 for males and females by 29% and 32%, respectively.

6. Mortality - All animals were observed twice daily for deaths. The following table (DER 008336) summarizes the mortality rates in this study. Mortality among the control, 25, 100 and 400 ppm levels was 20, 13, 20, 27% for males and 20, 19, 17, 25% for females, respectively. The statistical evaluation of survival indicated that males had a significant positive trend in mortality with incremental doses of amitraz. Female mice had no differential mortality with dose increments of amitraz.

Amitraz $B_6C_3F_1$ Mouse Study-Mortality Rates† and Cox or Generalized K/W Significant Test Results (Bernice Fisher, September 12, 1990)

Males	Week				
Dose(ppm)	1-26	27-52	53-78	79-104a	Total
0	0/100	0/100	2/100	18/98	20/100(20)*
25	0/75	2/75	1/73	7/72	10/75 (13)
100	1/75	3/74	2/71	9/69	15/75 (20)
400	1/75	1/74	7/73	11/66	20/75 (27)

Females	Week				
Dose(ppm)	1-26	27-52	53-78	79-107a	Total
0	2/100	0/98	4/92	14/88	20/100(20)
25	0/75	2/75	2/73	10/71	14/75 (19)
100	0/75	0/75	3/75	10/72	13/75 (17)
400	1/75	1/74	1/73	16/72	19/75 (25)

† Number of animals that died during interval/number of animals alive at the beginning of the interval.
( ) Percent
a -Final sacrifices at weeks 105-106 for males and 105-107 for females.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
* $p<.05$, ** $p<.01$.

7. Terminal Findings: All surviving mice were killed by carbon dioxide asphyxiation on completion of the 104-week period.

   a. Bone marrow smears (femoral) were prepared from all test and control animals for an assessment of the myeloid/erythroid cell ratio.

      The bone marrow myeloid/erythroid ratio was significantly ($p<0.001$) reduced, as compared to controls, for males at the 400 ppm level by 24% and for females at the 100 and 400 ppm levels by 22 and 26%, respectively. Tables for bone marrow myeloid/erythroid ratios, from this report are attached.

   b. Macroscopic observations

      Female mice at the 400 ppm level were obeserved to have an increased incidence of enlarged livers and liver masses accompanied by a decrease in the incidence of uterine changes, including thickened and congestion of the uterine wall and distention of the uterus. An increased incidence of female mice with minimal adipose tissue was also observed at the 400 ppm level.

      Male mice at the 100 and 400 ppm level were observed to have an increased incidence of enlarged preputial gland and reduced adipose tissue as compared to the controls.

      Females at the 100 ppm level exhibited a decreased incidence of uterine changes as compared to the controls.

      Prominence of the limiting ridge of the stomach was observed among all test and control groups being of higher incidence for females fed the 25, 100 and 400 ppm levels and for males fed the 100 and 400 ppm levels as compared to the controls.

b. Macroscopic (con't) The following table* summarizes the incidence of macroscopic findings reported for those animals that died during the study or were killed at the termination of the study.

Dose (ppm)	Control	25	100	400
Male				
No. Examined	100	75	75	75
Stomach				
Limiting ridge thickened	36	33	49	42
Liver				
Masses	37	19	17	31
Enlarged	9	5	8	7
Preputial gland				
Enlarged	12	6	14	20
Adipose tissue				
Sparse	1	—	3	3
Female				
No. Examined	100	75	75	74
Stomach				
Limiting ridge thickened	34	37	51	40
Liver				
Masses	12	13	18	66
Enlarged	6	4	6	35
Uterus				
Wall, thickened	54	37	29	—
Wall, congested	22	16	11	—
Distended	41	34	26	10
Adipose tissue				
Sparse	—	—	—	8

* Data taken from Tables 9a and 9b, pages 46-51, of this report.

c. Histopathological findings - An interim sacrifice was not preformed and tissues were not weighed.

Tissues in the following table were prepared for histopathological evaluation from the control and 400 ppm level. In the 25 and 100 ppm levels, tissues of adrenals, liver, lung, ovaries, pituitary, spleen, sternum, stomach, thyroids and uterus were examined microscopically.

The checked (X) tissues are recommended by Subdivision F Guidelines of November 1989.

X Adrenals	X Liver	X Spleen
X Aorta	X Lungs	X Stomach
X Bone marrow (femur)	X Lymph nodes	X Testes
X Brain	X Mammary gland	Thymus
X Caecum	X Ovaries	X Thyroids and
X Duodenum	X Pancreas	parathyroids
X Esophagus	X Pituitary	X Trachea
X Eyes	Prostate	X Urinary bladder
Gall bladder	X Salivary gland	X Uterus
Harderian gland	Sciatic nerve	X Colon
Nasal cavity	Seminal vesicles	X Kidneys
X Heart	X Skeletal muscle	X Epididymis
X Ileum	X Skin	
X Jejunum	X Spinal cord	

i. Non-neoplastic findings

Incidence of focal hyperkeratosis of the forestomach and spleen hematopoiesis was increased in males fed the 25, 100 and 400 ppm levels as compared to the controls. Females fed all three dietary levels exhibited an increased incidence of hyperplastic nodules, basophilic and telangiectatic foci of the liver as compared to the controls. An increased incidence of hyperplastic nodules and telangiectatic foci was observed in males at the 400 ppm level.

The incidence of pituitary hyperplasia, ovarian cysts and uterine cystic glands was reduced in females fed the 400 ppm level as compared to the females of control or 25 and 100 ppm levels.

010608

The following table* summarizes the incidence of non-neoplastic findings reported for those animals that died during the study or were killed at the termination of the study.

Dose (ppm)	Control	25	100	400
Male				
No. Examined	100	75	75	75
Liver				
Hyperplastic nodules	16	11	7	19
Basophilic foci	2	6	2	4
Telangiectatic foci	5	—	—	8
Spleen				
Hematopoiesis	10	20	23	19
Stomach				
Hyperkeratosis	22	37	39	52
Female				
No Examined	100	75	75	75
Liver				
Hyperplastic nodules	3	7	11	46
Basophilic foci	1	4	5	8
Telangiectatic foci	1	2	5	5
Spleen				
Hematopoiesis	23	27	25	22
Stomach				
Hyperkeratosis	27	32	35	20
Pituitary				
Hyperplasia	8	6	5	—
Ovarian cysts	28	14	20	8
Uterus				
Cystic glands	79	61	43	15

* Data taken from Tables 12a and 12b, pages 58-60, of this report.

ii. Neoplastic findings - The following tables (DER 008336) summarizes the incidence of hepatocellular and lung tumors in male and female mice.

In females there were significant dose-related positive trends in hepatocellular adenomas, carcinomas, and in the combined group of adenomas and/or carcinomas. Female mice also had a significant difference in the pair-wise comparison of controls and the highest dose group in hepatocellular adenomas, carcinomas and in the combined group of adenomas and/or carcinomas. The incidence of hepatocellular adenomas and carcinomas was not increased in males

Hepatocellular Tumor Rates (†) in Male and Female B6C3F1 Mice
(Bernice Fisher, September 12, 1990)

	Dose (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Adenomas	6/99	3/72	4/70	6[a]/71	4/98	1/73	3/75	13[c]/73
Percent	6	4	6	8	4	1	4	18
p=	0.23	0.72[n]	0.51	0.28	0.00**	0.29	0.65	0.00**
Carcinomas	14/99	8/72	6/70	8[b]/73	2/98	0/73	1/75	15[d]/73
Percent	14	11	9	11	2	0	1	21
p=	0.77[n]	0.44[n]	0.72[n]	0.78[n]	0.00**	0.33	0.60	0.00**
Combined	20/99	11/72	10/70	14/73	6/98	1/73	4/75	28[a]/73
Percent	20	15	14	19	6	1	5	38
p=	0.58[n]	0.67[n]	0.83[n]	0.65[n]	0.00*	0.12	0.55	0.00**

† Number of tumor bearing animals/number of animals at risk (males were evaluated by Peto's Prevalence tests, females were evaluated by Cochran-Armitage Trend and Fisher Exact test) excluding those that died before first tumor was observed.
n-negative change
a-First liver adenoma observed at week 70, dose 400 ppm.
b-First liver carcinoma observed at week 68, dose 400 ppm.
c-First liver adenoma observed at week 84, dose 400 ppm.
d-First liver carcinoma obsreved at week 94, dose 400 ppm.
Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted at Dose level.
* p <.05, **p <.01.

The following table shows the background incidence of hepatocellular tumors in two other studies conducted concurrently on B6C3F1 mice in the same testing facilities (Huntingdon Research Center, 1983).

Incidence of Hepatocellular Adenomas and Carcinomas in Concurrent Controls

	Study A		Study B	
	Males (%)	Females (%)	Males (%)	Females (%)
Adenoma	17	5	30	13
Carcinoma	14	5	18	6
Combined	31	10	48	19

The incidence of hepatocellular adenomas, carcinomas and adenomas/carcinomas combined in the high-dose female B6C3F1 mice was higher than the incidence of these types of lesions in other concurrent controls.

The incidence of lung tumors in male and female B6C3F1 mice are presented in the following table.

In males there was a significant dose-related positive trend in lung adenomas. Also, in the pair-wise comparison of the controls and the highest dose group, there was a significant difference in this tumor type in the high-dose males when compared to conrrols.

Lung Tumor Ratest and Peto's Prevalence Test Results for Males and Cochran-Armitage Trend Test and Fisher's Exact Test Results for Females (Bernice Fisher, September 12, 1990).

Males	Dose (ppm)			
	0	25	100	400
Adenomas	9/95	12/71	8[a]/69	16/64
Percent	9	17	12	25
p=	0.01**	0.11	0.35	0.01**

Females	Dose (ppm)			
	0	25	100	400
Adenomas	7/98	8/73	4/75	10[b]/73
Percent	7	11	5	14
p=	0.08	0.27	0.44	0.12

† Number of tumor bearing animals/number of animals examined
a-First male lung tumor observed at week 87, dose 100 ppm.
b-First female lung tumor observed at week 98, dose 400 ppm.
Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted at Dose level. * $p< 0.05$, ** $p< 0.01$

The following table shows the background incidence of lung tumors in two other studies conducted concurrently on B6C3F1 mice in the same testing facilities (Huntingdon Research Center,1983).

Incidence of Lung Tumors in Concurrent Control $B_6C_3F_1$ Mice

	Study A (%)	Study B (%)
Male	13	7
Female	5	7

The incidence of lung tumors in the high dose male and female B6C3F1 mice was higher than those for other concurrent controls.

Discussion: In this study there was a reduction in body weight gain and a significant positive trend in mortality in male mice suggesting that the highest dose (400 ppm) was excessive. In females, however, the Peer Review Committee agreed that the 400 ppm level was high but not excessive, since it was not life-threatening (there were no significant differences in mortality as compared to controls).

By week 13, body weight gain in males and females at the 400 ppm level was reduced by 25 and 14%, respectively accompanied by reduced body weight gain in males at the 100 ppm level of 13%.

At the 400 ppm level, body weight gain was reduced by week 18 for males and females by 31%. By week 52 at the 400 ppm level, body weight gain was reduced for males and females by 29 and 55%, respectively as compared to control values.

At the 100 ppm level, body weight gain was reduced by week 52 for males and females by 29 and 32%, respectively.

Dietary administration of amitraz to female B6C3F1 mice was associated with a significant ($p<0.01$) increase in the incidence of hepatocellular adenomas (18%), carcinomas (21%), and adenomas/carcinomas combined (38%) in the high-dose females when compared to controls (adenomas, 4%; carcinomas, 2%; and adenomas/carcinomas combined, 6%) with a significant ($p<0.01$) positive dose-related trend.

A significant ($p<0.01$) increase in the incidence of lung adenomas (25%) in male mice at the 400 ppm level was reported as compared to controls (9%), with a significant ($p<0.01$) dose-related trend.

Conclusion: Classification of Data - Minimum

Deficiency - A systemic NOEL was not demonstrated

This study is acceptable and satisfies the guideline data requirement (83-2b) for a carcinogenicity study in mice.

Systemic NOEL = < 25 ppm (males 2.3 and females 2.6 mg/kg/day) with a dose-related increased incidence of hyperplastic nodules, basophilic and telangiectatic foci in the liver of females, accompanied by an increased incidence of stomach hyperkeratosis and spleen hematopoiesis in males at this level.

Carcinogenicity - Positive in females at the 400 ppm level (50.1 mg/kg/day) with a significant increased incidence of hepatocellular adenomas (18%), carcinomas (21%), and combined adenomas/carcinomas (38%), accompanied by a significant increased incidence of lung adenomas (25%) in males at the 400 ppm level (44.7 mg/kg/day).

Amitraz toxicology review

Page _____ is not included in this copy.

Pages 19 through 23 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

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

010608

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

004252

MAY 23 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Amitraz
TOX Chem. No. 374 A

TO: Jay Ellenberger, PM 21
Insecticide Branch/RD (TS-767)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) 5/22/84

FROM: Ray Landolt
Review Section #1
Toxicology Branch/HED (TS-769) 5-22-84

Registrant: The Upjohn Co. No. 1023-59
Nor-Am Chemicals No. 45639-49

Action Requested: Review 2-year mouse oncogenic study

Recommendation:

Defer a toxicological conclusion on the oncogenic potential of Amitraz until a quantititative risk assessment has been completed.

2-Yr. Oncogenic-Mouse
Huntingdon Research Center No. 153/8262
TOX/83/179-93, Dec. 1983 Acc. No. 252098-252102

A. Procedure

Seventy-five male and 75 female 33 day old B6C3F1 hybrid (Charles River) mice were fed dietary levels of 25, 100, 400 ppm amitraz for 104 weeks. The untreated controls consisted of 100 male and 100 female B6C3F1 hybrid (Charles River) 33 day old mice. Animals were housed with five mice per sex per cage. All animals were acclimated to the laboratory for 12 days before the start of the study. Dietary levels were prepared weekly with dietary stability determined on weeks, 13, 26, 39, 52, 65, 78, 91 and 104. Cages were observed twice daily for dead animals. Food consumption, body weight

and palpation of each animal was recorded weekly. All dead or moribund animals were subjected to a detailed macroscopic examination and all tissues preserved. Samples of the following tissues were reported taken from all animals and preserved in neutral buffered 10% formalin:

adrenals
aorta (thoracic)
bone (femur)
brain (medullary, cerebellar and cortical sections)
caecum
duodenum
eyes
gall bladder
Harderian gland
head+
heart
ileum
jejunum
liver (from median and left lobes)
lungs (all lobes and bronchi)
lymph nodes (cervical, mesenteric, axillary, mediastinal and inguinal)
mammary gland
mid-colon
oesophagus
ovaries
pancreas
pituitary
prostate
salivary gland (sub-maxillary)
sciatic nerve
seminal vesicles
skeletal muscle (biceps femoris)
skin
spinal cord (cervical and lumbar)
spleen
sternum (bone marrow)
stomach (glandular and non-glandular)
testes with epididymides
thymus
thyroids (with parathyroids)
trachea
urinary bladder
uterus (plus cervix)
bone marrow smear
blood smear

+ for nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx and middle ear.

To assess the myeloid - erythroid cell ratio, bone marrow smears were prepared from marrow extruded from the femur. All tissue from the high dose and control animals were examined microscopically. At the low and intermediate dosage levels, liver, pancreas, spleen, lung, stomach, pituitary, thyroid, adrenals, ovaries, uterus, sternum and all macroscopically abnormal tissues were examined microscopically.

B. Results

1. Stability

a. Test material concentration varied between 88.2 and 98% with a mean of 95.3% during the first 54 weeks followed by a range of 93.4 to 100.8% with a mean of 98% for the duration of the study.

b. Dietary Analysis every 13 weeks showed the following actual dietary levels fed in ppm.

	1	13	26	39	52	65	78	91	104
400 PPM	375.0	379.9	376.8	370.7	440.4	392.4	365.4	407.3	386.5
100 PPM	92.0	102.5	101.7	96.4	116.5	102.6	96.8	105.8	104.4
25 PPM	25.0	23.7	30.1	26.5	30.5	27.0	24.6	26.6	24.3

2. Gross Observations

a. Males at the 400 ppm level were hyperactive and were reported to exhibit aggressive behavior. The incidence of cutaneous ulceration and inflammation of the perigenital and perianal areas was greater at the 100 and 400 ppm level than observed for the 25 ppm level and controls. The incidence of urogenital swelling from all three dietary levels was greater than observed for the control animals.

b. Incidences of gross adverse effects for females were comparable between control and test levels.

3. Mortality

	Control No. Animals 100		25 PPM No. Animals 75		100 PPM No. Animals 75		400 PPM No. Animals 75	
	Moribund Sacrifice & Deaths	% Survival	Moribund Sacrifice & Deaths	% Survival	Moribund Sacrifice & Deaths	% Survival	Moribund Sacrifice & Deaths	% Survi
Female	20	80	14	81	13	83	19	75
Male	20	80	10	87	15	80	20	73

4. Food Consumption

A decrease in group mean food consumption was observed during the initial 13 weeks for males and 19 weeks for females fed the 100 and 400 ppm dietary levels. Followed by an increased group mean food consumption reported for males and females fed the 400 ppm level, as compared to controls, for the duration of the study. During the initial 24 weeks the food conversion ratio for males and females fed the 400 ppm level resulted in a lower food utilization as compared to the controls and two lower dietary levels.

5. Body Weight

The group mean body weight changes for 400 ppm level were comparable to controls over the initial 4 weeks for males and 7 weeks for females, then stablized at a signficiantly lower body weight gain, as compared to controls for the duration of the study. A significant decrease in body weight gain was observed for females fed the 100 ppm level over the last 74 weeks of the study.

6. Terminal Observations

a. Macroscopic

An increase in liver and lymph node involvement was apparent for males and females fed the 400 ppm level. The incidence of preputial gland enlargement of males fed the 400 ppm level was greater than observed for the control males. The incidence of prominence of the limiting ridge of the stomach was greater than controls for females fed the 25, 100 and 400 ppm levels and for males fed the 100 and 400 ppm levels.

Sex	Tissue	Finding	Number of Animals per Dosage Level Control 100	25 ppm 75	100 ppm 75	400 ppm 75
Female	Liver	Enlarged	4	4	5	34
		Mass	12	12	18	65
	Lymph	Enlarged	19	6	14	22
		Congested	4	6	10	14
	Stomach	Limiting Ridge Prominent	34	37	51	40
Male	Liver	Enlarged	7	6	5	7
		Mass	36	16	17	31
	Lymph	Enlarged	25	18	33	24
		Congested	16	21	16	22
	Preputial Gland	Enlarged	12	6	13	20
	Stomach	Limiting Ridge Prominent	35	30	44	41

b. Bone Marrow - myeloid/erythroid ratio.
A decrease in the production of myeloid elements accompanied by an increase in erythroid elements resulted in a significant decrease in the myeloid/erythroid ratio for males fed the 400 ppm level and females fed the 100 and 400 ppm levels.

c. Microscopic

The incidence of hepatocellular tumors (carcinoma and adenoma) in females fed the 400 ppm level were greater than those observed in the control group. Females fed all three dietary levels exhibited a greater incidence of liver hyperplastic nodules,

telangiectatic and basophilic focus than controls. Males fed the 400 ppm level exhibited a greater incidence of liver hyperplastic nodules, telangiectatic and basophilic focus than controls. The incidence of spleen hematopoiesis in males and stomach focal hyperkeratosis in males and females fed all three dosage levels were greater than those observed in the control groups. Enlargement of the preputial gland in males fed the 400 ppm levels was greater than observed for the control group.

Sex	Tissue	Finding	Number of Animals per Dosage Level Control 100	25 ppm 75	100 ppm 75	400 ppm 75
Female	Liver	Hepatocellular carcinoma	2	0	1	15
		adenoma	4	1	1	16
Male	Liver	Hepatocellular carcinoma	14	8	6	7
		adenoma	3	3	1	3
Female	Liver	Hyperplastic nodule	2	7	11	41
		hyperplastic focus	1	0	0	12
		telangiectatic focus	1	2	4	6
		basophilic focus	1	4	5	8
Male	Liver	Hyperplastic nodule	16	9	7	19
		hyperplastic focus	0	0	0	2
		telangiectatic focus	4	0	0	8
		basophilic focus	2	6	2	4
Female	Spleen	hematopoiesis	23	27	25	22
Male	Spleen	hematopoiesis	10	20	23	19
Female	Stomach	focal hyperkeratosis	28	31	29	40
Male	Stomach	focal hyperkeratosis	22	34	39	53
Male	Preputial Gland	Enlarged	12	6	13	20

Tumor Incidence in Concurrent B6C3F1 Control Mice

	Study A		Study B	
	Female	Male	Female	Male
Hepatocellular carcinoma	5/104	14/104	6/104	19/104
Hepatocellular adenoma	5/104	18/104	14/104	31/104

C. Conclusion

1. There is apparent liver involvement of females with an increased incidence, as compared to controls, in heptocellular tumors at the 400 ppm level accompanied by a dose response increase in the incidence of hyperplastic nodules, telangiectatic and basophilic focus of the liver at the 25, 100 and 400 ppm levels. Males at the 400 ppm level exhibited an apparent increase as compared to controls, in the incidence of hyperplastic nodules, telangiectatic and basophilic focus. Spleen hematopoiesis of males fed the 25, 100 and 400 ppm levels appears to be greater than the incidence observed in the control group. The incidence of stomach hyperkeratosis of males and females fed all three dietary levels was greater than the incidence observed with the control groups.

2. Classification of Data - Minimum.

   a. Deficiency - A systemic no effect level was not established. However, this study scientifically fulfills the intent of the oncogenic testing guidelines permitting a determination of the oncogenic potential of amitraz in mice.

TS-769:LANDOLT:sll:x73710:4/30/84 card

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 6/17/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. YMI 6/15/93
Section I, Toxicology Branch II (H7509C)

ADDENDUM TO DER FOR BTS 27 419: 90 - DAY TOXICITY STUDY IN RATS

Amitraz
DER 001124
Chem. No. 106201
Study No. 71548
MRID 0051784

RESULTS

A. Mortality and Clinical Signs

The following clinical signs were extracted from the commentary in the study report; no raw data were provided.

200 mg/kg/day: Animals became irritable and shed red tears when dosing commenced, after which they were hunched, lethargic, weak, emaciated, and squealed when handled. They were euthanized after receiving 7 doses because of their poor condition.

50 mg/kg/day: After two days of dosing, the animals became excitable, aggressive, continually squealed and shed red tears. Dosing was discontinued after 7 days. Their behavior returned to normal within 7 days of cessation of treatment. On week 13 of the study, dosing was reinstituted for 7 days and the animals showed similar toxic reactions. One male and one female died.

12 mg/kg/day: Occasional irritability and excitability were reported.

3 mg/kg/day: There were no clinical signs of toxicity in this group.

Control: Three animals shed red tears on several occasions.

B. Body Weight and Body Weight Gain

Body weight in the 200 mg/kg males and females at euthanasia (after 7 days of dosing) was 85% of their original weight. Body weight gain in the 50 mg/kg males and females was reduced in comparison to the control. After treatment was discontinued, weight gain in these animals was comparable to the control. However, when treatment was reinstituted at week 13, they lost weight. Overall body weight gain was significantly reduced ($p < 0.05$) in the 12 mg/kg group males.

Table 1
Mean Body Weight Change in Rats
Treated with BTS 27 419 for 90 Days[a]

Dose Level (mg/kg/day)	0 - 13		Recovery Period▼	
	Males	Females	Males	Females
Control	259.2	101.7	27.1	11.4
3	254.3	103.2	26.2	9.1
% Control	98	101	97	80
12	239.3*	94.7	27.5	14.1
% Control	92	93	101	124
50▲	417.9	248.1	-43.3***	19.5***
% Control	161	243	-	171

[a] Extracted from Table 1 (page 6) of the study report; week 0 - 1 and % control calculated by the reviewer.
▼ Period of three weeks after 90-day treatment period.
▲ Dosed for 7 days, then untreated until week 13 when dosed for 7 days again.
* Significantly different from control, $p < 0.5$
*** Significantly different from control, $p < 0.001$

C. Food Consumption

Food consumption was not recorded.

D. Clinical Pathology

Hematology and clinical chemistry parameters in the treated groups were comparable to the control group except for females in the 50 mg/kg group which had significant increases in potassium and sodium at the terminal evaluation; the significance of these alterations is unknown.

E. Post-mortem Findings

Organ Weights

The absolute weights of the majority of the organs from the 50 mg/kg group were decreased in comparison to the control, most likely due to the significant decrease in body weight. Therefore, only the relative weights will be discussed. The study report indicates that the relative weight of the adrenals, liver and seminal vesicles with prostate in the 50 mg/kg group may have been influenced by treatment. In males, the relative weight of the liver

influenced by treatment. In males, the relative weight of the liver was decreased by 9%, the seminal vesicles with prostate was increased by 32%; in females, the adrenals and liver were increased by 45% and 14%, respectively. In the 50 mg/kg group that was sacrificed after the three-week recovery period, the relative weight of the adrenals and liver was closer to the control value; the seminal vesicles with prostate no longer differed from the control. The organs for which there was a significant weight change from the control group are summarized in Table 2.

Table 2
Relative Weight of Selected Organs
from Rats Treated with BTS 27 419 for 90 Days[a]

	Dose Levels (mg/kg/day)							
	Control		3		12		50	
	Males	Females	Males	Females	Males	Females	Males	Females
Terminal Necropsy								
Body Weight	462.2	252.2	462.1	254.0	453.8	250.7	398.1 ***	231.8 *
Adrenals	9.1	22.3	10.1	22.4	10.6 *	22.4	9.9	32.4 ***
Brain	0.414	0.680	0.416	0.684	0.432	0.705	0.485 ***	0.751 **
Liver	3.943	3.723	3.765 *	3.687	3.678 **	3.639	3.600 ***	4.615 ***
Lungs	0.351	0.451	0.363	0.475*	0.347	0.450	0.379 *	0.423 *
Testes	0.762		0.753		0.738		0.824 *	
Prostate and seminal vesicles	0.504		0.472		0.514		0.669 ***	
Spleen	0.168	0.229	0.179	0.235	0.180	0.216	0.186 *	0.225
Necropsy After Three-Week Recovery Period								
Body Weight	497.4	267.8					384.5 ***	261.9
Adrenals	6.0	24.1					10.5 **	27.8
Liver	3.791	3.689					3.655	4.002 *
Prostate and seminal vesicles	0.502						0.453	

[a] Extracted from Tables 6-8 (pages 11-12) of the study report.

* Significantly different from control, $p < 0.5$.

** Significantly different from control, $p < 0.01$.

*** Significantly different from control, $p < 0.001$

Gross Examination

The study report indicates that the stomachs of the rats in the 50 and 200 mg/kg groups were distended with compressed food. No other lesions were considered to be treatment-related. No raw data are provided.

Microscopic Examination

Treatment-related microscopic changes seen in the 50 and 200 mg/kg groups included the following: congestion of various organs, especially the spleen, heart and pituitary; fatty infiltration of the adrenals, especially in the 200 mg/kg group males; swollen acinar cells in the salivary gland in the 50 mg/kg group; early thymic involution; vacuolation of the liver, especially in the 200 mg/kg groups; and epithelial degeneration and eosinophilia in the epithelium of the trachea in 200 mg/kg females. The incidence of these lesions is summarized in Table 3.

Table 3
Incidence of Selected Microscopic Lesions
in Rats Treated with BTS 27 419 for 90 Days[a]

	Dose Levels (mg/kg/day)							
	Control		12		50		200	
	M	F	M	F	M	F	M	F
Number Examined	12	12	12	12	12	11	21	21
Splenic congestion	0	0	2	2	10	4	11	13
Cardiac congestion	0	0	1	0	3	1	14	20
Pituitary congestion	1	0	1	0	2	0	1	7
Fatty infiltration of adrenals	0	0	1	1	1	0	15	3
Swollen acinar cells or loss of acinar structure in salivary glands	0	0	0	0	7	2	0	0
Early thymic involution	0	0	0	0	4	7	5	19
Liver - vacuolation	0	0	0	0	2	1	11	6
Trachea - epithelial degeneration	0	0	0	0	0	0	0	4
Trachea - eosinophilia in epithelium	0	0	0	0	0	0	0	8

[a] Compiled by the reviewer from Tables 9 and 10 (pages 13 - 20) in the study report.

Microscopic examinations of the organs from animals in the control and 50 mg/kg groups that were sacrificed after a three-week recovery revealed the following lesions:

	Dose Levels (mg/kg/day)			
	Control		50	
	Males	Females	Males	Females
Number Examined	9	9	8	8
Salivary gland - swollen acini	0	0	7	0
Spleen - congestion	0	0	7	5
Thymus - early involution	0	0	3	0
Adrenals - congestion	0	0	1	0

No histopathology data were provided for the 3 mg/kg/day group.

F. DISCUSSION/CONCLUSIONS

BTS 27 419 was administered to rats by oral intubation at dosages of either 0, 3, 12, 50 or 200 mg/kg/day for 90 days. At the end of the treatment period, the animals were sacrificed except for some from the control and 50 mg/kg/day groups which were allowed to recover from the treatment before termination. Animals in the 200 mg/kg group were euthanized after seven days of dosing due to their poor condition. They were observed to be irritable and shed red tears immediately after dosing and then to be hunched, lethargic, weak, emaciated and squealed when handled. Treatment for the 50 mg/kg group was discontinued after 7 days due to similar signs of toxicity. These animals recovered within 7 days. When dosing was reinstituted on week 13 of the study, the signs recurred; one male and one female died. Occasional irritability and excitability were reported in the 12 mg/kg group. There were no clinical signs of toxicity in the 3 mg/kg group.

Body weight and body weight gain were confounded by the deaths of the animals in the 200 mg/kg group and the discontinuation of treatment in the 50 mg/kg group. Overall body weight gain was significantly reduced in the 12 mg/kg group males. The absolute weights of the majority of the organs from the 50 mg/kg group were decreased, most likely due to the decrease in body weight. No data were provided for the 200 mg/kg group. The relative weights of several organs significantly differed from the control including:

adrenals - increased in the 12 mg/kg males and 50 mg/kg females
brain - increased in the 50 mg/kg males and females
liver - decreased in the 3, 12 and 50 mg/kg males; increased in the 50 mg/kg females
lungs - increased in the 3 mg/kg females and 50 mg/kg males; decreased in 50 mg/kg females
testes - increased in the 50 mg/kg males
prostate and seminal vesicles - increased in the 50 mg/kg males
spleen - increased in the 50 mg/kg males

The weights of the adrenals and liver were increased in the 50 mg/kg males and females, respectively, for the group of animals which were allowed to recover after treatment.

On microscopic examination of the organs, there was an increase in the incidence of a number of lesions which were probably treatment-related. The incidence of the following was increased in the 50 and 200 mg/kg groups: congestion of the spleen, heart and pituitary; early thymic involution; and vacuolation of the liver. Fatty infiltration of the adrenals and epithelial degeneration and eosinophilia in the epithelium of the trachea were increased in the 200 mg/kg group. Swollen acinar cells or loss of acinar cells in the salivary gland were increased in the 50 mg/kg group. Swollen acini in the salivary gland, congestion of the spleen and early involution of the thymus were increased in the 50 mg/kg group animals which were sacrificed after a three-week recovery period.

The No Observed Effect Level = 3 mg/kg/day (males and females)

The Lowest Observed Effect Level = 12 mg/kg/day (males and females)

G. Classification - Supplementary (No raw data were submitted for the clinical signs or gross necropsy examinations) - This study does not satisfy the guideline requirements (82-1) for a subchronic oral toxicity study in rodents.

010608

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/6/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 7/15/93
Section I, Toxicology Branch II (H7509C)

ADDENDUM TO DER FOR BTS 27 419: 90 - DAY TOXICITY STUDY IN DOGS

Anitraz
DER 001124
Chem. No. 106201
Study No. P71547
MRID 0040345

RESULTS

A. Clinical Signs

The following table was constructed from the commentary on clinical signs provided in the study report; no raw data were submitted. Number of affected animals is included if it was reported in the commentary.

Table 1
Clinical Signs Observed in Dogs
Treated with BTS 27 419 for 90 Days

	Dose Levels (mg/kg/day)		
	0.25	1	4
CNS depression	slight in 1 male 3 hr after dosing on week 8	within 3 hrs of dosing on first 3 days - "less marked and of shorter duration" thereafter	4/4 dogs - within 3 hrs of dosing on first 3 days - lasted 6 hrs - subdued after dosing thereafter
Ataxia			within 3 hrs of dosing on days 2 & 3 - lasted 3 hrs - "almost impossible to detect" thereafter
Vomiting			females - day 1 males - day 2
Subnormal rectal temperature		"consistently throughout the period of dosing"	within 3 hrs of dosing "at intervals during the treatment period" - lasted 6-24 hrs
Slow pulse rate			
Acute catarrhal conjunctivitis	"little conjunctivitis"	"little conjunctivitis"	varying degrees from week 8 onwards

Serial BUN, sodium and potassium levels done at selected times during the study remained stable after dosing.

Urinalysis

Glucose was present in the urine of one female dog in the 1 mg/kg group at weeks 2 and 3 of dosing; in two males in the 4 mg/kg group at week 2 of dosing; and in one male in the 4 mg/kg group at week 3 of dosing.

D. Post-mortem Findings

Organ Weight

The absolute and relative weights of the liver were increased in the treated groups as compared to the control. No analyses were done to determine if the differences were statistically significant.

	Dose Levels (mg/kg/day)			
	Control	0.25	1	4
Mean Absolute Liver Weight	308.3	367.5	379.9	421.3
Mean Relative Liver Weight	2.502	2.759	2.853	3.193

Note: Calculated from Tables 33 and 34 (pages 37-38) of the study report.

Gross Examination

There were no treatment-related effects seen on gross examination.

Microscopic Examination

A variety of lesions was seen in the liver in both the treated and control groups, however the extent of the lesions appeared to be increased in the 4 mg/kg group. Enlargement of the central and midzonal hepatocytes was seen in all of the treated animals but the incidence was not dose-related. According to the study report, the area affected was enlarged in the two higher dose levels. Hyperplasia of the small periportal hepatocytes and increased numbers of binucleate cells were seen at the 4 mg/kg dose. A list of the observations and the number of times each was reported follows; multiple lesions were reported from each specimen.

were pronounced on the first three days of dosing but subsided for the remainder of the study. Dogs in the 1 mg/kg/day group had CNS depression on the first three days. Subnormal rectal temperature and slow pulse rate were reported consistently throughout the dosing period in the 1 and 4 mg/kg/day groups. Serial blood glucose levels taken after dosing on days 1, 38 and 78 showed a sharp increase at six hours post-dosing in the 4 mg/kg/day group; the values returned to normal by 24 hours. Glucose was present in the urine of 1 female in the 1 mg/kg/day group at weeks 2 and 3; in two males in the 4 mg/kg/day group at week 2; and in one male in the 4 mg/kg/day group at week 3. There was a dose-responsive increase in the absolute and relative weights of the liver in the treated groups. A variety of lesions was seen on microscopic examination of the liver; the extent of the lesions appeared to be increased in the 4 mg/kg/day group. In addition, the following lesions were reported in the treated groups but not in the control: thinning of the zonae fasciculata and reticularis in the adrenals; neutrophilia in the bone marrow; and glandular hypoplasia of the prostate in male dogs.

The No Observed Effect Level (NOEL) = 0.25 mg/kg/day (males and females)

The Lowest Observed Effect Level (LEL) - 1 mg/kg/day

G. Classification: Supplementary (See DEFICIENCIES) - This study does not satisfy the guideline requirements (82-1) for a subchronic oral toxicity study in nonrodents.

Haematology - erythrocyte, leukocyte, differential leukocyte and thrombocyte count, Hb concentration, packed cell volume, erythrocyte sedimentation rate and thrombotest activity.

Blood chemistry - bilirubin, sugar, urea-N, GPT, GOT, alkaline phosphatase, Na and K.

Urine analysis - bilirubin, protein glucose, total reducing substance, blood.

The dogs were killed by an IV injection of sodium pentobarbitone and exsanguinated from the carotid artery. Organ weights were recorded of the adrenals, brain, gonads, heart, kidneys, liver, lungs, pancreas, pituitary, secondary sex organs, spinal cord, spleen and thyroids, and samples of these organs and of the aorta, bladder, bone marrow, colon, duodenum, eyes, gall bladder, ileum, jejunum, lymph nodes, esophagus, optic nerve, mammary gland, salivary gland, sciatic nerve, skeletal muscle, skin, stomach, tongue, trachea, thymus and vagina were taken for histological examination.

Results-

4 mg/kg - CNS depression/ataxia 3 hrs following dosing (persisted for 6 hrs); vomiting; subnormal rectal temperatures and pulse rates 3 hrs. after dosing (returned to normal within 24 hrs); acute catarrhal conjunctivitis (started at 8 wks); increased blood sugar (maximal at 6 hrs. post dosing, normal at 24 hr); small amount of glucose in urine;

PATHOLOGY - small uteri; increased liver weight (hyperplasia of the small periportal hepatocytes and increase in binucleate cells); hyperplasia of zona glomerulosa (subsequent w/ decrease in zona fasciculata and reticularis); neutrophilia in bone marrow; pigment noted in bone marrow and spleen.

1 mg/kg - CNS depression; decrease in rectal temperature and pulse rate at 3 hr. after dosing; similar but less pronounced changes in blood sugar concentration from the 4 mg/kg group; small amount of glucose in urine in one dog;

PATHOLOGY - slight enlargement of central and midzonal hepatocytes in liver; hyperplasia of zona glomerulosa w/ decrease in zona fasciculata and reticularis; neutrophilia in bone marrow

Small portions of these tissues together with the following were prepared for microscopic examination:

aorta	duodenum
trachea	jejunum
lymph nodes	ileum
gall bladder	colon
urinary bladder	skin
salivary gland	mammary gland
tongue	skeletal muscle
oesophagus	bone marrow
stomach	peripheral nerve
eye	optic nerve

Results -

No adverse effects were noted as pertains to bodyweight, food consumption, water consumption, ophthalmoscopy.

Decreased rectal temperature were noted in the 0.25 and 1.0 mg/kg groups for 1 to 2 hrs. following dosing.

There was a significant reduction in heart rate for the 1.0 mg/kg group one to two hrs. after dosing on days 41 and 83 (only checked 3 times).

Haematology, biochemistry and urinalysis were within normal limits and there was no dose relationship for the exceptional cases that occurred.

No morphological change or variation from normal was seen in any of the tissues or organs examined that is considered to be associated with administration of BTS 27271.

NEL = 0.25 mg/kg

NOTE: Dr. Kent J. Davis was consulted concerning the aforementioned 90-DAY feeding studies in dogs and confirms no significant dose related pathology.

010608

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/13/93
Senior Pharmacologist, Review Section I, TBII/HED H7509C
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Yan 7/14/93
Section Head, Review Section I, TBII/HED H7509C

DATA EVALUATION RECORD

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

EPA Identification No.s: EPA MRID No. 00029959
EPA Pesticide Chemical Code 106201
Toxicology Chemical Code 374A

Test Material: BTH 27 419

Synonyms: Amitraz, 1-di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene

Sponsor: Boots Hercules Agrochemicals Co.

Testing Facility: Boots

Title of Report: BTS 27 419: Teratogenicity in the Rat

Study Number(s): TX 73028

Author(s): M. M. Sutton

Report Issued: 1973

Conclusions: Boots-Wistar rats received 0, 1, 3, or 12 mg/kg/day by oral gavage (assumed) from gestation days 8 through 20. No treatment related effects were noted. However, based on data provided in the study MRID# 00029960 (*BTS 27 419: Effect on Pregnancy, Parturition and Care of the Young in Rats*, Boots, Study No. TX 73031, 1973) where maternal toxicity was noted at 12 mg/kg/day, the following are the NOEL/LOEL for this study (MRID# 00029959):

Maternal Toxicity NOEL = 3 mg/kg/day
Maternal Toxicity LOEL = 12 mg/kg/day
Developmental Toxicity NOEL ≥ 12 mg/kg/day
Developmental Toxicity LOEL > 12 mg/kg/day

This study showed no specific evidence of developmental toxicity.

Core Classification: Core Supplementary Data.
This study does not satisfy the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 83-3a) for a developmental toxicity (teratology) study in rats.

Dose Administration:

All doses were administered in a volume of 1 ml/100 g of body weight/day prepared at unknown intervals during the dosing period. The dosing solutions were apparently not analyzed for concentration and stability. Dosing was based on a 9-day study in rats where presumably *transient depression in growth rats and behavioural changes...* were noted.

Observations

There was no indication if animals were checked for mortality or abnormal condition during any specific intervals. Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of: examination of uterine contents, the number of live, dead and resorbed fetuses recorded, and the number of corpora lutea recorded (difficult if animals were primiparous).

The fetuses were examined in the following manner: examined externally, weighed, dissected, head removed and preserved in Bouin's fixative for free hand sectioning, and the carcasses were processed for skeletal examination (procedure not provided).

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

Statistical analysis

The following statistical analysis methods were employed:

Chi-square test

Compliance

None provided, study is from 1973.

Gross Pathological Observations

No abnormal gross pathological observations were reported.

Cesarean Section Observations

Table III: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	11	12	13	11
#Animals Mated/Inseminated	11	12	13	11
#Animals Pregnant	11	12	13	11
Pregnancy Rate (%)	100	100	100	100
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	0	0	0	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	134	146	148	137
Corpora Lutea/dam[1]	12.2	12.2	11.4	12.5
Total Implantations[1]	113	129	139	129
Implantations/Dam[1]	10.3	10.8	10.7	11.7
Total Live Fetuses	105	116	136	122
Live Fetuses/Dam	9.5	9.7	10.5	11.1
Total Resorptions	7	12	3	8
Resorptions/Dam[1]	0.6	0.9	0.2	0.7
Total Dead Fetuses	1	1	0	0
Mean Fetal Weight (gm)	3.02	2.90	3.05	2.77
Preimplantation Loss(%)[1]	15.7	11.6	6.1	5.8
Postimplantation Loss(%)[1]	7.1	10.1	2.2	5.4
Sex Ratio (% Male)[1]	44.8	49.1	49.3	62.3

[1] = calculated by reviewer
a = Data extracted from TX 73028, Tables 3 and 4.

No treatment related effects were noted in the above data.

E. Core Classification: Core Supplementary Data.

No treatment related effects were noted in this study. However, based on data provided in the study MRID# 00029960 (*BTS 27 419: Effect on Pregnancy, Parturition and Care of the Young in Rats*, Boots, Study No. TX 73031, 1973) where maternal toxicity was noted at 12 mg/kg/day, the following are the NOEL/LOEL for this study (MRID# 00029959):

Maternal Toxicity NOEL = 3 mg/kg/day
Maternal Toxicity LOEL = 12 mg/kg/day
Developmental Toxicity NOEL ≥ 12 mg/kg/day
Developmental Toxicity LOEL > 12 mg/kg/day

This study showed no specific evidence of developmental toxicity.

This study does not satisfy the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 83-3a) for a developmental toxicity (teratology) study in rats.

F. Risk Assessment:

None at this time.

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/13/93
Senior Pharmacologist, Review Section I, TBII/HED H7509C
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. 7/14/93
Section Head, Review Section I, TBII/HED H7509C

DATA EVALUATION RECORD

Study Type: One-Generation Reproduction
Species: Rat; Guideline: none

EPA Identification No.s: EPA MRID No. 00029960
EPA Pesticide Chemical Code 106201
Toxicology Chemical Code 374A

Test Material: BTH 27 419

Synonyms: Amitraz, 1-di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene

Sponsor: Boots Hercules Agrochemicals Co.

Testing Facility: Boots

Title of Report: BTS 27 419: Effect on Pregnancy, Parturition and Care of the Young in Rats

Study Number(s): TX 73031

Author(s): M. M. Sutton

Report Issued: 1973

Conclusions: Boots-Wistar rats received 0, 1, 3, or 12 mg/kg/day by oral gavage (assumed) from gestation days 1 through lactation day 21. For the maternal animals the high dose group gained less body weight than the control group. No other observations were reported. No treatment related effects were noted on the pups.

Maternal Toxicity NOEL = 3 mg/kg/day
Maternal Toxicity LOEL = 12 mg/kg/day
Reproductive/Developmental Toxicity NOEL ≥ 12 mg/kg/day
Reproductive/Developmental Toxicity LOEL > 12 mg/kg/day

Core Classification: Core Supplementary Data.

This study is not a 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340). This study is used to support MRID# 00029959 (*BTS 27 419: Teratogenicity in the Rat*, Boots, Study Number: TX 73028, 1973).

Dose Administration:

All doses were administered in a volume of 1 ml/100 g of body weight/day prepared at unknown intervals during the dosing period. The dosing solutions were apparently not analyzed for concentration and stability. It was not indicated what the dosing was based on.

Observations

There was no indication if animals were checked for mortality or abnormal condition during any specific intervals. Body weights were recorded during gestation and lactation periods. Abnormal condition and behavior were recorded during gestation, lactation and weaning. The numbers of pups born (alive and dead), number of pups alive on lactation day 4 and weaning at day 21 were recorded. Dead animals were examined externally and dissected. At weaning day 21, the pups were sacrificed, examined externally, body weights recorded, sexed, dissected and samples of brain, eyes, gonads, kidneys, liver and lungs were removed for microscopic examination.
The carcasses were processed for skeletal examination by an alizarin-red method (method was not provided).

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

Statistical analysis

The following statistical analysis methods were employed:

Chi-square test

Compliance

None provided, study is from 1973.

Lactation Period Observations

The investigators provided group mean and individual animal data, the following table is from the investigators report:

TABLE 4

Effect of BTS 27 419 on pregnancy and survival of the young of rats dosed from day 1 of pregnancy until day 20 post partum.

BTS 27 419 mg/kg/day	No. of litters: Born	Day 4	Day 21	Mean gestation period days	Total no. of young: Born	Day 4	Day 21	Mean no. of young: Born	Day 4	Day 21	Viability index a %	Lactation index b %	Sex ratio M/F	Mean foetal weight (g): M	F	M&F
Control	13	13	11	23.2	177	129	74	10.5	9.9	6.7	94.2	57.4	34/40	32.0	30.8	31.4
1	13	13	13	23.1	175	124	95	10.4	9.5	7.3	91.9	76.6	49/53	32.3	31.3	31.8
3	13	13	13	23.2	173	123	96	10.2	9.5	7.4	92.5	78.1	51/55	32.9	30.5	30.7
12	14	14	14	23.4	129	117	91	9.2**	8.4**	6.5**	90.7	77.8	44/47	31.2	27.9	29.0

** Significantly different from control $p < 0.01$

a. Number of young alive on day 4 as a percentage of those born.

b. Number of young alive on day 21 as a percentage of those alive on day 4.

There was no indication of a treatment related effect; although the high dose group had a slightly lower mean litter size at birth and lactation day 4, at lactation day 21 (weaning) litter sizes were equivalent between treated groups and controls.

Attached Tables 5 and Appendices 4, 6, 7, and 8 present the observations on the pups. No treatment related observations were noted.

Amitraz toxicology reviews

Page _____ is not included in this copy.

Pages 48 through 51 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

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

010608

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/13/93
Senior Pharmacologist, Review Section I, TBII/HED H7509C
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. 7/14/93
Section Head, Review Section I, TBII/HED H7509C

DATA EVALUATION RECORD

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

EPA Identification No.s: EPA MRID No. 00029959 61
EPA Pesticide Chemical Code 106201
Toxicology Chemical Code 374A

Test Material: BTH 27 419

Synonyms: Amitraz, 1-di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene

Sponsor: Boots Hercules Agrochemicals Co.

Testing Facility: Boots

Title of Report: BTS 27 419: Teratogenicity in the Rabbit

Study Number(s): TX 73029

Author(s): M. M. Sutton

Report Issued: 1973

Conclusions: New Zealand White Rabbits (unknown source) received 0, 1, 5, or 25 mg/kg/day by oral gavage (assumed) from gestation days 6 through 18. The high dose group maternal animals gained less weight than the control group and 4 high dose animals aborted. Developmental toxicity was noted in the high dose group in the form of decreased litter size, decreased implantations, increased postimplantation loss, and decreased mean fetal body weight.

Maternal Toxicity NOEL = 5 mg/kg/day
Maternal Toxicity LOEL = 25 mg/kg/day
Developmental Toxicity NOEL = 5 mg/kg/day
Developmental Toxicity LOEL = 25 mg/kg/day

This study has evidence of developmental toxicity.

Core Classification: **Core-Supplementary Data.**
This study does not satisfy the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 83-3b) for a developmental toxicity (teratology) study in rabbits.

Second (primary) Study:

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	vehicle	10
Low Dose	1	8
Mid Dose	5	9
High Dose	25	10

Dose Administration:

All doses were administered in a volume of 0.5 ml/kg of body weight/day prepared at unknown intervals during the dosing period. The dosing solutions were apparently not analyzed for concentration and stability.

Observations

There was no indication if animals were checked for mortality or abnormal condition during any specific intervals. Animals were weighed ***regularly***, no interval specified. Dams were sacrificed on day 30 of gestation. Examinations at sacrifice consisted of: macroscopic examination, an examination of uterine contents, the number of live, dead and resorbed fetuses recorded, and the number of corpora lutea recorded.

The fetuses were examined in the following manner: live fetuses were weighed, examined externally, and, dissected for the rangefinding study. For the primary study, in addition to the aforementioned observations, samples of the brain, eye, genitals, kidneys, liver and lungs were taken for microscopic examinations and the carcasses were then processed for skeletal examination (no procedure provided).

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

Statistical analysis

No statistical analysis methods were employed.

Compliance

None provided, study is from 1973.

Gross Pathological Observations

One 100 mg/kg/day animal had lung congestion. One 50 mg/kg/day animal had necrotic tissue below the tongue and pyometra. One control animal had gavage error.

Cesarean Section Observations

Table II: Cesarean Section Observations[a]

Dose:	Control	1	5	25	50	100
#Animals Assigned	4	3	2	4	2	2
#Animals Mated/Inseminated	4	3	2	4	2	2
#Animals Pregnant	4	3	2	4	2	2
Pregnancy Rate (%)	100	100	100	100	100	100
Maternal Wastage						
#Died	1	0	0	0	1	2
#Died/pregnant	1	0	0	0	1	2
#Non pregnant	0	0	0	0	0	0
#Aborted	0	0	0	1	1	0
#Premature Delivery	0	0	0	0	0	0
#total litter loss	0	1	0	0		
# Litters	3	2	2	3		
Total Corpora Lutea	35	31	28	37		
Corpora Lutea/dam[1]	11.7	10.3	14.0	12.3		
Total Implantations[1]	27	17	25	27		
Implantations/Dam[1]	9.0	8.5	12.5	9.0		
Total Live Fetuses	25	14	17	25		
Live Fetuses/Dam	8.3	7.0	8.5	8.3		
Total Resorptions[1]	0	1	6	1		
Total Dead Fetuses[1]	0	2	2	1		
Mean Fetal Weight (gm)	40.5	41.8	49.3	39.8		
Preimplantation Loss(%)[1]	22.9	15.0	10.7	27.0		
Postimplantation Loss(%)[1]	7.4	17.7	32.0	7.4		

[1] = calculated by reviewer
a = Data extracted from TX 73029, Tables 3 and 5.

Treatment related effects were noted at 50 mg/kg/day and above in the provided data.

Gross Pathological and Microscopic Observations

The investigators provided individual animal data; the following table presents the provided data:

Table II: Autopsy Observations[a]

Dose:	Control	LDT	MDT	HDT
# Examined gross/micro	10/9	8/8	9/9	10/4
Observation				
Liver:				
Pale	1	0	0	0
Spots, etc.	1	0	1	4
Neutrophilia	3	1	0	1
Lungs:				
Pale	0	0	0	1
Spots, etc.	0	2	0	5
Neutrophilia	8	1	0	1
Spleen:				
Congestion	3	2	0	1
Neutrophilia	3	0	0	1

a = Data extracted from TX 73029, Tables 8 and 9.

No specific treatment related effects were observed.

2. **Developmental Toxicity**

External Examinations

One mid dose fetus had a combination of cleft palate, small ears, meningocoele, and one toe displaced, 1 high dose fetus had gastroschisis and along with a litter mate had hydramnios and 1 low dose fetus had an absence of lower incisors.

Visceral Examinations

One low dose fetus had a double gall bladder and 1 mid dose and 1 high dose fetus had an absence of the postcaval lobe of the lung.

Skeletal Examinations

One mid dose fetus had fused sternebrae (no indication of which sternebrae). Data were also provided for *Calcification of the sternum of fetuses...*(for uncalcified centrae), no treatment related effects were noted. The investigators stated that: *Skeletal development of the fetuses from the dosed groups was no different from that in the control fetuses, as judged by progress in calcification of the sternebrae, and no major skeletal defects were seen in fetuses from any of the groups.* Further, *Common minor variants in soft tissue and skeletal formation were seen in a few fetuses but were unrelated to treatment.* This was supported by the provided data.

D. **Discussion/Conclusions**

a. **Maternal Toxicity**:

The high dose group gained less weight than that of the control group and 4 high dose animals aborted.

b. **Developmental Toxicity**:

i. **Deaths/Resorptions**:

The high dose group presented with a decreased litter size, decreased implantations, increased postimplantation loss (and pre implantation loss, which may indicate that treatment started before implantation occurred), and decreased mean fetal body weight.

ii. **Altered Growth**:

See above.

Amitraz toxicology reviews

Page 57 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

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

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 6/4/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 7/15/93
Section I, Toxicology Branch II (H7509C)

DER 001124
Chem No. 106201
Study No. TX 73035
MRID 0044586

ADDENDUM TO DER FOR: Amitraz

BTS 27 419: TWO-YEAR ORAL TOXICITY STUDY IN DOGS

RESULTS

A. Clinical Signs

The study report states that all eight dogs given 1 mg/kg BTS 27 419 exhibited slight CNS depression 3 hours after dosing on days 1 and 2 and all appeared normal by the following morning. One male dog in this group had a slightly subnormal temperature (99° F) at three hours which returned to normal by 24 hours. One female was slightly hypothermic three hours after dosing on weeks 52 and 79 (99.4 and 98.4° F, respectively).

No data on clinical signs are included with this study. According to the study report, the dogs were examined clinically before and immediately after dosing on the first two days and again during weeks 4, 13, 26, 39, 52, 78 and 102.

While most of the mean body temperatures were within normal limits (≈ 100.5 - 102.5° F), there was a fairly consistent dose-responsive decrease in temperature that lasted at least six hours. The following table shows how the mean temperatures (° ↑ or ↓) at 1.5 (two time points), 3 and 6 hours compare to the pre-dosing mean temperatures.

Table 1
Change in Body Temperature in Dogs
Dosed with BTS 27 419 for Two Years[a]

		Hours After Dosing		
time	mg/kg/day	1.5	3	6
Day 1	control		--	
	0.1		↑ 0.3	
	0.25		↑ 0.3	
	1.0		↓ 0.6	
Week 39	control	--	--	--
	0.1	↓ 0.1	↓ 0.1	↓ 0.1
	0.25	↓ 0.2	↓ 0.4	↓ 0.3
	1.0	↓ 1.1	↓ 1.2	↓ 0.8
Week 52	control		↓ 0.2	↓ 0.6
	0.1		↑ 0.3	↓ 0.1
	0.25		↓ 0.7	↑ 0.4
	1.0		↓ 1.2	↓ 0.5
Week 79	control		↓ 0.3	↓ 0.2
	0.1		↓ 0.7	↓ 0.3
	0.25		↓ 0.5	↑ 0.3
	1.0		↓ 1.9	↓ 0.8
Week 103	control	--	↑ 0.2	--
	0.1	↓ 0.6	↓ 0.7	↓ 1.0
	0.25	↓ 0.7	↓ 0.2	↓ 0.2
	1.0	↓ 1.5	↓ 0.8	↓ 0.6

[a] Calculated by the reviewer from Table 2 (page 5) of the study report.
-- No change from pre-dosing.

Body Weight

Body weight and body weight gain of the treated animals were comparable to the control.

Clinical Pathology

All the hematology, clinical chemistry and urinalysis parameters of the treated groups were comparable to the control. Serial blood glucose levels taken 0.75, 1.5, 3, 6 and 24 hours after dosing at Weeks 40 and 53 showed an increase in the values of the 1.0 mg/kg

group at 3 hours, but the changes were not biologically significant.

Gross and Microscopic Pathology

Organ weights were within normal limits. There were no treatment-related changes in gross or microscopic pathology.

DISCUSSION/CONCLUSIONS

BTS 27 419 was administered in gelatin capsules to beagle dogs at dosages of either 0, 0.1, 0.25 or 1.0 mg/kg/day for two years. The study report states that all eight dogs in the 1.0 mg/kg group had CNS depression on the first two days of dosing, however the data on clinical signs have not been submitted. There was a dose-responsive decrease in body temperature after dosing that lasted approximately six hours, but the temperatures remained essentially within the normal range. None of the other pre- or post-mortem parameters were affected by the chemical.

No Observed Effect Level (NOEL) = 0.25 mg/kg/day
Lowest Observed Effect Level (LEL) = 1.0 mg/kg/day

CLASSIFICATION: Minimum - No data has been submitted on clinical signs.

010608

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 9/28/93
Senior Pharmacologist, Review Section I, TBII/HED H7509C

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y.M.I 9/28/93
Section Head, Review Section I, TBII/HED H7509C

DATA EVALUATION RECORD

Study Type: Multigeneration Reproduction
Species: Rat; Guideline: §83-4

EPA Identification No.s: EPA MRID No. 00029962
EPA Pesticide Chemical Code 106201
Toxicology Chemical Code 374A

Test Material: BTH 27 419

Synonyms: Amitraz, 1-di(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene

Sponsor: Boots Hercules Agrochemicals Co.

Testing Facility: Boots

Title of Report: BTS 27 419: Multigeneration Feeding Test in Rats

Study Number(s): TX 73036

Author(s): Margaret M. Sutton

Report Issued: 1973

Conclusions: Boots-Wistar Rats (Boots-source) received 0, 15, 50, or 200 ppm in the diet. Systemic toxicity was noted as decreased body weight gain and food consumption in the high dose F_0 animals. Reproductive toxicity was not as an almost complete loss of the high dose group during the $_1$ generation; there were not enough animals left for subsequent matings. The mid dose group (50 ppm) showed reduced litter size and pup survival in all 3 generations, and a slight reduction in pup weights in the F_1 and F_2 generations. No specific treatment related developmental toxicity was noted in the provided data.

Systemic Toxicity NOEL = 50 ppm
(Males, 4.84 mg/kg/day, Females, 5.22 mg/kg/day)
Systemic Toxicity LOEL = 200 ppm
(Males, 16.41 mg/kg/day, Females, 20.05 mg/kg/day)
Reproductive Toxicity NOEL = 15 ppm
(Males, 1.47 mg/kg/day, Females, 1.58 mg/kg/day)
Reproductive Toxicity LOEL = 50 ppm
(Males, 4.84 mg/kg/day, Females, 5.22 mg/kg/day)

This study shows evidence of developmental/ reproductive toxicity and should be recommended to the Reproductive/Developmental Toxicity Peer Review Committee.

Core Classification: Core-Supplementary Data, this study does not satisfy the 1984 Pesticide Assessment Guideline (40 CFR 158.340, §83-4) for a multigeneration study in rats.

I. Study Conduct

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

Test Compound: Purity: not provided
Density: not provided
Description: not provided
Batch No.: 2099DR
Receipt date: not provided
other provided information: none
Contaminants: not provided

Vehicle(s): unknown

Test Animal(s): Species: Rat
Strain: Boots-Wistar
Source: Boots
Age: newly weaned
Body Weight:

Newly weaned male (12/group) and female (24/group) Boots-Wistar strain rats were obtained for the first parental generation of the study from the colony at Boots. The rats were acclimated for a period of 7 days before they were placed into the study, and were kept under standard animal care conditions. They received Oxoid breeding diet and water during this period, *ad libitum*.

Diet preparation:

Test diets were apparently not analyzed for homogeneity of mixtures and chemical stability in dietary mixtures .

B. Procedures and Study Design

Mating:

After the 1 week acclimation period, the rats received test diets for 10 weeks. At that time 10 males and 20 females per group were chosen at random. One male was caged with 2 females from the same test group for 1 week and then crossed over to a second pair of females for another week. There was no indication if brother-sister matings were avoided initially; however, they were avoided during the F_1 mating. Vaginal smears were examined daily for the 14 day mating period to check estrus cycles and to determine if copulation occurred (detection of sperm considered beginning of gestation).

After successful mating, each pregnant female was individually placed into a cage with a solid bottom and an unknown bedding where they were kept through gestation and lactation.

Mating schedule:

The F_0 parental animals were given test diets for 10 weeks before they were mated, and the F_1 parental animals were selected from the F_1 litters at 3 to 6 weeks old; it was assumed that they received diet for a similar period of time as for the F_0 animals.

Animal assignment:

F_0 animals were randomly assigned to test groups as follows:

Test groups No.	Designation	Dose (ppm)*	Animals per group ** Males	Females
1	Control	control diet	10	20
2	Low (LDT)	15	10	20
3	Mid (MDT)	50	10	20
4	High (HDT)	200	10	20

* Diets were administered from the beginning of the study until the animals were sacrificed.
** The same number of animals were picked from the F_1 litters as parents for the F_2 generation.

C. **Observation Schedule**

Parental animals:

Observations and the schedule for those observations is summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	not clear	not clear
Detailed clinical observations	not clear	not clear
Body weight	All	Weekly until animals housed for mating then - not clear
Food consumption	All	As above

Reproductive performance:

Parental reproductive performance was assessed from breeding and parturition records of animals in the study. A mating was considered successful if sperm was found in the vaginal smears.

The following index was calculated (method not provided):

Pregnancy index

Litter observations:

According to the report, the following litter observations were made:

	Time of observation (lactation day)		
Observation	**Birth**	**Day 4**	**Day 21**
Number of live pups	X	X	X
Pup weight			X
External alterations			X
Number of dead pups	X	X	X
Sex of each pup			X

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

The following indexes were calculated (methods not provided):

Viability index
Lactation index

Necropsy

Parental animals:

All surviving parental males were sacrificed as soon as possible after the last litters in each generation were produced. Maternal animals were sacrificed after the last litter of each generation was weaned. These animals were subjected to a post mortem examination (no details were provided).

Offspring:

The F_1, and F_2 offspring were sacrificed at 21 days of age. These animals were subjected to post mortem examination (no details were provided); however, the F_3 (F_2 offspring) were examined as follows (see next page).

Necropsy observations:

Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

The following tissues were prepared for microscopic examination: liver, kidneys, brain, eyes and gonads.

Data Analyses

Statistical analyses used were not reported.

II. REPORTED RESULTS

A. Analysis of test diets:

Apparently these were not conducted.

B. Parental animals

Mortality and clinical signs:

No animals were reported to have died and apparently no clinical signs were recorded.

Body weight and food consumption:

Reported body weight and selected food consumption results are summarized as follows:

Dose group:	Control	Low	Mid	High
Observation and study week				
$F_0(P_1)$ Generation Males - Pre-mating				
Mean body weight (g)				
0	58.6	59.4	61.3	62.5
10	241.8	247.5	251.2	232.3
Mean weight gain (g)				
0 - 10	183.2	188.2	189.9	169.8
Mean food consumption (g/rat/day)				
0	11.7	11.5	11.3	11.4
1	11.4	11.1	11.7	10.0
10	6.0	5.6	6.5	5.0

continued

Dose group: Observation and study week	Control	Low	Mid	High
$F_0(P_1)$ Generation Females - Pre-mating				
Mean body weight (g)				
0	55.9	57.9	56.7	57.8
10	155.6	156.7	157.1	143.3
Mean weight gain (g)				
0 - 10	99.7	98.8	100.4	85.5*
Mean food consumption (g/rat/day)				
0	11.9	11.9	11.6	12.4
1	11.2	11.4	11.3	9.9
4	9.7	9.9	9.4	9.2
5	8.9	9.6	9.2	9.3
6	8.6	9.0	8.7	8.0
7	9.0	9.0	9.1	8.3
8	10.4	10.1	9.5	9.7
10	1-.-	12.6	12.8	14.6
F_1 Generation Males - Pre-mating				
Mean body weight (g)				
1	74.8	57.2	60.4	
8	222.0	218.8	213.7	
Mean weight gain (g)				
1 - 8	147.2	161.6	153.3	
Mean food consumption (g/rat/day)				
1	18.1	20.3	19.6	
2	14.0	16.9	15.8	
8	9.0	9.3	9.3	
F_1 Generation Females - Pre-mating				
Mean body weight (g)				
1	73.0	63.3	61.5	
8	149.3	150.2	150.4	
Mean weight gain (g)				
1 - 8	76.3	86.9	88.9	
Mean food consumption (g/rat/day)				
1	15.8	17.1	17.5	
2	12.4	13.6	13.4	
3	7.1	7.2	6.4	
6	8.2	8.3	7.9	
7	not determined			
8	not determined			
F_2 Generation Males - Pre-mating				
Mean body weight (g)				
1	112.2	111.0	109.3	
9	226.3	243.5	230.8	
Mean weight gain (g)				
1 - 9	114.1	132.5	121.5	
Mean food consumption (g/rat/day)				
1	12.9	13.0	12.5	
2	8.3	8.3	8.4	
9	8.9	8.7	8.8	

continued

Dose group:	Control	Low	Mid	High
Observation and study week				
F_2 Generation Females - Pre-mating				
Mean body weight (g)				
1	94.7	97.9	94.4	
9	154.0	158.8	147.1	
Mean weight gain (g)				
1 - 9	59.3	60.9	52.7	
Mean food consumption (g/rat/day)				
1	13.4	12.8	13.0	
2	7.3	7.4	7.5	
3	11.0	10.4	10.7	
6	9.4	9.1	9.2	
7	11.0	10.6	11.0	
8	not determined			
9	not determined			

Data extracted from Report TX 73036, Tables 2, 3, 4, 5, 6, and 7.
*** = Statistically significantly different from control, $p<0.05$.**

The high dose group of the F_0 animals gained less weight than that of the control group, the food consumption in the males was slightly reduced at 8 weeks; however, the females were unaffected. No data were provided for group mean body weights and food consumption values for pregnant or nursing dams.

Test Substance Intake:

Based on food consumption and body weight (dietary analyses were not conducted) results, the doses expressed as mg test substance/kg body weight were as follows during the pre-mating period:

	Males	**Females**
	15, 50, 200 ppm	**15, 50, 200 ppm**
P_1	1.29, 4.36, 16.41	1.58, 5.09, 20.05
F_1	1.77, 5.61	1.67, 5.46
F_2	1.35, 4.56	1.51, 5.11
MEAN	**1.47,4.84, 16.41**	**1.58, 5.22, 20.05**

Data extracted from Report TX 73036, Tables 8, 9, and 10.

Reproductive performance:

Results for the parental animals are summarized from the report as follows:

Observation	Control	Dose group Low	Mid	High
		$F_0(P_1)$ Generation		
		Males		
Mated	10	10	10	10
Fertile (Fertility not determined)				
		Females		
Number mated	20	20	20	20
Number fertile	18	18	17	17
Gest. interval (days)	22.9	22.9	23.0	23.2
Number of litters	18	18	17	17
Total litter losses	0	0	0	0
Mean litter size (Day 1)	7.9	9.1	9.1	6.7
Mean litter size (Day 4)	7.4	8.3	8.3	5.5
Mean litter size (Day 21)	6.8	7.0	5.9	2.5
Number of pups (Day 1)	142	164	155	114
Number of pups (Day 4)	134	149	141	55
Number of pups (Day 21)	115	119	101	5
Pup deaths (Days 4-21)	19	30	40	50
Mean pup wgt(g)(Day 21)	25.1	23.1	23.3	29.6
		F_1 Generation		
		Males		
Mated	10	10	10	
Fertile (Fertility not determined)				
		Females		
Number mated	19	20	20	
Number fertile	17	20	19	
Gest. interval (days)	22.9	23.1	22.9	
Number of litters	17	20	19	
Total litter losses	0	0	0	
Mean litter size (Day 1)	8.6	7.9	9.1	
Mean litter size (Day 4)	8.4	7.3	8.3	
Mean litter size (Day 21)	8.3	7.1	7.5	
Number of pups (Day 1)	146	158	172	
Number of pups (Day 4)	142	146	158	
Number of pups (Day 21)	141	141	135	
Pup deaths (Days 4-21)	1	5	23	
Mean pup wgt(g)(Day 21)	32.0	33.3	29.9	

continued

Observation	Control	Dose group Low	Mid	High
	F_2 Generation			
		Males		
Mated Fertile (Fertility not determined)	12	12	12	
		Females		
Number mated	23	24	24	
Number fertile	22	21	20	
Gest. interval (days)	23.1	23.0	23.2	
Number of litters	22	21	20	
Total litter losses	0	0	0	
Mean litter size (Day 1)	9.3	8.8	9.2	
Mean litter size (Day 4)	9.0	7.9	8.2	
Mean litter size (Day 21)	7.5	6.8	5.5*	
Number of pups (Day 1)	204	185	183	
Number of pups (Day 4)	189	166	164	
Number of pups (Day 21)	142	108	71	
Pup deaths (Days 4-21)	47	58	93	
Mean pup wgt(g)(Day 21)	30.5	32.6	28.6	

Data extracted from Report TX 73036, Tables 1, 14, 15, and 16.
* = Statistically significantly different from control, $p<0.05$.

There was an almost complete loss of the high dose group during the F_1 generation; not enough animals were left for subsequent matings. The mid dose group also showed reduced litter size and pup survival in all 3 generations, and a slight reduction in pup weights in the F_1 and F_2 generations.

Necropsy results

Organ weights

Organ weights were not measured.

Pathology

Macroscopic examination:

None were conducted on the adult animals.

Microscopic examination:

None were conducted on the adult animals. It was stated that the ***HISTOPATHOLOGY IS NOT COMPLETE AND WILL BE REPORTED SEPARATELY.***

C. Offspring

Viability and clinical signs:

Viability results from pups during lactation are summarized from the report as follows:

Observation	Control	Dose group Low	Mid	High
		F_1 Generation		
Viability Index	94	91	91	48
Lactation Index	86	80	72	9
		F_2 Generation		
Viability Index	97	92	92	
Lactation Index	99	97	85	
		F_3 Generation		
Viability Index	93	90	90	
Lactation Index	75	55	43	

Changes in mean litter sizes were discussed under the adult observations. No clinical observations were reported. Group mean body weights were also discussed above, only those from lactation day 21 were available.

Necropsy results

Organ weights:

Organs were not weighed.

Pathology

Macroscopic examination:

The investigators reported the following observations:

> Two rats with congenital defects were found among those that died or were killed before weaning. One in the 15 ppm group (F2) had hydrocephalus and situs inversus viscerum and the other in the 50 ppm group (F1) was severely jaundiced within 5 days of birth. After weaning one animal in each of the control (F1), 15 ppm (F2) and 50 ppm (F2) groups was found to have dilated pelvis of the right kidney; there were single cases of unilateral anophthalmia , 15 ppm (F1), diaphragmatic hernia, 15 ppm (F3), and hydrocephalus, 50 ppm (F1). Examination of the stained skeletons of F3 generation rats revealed two cases of misshapen sternebrae, one in each of the 50 ppm and control groups, and one control rat with an extra calcification centre in the sternum.
>
> Some other abnormalities found, such as haemorrhage in the skull and subcutaneous gas were probably traumatic in origin, while the remainder were not associated with any treatment group or generation and their occurrence is considered to be fortuitous.

No specific treatment related effects were noted in the above observations.

Study Deficiencies:

The percent active ingredient of the test substance was not provided.
Very limited data were provided.
Mating was not 1 male to 1 female (only 10 males used per dose group/ per generation).
No data on reproductive organs were provided.
Litter data were only provided for a few time points.
Histopathology data were not provided

III. DISCUSSION

Systemic toxicity was noted as decreased body weight gain in the high dose F_0 animals. Food consumption in the F_0 males was slightly reduced at 8 weeks; however, the females were unaffected. Reproductive toxicity was noted as an almost complete loss of the high dose group during the F_1 generation; there were not enough animals left for subsequent matings. The mid dose group showed reduced litter size and pup survival in all 3 generations, and a slight reduction in pup weights in the F_1 and F_2 generations. No specific treatment related developmental toxicity was noted in the provided data.

Systemic Toxicity NOEL = 50 ppm
(Males, 4.84 mg/kg/day, Females, 5.22 mg/kg/day)
Systemic Toxicity LOEL = 200 ppm
(Males, 16.41 mg/kg/day, Females, 20.05 mg/kg/day)
Reproductive Toxicity NOEL = 15 ppm
(Males, 1.47 mg/kg/day, Females, 1.58 mg/kg/day)
Reproductive Toxicity LOEL = 50 ppm
(Males, 4.84 mg/kg/day, Females, 5.22 mg/kg/day)

This study shows evidence of developmental/ reproductive toxicity and should be recommended to the Reproductive /Developmental Toxicity Peer Review Committee.

Core Classification: Core-Supplementary Data, this study does not satisfy the 1984 Pesticide Assessment Guideline (40 CFR 158.340, §83-4) for a multigeneration study in rats.

Amitraz toxicology reviews

Page _____ is not included in this copy.

Pages 74 through 75 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

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

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 9/28/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I. 9/28/93
Section I, Toxicology Branch II (H7509C)

ADDENDUM TO DER 001124 FOR AMITRAZ:

BTS 27 419 : Carcinogenicity and Long-term Toxicity Study in Rats

EPA ID NUMBERS: Chem. No. 106201
Study No. TX 73043
MRID No. 0044585

RESULTS

A. Mortality

Survival rates for the treated and control groups were similar.

B. Clinical Signs

The number of rats which were reported to be either excitable, nervous or aggressive was increased in the 200 ppm group; the females were more frequently affected. The number of animals in which either of these signs was reported and the mean day of the first report are summarized below.

	Dosage Levels (ppm)			
	0	15	50	200
Males				
Number of Animals Affected	3	0	3	10
Mean Day of First Report	49	--	50	42
Females				
Number of Animals Affected	6	7	17	24
Mean Day of First Report	43	39	41	26

Note: Calculated by the reviewer from Appendix 1 (pages 35 - 71) of the study report.

C. Food Consumption and Dosage

Food Consumption

The study report states that food consumption of both males and

females fed 200 ppm was reduced the first week of treatment and remained less than that of the controls until the fourth week, but afterwards there was no difference. Table 1 summarizes food consumption for the first four weeks.

Table 1
Initial Food Consumption (g/100 g body weight) in Rats Treated with BTS 27 419 in the Diet for Two Years*

		Week of Study				
Dosage	Sex	-1	1	2	3	4
0	M	9.9	9.1	8.6	7.7	7.6
	F	10.5	9.8	9.8	8.7	8.9
15	M	10.0 (101)+	9.2 (101)	8.4 (98)	7.7 (100)	7.4 (97)
	F	10.6 (101)	9.6 (98)	9.2 (94)	8.5 (98)	8.7 (98)
50	M	10.0 (101)	9.0 (99)	7.2 (83)	7.7 (100)	7.4 (97)
	F	10.5 (100)	9.7 (99)	7.2 (72)	8.2 (94)	8.4 (94)
200	M	9.9 (100)	7.6 (84)	6.2 (72)	7.1 (92)	7.1 (93)
	F	10.1 (96)	8.4 (86)	8.2 (84)	7.9 (91)	7.8 (88)

* Extracted from the Table 2 (page 21) of the study report; percentage of the control value calculated by the reviewer.
+ Percentage of the control value.

Dosage

The actual dosages received by the animals are as follows:

Dose (ppm)	Nominal (mg/kg/day)	Average Intake (weeks 1-104) (mg/kg/day)		Percentage of Nominal	
		males	females		
15	0.75	0.77	0.97	103	129
50	2.5	2.5	3.13	100	125
200	10	10.18	12.59	102	126

D. Body Weight and Body Weight Gain

There were no statistically significant differences in body weights

between the treated and control groups. Body weight gain in the 200 ppm males and females was significantly lower than the control group at several time points - weeks 0-12 for both males and females, weeks 12-24 for females, weeks 24-36 for males and weeks 0-104 for males.

Table 2
Mean Body Weight Change (G) in Rats Treated with BTS 27 419 in the Diet for Two Years[a]

		Week of Study						
Dosage	Sex	0-12	12-24	24-36	36-48	48-72	72-96	0-104
Control	M	203.0	49.2	50.3	25.8	23.4	-10.7	331.0
	F	84.9	24.9	13.7	14.2	21.2	13.6	168.0
15	M	204.5	51.4	51.7	26.6	26.2	-17.6	309.3
	F	82.9	20.6	18.2	16.2	30.0	14.6	172.9
50	M	204.5	50.0	51.6	31.2	24.1	-13.7	323.3
	F	80.7	21.8	16.7	16.8	35.0*	25.9*	184.6
200	M	173.5 ***	45.1	35.3 ***	21.6	26.2	-17.8	286.5 **
	% control value	85	92	70	84	112		87
	F	68.1 ***	18.6 **	16.5	14.3	25.9	23.5	166.4
	% control value	80	75	120	101	122	173	99

[a] Extracted from Table 5 (page 24) of the study report.
* Significantly different from control , $p < 0.05$
** Significantly different from control, $p < 0.01$
*** Significantly different from control, $p < 0.001$

E. Clinical Pathology

There was no evidence of a treatment-related effect on any of the parameters measured.

F. Necropsy Findings

Organ Weights

There was no alteration in the organ weights of the treated animals in comparison to the controls.

Gross and Microscopic Examinations

The findings on gross and microscopic examination were considered common to aged rats. The incidences between the treated and control groups were comparable.

Tumor Incidence

Pituitary adenoma was the most frequently reported tumor type in both male and female rats; mammary fibro-adenoma was also frequently seen in the females. The incidence of all tumors was comparable between the treated and control groups.

G. DEFICIENCIES

1. The study deviated from the Pesticide Assessment Guidelines, Subdivision F, in the following:

a. The number of animals in each group, 40 males and 40 females, did not meet the guideline requirement of at least 100 animals, 50 males and 50 females.

b. For the clinical pathology examinations, the number of animals tested and the range of clinical chemistry parameters measured did not meet the guideline suggestions. Additionally, urinalyses were not done.

c. Ophthalmological examinations were not done.

2. No analyses were done to determine the homogeneity and concentration of the test chemical in the diet. The study report states that it was ascertained that the compound was stable in the diet.

H. DISCUSSION/CONCLUSIONS

BTS 27 419 was administered in the diets of male and female rats at dosages of either 15, 50 or 200 ppm for two years. The actual dosages received by the males in the 15, 50 and 200 ppm groups were 0.77, 2.5 and 10.18 mg/kg/day, respectively. The dosages received by the females were 0.97, 3.13 and 12.59 mg/kg/day, respectively. There was no treatment-related effect on survival. The number of rats reported as excitable, nervous or aggressive was increased in the 50 (females) and 200 ppm groups (males and females); the females were more frequently affected. Body weight gain was decreased in the 200 ppm group at weeks 0-12 for both males and females, weeks 12-24 for females, weeks 24-36 and 0-104 for males. Food consumption was reduced in both the males and females in the 200 ppm group at the beginning of treatment but then was comparable to the control group. There were no treatment-related effects on clinical pathology parameters or necropsy findings. The incidence of tumors was comparable between the treated and control groups.

The No Observed Effect Level (NOEL) = 50 ppm (males) and 15 ppm (females).

The Lowest Observed Effect Level (LEL) = 200 ppm (males - based on clinical signs and decreased body weight gain) and 50 ppm (females - based on clinical signs).

The Maximum Tolerated Dose (MTD) = 200 ppm (males and females) based on clinical signs and decreased body weight gain.

I. CLASSIFICATION - Minimum (See DEFICIENCIES) - This study satisfies the guideline requirements (83-5) for a combined chronic toxicity/carcinogenicity study in rats.