1/27/1998

DATA EVALUATION REPORT

012483

G-28279 TECHNICAL

STUDY TYPE: SUBCHRONIC FEEDING - RAT (82-1)

Prepared for

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

Prepared by

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Task Order 94-5

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Disclaimer

This final DER may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

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Toxicology Branch II (7509C)

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic Feeding - Rat (82-1)

TOX. CHEM, NO: 063

P.C.CODE.: 080803

MRID NO.: 430132-05

TEST MATERIAL: G-28279 technical

SYNONYMS: 4-Chloro, 2-amino, 6-isopropylamino-s-triazine; deethylatrazine; de-ethylated atrazine

STUDY NUMBER: 901261

SPONSOR: CIBA-GEIGY Limited, Agricultural Division, 4002 Basle, Switzerland

TESTING FACILITY: CIBA-GEIGY Limited, Short/Long-term Toxicology, 4332 Stein, Switzerland

TITLE OF REPORT: 90-Day Oral Toxicity Study in Rats; 3-Month Oral Toxicity Study in Rats (administration in food)

AUTHOR: M. Schneider

REPORT ISSUED: May 8, 1992 (study completion date)

EXECUTIVE SUMMARY: Groups of 10 male and 10 female RAIf (SPF) rats were fed diets containing G-28279 technical (purity 96.7%; Lot #FL-901747) at concentrations of 0, 10, 50, or 500 ppm for 13 weeks. The average consumption of test material was 0.602, 3.20, or 34.9 mg/kg/day (males) and 0.641, 3.34, or 37.5 mg/kg (females). All animals survived to study termination. No treatment-related clinical signs or ocular lesions were seen at any dose level. At 10 ppm, there were no effects on body weight, clinical chemistry parameters, hematologic values, or organ weights, nor were macroscopic or histopathologic changes observed. Slightly decreased body weights and body weight gains were seen in males administered 50 or 500 ppm of the test material. At week 13, the body weights of males were 93% and 88% of control values at 50 and 500 ppm, respectively, and the body weight gains were 96, 90, or 84% of control values at 10, 50, or 500 ppm, respectively. For female rats exposed to 500 ppm, the body weights were 9-12% below those of controls from treatment week 2 to 13, and the mean body weight gain was 80% of

controls at the end of the treatment period. Food consumption was not significantly affected by treatment with G-28279 technical. Organ weight changes (absolute and relative to body weight) in males at 500 ppm included increased relative kidney weights (14% above control values), decreased absolute heart weights (-10%), increased relative testes weights (+21%), and increased relative brain weights (+18%). In the absence of histopathologic lesions in the corresponding organs, these organ weight changes are of uncertain toxicological significance and may not be treatment-related. Increased relative liver weights (+13%) and extramedullary hematopoiesis of the liver seen in female rats at 500 ppm indicate slight hepatotoxicity. Minimal to moderate fatty changes of adrenal cortex and slight hypertrophy of the thyroid follicular epithelium in males at 500 ppm suggest treatment-related effects. Additionally, extramedullary hematopoiesis of the spleen in females at 500 ppm and hypertrophy of pituitary cells in males at 500 ppm may suggest treatment related effects.

The LOEL is 500 ppm (3.20 mg/kg/day for males, 3.34 mg/kg/day for females), based on decreased body weights and body weight gains in both sexes. Possible histopathologic effects in the liver, adrenal cortex, and thyroid were seen at 500 ppm. The corresponding NOEL is 50 ppm (0.602 mg/kg/day for males, 0.641 mg/kg/day for females).

Classification: This study is classified as Core-Guideline and satisfies the guideline requirement for a subchronic dietary toxicity study (82-1) in rats.

Special Review Criteria (40 CFR 154.7) None

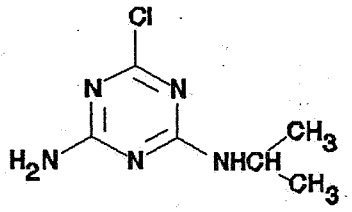
Flagging Statement (40 CFR 158.4): The study, conducted with an atrazine metabolite, meets or exceeds the flagging criteria (report code 11) for a subchronic feeding study, based on the current ADI for atrazine. No ADI has been established for this metabolite.

A. MATERIALS

1. Test Material: G-28279 technical

Description: solid
Lot/Batch #: FL-901747
Purity: 96.7% a.i.
Stability of compound: 5 weeks in the diet (50 and 500 ppm; 10% loss at 10 ppm)
CAS #: not available

Structure:



2. Vehicle and/or positive control

Dry test material was mixed with feed (pelleted, certified standard diet, Nafag No. 890 Tox); therefore, no vehicle was required. A positive control was not included.

3. Test animals

Species: rat
Strain: Tif: RAIf (SPF), hybrids of RII/1 x RII/2
Age and weight at study initiation: 5-6 weeks; 106.7-128.6 g (males), 100.1-120.2 g (females) (at week -1)
Source: Animal Production, CIBA-GEIGY Limited, 4332 Stein, Switzerland
Housing: housed in groups of 5 in macrolon cages and wood bedding
Environmental conditions:
Temperature: 22±2°C
Humidity: 55±10%
Air changes: 16-20/hr
Photoperiod: 12 hr day/12 hr night
Acclimation period: 7 days

B. STUDY DESIGN

1. Animal assignment

Animals were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN					
Dose Group	Conc. in Diet (ppm)	Dose (mg/kg/day)[a]		No. of Animals	
		Male	Female	Male	Female
1 Control	0	0	0	10	10
2 Low (LDT)	10	0.602	0.641	10	10
3 Mid (MDT)	50	3.20	3.34	10	10
4 High (HDT)	500	34.9	37.5	10	10

Data taken from pp. 21 and 39-40, MRID No. 430132-05.
[a]Based on the analytically determined value in the diet.

Dose selection rationale: The doses used in this study were based on the results of a 28-day feeding study with rats (Project No. 252090; RCC, Research and Consulting Company AG, Itingen). Male and female Wistar rats were administered 50, 500, or 2000 ppm G-28279 technical, corresponding to an average daily intake of 4.6, 46.5, or 161.2 mg/kg for males and 4.6, 49.7, or 164.5 mg/kg for females, respectively. The following effects were reported: Food consumption was decreased in males and females at 2000 ppm and a dose-related decrease in body weights was seen in males and females. Males and females at 2000 ppm exhibited restlessness during the last week of treatment. Slight changes in hematocrit, MCV, MCHC, and platelet counts were seen at 500 and 2000 ppm (sex not reported), and increased reticulocyte counts were seen in male and female rats at 2000 ppm. Glucose, calcium, chloride, albumin, total protein, and globulin levels were slightly decreased, and phosphorus, sodium, potassium levels, and albumin/globulin ratios were increased at 500 and/or 2000 ppm (sex not reported). Absolute and relative thymus weights were decreased in males at 500 and 2000 ppm and in females at 2000 ppm. The no-observed effect level (NOEL) was considered to be 50 ppm, corresponding to an average daily intake of 4.6 mg/kg body weight.

2. Diet preparation and analysis

Diet was prepared at monthly intervals by mixing appropriate amounts of test substance with pulverized food; 25% water was added before pelleting to ensure necessary pellet quality. The pellets were air-dried and stored at room temperature in stainless steel containers. Content and homogeneity of test material was determined in triplicate samples taken from the first, middle, and final discharge of

the mixing blender at the nominal concentrations of 10, 50, and 500 ppm during study days 1-29. Test material content was also measured in duplicate samples during study days 57-93. Stability was measured and reported separately (FAR 704/91, Test No. 901261); details are not provided in the study report.

Results -

a. Homogeneity analysis - The distribution of a.i. in rodent feed was found to be homogeneous.

b. Stability analysis - Stable in rodent fed at concentrations of 50 and 500 ppm for 5 weeks at room temperature; 10% loss of test material at 10 ppm during same time period.

c. Concentration analysis - The mean concentrations of test material in the diet were 82.9, 87.4, and 90.8% for nominal concentrations of 10, 50, and 500 ppm, respectively.

3. Diet

Animals were fed a pelleted, certified standard diet (Nafad No. 890 Tox) and watered ad libitum.

4. Statistics

Univariate statistical analysis was performed for each time point and parameter. Nonparametric methods were applied to allow for non-normal as well as normal data distribution. Each treated group was compared to the control group by Lepage's two-sample test [Y. Lepage, Biometrika 58: 213-217 (1971)]. This test, a combination of the Wilcoxon and Ansari-Bradley tests, is a combined test for location and dispersion. Levels of significance were set at $p<0.05$ or $p<0.01$. Trend analysis was performed using Jonckheere's test for ordered alternatives [A.R. Jonckheere, Biometrika 41: 133-145 (1954)], with a level of significance set at 1% for positive or negative trends.

5. Signed and dated GLP and quality assurance statements were present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily on working days and once daily on weekends and holidays for mortality. Observations for signs of toxicity were conducted daily and recorded at least weekly.

Results - There was no treatment-related effect on survival.

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One male rat administered 500 ppm died accidentally after blood sampling on study day 92. No clinical findings attributable to the test material were noted for either male or female rats. Eye exudate was observed in one male rat exposed to 10 ppm and in two male rats exposed to 500 ppm.

2. Body weight

Animals were weighed during the acclimatization period and weekly during the experimental period.

Results - Mean body weights at weekly intervals are presented in Table 2. No treatment-related effects were noted for either male or female rats administered 10 ppm. Slightly decreased body weights compared to controls were seen in males administered 50 or 500 ppm of the test material during the study period. At week 13, the body weights were 93% (50 ppm) and 88% (500 ppm) of control values. The body weight gain was 96, 90, or 84% of control values for males receiving 10, 50, or 500 ppm, respectively. The authors noted, however, that two male control animals had a very high body weight gain compared with historical controls (historical control data not provided). Excluding these two animals from the calculations resulted in lower mean values for body weight and body weight gain in control animals. Thus, compared with recalculated control values, the mean body weights of males exposed to 50 ppm were only 3-4% lower (mainly during the second half of the study) and those of males exposed to 500 ppm were 6-9% lower (from week 3 on). The mean body weight gain was 4% and 10% below the recalculated control values for males treated with 50 and 500 ppm, respectively. For female rats exposed to 500 ppm, the body weights were 9-12% below those of controls from treatment week 2 to 13, and the mean body weight gain was 20% below that of controls at the end of the treatment period. The body weights of females treated with 50 ppm did not differ significantly from controls.

TABLE 2. GROUP MEAN BODY WEIGHTS (g) AT WEEKLY INTERVALS OF MALE AND FEMALE RATS FED G-28279 TECHNICAL FOR 13 WEEKS

Week of Study	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	10	50	500	0	10	50	500
-1	114.9	114.6 (100)[a]	116.8 (102)	116.2 (101)	111.3	107.3* (96)	109.0 (98)	110.0 (99)
1	177.8	176.2 (99)	173.6 (98)	171.9 (97)	149.0	146.1 (98)	148.9 (100)	144.5 (97)
2	238.0	234.3 (98)	230.6 (97)	223.5 (94)	180.2	176.8 (98)	181.6 (101)	164.8** (91)
3	300.0	288.9 (96)	284.5 (95)	271.7* (91)	205.4	203.7 (99)	199.5 (97)	184.3** (90)
4	333.3	326.7 (98)	318.4 (96)	302.9* (91)	227.1	224.9 (99)	219.3 (97)	199.1** (88)
5	366.0	360.6 (99)	347.3 (95)	328.7 (90)	243.6	240.3 (99)	235.8 (97)	213.6** (88)
6	397.3	395.3 (99)	374.8 (94)	356.7* (90)	258.8	253.8 (98)	250.3 (97)	226.4** (87)
7	426.8	421.2 (97)	405.4 (95)	381.1 (89)	271.8	268.0 (99)	261.1 (96)	235.6** (87)
8	447.2	440.4 (98)	416.7 (93)	399.9 (89)	279.6	275.3 (98)	271.5 (97)	243.6** (87)
9	463.7	455.0 (98)	430.2 (93)	413.8 (89)	285.6	281.9 (99)	279.4 (98)	248.4** (87)
10	484.5	470.6 (97)	445.1 (92)	425.5 (88)	290.0	295.0 (102)	290.9 (100)	255.7** (88)
11	507.4	488.7 (96)	463.7 (91)	448.1 (88)	305.2	302.6 (99)	299.4 (98)	265.3** (87)
12	516.0	497.9 (96)	470.1 (91)	447.1* (87)	301.0	301.0 (100)	301.4 (100)	265.5** (88)
13	521.4	504.7 (97)	482.9 (93)	458.5 (88)	296.2	290.5 (98)	296.7 (100)	257.3** (87)
Body Weight Gain[b]	406.5	390.1 (96)	366.1 (90)	342.3 (84)	184.9	183.2 (99)	187.7 (102)	147.3 (80)

Data taken from table on p. 43, MRID No. 430132-05.
[a] Calculated by reviewer; numbers in parenthesis are percent of control weight.
[b] Calculated by reviewer; numbers in parenthesis are percent of control weight gain.
*Significantly different from control, $p<0.05$ (Lepage's two-sample test).
**Significantly different from control, $p<0.01$ (Lepage's two-sample test).

3. Food consumption and compound intake

Food consumption was determined weekly from the food consumption per cage and the number of animals present; therefore, no statistical analysis was performed. Mean daily diet consumption was calculated as g food/animal/week. Food efficiency [(body weight gain in g/food consumption in g per unit time) X 100] was not calculated by the study author. Compound intake (mg/kg/day) was calculated based on food consumption ratios and actual amount of test material in the diet. The method for calculating compound intake is presented in the Appendix of this DER.

Results -

a. Food consumption - Data at weekly intervals are presented in Table 3. For males, the overall food consumption for the entire study period was 4%, 7%, or 6% lower for groups receiving 10, 50, or 500 ppm, respectively. In the absence of a clear dose-response and taking into account the high body weight of two male control rats, the differences in food consumption between controls and treated animals were considered to reflect normal biological variations. In females at 500 ppm, the overall food consumption during weeks 1 to 7 was 7% lower than that seen in controls, but was similar to control values for the remainder of the study period.

b. Compound consumption (time-weighted average) - Males received doses of 0.602, 3.20, or 34.9 mg/kg/day and females 0.641, 3.34, or 37.5 mg/kg/day for dietary concentrations of 10, 50, or 500 ppm, respectively.

c. Food efficiency - Based on total weight gain and on the total amount consumed, the overall efficiency calculated by the reviewer was 16.53, 16.51, 15.95, or 14.81 g body weight gain/g food for males and 10.92, 10.89, 11.33, or 9.03 g body weight gain/g food for females at dietary concentrations of 0, 10, 50, or 500 ppm, respectively. Thus, male rats exhibited a slight decreased food efficiency that may have been a treatment-related effect at the high dose. In female rats, food efficiency was increased at 10 and 50 ppm relative to controls, but was decreased at 500 ppm.

4. Water consumption

Water consumption was recorded weekly from the water consumption per cage; therefore, statistical analysis was not performed.

Results - Compared with controls, the water consumption of males receiving 500 ppm was increased by 11% from week 5 on. In females given the same dose, water intake was increased by 31%. No effects on water consumption

TABLE 3. GROUP MEAN FOOD CONSUMPTION (g/ANIMAL/WEEK) AT WEEKLY INTERVALS OF MALE AND FEMALE RATS FED G-28279 TECHNICAL FOR 13 WEEKS

Week of Study	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	10	50	500	0	10	50	500
-1	122.0	120.0	125.0	127.0	102.8	101.6	102.9	104.4
1	154.6	150.0	145.0	145.7	112.2	111.2	105.9	102.3
2	184.9	176.5	173.3	175.5	130.9	128.6	125.2	123.0
3	200.6	189.2	185.2	188.1	139.6	138.4	134.1	125.7
4	195.9	191.2	186.0	185.9	145.3	141.6	138.2	134.1
5	191.6	189.3	183.9	180.0	141.5	138.2	136.4	129.0
6	197.7	193.4	183.6	181.7	137.3	134.4	134.2	136.6
7	195.1	189.8	183.2	186.2	142.5	138.2	133.4	129.1
8	190.1	185.7	179.8	185.2	131.9	130.5	134.3	127.9
9	189.6	182.5	176.8	176.1	121.1	131.1	130.4	126.2
10	185.7	176.9	172.8	174.2	123.3	128.7	126.1	129.5
11	188.5	177.9	175.0	177.3	127.6	127.7	124.0	126.8
12	189.2	175.4	172.8	170.9	117.7	115.9	124.9	115.5
13	196.1	185.6	177.7	184.1	122.3	117.7	109.4	125.1
Week 1-13[a]	2,459.6	2,363.4	2,295.1	2,310.9	1,693.2	1,682.2	1,656.5	1,630.80

Data taken from table on p. 47, MRID 430132-05.
[a]Total food consumption (g)

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were noted in controls or animals exposed to 10 or 50 ppm.

5. Ophthalmoscopic examination

The eyes of controls and animals receiving 500 ppm were examined before treatment (day -2) and during the last week of treatment (day 85).

Results - No treatment-related ocular effects were noted.

6. Blood was collected at the termination of the study for hematology and clinical analysis from all surviving animals. Following overnight fasting, the animals were anesthetized with ether and blood was collected from the orbital sinus. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements	X	Red cell vol. distribution width
	(Thromboplastin time)	X	HGB conc. distribution width
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies

Results - Slightly, but statistically significant differences between control and treated groups were noted in several red blood cell (RBC) parameters: increased MCHC in males at 500 ppm (+4%, $p<0.01$); decreased MCV in females at 500 ppm (-3%, $p<0.05$); and decreased MCH in females at 10 (-2%, $p<0.05$) and 50 ppm (-1%, $p<0.05$). Prothrombin time increased by 14% in males at 50 ppm ($p<0.01$) and by 4% at 500 ppm ($p<0.05$). In the absence of other biologically significant changes in RBC parameters, the observed effects on hematologic parameters could be related to test-material exposure.

b. Clinical chemistry

X	Electrolytes
X	Calcium*
X	Chloride*
	Magnesium*
X	Phosphorus*
X	Potassium*
X	Sodium*
	Enzymes
X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
	Creatinine phosphokinase*
	Lactic acid dehydrogenase (LDH)*
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*
X	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

X	Other:
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total bilirubin
X	Total serum protein (TP)*
	Triglycerides
	Serum protein electrophoresis
X	A/G ratio

* Required for subchronic studies

Results - Statistical analysis revealed significant differences in some parameters between treated rats and controls. In males, they included increased creatinine at 10 ppm (+10%, $p<0.05$); decreased albumin at 500 ppm (-2%, $p<0.05$); increased albumin/globulin at 50 ppm (+5%, $p<0.05$); increased cholesterol at 500 ppm (+19%, $p<0.05$); decreased calcium at 500 ppm (-3%, $p<0.01$); and increased SGPT at 500 ppm (+27%, $p<0.01$). In female rats, creatinine wassignificantly increased at 10 ppm (+23%, $p<0.05$) and 500 ppm (+26%, $p<0.01$), but not at 50 ppm. Except for increased SGPT activity at all dose levels, a dose-related trend was not observed. Although the above effects were statistically significant, the magnitude of these changes relative to controls were too low to be biologically significant.

7. Urinalysis*

Urine was collected overnight from individual animals housed in special metabolism cages. Food and water were withheld during the time of urine collection. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Not required for subchronic studies

Results - At the end of the treatment period, the urine volume was decreased (-31% for males, -27% for females, $p<0.05$) at 10 ppm, and the urine density was slightly increased in males receiving 10 ppm and in females receiving 10 or 50 ppm of the test material (all $p<0.05$). At 500 ppm, the urine volume of females was slightly decreased ($p<0.05$). Trace amounts of ketones, possibly related to stress, were found in all male groups, in female controls, and in one female each treated with 10 or 500 ppm. The magnitude of the above changes relative to controls were too low to be biologically relevant. Protein, ranging from trace quantities to "large amounts" was detected in all groups of male and female rats and may be characteristic for this strain of rat.

8. Sacrifice and pathology

With the exception of a male rat that died accidentally on study day 92, all animals were sacrificed under ether anesthesia at the end of the test period. Gross pathological examinations were conducted and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

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

* Required for subchronic studies
\+ Organ weight required in subchronic and chronic studies.
++ Listed as small intestine
+++ Listed as large intestine

Results -

a. Organ weight - Feeding of the test material resulted in a few statistically significant changes in absolute and/or relative (to body weight) organ weights at the highest dose, 500 ppm (Table 4). Increased relative liver weight in females (113% of controls, $p<0.05$) was the only deviation from control values that was attributed to treatment with the test material. A dose-related trend was not observed. this weight change may

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also reflect the reduced body weight gain.

TABLE 4. MEAN ABSOLUTE WEIGHTS (g) AND RELATIVE WEIGHTS (% of BODY WEIGHT) OF AFFECTED ORGANS IN MALE AND FEMALE RATS FED G-28279 TECHNICAL FOR 13 WEEKS

Organ/Sex		Exposure Level (ppm)							
		0		10		50		500	
		ABS	REL	ABS	REL	ABS	REL	ABS	REL
Heart	M	1.559	3.089	1.504	3.112	1.435	3.114	1.396*	3.247
Brain	M	2.416	4.816	2.412	5.019	2.357	5.122	2.434	5.681**
Kidneys	M	3.187	6.283	2.905	6.034	2.861	6.209	3.071	7.136*
Testes	M	4.352	8.622	4.107	8.542	4.034	8.766	4.466	10.40**
Liver	F	10.39	35.98	10.38	36.43	10.24	35.99	10.52	40.72**

Data from pp. 77-80, MRID No. 430132-05.
* Significantly different from control, $p<0.05$ (Lepage's two-sample test)
** Significantly different from control, $p<0.01$ (Lepage's two-sample test)

Decreased absolute heart weights (-10%, $p<0.05$), increased relative kidney weights (+14%, $p<0.05$), and increased relative testes weights (+21%, $p<0.05$) in males exposed to 500 ppm were attributed to decreased body weight gain and were not considered of toxicologic relevance. The authors did not offer an explanation for the significantly increased relative brain weight (+18%, $p<0.01$) in males at 500 ppm,

b. Gross pathology - Macroscopic findings, limited to a thymus with mottled appearance in a male rat exposed to 500 ppm and a liver nodule in a control female, were not considered treatment-related.

c. Microscopic pathology -

1) Non-neoplastic - Increased incidences of nonneoplastic lesions, classified as minimum and/or moderate, occurred in the adrenal gland, thyroid gland, and pituitary gland of males and in the spleen and liver of females (Table 5). Male rats exposed to

500 ppm exhibited minimal to moderate fatty changes in the adrenal cortex. Minimal hypertrophy of the thyroid follicular epithelium occurred in males at all dose levels, including controls, but the incidence was higher at 500 ppm. Hypertrophy of pituitary cells in the adenohypophysis, considered to represent TSH-producing cells, was seen in males exposed to 50 ppm (minimal) and 500 ppm (minimal to moderate). In female rats, there was an increased incidence of minimal and/or moderate extramedullary hematopoieis

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TABLE 5. TREATMENT-RELATED MICROSCOPIC CHANGES IN ORGANS OF MALE AND FEMALE RATS FED G-28279 TECHNICAL FOR 13 WEEKS

Organ/Effect	Treatment Group (No. of Animals)			
	Control (10)	10 ppm (10)	50 ppm (10)	500 ppm (10)
	Males			
Adrenal gland, fatty change of adrenal cortex minimal moderate total	1 0 1	0 0 0	0 1 1	6 1 7 (p<0.01)*
Thyroid gland, hypertrophy of follicular epithelium minimal	2	2	2	8 (p<0.01)*
Pituitary gland, hypertrophy of pituitary cells in adenohypophysis minimal moderate total	0 0 0	0 0 0	1 1	4 1 5 (p<0.02)*
	Females			
Spleen, extramedullary hematopoiesis minimal moderate total	4 0 0	3 0 0	0 5 5	3 7 10 (p<0.005)*
Liver, extramedullary hematopoiesis minimal	0	0	0	3 (p<0.11, N.S.)*

Data taken from pp. 97-99, MRID No. 430132-05.
* Fisher exact test, calculated by reviewer.

of the spleen in all treated groups as well as controls. Female rats treated with 500 ppm exhibited minimal extramedullary hematopoieis of the liver. The authors noted that other microscopic changes, commonly found in this colony of rats, were not related to treatment with the test material.

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2) Neoplastic - There was no evidence of neoplastic lesions in any of the treated or control rats.

D. DISCUSSION

Groups of 10 male and 10 female rats were used to assess the toxicity of G-28279 technical, a metabolite of atrazine, following 90-day dietary administration of doses of 10, 50, or 500 ppm. Groups of 10 male and 10 female rats were treated similarly, but given only the rodent diet without the test material. All animals survived the test period and no clinical signs of toxicity were observed. However, effects on growth, liver weights, and development of histopathologic lesions occurred in treated rats, primarily at the highest dose. Effects on growth were manifested by slightly decreased mean body weights and body weight gains in both sexes, with the greatest effect occurring at 500 ppm. Growth depression fell below 10% relative to controls only at 500 ppm. Food consumption in males was not significantly affected by treatment with the test material and females consumed slightly less food only during the first few weeks of the study. The slightly decreased food efficiency in males at 50 and 500 ppm and a more pronounced decrease in females at 500 ppm suggest that the depressed growth was due to a toxic effect of the test material. Water consumption was slightly increased (+11%) for males and considerably increased (+31%) for females at 500 ppm compared with controls. Although slightly increased relative kidney weights in males and increased serum creatinine levels in females may be indicators of potential renal toxicity, the magnitude of these changes relative to controls were too low to be biologically relevant. In addition to increased relative kidney weights, organ weight changes in males at the high dose included decreased absolute heart weights, increased relative testes weights, and increased relative brain weights. Decreased body weight gain may have accounted for some of the organ weight changes. In the absence of histopathologic lesions in the corresponding organs, the weight changes are not necassarily indicative of toxicity. Female rats exhibited slightly increased relative liver weights (13% above control value) and extramedullary hematopoiesis of the liver at 500 ppm, indicating slight hepatotoxicity. However, there were no biochemical correlates; a dose-related increase of SGPT activity was seen only in male, but not in female rats. Minimal to moderate fatty changes of adrenal cortex in males and slight hypertrophy of the thyroid follicular epithelium in males at 500 ppm suggest treatment-related effects. Extramedullary hematopoiesis of the spleen in females at 500 ppm may be attributed to a yreatment related effect in female rats and hypertrophy of pituitary cells in males at the same dose levels may reflect abnormally high hormonal activity. Thus, the splenic and pituitary lesions were not considered treatment-related effects.

The doses for the 13-week study were selected from the results

of a feeding study in which rats were administered 50, 500, or 2000 ppm G-28279 technical for 28 days. Administration of the test material produced dose-related decreases in body weight and decreased food consumption at 2000 ppm in both sexes. Also observed were slight changes in several hematologic and clinical chemistry parameters. Decreased absolute and relative thymus weights were seen in males at ≥500 ppm and in females at 2000 ppm. Based on the findings of this range-finding study, the doses selected for the 13-week study appear to be justified.

E. STUDY DEFICIENCIES

For the clinical chemistry analysis, magnesium, creatinine phosphokinase, and LDH were not determined. LDH activity levels may have provided a biochemical correlate to confirm liver damage.

Food efficiency was not calculated.

The following tissues were subjected to macroscopic examination, but were not evaluated histologically: spinal cord, eyes, mammary gland, muscle, and skin.

The above deficiencies did not affect the outcome of the study.

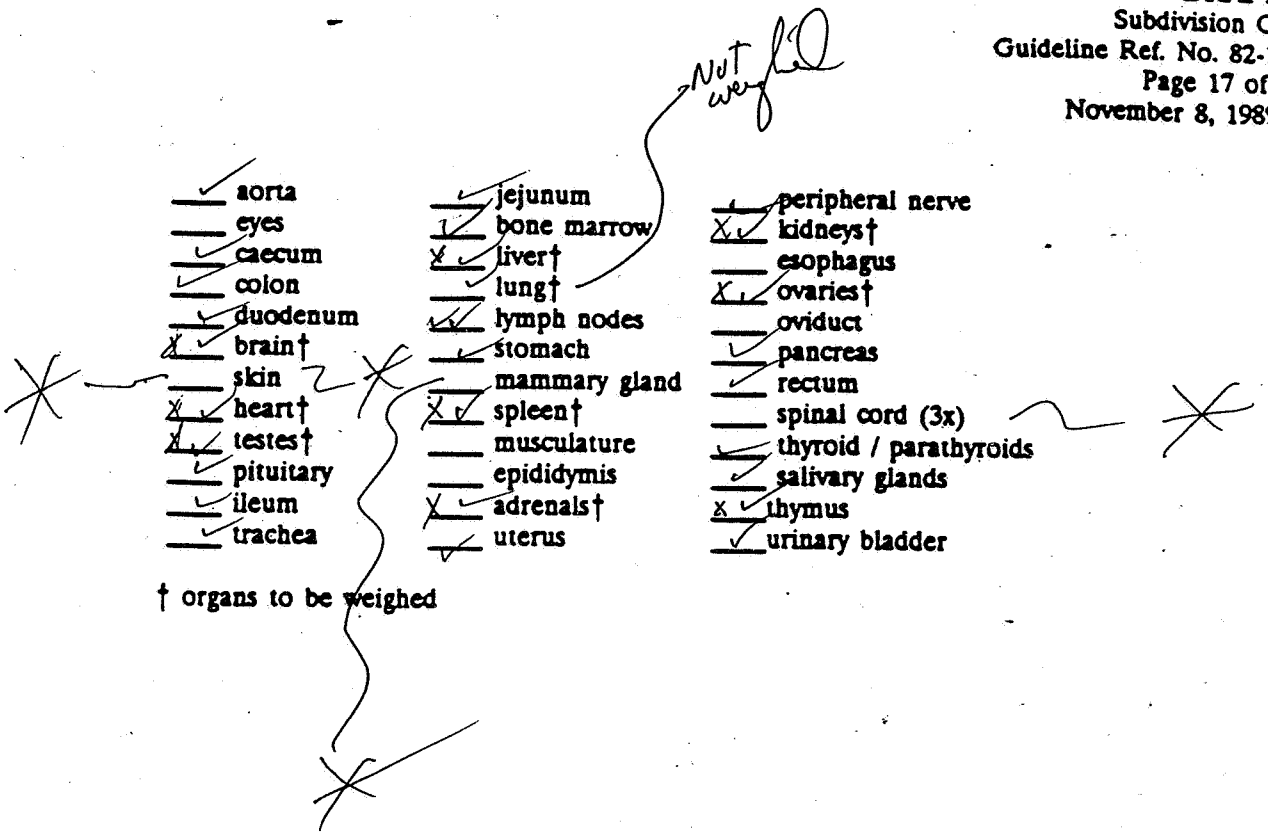
82-1 Subchronic Feeding in the Rodent and Nonrodent

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 10 rodents or 4 nonrodents/sex/group (3 test groups and control group).
3. ✓ Dosing duration daily for 90-days or 5 days/week for 13 weeks.
4. ___ Doses tested include signs of toxicity at high dose but no lethality in nonrodents or a limit dose if nontoxic (1000 mg/kg).
5. ✓ Doses tested include a NOEL.
6. • ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual or cage food consumption.
10. • ✓ Opthalmoscopic examination (at least pretest and at term) control and high dose.
11. ✓ Clinical pathology data of 12 & 13 at termination for rodents, before, monthly or midway and at termination for nonrodents.
12. ___ Hematology.
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (~~or~~ clotting measure)
13. ___ Clinical chemistry.
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - • ___ Creatinine kinase
    - ___ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ✓ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea- urea nitrogen (BUN)
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
14. • ✓ Urinalysis, only when indicated by expected or observed activity. As scheduled in 11.
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ✓ Total bilirubin
    - • ✓ Urobilirubin
    - ___ Sediment
    - ✓ Specific gravity (osmolality)
    - • ✓ Volume
15. ✓ Individual necropsy of all animals.
16. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.

Criteria marked with a • are supplemental and may not be required for every study.



† organs to be weighed

Criteria marked with a * are supplemental and may not be required for every study.