12-12-98

DATA EVALUATION REPORT

DIAMINOCHLOROTRIAZINE (G-28273)

STUDY TYPE: 90-DAY SUBCHRONIC DIETARY TOXICITY STUDY IN RATS (82-1)

Prepared for

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

Prepared by

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PC 080803

[DIAMINOCHLOROTRIAZINE (G-28273)] Subchronic Oral Study (82-1)

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic Feeding ▒ Rats (82-1)

TOX. CHEM, NO: 080803

P.C.CODE.: 063

MRID NO.: 430132-07

TEST MATERIAL: Diaminochlorotriazine (G-28273)

SYNONYMS: 2-Chloro, 4,6-diamino-s-triazine; diaminoatrazine; DACT

STUDY NUMBER: F-00006

SPONSOR: CIBA-GEIGY Corporation, Agricultural Division, P.O. Box 18300, Greensboro, NC 27419

TESTING FACILITY: CIBA-GEIGY Environmental Health Center, 400 Farmington Avenue, Farmington, CT 06032

TITLE OF REPORT: 90-Day Oral Toxicity Study in Rats

AUTHORS: J.C. Pettersen, A.D. Richter, and P.A. Gilles

REPORT ISSUED: November 5, 1991 (study completion date)

EXECUTIVE SUMMARY: Groups of 15 male and 15 female CD Sprague-Dawley rats were fed diets containing diaminochlorotriazine (G-28273) (purity 98.2%) at concentrations of 0, 10, 100, 250, or 500 ppm for 13 weeks. The average consumption of test material was 0.7, 6.7, 16.7, or 34.1 mg/kg/day (males) and 0.7, 7.6, 19.7, or 40.2 mg/kg/day (females). All animals survived to study termination. No treatment-related clinical signs of toxicity including ocular lesions were seen at any dose level. At 500 ppm, mean body weights of male rats were lower than controls during most of the study period, decreasing to 87% of controls at week 12. Body weight gain at week 12 was 82% of controls for males receiving 500 ppm, and 85% and 83% of controls for females receiving 250 and 500 ppm, respectively. No treatment-related effects on body weight or body weight gain were seen at the lower doses in either sex. Food consumption was not affected by administration of the test material. There were no biologically significant effects on hematology, clinical chemistry, urinalysis, and gross or histopathology at any dose

level. Although several organ weight changes were observed, there were no histologic or functional correlates. Estrous cycle data indicated a treatment-related effect at doses of ≥100 ppm. The effects, generally more pronounced on days 70-85 than on days 14-28 and 42-56, included lengthening of the estrus cycle and/or an increased incidence of rats exhibiting cycles with persistent estrus and/or diestrus. There were no apparent effects on serum levels of estradiol, progesterone, prolactin, and corticosterone. **Based on estrous cycle effects in female rats, this study provided a NOEL = 10 ppm (0.7 mg/kg/day) and a LOEL = 100 ppm (7.6 mg/kg/day).**

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

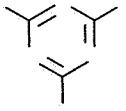
Special Review Criteria (40 CFR 154.7): None

Flagging Statement (40 CFR 158.34): The study, conducted with a metabolite of atrazine, 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: Diaminochlorotriazine (G-28273)

Description: white powder
Lot/Batch #: FL-871776, EHS Code No. 88-009
Purity: 98.2 % a.i.
Stability of compound: at least 3 weeks in the diet
CAS #: not available
Structure:



2. Vehicle and/or positive control

Dry test material was mixed with feed (Purina Certified Rodent Chow #5002 ground meal); therefore, no vehicle was required. A positive control was not included.

3. Test animals

Species: rat
Strain: Crl:CD (SD) BR rats
Age and weight at study initiation: 6 weeks; 180-184 g (males), 144-147 g (females), mean body weight
Source: Charles River Laboratories, Inc., Kingston, NY
Housing: Housed individually in polycarbonate cages (2 animals/sex/cage during acclimation period)
Environmental conditions:
Temperature: 19-24°C
Humidity: 40-60%
Air changes: 15/hr
Photoperiod: 12 hr day/12 hr night
Acclimation period: 12 days (males), 14 days (females)

B. STUDY DESIGN

1. Animal assignment

Animals were assigned to the test groups so that all groups of the same sex had mean body weights which did not differ significantly at the time of assignment (Table 1).

TABLE 1: STUDY DESIGN[a]

Dose Group	Conc. in diet (ppm)	Dose (mg/kg/day)		No. of Animals	
		Male	Female	Male	Female
1 Control	0	0	0	15	15
2 Low (LDT)	10	0.7	0.7	15	15
3 Mid-1 (MDT-1)	100	6.7	7.6	15	15
3 Mid-2 (MDT-2)	250	16.7	19.7	15	15
4 High (HDT)	500	34.1	40.2	15	15

[a]Data taken from p. 22 of study report, MRID No. 430132-07.

Dose selection rationale: The doses used in this study were based on the results of a 4-week dietary range-finding study (No. MIN 872283) with rats in which large body weight decreases were observed at dose levels of ≥1000 ppm of the test material. Body weight decreases were projected to be 10

to 15% for male rats administered 500 ppm, a concentration that was considered to be the maximum tolerated dose. The LOEL was projected to be 10 ppm or possibly 100 ppm.

2. Diet preparation and analysis

Diet was prepared every two to three weeks by mixing appropriate amounts of test material with Purina Certified Rodent Chow #5002 ground meal in a blender; the prepared diet was stored at room temperature. During the study, six samples from each dose level (two each taken from the bottom, middle, and top layer of each batch) were analyzed for concentration and homogeneity. Samples were taken before the initiation of the study and at weeks 2, 5, 8, 10, and 12. The control feed was also analyzed for test material concentration. Stability of the test material in rodent feed was not measured in this study, but was demonstrated in work performed at the CIBA-GEIGY, Summit, NJ, facility.

Results -

a. Homogeneity Analysis ▒ Analysis of blended diets indicated acceptable homogeneity.

b. Stability Analysis ▒ Stable in rodent feed for up to 21 days at room temperature at 10 and 1000 ppm.

c. Concentration Analysis ▒ All values except one of six 250 ppm blends were within ± 10% of the target concentration. The measured concentration of the 250 ppm blend was 215 ppm, 14% lower than the target concentration.

3. Diet

Animals were fed Certified Rodent Chow (#5002, Purina Mills, Inc.) and watered *ad libitum*.

4. Statistics

Body weight and clinical laboratory test values (hematology, clinical chemistry, and urinalysis) were analyzed by one-way analysis of variance and Dunnett's t-test. Nonparametric data were analyzed by Fisher's Exact test with Bonferroni's correction for multiple group comparison. Levels of significance were set at $p<0.05$ or $p<0.01$.

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

C. METHODS AND RESULTS

1. Observations

Animals were inspected at least twice daily for signs of toxicity and mortality. In addition, all animals were given a detailed physical examination each week, including palpation for the presence of tumors.

Results - No animals died during the study period, and no clinical signs of toxicity attributable to the test material were seen in either male or female rats. Incidental findings were noted for all groups.

2. Body weight

Animals were weighed weekly and at termination of the study.

Results - Group mean weekly body weights are presented in Table 2. There were no significant body weight decreases at any time during the study in male rats given 10, 100, or 250 ppm. At 500 ppm, the body weights of males were significantly lower than controls through most of the study period, gradually decreasing from 92% ($p<0.05$) of controls at week 2 to 87% ($p<0.01$) of controls at week 12. Slightly lower body weights were also seen in females at the higher doses (250 and 500 ppm), but the decreases did not significantly differ from controls at any dose level. The cumulative weight gain from initiation of the study to week 12 was significantly decreased in males fed 500 ppm (81% of control, $p<0.01$) and in females fed 250 ppm (85% of control, $p<0.05$) and 500 ppm (83% of control, $p<0.01$). The authors noted that the body weight changes are suggestive of mild to moderate toxicity of the test material.

TABLE 2. GROUP MEAN BODY WEIGHTS (g) OF MALE AND FEMALE RATS FED DIAMINOCHLOROTRIAZINE (G-28273) FOR 13 WEEKS[a]

Week of Study	Exposure Level (ppm)									
	Males					Females				
	0	10	100	250	500	0	10	100	250	500
0	181	180	182	184	180	146	147	147	144	147
1	248	248	251	247	235	173	172	169	163	164
2	306	306	299	301	282*	196	196	192	184	186
3	348	349	342	343	324	210	209	207	199	199
4	381	389	381	377	352*	225	225	221	216	211
5	414	420	409	405	378**	239	237	235	226	224
6	442	446	441	434	402*	251	248	247	239	237
7	461	472	460	459	421*	258	254	253	241	244
8	488	501	497	482	446*	264	259	260	251	247
9	505	522	512	504	456*	277	271	272	262	256
10	516	534	528	516	469	284	276	275	266	266
11	541	556	554	534	477**	289	280	280	270	269
12	559	571	572	550	488**	292	281	281	268	268
Mean body weight gain	378	391 (103)[b]	390 (103)	366 (97)	308** (81)	146	134 (92)	134 (92)	124* (85)	121** (83)

[a] Data taken from Tables 3, 4, and 5 (pp. 40-44) of study report, MRID No. 430132-07.
* Significantly different from control, $p<0.05$ (Dunnett's test)
** Significantly different from control, $p<0.01$ (Dunnett's test)
[b] Numbers in parenthesis are percent of control weight gain calculated by the reviewer

3. Food consumption and compound intake

Food consumption for each animal was determined weekly and at termination of the study. Food consumption was calculated as g/rat/day. Compound consumption (time-weighted average) was calculated from group mean body weights, food consumption data, and concentration of the test material in the diet. Food efficiency [(body weight gain in g/food consumption in g per unit time) x 100] was not calculated.

Results -

a. Food consumption ▒ Data at weekly intervals are presented in Table 3. Except for a significantly ($p<0.05$) decreased food consumption in male rats fed 500 ppm at week 1, the amount of food consumed was generally similar in rats receiving the treated food and control diet. Weekly fluctuations showing no consistent pattern were noted in all groups.

b. Compound consumption (time-weighted average) ▒ Males received doses of 0.7, 6.7, 16.7, or 34.1 mg/kg/day and females received doses of 0.7, 7.6, 19.7, or 40.2 mg/kg/day for dietary concentrations of 10, 100, 250, or 500 ppm, respectively.

c. Food efficiency ▒ Efficiency was not calculated by the study authors. Based on total weight gain and on the total amount consumed, the overall food efficiency calculated by the reviewer was 16.7, 16.5, 16.5, 16.0, or 13.7 g body weight gain/g food for male rats and 9.2, 8.6, 8.4, 8.0, or 7.5 g body weight gain/g food for female rats for dietary concentrations of 0, 10, 100, 250, or 500 ppm, respectively. Thus, male rats showed a treatment-related effect on food efficiency that was more pronounced at 500 ppm. A dose-related decrease of food efficiency was seen in female rats.

4. Ophthalmoscopic examination

Eyes were examined prior to administration of the test material and at termination of the study.

Results - No ocular lesions attributable to the test material were noted.

5. Estrous cycle determination and hormone analysis

a. Estrous cycle ▒ Vaginal smears were prepared and examined on study days 14 through 28 for control and females fed 500 ppm and on study days 42 through 56 and days 70 through 85 for all females. Smears were prepared between 8:00 and 10:00 a.m. The cell types (cornified cells, leukocytes, and nucleated epithelial cells) and their relative proportionality were recorded to determine the stage of estrus. Cycle lengths were defined as the number of days between

successive estrous-type smears. The most common normal pattern was 4 days in length; a few animals had 5-day cycles which were also considered normal. Irregularities of the cycle included early or intermittent proestrus; inconsistent cycle length (3-day cycle followed by 5-day cycle); persistent, early, or unexpected estrus; and persistent or prolongation of diestrus beyond 3 days. Cycle length was considered indeterminate when it varied considerably, or the animal(s) were stuck in a particular stage of estrus.

An additional evaluation of the estrus cycle data was conducted by an external consultant, Dr. Paul Terranova (University of Kansas Medical Center, Kansas City, KS).

TABLE 3. GROUP MEAN FOOD CONSUMPTION (g/DAY) AT WEEKLY INTERVALS OF MALE AND FEMALE RATS FED DIAMINOCHLOROTRIAZINE (G-28273) FOR 13 WEEKS[a]

Week of Study	Exposure Level (ppm)									
	Males					Females				
	0	10	100	250	500	0	10	100	250	500
1	23	24	24	22	21*	15	16	16	16	16
2	25	26	25	25	24	18	17	17	17	17
3	25	26	26	26	24	17	17	18	18	17
4	25	26	26	26	25	17	18	18	17	18
5	25	26	25	26	26	19	18	18	18	18
6	25	25	27	25	25	20	19	19	19	19
7	25	27	26	26	25	17	16	18	17	18
8	26	26	27	26	26	17	16	18	17	18
9	25	26	26	26	25	19	17	18	18	18
10	26	29	28	25	26	18	18	18	18	19
11	26	25	28	26	26	18	19	17	17	18
12	26	26	27	26	26	17	16	17	16	19
Total Food Consumption[b]	2268	2367	2367	2286	2250	1593	1557	1593	1557	1611

[a] Data taken from Tables 6 and 7 (pp. 45-48) of study report, MRID No. 430132-07.
[b] Calculated by reviewer (by multiplying average daily food consumption by 90 days).
* Significantly different from control, $p<0.05$ (Dunnett's test)

b. Serum hormones ▒ Blood samples were obtained (up to 10 females/dose level) from the intraorbital sinus of animals under Metofane® anesthesia. For the determination of serum estradiol, progesterone, and prolactin levels, samples were collected only between 11:15 and 11:45 a.m. from animals believed to be in proestrus on the day of collection. In addition to animals in proestrus, several animals with abnormal cycles were sampled. Corticosterone levels were averaged without regard to the stage of the estrous cycle. Serum samples were prepared and sent to Dr. Charles Eldridge (Bowman Gray, School of Medicine, Winston- Salem, NC) for determination of hormone levels.

Results -

a. Estrous cycle ▒ Estrous cycle effects for the three evaluation periods are presented in Table 4. On study days 14-28, the effects on the estrus cycle on the rats treated with 500 ppm were minimal, consisting of increased incidence of animals with 5-day cycles and an increased incidence of persistent diestrus compared with controls. These early changes preceded more severe changes at the next two intervals (days 42-56 and days 70-85).

At days 42-56, estrus cycle effects were observed at ≥ 100 ppm and typically included an increased incidence of variable, indeterminate, <4 or >5 day cycles. Also seen at this interval was persistent estrus in several animals treated with 250 and 500 ppm. However, the incidence of animals exhibiting variable, indeterminate, <4 or >5 day cycles or animals with prolonged or persistent estrus was considerably higher at 250 ppm than at 500 ppm.

At days 70-85, a further increase of estrous cycle effects was noted. The effects included an increased incidence of animals exhibiting variable, indeterminate, or <4 or >5 day cycles at 500 ppm, an increased incidence of prolonged or persistent estrus at 250 and 500 ppm, and an increased incidence of persistent diestrus at 500 ppm. Effects at 100 ppm observed on days 42-56 were not apparent at days 70-85. At 10 ppm, the incidence of animals exhibiting persistent estrus and diestrus on days 70-85 was higher than that seen at 100 ppm. Because of the lack of a clear dose-response and considerable variability of the data, the authors did not consider the effect at 10 ppm treatment-related.

The consultant's analyses of estrous cycle data which included statistical analysis of the data agreed with those conducted by the study authors. The consultant concluded that there were treatment-related effects on estrous cycle at concentrations of ≥100 ppm. These effects, more pronounced during study days 70 to 85 than during days 42 to 56, included lengthening of the estrous cycle with a tendency towards an increased incidence of persistent estrus. Statistically significant effects were attained only at doses of ≥250 ppm after 70 days of treatment. They indicated that individual variability of estrous cycle data, which tended to be greater in the treated groups, precluded a more exact determination of the estrus cycle effects.

It should be noted that the group incidence values of estrous cycle effects calculated by the consultant (Table A-1, p. 429) are not the same values as those presented in the study report.

TABLE 4. ESTROUS CYCLE LENGTH AND INCIDENCE OF PERSISTENT ESTRUS OR DIESTRUS IN FEMALE RATS FED DIAMINOCHLOROTRIAZINE (G-28273)[a]

Dose Level (ppm)	Study Days	Cycle Length		Persistent Estrus	Persistent Diestrus
		4 to 5 Days	Variable[b]		
0	14-28[c]	15/15	0/15	0/15	1/15
500	14-28	13/15	2/15	0/15	3/15
0	42-56	13/15	2/15	1/15	1/15
10	42-56	13/15	2/15	3/15	2/15
100	42-56	8/15	7/15*	3/15	4/15
250	42-56	3/15	12/15***	8/15**	4/15
500	42-56	8/15	7/15*	5/15	1/15
0	70-85	15/15	0/15	0/15	0/15
10	70-85	12/15	3/15	4/15*	3/15
100	70-85	12/15	3/15	2/15	1/15
250	70-85	3/15	12/15***	11/15***	2/15
500	70-85	4/15	11/15***	7/15**	5/15

[a] Data taken from Tables 10a and 10b (pp. 53-54) of study report, MRID No. 430132-07.
[b] Includes indeterminate and <4 days and >5 days
[c] Only animals administered 500 ppm and controls were examined on days 14-28.
* Significantly different from control, $p<0.05$ (Fisher Exact test), calculated by reviewer.
** Significantly different from control, $p<0.01$ (Fisher Exact test), calculated by reviewer.
*** Significantly different from control, $p<0.001$ (Fisher Exact test), calculated by reviewer.

b. Serum hormones ▒ Based on the data provided in the study report, serum levels of estradiol, progesterone, prolactin, and corticosterone appeared

to be unaffected by treatment with the test material (Table 5). The authors noted, however, that due to the disruptive effects of the test material on the estrous cycle, only one blood sample was available from rats given 250 ppm (not presented in Table 5) and four samples from rats given 500 ppm for comparison of estradiol, progesterone, and prolactin levels.

TABLE 5. SERUM HORMONE LEVELS IN FEMALE RATS FED DIAMINOCHLOROTRIAZINE (G-28273)[a]

Dose Level (ppm)	Estradiol (pg/mL)	Progestrone (ng/mL)	Prolactin (ng/mL)	Corticosterone (ng/mL)
0	24.6±5.7[b] (10)[c]	7.41±2.49 (10)	2.2±2.2 (10)	456.7±77.6 (10)
10	26.4±9.0 (8)	7.64±1.37 (7)	3.7±2.7 (7)	447.5±109.2 (10)
100	24.2±4.5 (9)	8.53±1.89 (9)	1.5±1.4 (8)	408.3±154.8 (10)
250	ND	ND	ND	438.3±145.9 (10)
500	19.1±3.9 (4)	9.58±1.49 (4)	3.0±1.5 (4)	424.8±104.1 (10)

[a] Data taken from Table K3 (p. 419) of study report, MRID No. 430132-07.
[b] Group mean values ±S.D.
[c] Numbers of animals tested.
ND = no data

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

a. Hematology

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

* Required for subchronic studies

Results - Examination of hematologic data revealed no treatment-related biologically significant effects.

b. Clinical Chemistry

X	Electrolytes
X	Calcium*
X	Chloride*
	Magnesium*
X	Phosphorus*
X	Potassium*
X	Sodium*

X	Enzymes
X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
X	Bilirubin
X	A/G ratio
X	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	Sorbitol dehydrogenase

X	Other
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total serum protein (TP)*
X	Triglycerides
	Serum protein electrophoresis

* Required for subchronic studies

Results - Pertinent clinical chemistry values are summarized in Table 6. Statistical analysis revealed significant differences in some parameters: decreased serum calcium values in males and females (500 ppm); increased chloride values in males (100 ppm); increased serum GGT activity in males (10 and 100 ppm) and in females (10 and 500 ppm); decreased total protein values in males (250 and 500 ppm); and decreased globulin values in males (500 ppm). Although statistically significant,

the magnitude of these changes relative to controls were too low to be biologically significant. A dose-related decrease in globulin and total protein values was noted in male rats that may have been related to treatment, but the magnitude of the reduction does not suggest that the effect is biologically significant.

TABLE 6. SELECTED CLINICAL CHEMISTRY VALUES IN MALE AND FEMALE RATS FED DIAMINOCHLOROTRIAZINE (G-28273) FOR 13 WEEKS[a]

Parameter	Exposure Level (ppm)									
	Males					Females				
	0	10	100	250	500	0	10	100	250	500
Calcium (mg/dL)	10.3 ±0.3[b]	10.2 ±0.2	10.3 ±0.3	10.1 ±0.3	10.0* ±0.4	10.3 ±0.3	10.3 ±0.3	10.2 ±0.3	10.1 ±0.3	9.9** ±0.3
Chloride (mmol/L)	101 ±2	102 ±1	103** ±2	102 ±1	102 ±2	104 ±2	104 ±2	105 ±2	104 ±1	103 ±2
GGT[c] (I.U./L)	5 ±1	6** ±1	6** ±1	5 ±1	5 ±1	5 ±1	6** ±1	5 ±1	5 ±0	6** ±1
Globulin (g/dL)	3.1 ±0.3	3.0 ±0.3	2.9 ±0.2	2.9 ±0.3	2.8* ±0.3	3.0 ±0.4	3.0 ±0.3	2.9 ±0.2	3.0 ±0.5	3.1 ±0.3
Total protein (g/dL)	6.5 ±0.3	6.4 ±0.3	6.3 ±0.3	6.2* ±0.3	6.1* ±0.4	6.6 ±0.4	6.7 ±0.5	6.7 ±0.5	6.6 ±0.6	6.7 ±0.4

[a] Data taken from Tables 14 and 15 (pp. 60-66) of study report, MRID No. 430132-07.
[b] Group mean values ±S.D.
[c] Gamma-glutamyl transferase
* Significantly different from control, $p<0.05$ (Dunnett's test)
** Significantly different from control, $p<0.01$ (Dunnett's test)

7. Urinalysis*

Urine was collected from fasted animals prior to sample collection and sacrifice. The CHECKED (X) parameters were examined.

X	
X	Appearance
X	Volume
X	Specific gravity
X	Sediment (microscopic)**
X	Protein**

X	
X	Glucose**
X	Ketones**
X	Bilrubin
X	Blood**
X	Nitrate
X	Urobilinogen**

* Not required for subchronic studies
** Semi-quantitative analysis

Results - Examination of urinalysis data revealed no treatment-related effects.

8. Sacrifice and pathology

All animals were sacrificed by lethal injection (i.p.) with sodium pentobarbital. Prior to sacrifice, the animals were fasted overnight. Gross pathological examinations were conducted and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	Digestive system
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Pancreas*
	Gallbladder*

	Respiratory
X	Trachea*
XX	Lung*

X	Cardiovasc./Hemat.
X	Aorta
X	Heart*
X	Bone marrow*
X	Lymph nodes*
XX	Spleen
XX	Thymus*

	Urogenital
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
X	Epididymides
X	Prostate
X	Seminal vesicle
XX	Ovaries*+
X	Uterus*

X	Neurologic
XX	Brain*+
X	Periph. nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic n.)*

	Glandular
XX	Adrenal gland*
	Lacrimal gland
X	Mammary gland*
X	Parathyroids*
X	Thyroids*

	Other
X	Bone*
X	Skeletal muscle*
X	Vagina
X	Harderian gland
X	Skin*

X	Nose	X	All gross lesions and masses*
	Pharynx		
	Larynx		

* Required for subchronic
† Organ weight required in subchronic and chronic studies.

Results -

a. Organ weight ▒ Feeding of the test material resulted in several minor changes in absolute and/or relative organ weights, primarily at the two higher doses (Table 7). Statistically significant changes included the following: decreased absolute thymus weights in males and females at 500 ppm; increased relative spleen weights in males and females at 500 ppm; increased relative kidney weights in males and females at 500 ppm and in females at 250 ppm; decreased absolute liver weights in males and increased relative liver weights in females at 500 ppm; increased relative lung weights in males at 500 ppm and females at 250 and 500 ppm; increased relative adrenal weights in males at 500 ppm and in females at 250 ppm; increased relative testes weights in males at 250 ppm and 500 ppm; and decreased absolute and

TABLE 7. ABSOLUTE WEIGHTS (g) AND RELATIVE WEIGHTS (% of BODY WEIGHT) OF AFFECTED ORGANS IN MALE AND FEMALE RATS FED DIAMINOCHLOROTRIAZINE (G-28273) FOR 13 WEEKS[a]

Organ/Sex		Exposure Level (ppm)									
		0		10		100		250		500	
		ABS	REL	ABS	REL	ABS	REL	ABS	REL	ABS	REL
Adrenals	M	0.0548 ±0.0092	0.0104 ±0.0020	0.0583 ±0.0134	0.0105 ±0.0016	0.0605 ±0.0087	0.0113 ±0.0016	0.0577 ±0.0088	0.0110 ±0.0017	0.0557 ±0.0059	0.0121* ±0.0014
	F	0.0662 ±0.0088	0.0238 ±0.0032	0.0660 ±0.0099	0.0243 ±0.0038	0.0677 ±0.0112	0.0254 ±0.0045	0.0733 ±0.0113	0.0287* ±0.0053	0.0654 ±0.0079	0.0262 ±0.0034
Brain	M	2.123 ±0.107	0.401 ±0.041	2.121 ±0.082	0.389 ±0.044	2.149 ±0.074	0.401 ±0.040	2.082 ±0.090	0.398 ±0.043	2.040* ±0.065	0.443* ±0.029
Kidneys	M	3.438 ±0.444	0.648 ±0.088	3.424 ±0.492	0.621 ±0.053	3.390 ±0.417	0.627 ±0.038	3.373 ±0.418	0.640 ±0.058	3.307 ±0.329	0.714* ±0.046
	F	1.755 ±0.197	0.629 ±0.057	1.732 ±0.197	0.653 ±0.050	1.709 ±0.143	0.640 ±0.046	1.748 ±0.153	0.681* ±0.054	1.820 ±0.187	0.727** ±0.063
Liver	M	14.686 ±1.847	2.754 ±0.222	15.095 ±1.910	2.740 ±0.183	14.946 ±2.364	2.757 ±0.207	14.635 ±2.761	2.750 ±0.187	12.317* ±1.514	2.657 ±0.216
	F	7.068 ±0.694	2.532 ±0.186	7.011 ±0.913	2.611 ±0.373	6.948 ±0.733	2.598 ±0.241	7.021 ±0.826	2.728 ±0.203	7.224 ±0.929	2.875** ±0.205
Lungs	M	1.640 ±0.146	0.309 ±0.030	1.659 ±0.155	0.303 ±0.029	1.710 ±0.104	0.319 ±0.032	1.714 ±0.168	0.326 ±0.023	1.675 ±0.106	0.363* ±0.027
	F	1.143 ±0.103	0.411 ±0.042	1.193 ±0.102	0.455* ±0.029	1.153 ±0.097	0.433 ±0.048	1.187 ±0.099	0.464** ±0.045	1.243 ±0.126	0.497** ±0.049

TABLE 7. (Continued)

Organ/Sex		Exposure Level (ppm)									
		0		10		100		250		500	
		ABS	REL	ABS	REL	ABS	REL	ABS	REL	ABS	REL
Spleen	M	0.835 ±0.115	0.157 ±0.024	0.894 ±0.175	0.162 ±0.019	0.875 ±0.104	0.163 ±0.019	0.912 ±0.157	0.173 ±0.020	0.899 ±0.151	0.195** ±0.033
	F	0.564 ±0.093	0.202 ±0.034	0.563 ±0.096	0.217 ±0.030	0.528 ±0.072	0.199 ±0.032	0.582 ±0.084	0.226 ±0.018	0.611 ±0.076	0.244** ±0.032
Testes	M	3.375 ±0.272	0.636 ±0.064	3.643 ±0.353	0.676 ±0.073	3.553 ±0.400	0.661 ±0.072	3.672 ±0.300	0.701* ±0.085	3.602 ±0.189	0.777** ±0.062
Thymus	M	0.294 ±0.082	0.056 ±0.016	0.311 ±0.082	0.058 ±0.017	0.294 ±0.082	0.054 ±0.012	0.302 ±0.078	0.057 ±0.016	0.224* ±0.038	0.048 ±0.008
	F	0.255 ±0.046	0.092 ±0.017	0.247 ±0.049	0.093 ±0.023	0.229 ±0.047	0.086 ±0.020	0.220 ±0.032	0.086 ±0.014	0.212* ±0.039	0.084 ±0.013

[a] Data from Tables 18 and 19 (pp. 68-71) of study report, MRID No. 430132-07.
[b] Group mean values ±S.D.
* Significantly different from control, $p<0.05$ (Dunnett's test)

increased relative brain weights in males at 500 ppm. There were no clear indications of a dose-related trend. The low magnitude of the body weight changes relative to controls and the absence of microscopic changes in the same organs suggest the changes were of uncertain biological relevance.

b. Gross pathology ▒ No treatment-related gross lesions were observed in either male or female rats receiving the test material.

c. Microscopic pathology ▒

1) Non-neoplastic ▒ There were no treatment-related systemic microscopic changes in tissues or organs of any of the test animals. The number and types of histopathologic lesions observed were comparable for controls and animals treated with the test material.

2) Neoplastic ▒ Except for one benign adenoma of the pituitary gland seen in a female rat receiving 250 ppm, no neoplastic lesions were observed in either male or female rats receiving the test material or in controls.

D. DISCUSSION

Groups of 15 male and 15 female rats were used to assess the toxicity of diaminochlorotriazine (G-28273) following 90-day dietary administration of doses of 10, 100, 250, or 500 ppm. A group of 15 male and 15 female rats were treated similarly but given only the rodent diet without the test material. All animals survived the test period. No signs of toxicity were observed as determined by clinical or biochemical assessments or pathological effects on tissues or organs. Administration of the test material produced decreased absolute body weights and decreased body weight gains in males administered 500 ppm and decreased body weight gains in females at 250 and 500 ppm, but had no observable effect on food consumption. The decreased food efficiency suggests that the depressed growth was due to a toxic effect of the test material. Although changes in absolute and/or relative weight of several organs were observed, there were no gross, histopathologic, or functional corrolates. The organ weight changes, therefore, are of uncertain toxicologic significance. In addition to the effects on body weight, dietary administration of diaminochlorotriazine (G-28273) produced disturbances of the estrous cycle, affecting cycle length and frequency. The effects were variable with regard to dose level as well as to evaluation period and lacked a clear dose-response. For example, the incidence of animals

exhibiting variable, indeterminate, or < 4 or >5 day cycles or prolonged or persistent estrus was higher at 250 ppm than at 500 ppm on the second (days 42-56) and third (days 70-85) evaluation periods. Although the estrous cycle effects were generally more pronounced on days 70-85, the effects at 100 ppm were less pronounced on days 70-85 than on days 42-56. Serum levels of estradiol, progesterone, prolactin, and corticosterone appeared to be unaffected by treatment with the test material and are not supportive of estrus cycle alterations. However, only one blood sample per animal was obtained and no or few samples were obtained from the 250 ppm or 500 ppm groups, respectively. Additionally, the authors noted that estradiol, progesterone, and prolactin levels fluctuate throughout the day and throughout the estrus cycle; and anesthesia prior to blood sampling may affect hormone levels in Sprague-Dawley rats [see Roberts, S., et al. The alteration of serum hormone levels by single and repeated ether anesthesia. Toxicologist 9: 275 (1989)]. Based on estrous cycle effects in the absence of a clear dose-response and the noted variability of these effects, 10 ppm (0.7 mg/kg/day) was identified as the NOEL and 100 ppm (7.6 mg/kg/day) was identified as the LOEL for 90-day exposure to the diaminochlorotriazine (G-28273).

The doses for the 13-week study were selected from the results of a dietary range-finding study in which rats were administered diaminochlorotriazine (G-28273) for 4 weeks. Doses of ≥1000 ppm produced unspecified large reductions in body weights. Although the range-finding study provided few details, the doses selected for the 13-week study appear to be justified.

E. STUDY DEFICIENCIES

Only a very limited description of the range-finding study is provided. The study report gave no explanation why the effects on estrus cycle and hormone levels, a major portion of the study, were examined. Food efficiency was not calculated. Lactic acid dehydrogenase and magnesium were not determined; this omission, however, does not compromise the integrity of the study, because there was no evidence of systemic toxicity as determined by histopathologic or biochemical indices.