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CASWELL FILE

009497

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

MAY 11 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

SUBJECT: Review of the Developmental Toxicity Study in Rats; and the One-Year Chronic Study in Dogs with the Atrazine Metabolite, Diaminochlorotriazine.

Tox. Chem No. 264B/063
HED Project No. 0-0812
Rec No. 260480 & 260481

TO: Kathy Pearce
PM 76
SRB
SRRD (7508W)

FROM: Henry Spencer, Ph.D. 4/22/92
Acting Section Head, Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

THRU: Karl Baetcke, Ph.D. 4/24/92
Chief
Toxicology Branch I
Health Effects Division (H7509C)

ACTION: The registrant has submitted for review, two studies--a chronic feeding study in dogs MRID No. 41392401 and a rat developmental toxicity study in rats MRID No. 41392402 - on the atrazine metabolite, diaminochlorotriazine (DACT).

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CONCLUSIONS:

1. Both of these studies are of a supplemental nature because they are only providing nonrequired information. However, because of the potential impact on dietary exposures , the data are being forwarded to the RFD Peer Review Group of HED for evaluation.

2. Doses of (DACT) were provided in the diets of dogs at levels of 0, 5, 100 or 1500/750 ppm for 13 weeks or 52 weeks. Severe toxicity occurred at 1500 ppm (HDT) and females were placed on treatment of 750 ppm after 6 weeks. Males were changed to 750 ppm after 6 weeks and removed to control diets from weeks 9-13 after which they were again given the 750 ppm treatment.

Results: 750 ppm (24.1 mg/kg for males and 32.7 mg/kg for females), Several of each sex were sacrificed in moribund condition. Heart function was effected with thickened valves, thrombosis, chronic myocarditis, necrosis, inflammation, hemorrhage and enlargement.

Fluid accumulation was noted in the pericardial, thoracic and abdominal spaces.

Livers of treated animals showed congestion, centrilobular fibrosis, atrophy, necrosis, bile stasis, hemosiderosis, adhesions and mottling.

Testes were affected by hypospermatogenesis, and hypospermia. Thymus glands were atrophied and bone marrows were hyperplastic. Decreased body wts and weight gains occurred at 1500 ppm after 6 weeks. Males continued to lose weight at 750 ppm. Hematological parameters effected were reversible. Tremors at the highest doses of 1500 and 750 ppm occurred in most animals. One female at 100 ppm also exhibited tremors in one time period.

An LEL-threshold for tremors in females is considered to be 100 ppm (3.43 mg/Kg/day) with a frank-LEL at 750ppm. A NEL can be established in female dogs at 5 ppm (0.195 mg/kg/day).

3. The Toxicology Branch notes that the doses chosen by registrant of 0, 5, 100, or 1500 ppm are unusual in that they are extremely far apart at the 5 and 100 ppm levels (ie. 20 fold diffent) and makes the use of the 5 ppm as a NEL much more disparate than the ususal 10 fold normally seen.

4. The developmental study MRID No. 4139202 with gavaged

doses of 0, 2.5, 25, 75, or 150 mg/kg/day is sufficient to show that the maternal NOEL is 25 mg/kg/day and LEL is 75 mg/kg/day based on reduced body wt. gain and reduced food consumption at 75 and 150 mg/kg/day.

The main developmental effects reported were of bones being incompletely ossified at 25 mg/kg/day. There was also a tendency for increasing incidences of shortened or absent renal papilla in the recovered fetuses at 25 mg/Kg/day. Toxicity was also noted as reduced fetal weights at 75mg/kg/ day and above.

5. An additional submission, MRID 42165502, was related by the registrant to show the purity of the test material to be 98.2 % ai. which was used in the rat developmental toxicity study.

6. The DERS are submitted to the files.

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EPA No.: 68D80056
DYNAMAC No.: D992-112 A1-A2
TASK No.: 1-12A1-A2
August 15, 1990

DATA EVALUATION RECORD

DIAMINOCHLOROTRIAZINE

13/52-Week Oral Toxicity Study in Dogs

REVIEWED BY:

John J. Liccione, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: _____
Date: _____

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Signature: Karl W. Pfeifle
Date: Received 4/24/92

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DATA EVALUATION RECORD

GUIDELINE § 83-1

STUDY TYPE: Dietary toxicity in dogs.

MRID NUMBER: 413924-01.

TEST MATERIAL: Diaminochlorotriazine.

SYNONYM(S): DACT.

STUDY NUMBER(S): 872151.

SPONSOR: Agricultural Division, CIBA-GEIGY Corporation, Summit, NJ.

TESTING FACILITY: Division of Toxicology/Pathology, CIBA-GEIGY Corporation, Summit, NJ.

TITLE OF REPORT: 13/52-Week Oral Toxicity Study in Dogs.

AUTHOR(S): Thompson, S. S., Batastini, G. G., and Arthur, A. T.

REPORT ISSUED: January 17, 1990.

CONCLUSIONS:

Diaminochlorotriazine was fed to male and female dogs at dietary levels of 0, 5, 100, or 1500 ppm for 13 or 52 weeks. Because of severe toxicity at the highest dose, which was evident after 6 weeks of treatment, the high-dose dogs were fed a diet containing 750 ppm. Females tolerated this dose level and received 750 ppm until termination at 13 or 52 weeks, or through 13 weeks followed by a 39-week recovery period. Since males continued to exhibit signs of toxicity at 750 ppm, they were fed untreated diet for weeks 9 through 13. Four male dogs were then placed again on a diet containing 750 ppm until termination at 52 weeks.

The mean daily doses for male dogs receiving dietary levels of 5, 100, and 1500/750 ppm for 52 weeks were 0.187, 3.61, and 24.1 mg/kg/day, while the doses were 0.195, 3.43, and 32.7 mg/kg/day for females receiving the same dietary levels. Among the high-dose dogs, five males and two females were sacrificed moribund during the treatment period. Moribundity was attributed to impairment of heart function, the primary treatment-related effect of diaminochlorotriazine, which was accompanied by several clinical and pathological changes. Pathological cardiac findings included enlargement, softness, thickened valves, lesions, distension, red/dark color, thrombosis, chronic myocarditis, necrosis, inflammation, hemorrhage, and hemosiderosis. Secondary treatment-related changes in the high-dose animals were seen in the liver (enlargement, congestion, centrilobular fibrosis/atrophy, bile stasis, necrosis, hemosiderosis, red/dark color, lesions, adhesions, mottling, and rough texture); testes (hypospermatogenesis and hypospermia); thymus (atrophy); bone marrow (hyperplasia); and pericardium, thoracic, and abdominal cavities (fluid accumulation). Recovery females did not exhibit any clinical ophthalmological signs of cardiac impairment. Other effects at the high dose included decreased body weight and body weight gains in males and females dosed for 6 weeks; increased mean spleen, liver, and kidney weights; anemia with accompanying reticulocytosis (a reversible effect); decreases in albumin, calcium, and total cholesterol levels; nonsignificant increases in lactic acid dehydrogenase activity; and elevations in platelet levels. High-dose males continued to lose weight when the dose was lowered to 750 ppm. Severe anemia with reticulocytosis was noted in only one of the two recovery females. The effects of diaminochlorotriazine on cholesterol levels and erythroid parameters were reversible as suggested by the findings noted in the recovery females. Tremors were noted in almost all animals of both sexes beginning at week 6 and extending until week 14. No adverse effects were observed at dietary levels of 5 or 100 ppm. with the exception of a threshold for the tremors a 100 ppm. a NOEL = 5 ppm. (.195 mg/kg/day) HK 3/20/
Although there was appreciable mortality at the highest dose of 1500 ppm, a sufficient number of animals were at risk to evaluate histopathology. Administration of dietary levels of ≥750 ppm to dogs is associated with symptomatology of cardiac impairment. The NOEL is 100 ppm.

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Classification: This study satisfies the Guideline requirements for a chronic dog study (83-1).

A. MATERIALS:

1. Test Compound: Diaminochlorotriazine; description: white powder; batch No.: FL 871423; purity: 98.7%.

2. Test Animals: Species: dog; strain: beagle; all dogs were approximately 8 months of age at initiation of treatment; weight at initiation of treatment: males-- 6.7-10.3 kg, females -- 6.3-9.3 kg; source: Marshall Farms, North Rose, New York.

B. STUDY DESIGN:

1. Animal Assignment: Dogs were acclimated to laboratory conditions for approximately 6 weeks prior to treatment. Prior to initiation of treatment, 36 males and 36 females were randomly assigned to the following groups using a computer-generated randomization procedure that stratified the animals within each sex by body weight:

Test Group	Dietary Level (ppm)	Main Study (52 weeks) Males	Main Study (52 weeks) Females	Interim Sacrifice (13 weeks) Males	Interim Sacrifice (13 weeks) Females	Interval: Dose and Weeks (wk)
1 Control	0	4	4	6	6[c]	-- --
2 Low	5	4	4	4	4	-- --
3 Mid	100	4	4	4	4	-- --
4 High	1500/750[a]	--	--	4[b]	--	-- --
		--	--	2	--	1500 ppm (1-6 wk) 750 ppm (7-8 wk) 0 ppm (9-13 wk)
		4	---	---	--	1500 ppm (1-6 wk) 750 ppm (7-8 wk) 0 ppm (9-13 wk) 750 ppm (14-52 wk)
		--	--	--	4	1500 ppm (1-6 wk) 750 ppm (7-13 wk)
		--	4	--	--	1500 ppm (1-6 wk) 750 ppm (7-52 wk)
		--	--	--	2[d]	1500 ppm (1-6 wk) 750 ppm (7-13 wk) 0 ppm (14-52 wk)

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[a]The high-dose animals received 1500 ppm for 6 weeks, but because of toxicity, the dose was lowered to 750 ppm beginning with week 7. Females continued receiving 750 ppm until termination at 13 or 52 weeks, or through 13 weeks followed by a 39-week recovery period. In contrast to females, the males received 750 ppm through week 8, and because of continued toxicity at this dose, they were placed on diets containing 0 ppm for weeks 9 through 13. Two of the high-dose males were sacrificed as scheduled after week 13, while four other dogs were placed again on a diet containing 750 ppm until termination at 52 weeks.

[b]Two dogs received 1500 ppm until sacrificed moribund during week 6, while two other dogs received 1500 ppm for the first 6 weeks, then 750 ppm until sacrificed moribund during week 9.

[c]Two control females were designated as recovery controls (13-week and 39-week recovery).

[d]Two high-dose females designated as recovery females were treated for 13 weeks and were then allowed a 39-week recovery period.

Dose levels were selected on the basis of the results of a previous 4-week study, which indicated gross and microscopic hemorrhagic changes in the right atrium in dogs receiving oral doses greater than or equal to 1500 ppm. The 4-week study was not available for review.

In the present study, the animals were housed individually in cages within rooms with temperature maintained at 69 ± 5°F, a relative humidity of 50 ± 20%, and a 12-hour light/dark cycle. Animals were identified by individual ear tatoo numbers.

2. Diet Preparation: An appropriate amount of test substance was mixed with a small amount of feed to form a premix. This premix was then adjusted to the desired concentration by adding the appropriate amount of feed. Diets containing the test substance were prepared weekly. Stability of the test substance in the feed (stored at room temperature) was determined prior to initiation of the study and for a period of 20 days (3000 ppm), 31 days (5 ppm), and 45 days (3000 ppm). At selected weekly intervals during the study, samples were analyzed for homogeneity and level of test compound.

Results: Results of samples analyzed to determine homogeneity of the test substance in the diet at levels of 5, 100, 750, and 1500 ppm indicated a homogeneous mix. The relative standard deviations of homogeneity ranged from 0.2% to 8.5% for all groups (eight intervals of analysis). The concentrations of the test material in the diets (16 intervals of analysis) were generally within 5% of nominal concentrations, ranging from 87% to 109%. Results of stability analysis indicated that the test substance, at concentrations of 5 ppm and 3000 ppm, was chemically stable for at least 31 days at room temperature.

3. Food and Water Consumption: Animals received food (Certified Purina Lab Canine Diet No. 5007) and water ad libitum.

4. Statistics: The following statistical methods were used to

analyze body weight, food consumption, clinical laboratory, and organ weight data. The F-test was used to assess the equality of variances between dosed and control groups. The Dunnett test was applied to determine the level of significance if the data displayed normal distribution. If the data did not follow a normal distribution, then appropriate statistical analyses were performed which included transformation of data, nonparametric tests, and multiple comparison procedures with unequal variances. Nonparametric data were evaluated by the Kruskal-Wallis and Chi-square tests.

5. Quality Assurance: A quality assurance statement was signed and dated January 17, 1990.

C. METHODS AND RESULTS:

1. Observations: All animals were observed daily for appearance, mortality, and clinical status. All animals received physical/auditory examination prior to initiation of treatment (week -3) and at weeks 12, 25, 39, and 52. In addition, the high-dose animals received these examinations at weeks 6 and 9. All animals also underwent electrocardiography recordings prior to initiation of treatment, and at weeks 5-6, 9-11, 13-14, 26-27, and 51-52. Electrocardiograms were performed approximately 4 to 6 hours after dosing.

Results: There was no mortality among male and female dogs receiving 0, 5, or 100 ppm. Dosing of 1500 ppm resulted in moribundity in 2 out of 10 males during week 6; after lowering the dose to 750 ppm, 2 additional males were moribund at week 9, and there was another death at week 26. One female receiving 1500 ppm was found moribund at week 6 and one at week 51. The moribund condition in these dogs was attributed by the study authors to severe compound-related clinical signs associated with impaired cardiac function. Survival rates at termination of the study were 37.5% for the high-dose males and 60.0% for the high-dose females. Treatment-related clinical signs (summarized in Table 1) were noted only in the high-dose animals. Mucoid, bloody diarrhea, few feces, or soft feces, which became apparent at week 2, occurred with a higher frequency in high-dose animals. Inappetence, hypothermia, labored

TABLE 1. Representative Clinical Findings in Dogs Fed Diaminochlorotriazine for 13/52 Weeks[a,b]

Clinical Findings	Dietary Level (ppm)							
	Males				Females			
	0	5	100	1500/750	0	5	100	1500/750
(No. Animals Examined)	(10)	(8)	(8)	(10)	(10)	(8)	(8)	(10)
Abdominal distention	0	0	0	3	0	0	0	2
Aggressive	0	1	0	1	0	0	0	0
Appendage/nonweight-bearing	0	0	0	0	0	0	0	0
Cachexia	0	0	0	2	0	0	0	2
Dermatitis	0	0	1	1	1	0	1	2
Emaciation	0	0	0	3	0	0	0	2
Emesis	0	3	1	3	0	1	2	4
Emesis/bloody	0	0	1	1	0	0	0	0
Feces/mucoid	3	5	3	10	3	0	3	10
Feces/bloody	1	0	1	4	1	0	0	0
Feces/diarrhea	0	1	2	3	1	2	1	5
Feces/few	2	1	3	8	0	0	1	8
Feces/soft	1	0	2	6	0	0	0	5
Foot/sore	0	0	0	0	0	3	0	0
Gait/abnormal	0	0	0	1	0	0	0	0
Hunched Posture	0	0	0	1	0	0	0	0
Hypothermia	0	0	0	1	0	0	0	0
Inactive	0	0	0	6	0	0	0	3
Inappetence 100%	0	0	0	2	0	0	0	1
Labored breathing	0	0	0	2	0	0	0	1
Lethargy	0	0	0	0	0	0	0	1
Pale	0	0	0	5	0	0	0	2
Recumbent	0	0	0	0	0	0	0	1
Swollen/appendage	0	1	0	0	0	0	0	0
Tremors	0	0	0	10	0	0	1	10
Vocalization	0	0	0	1	0	0	0	0

[a]The number in parentheses indicates the number of animals examined.

[b]The incidence reported is based on the number of animals displaying the clinical sign on at least one occasion during the study.

breathing, hunched posture, abnormal gait, lethargy, recumbency, and vocalization were seen only in dogs sacrificed moribund. Inactivity, paleness, cachexia, abdominal distention, and emaciation were noted in high-dose dogs that were sacrificed on schedule and in high-dose dogs sacrificed moribund. The clinical observations in the dogs were not considered by the study authors to represent a direct effect of the test substance but rather to be of secondary origin to impaired cardiac function. Clinical signs were not detected in the two high-dose recovery females from week 13 to the termination of the 39-week recovery period.

Tremors were noted in almost all high-dose dogs beginning at week 5. The tremors persisted in these dogs even when the dose of 1500 ppm was lowered to 750 ppm. In males, tremors continued to week 11, while in females the tremors persisted to week 15. Although one female receiving 100 ppm experienced tremors, this condition was not considered by the study authors to be related to treatment. Treatment-related physical examination findings (summarized in Table 2) were noted in the high-dose animals and included irregular, rapid heart rate, precordial thrill, pulse deficit, abdominal ascites, and emaciation. These findings began at week 6 and were considered by the study authors to be consistent with impaired cardiac function. Sporadic findings in four high-dose males included tachypnea, pale mucous membranes, hunched posture, stiff gait, and abdominal pain. These findings were also considered by the study authors to be secondary in origin to impaired cardiac function and overall body deterioration.

Pulse deficits were palpated in the two high-dose recovery females at week 6, a finding which was not evident at week 12 (one week prior to the termination of the treatment period for the recovery dogs) or during the recovery period of 39 weeks. The study authors considered these results to indicate an intermittent and/or reversible atrial fibrillation in the recovery dogs. Results of electrocardiography indicated a treatment-related atrial fibrillation in high-dose dogs. At week 5, atrial fibrillation was noted in three high-dose males that were eventually sacrificed moribund at week 6 or 9, in one high-dose female sacrificed moribund at week 6, and in one high-dose female sacrificed at week 13. At later times in the study, other high-dose animals exhibited atrial fibrillation. These included one high-dose male sacrificed moribund at week 26, two high-dose males sacrificed at week 53, one high-dose female sacrificed moribund at week 51, and one high-dose female sacrificed at week 53. The atrial fibrillation correlated with pathological cardiac changes.

TABLE 2. Representative Physical Examination Findings in High-Dose Dogs Fed Diaminochlorotriazine for 13/52 Weeks[a]

Study Week	Finding: Irregular Heartbeat	Precordial Thrill	Pulse Deficit	Abdominal Ascites	Emaciation
			Males		
6	6/10	2/10	4/10	0/10	0/10
9	2/7	0/7	2/7	1/7	0/7
12	0/6	0/6	0/6	0/6	0/6
25	1/4	0/4	1/4	1/4	1/4
39	1/3	0/3	1/3	0/3	0/3
52	1/3	0/3	1/3	1/3	0/3
			Females		
6	5/10	1/10	4/10	0/10	0/10
9	1/9	0/9	1/9	0/9	0/9
12	2/0	0/9	1/9	1/9	0/9
25	0/6	0/6	0/6	0/6	1/6
39	1/6	0/6	1/6	0/6	0/6
52	1/5	0/5	1/5	1/5	0/5

[a]Data presented as numbers with finding/number of dogs examined.

2. Body Weight: Dogs were weighed prior to treatment (days -14 and -7), weekly for weeks I-13, monthly thereafter, and at termination.

Results: Tables 3 and 4 summarize data on mean body weights and mean body weight gains in dogs treated for 13 weeks (interim sacrifice) and 52 weeks (main study). Mean body weights were decreased relative to baseline values (Day 0) in males and females receiving 1500 ppm during the first 6 weeks of the study. For the first 6 weeks of the study, the mean body weight gain was decreased by 8.7% in the high-dose males and 11.1% in the high-dose females as compared with pretest values. The high-dose males continued to lose weight after the dose level of 1500 ppm was lowered to 750 ppm at week 7. Because toxicity was still evident in males receiving 750 ppm, these dogs were fed a control diet from weeks 9 through 13; mean body weights for these males returned to baseline levels by week 12. When these dogs were placed again on diets containing a dose level of 750 ppm at week 14, the body weights were maintained at a generally constant level. In contrast to the males, mean body weights did not fluctuate much from baseline values in the high-dose females when the dose level of 1500 ppm was lowered to 750 ppm at week 7. Mean body weights were significantly ($p \leq 0.05$) lower (by approximately 17%) than controls in females receiving the high dose between weeks 7 and 13. At termination, mean body weight gains (calculated from the difference between % change at week 51 and week 0) in the high-dose males and females (excluding recovery females) were 109% and 9.1% lower than controls, respectively. The recovery high-dose females exhibited weight gains through the end of the study; at termination, mean body weight and body weight gain in these females surpassed those of controls.

3. Food Consumption and Compound Intake: Food consumption was determined prior to initiation of treatment at day 0, weekly during weeks 1 through 13 of the treatment period, monthly thereafter, and 1 week prior to termination.

Results: Table 5 summarizes data on food consumption at selected intervals. For the first 8 weeks of treatment, the food intake of the high-dose males was up to 35% lower than that of controls. When these dogs were placed

TABLE 3. Mean Body Weights at Selected Intervals for Dogs Fed Diaminochlorotriazine for 13/52 Weeks[a]

Mean Body Weights (kg ± S.E.) at Selected Study Weeks; Overall Body Weight Gains							
0	6	13	25	37	51	Total Weight Change	Percentage Change[b]
			Males				
8.930 ± 0.206	9.150 ± 0.349	9.800 ± 0.393	10.925 ± 0.232	11.425 ± 0.317	11.500 ± 0.255	2.570	100%
8.462 ± 0.448	8.725 ± 0.539	9.475 ± 0.516	10.425 ± 0.743	10.550 ± 0.908	10.950 ± 0.877	2.488	96.8
8.675 ± 0.306	8.775 ± 0.331	9.200 ± 0.364	9.600 ± 0.772	9.450 ± 0.849	9.850 ± 0.971	1.175	45.7
8.870 ± 0.244	8.100 ± 0.406	9.067 ± 0.406	9.250 ± 0.544	8.867 ± 0.393	8.633 ± 0.677	-0.237	-9.2
			Females				
7.730 ± 0.273	8.080 ± 0.402	8.570 ± 0.440	8.383 ± 0.555	8.550 ± 0.613	8.717 ± 0.705	0.987	100%
7.750 ± 0.321	8.137 ± 0.272	8.437 ± 0.269	9.150 ± 0.166	9.775 ± 0.335	9.775 ± 0.382	2.025	205.2
7.887 ± 0.0214	8.087 ± 0.223	8.500 ± 0.312	8.875 ± 0.229	9.325 ± 0.364	9.225 ± 0.165	1.338	135.6
7.670 ± 0.186	6.822* ± 0.230	7.111* ± 0.306	7.825 ± 0.586 (8.250 ± 0.050)	7.975 ± 0.489 (8.950 ± 0.150)	8.567 ± 0.145 (9.000 ± 0.300)	0.897 --	90.9 --

[a] Includes all animals treated for 13 weeks (interim sacrifice including recovery period) and 52 weeks (main study).

[b] Body weight gain relative to control (based on survivors) calculated by the study authors according to the equation:

$$\% \text{ Gain} = \frac{\text{Weight change of group}}{\text{Weight change of control}} \times 100.$$

( ) in parentheses indicates data for recovery females. Data for recovery females were not analyzed statistically.

* Statistically different from control value, $p \leq 0.05$.

TABLE 4. Mean Body Weight Gains at Selected Intervals in Dogs Fed Diaminochlorotriazine for 13/52 Weeks[a]

	Mean Body Weight Gain (percent of pretest weight ± S.E.) at Study Week:					
	1	7	13	25	37	51
			Males			
	2.501 ± 0.734	2.273 ± 2.797	9.545 ± 3.359	22.669 ± 4.059	28.166 ± 3.684	29.037 ± 3.424
	0.062 ± 0.601	2.888 ± 2.400	12.224 ± 3.218	24.614 ± 4.512	25.875 ± 5.903	30.717 ± 5.455
	1.195 ± 0.334	1.207 ± 2.060	6.403 ± 3.802	14.346 ± 6.363	12.547 ± 7.631	17.466 ± 9.872
	-0.307 ± 1.705	-7.921* ± 3.352	3.799 ± 1.948	6.880 ± 6.159	2.678* ± 4.864	0.041* ± 8.732
			Females			
	2.067 ± 0.704	4.129 ± 2.021	10.410 ± 2.529	15.207 ± 3.712	17.436 ± 4.450	19.508 ± 5.324
	1.378 ± 0.797	5.393 ± 2.252	9.584 ± 3.636	10.641 ± 5.756	18.113 ± 6.552	17.911 ± 5.726
	0.833 ± 0.768	2.596 ± 1.537	7.798 ± 2.983	13.401 ± 2.941	19.039 ± 3.661	18.333 ± 6.389
	-0.875* ± 0.693	-11.479** ± 1.269	-7.788** ± 2.686	-1.614* ± 5.756 (15.696 ± 6.363)	0.397 ± 4.623 (25.373 ± 4.039)	3.503 ± 4.850 (25.971 ± 1.971)

[a] Data represent weight gains over the baseline values (Day 0) during the treatment period and include all animals treated for 13 weeks (interim sacrifice including 39-week recovery period) and 52 weeks (main study).

[b] The numbers in parentheses indicate data for recovery females. Data for recovery females were not analyzed statistically.

*Significantly different from control value, $p \leq 0.05$.

**Significantly different from control value, $p \leq 0.01$.

on untreated diet at weeks 9 through 13, food intake returned to levels similar to that of controls. From week 17 (when the diet containing 750 ppm recommenced) to the termination of the study, the food intake of the high-dose males continued to be lower than that of controls; the reduction in food consumption was statistically significant ($p \leq 0.05$) at weeks 17 (25% lower), 21 (22% lower), and 25 (22% lower). The food intake of the high-dose females was significantly lower ($p \leq 0.05$) than that of controls during weeks 3 through 7 (up to 23% lower) and 13 (19% lower). The food intake of the high-dose females from week 18 to termination was similar to controls. The food intake of the high-dose recovery females was slightly but nonsignificantly lower than that of either controls or other high-dose females during the recovery period. The mean daily doses for male dogs receiving dietary levels of 5, 100, and 1500/750 ppm for 52 weeks were 0.187, 3.61, and 24.1 mg/kg/day, while the doses were 0.195, 3.43, and 32.7 mg/kg/day for females receiving the same dietary levels.

4. Ophthalmological Examinations: Ophthalmoscopic examinations were performed at predose and at weeks 13, 26, and 52.

Results: There was no effect of treatment on ophthalmology.

5. Hematology and Clinical Chemistry: Blood samples were collected from the jugular vein of each dog prior to treatment, and at weeks 13, 25, and 52. Samples were taken prior to feeding. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocyte count (RETIC)
X Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation: thromboplastin time (PT)
X Clotting time
X Heinz body formation

Clotting time was determined on days 85 and 362.

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 5. Mean Food Consumption Data at Selected Intervals for Dogs Fed Diaminochlorotrazine for 13/52 Weeks[a]

...ry level (ppm)	Mean Food Consumption (g/week ± S.E.) at Selected Study Weeks:						
	0	1	7	13	25	37	51
				Males			
	2236.6 ± 106.8	2439.2 ± 136.6	2358.9 ± 62.8	2354.9 ± 90.0	2334.3 ± 30.9	2398.9 ± 20.7	2085.1 ± 147.4
	1769.8 ± 178.7	2097.3 ± 211.5	2376.3 ± 144.1	2375.3 ± 61.8	2399.5 ± 106.4	2303.8 ± 150.9	2284.2 ± 12[illegible]
	2149.0 ± 111.8	2245.1 ± 89.1	2332.9 ± 110.8	2244.3 ± 113.5	2488.4 ± 123.5	2339.0 ± 158.6	2471.8 ± 98.7
)	2166.7 ± 118.6	1794.5 ± 169.5*	1681.2 ± 169.6**	2368.5 ± 74.3	1813.1 ± 165.5*	2169.1 ± 93.9	2076.9 ± 110.4
				Females			
	1632.1 ± 127.4	1891.9 ± 138.7	2126.9 ± 104.1	2022.9 ± 96.2	2043.4 ± 140.5	1811.2 ± 145.7	1978.8 ± 161.0
	1530.8 ± 79.4	1687.2 ± 101.1	1978.3 ± 122.5	2123.0 ± 114.4	2338.2 ± 149.1	2354.8 ± 139.7	2274.4 ± 178.1
	1788.5 ± 142.6	1860.8 ± 149.3	2010.9 ± 67.8	2000.9 ± 91.9	2217.6 ± 283.2	2319.2 ± 170.5	1997.7 ± 141.7
[illegible]0	1498.5 ± 103.6	1482.4 ± 100.1	1662.2 ± 136.7*	1636.7 ± 116.9*	2108.9 ± 250.3	2173.5 ± 164.2	2021.1 ± 199.3

[illegible]clude all animals treated for 13 weeks (interim sacrifice including 39-week recovery period) and 52 weeks (main study).

[illegible]ficantly different from control value, $p \leq 0.05$.

ficantly different from control value, $p \leq 0.01$.

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Results: Table 6 summarizes data on selected hematology parameters. Treatment-related anemia with an accompanying reticulocytosis was noted only in the high-dose animals. The effects included decreased erythrocyte count, hematocrit, and hemoglobin concentration; the decreases were statistically significant ($p \leq 0.05$ for hematocrit concentration, $p \leq 0.01$ for hemoglobin concentration and erythrocyte count) at weeks 13 and 25 in the females. Decreases in males were not as great as in females, and none reached a level of significance. Also, alterations in erythrocyte size or color and the presence of Howell-Jolly bodies or nucleated red blood cells were seen at weeks 13, 25, and/or 52 in several high-dose animals. Severe anemia with a pronounced increase in reticulocyte count was observed in one of the two high-dose recovery females at week 13. This particular dog recovered from the anemia by week 25 with the reticulocyte count returning to normal control level. Increases in the reticulocyte count were also seen in other high-dose females (significance of $p \leq 0.05$ at week 13) and in the high-dose males (significance of $p \leq 0.05$ at week 25). The reticulocyte count returned to control level, with red blood cell morphology becoming normal, by week 52. A treatment-related increase in platelet levels was noted in high-dose females (excluding the recovery females) at weeks 25 ($p \leq 0.01$) and 52 ($p \leq 0.05$). Other hematological alterations were regarded by the study author to be incidental and of normal biological variation.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†	X	Albumin/globulin ratio
	Magnesium†	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol (TCHOL)†
X	Sodium†		Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein†
X	Creatine phosphokinase†		Triglycerides
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 6. Selected Hematology Parameters (mean ± S.E.) for Dogs Fed Diaminochlorotriazine for 13/52 Weeks[a,b]

Parameter/ Interval	Dietary Level (ppm)							
	Males				Females[c]			
	0	5	100	1500/750	0	5	100	1500/750
RBC ($10^6/mm^3$)								
Day -14	6.323 ± 0.179	6.625 ± 0.155	5.732 ± 0.145	6.145 ± 0.139	6.672 ± 0.151	6.571 ± 0.265	6.369 ± 0.232	6.751 ± 0.192
Day 85	6.100 ± 0.154	6.179 ± 0.089	6.001 ± 0.098	5.203 ± 0.453	6.207 ± 0.249	6.306 ± 0.212	6.304 ± 0.157	5.048 ± 0.424*
Day 174	6.412 ± 0.275	6.870 ± 0.126	6.545 ± 0.273	5.515 ± 0.637	6.857 ± 0.129	6.537 ± 0.282	6.992 ± 0.036	4.865 ± 1.218* (6.675 ± 0.035)
Day 362	6.730 ± 0.263	7.477 ± 0.267	7.007 ± 0.302	5.587 ± 0.730	6.817 ± 0.094	6.762 ± 0.433	6.970 ± 0.256	6.183 ± 0.528 (7.530 ± 0.800)
HGB (g/dL)								
Day -14	15.790 ± 0.490	16.387 ± 0.490	14.675 ± 0.430	15.590 ± 0.342	17.240 ± 0.440	16.75 ± 0.575	16.03 ± 0.561	17.13 ± 0.441
Day 85	14.88 ± 0.360	15.13 ± 0.302	14.70 ± 0.169	12.83 ± 1.042	15.51 ± 0.589	15.49 ± 0.424	14.99 ± 0.239	12.48 ± 1.082**
Day 174	15.93 ± 0.504	16.93 ± 0.317	15.98 ± 0.680	13.20 ± 1.711	17.48 ± 0.494	16.88 ± 0.614	16.80 ± 0.122	12.10 ± 2.760** (17.20 ± 0.400)
Day 362	16.58 ± 0.405	18.28 ± 0.236	17.30 ± 0.696	13.53 ± 2.224	17.13 ± 0.275	17.80 ± 1.215	17.03 ± 0.520	15.30 ± 1.411 (19.56 ± 2.250)
HCT (%)								
Day -14	43.900 ± 1.394	45.125 ± 1.172	41.375 ± 1.068	43.900 ± 1.016	48.00 ± 1.145	47.13 ± 1.505	44.88 ± 1.517	47.80 ± 1.104
Day 85	43.30 ± 1.212	44.13 ± 0.972	42.75 ± 0.675	38.67 ± 2.499	44.90 ± 1.798	45.38 ± 1.295	43.38 ± 0.999	37.11 ± 2.898*
Day 174	44.75 ± 1.493	47.75 ± 0.629	45.75 ± 2.394	38.25 ± 4.131	47.67 ± 0.760	47.00 ± 1.958	47.50 ± 0.500	33.75 ± 6.303* (48.50 ± 1.500)
Day 362	47.25 ± 0.854	53.00 ± 0.707	49.00 ± 1.958	38.00 ± 5.686	49.00 ± 0.516	51.00 ± 0.516	48.75 ± 1.548	42.67 ± 3.930 (55.50 ± 6.500)

(continued)

TABLE 6. (continued)

Parameter/ Interval	Dietary Level (ppm)							
	Males				Females[c]			
	0	5	100	1500/750	0	5	100	1500/750
Platelets (10^3/mm^3)								
Day -14	292.9 ± 19.07	299.50 ± 20.57	267.4 ± 23.64	322.9 ± 17.15	289.0 ± 14.59	285.5 ± 24.98	307.6 ± 26.69	286.1 ± 26.27
Day 85	300.6 ± 40.67	332.5 ± 27.39	312.8 ± 30.10	277.7 ± 42.95	304.7 ± 19.39	281.6 ± 19.49	295.9 ± 29.01	345.4 ± 26.83
Day 174	280.0 ± 9.174	341.0 ± 30.05	293.5 ± 26.31	341.8 ± 53.60	255.7 ± 35.53	310.5 ± 16.95	286.3 ± 44.86	493.3 ± 58.17** (276.5 ± 13.50)
Day 362	261.8 ± 14.27	300.5 ± 30.63	295.0 ± 37.09	343.3 ± 56.46	284.7 ± 22.81	274.3 ± 26.67	249.5 ± 28.91	501.7 ± 52.21* (346.0 ± 64.00)
Retic. (%)								
Day -14	1.280 ± 0.165	0.950 ± 0.091	0.962 ± 0.153	1.290 ± 0.099	1.450 ± 0.135	1.787 ± 0.130	1.375 ± 0.167	1.380 ± 0.205
Day 85	1.180 ± 0.126	0.962 ± 0.092	0.812 ± 0.097	3.083 ± 0.983	0.910 ± 0.168	0.875 ± 0.182	0.962 ± 0.207	5.822 ± 2.822*
Day 174	0.900 ± 0.265	1.150 ± 0.155	1.125 ± 0.229	2.800 ± 1.042*	0.983 ± 0.202	1.400 ± 0.168	1.050 ± 0.189	3.375 ± 1.813 (0.800 ± 0.400)
Day 362	0.875 ± 0.347	ND[d]	ND	0.733 ± 0.240	1.050 ± 0.232	ND	ND	0.733 ± 0.067 (0.750 ± 0.250)

[a]Data include all animals treated for 13 weeks (interim sacrifice including 39-week recovery period) and 52 weeks (main study).

[b]Abbreviations are as follows:
Retic. = Reticulocyte count
HCT = Hematocrit
HGB = Hemoglobin

[c]Numbers in parentheses indicates data for recovery females.

[d]ND = Not determined.

*Significantly different from control value, $p \leq 0.05$.

**Significantly different from control value, $p \leq 0.01$.

Results: Table 7 summarizes data on selected clinical chemistry parameters. Several treatment-related changes were noted. Albumin levels were slightly but non-significantly decreased in the high-dose males and females (excluding recovery females) at weeks 25 and 52. The hypoalbuminemia was considered by the study authors to be consistent with the ascites and cardiac cachexia associated with impaired cardiac function. Significant ($p \leq 0.05$) decreases in serum calcium levels, considered by the study authors to be directly related to the hypoalbuminemia, were noted in the high-dose males at week 52 and in high-dose females (excluding recovery females) at week 13, and 25. Total cholesterol levels were significantly decreased in high-dose females (excluding recovery females) at weeks 13 ($p \leq 0.01$) and 25 ($p \leq 0.01$). Total cholesterol levels were near control values in the recovery females, indicating that this effect of diaminochlorotriazine may be reversible. Nonsignificant increases in the LDH activity were noted in the high-dose males (79.4% increase) and high-dose females (82.8% increase) at weeks 13 and in both sexes (53% increase in males; 50% increase in females) receiving the high dose at week 25. The increases in LDH activity were attributed by the study authors to two high-dose animals (one male and one female) that were sacrificed moribund with clinical and pathological symptoms of heart failure. Other statistically significant differences from control values were regarded by the study authors to be of little or no biological importance.

6. Urinalysis: Urine was collected from all dogs at pretest (day -8) and on days 83, 176, and 365. The CHECKED (X) parameters were examined:

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

Results: No effects of treatment with diamino-chloro-triazine on urinary parameters were seen.

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 7. Selected Clinical Chemistry Parameters (mean ± S.E.) for Dogs Fed Diaminochlorotriazine for 13/52 Weeks[a,b]

ameter/ erval	Dietary Level (ppm)							
	Males				Females			
	0	5	100	1500/750	0	5	100	1500/750
CHOL (mg/dL)								
Day -14	187.3 ± 9.139	222.0 ± 16.07	176.4 ± 15.98	211.0 ± 11.79	196.7 ± 9.250	194.8 ± 12.66	190.0 ± 10.59	176.7 ± 6.777
Day 85	145.3 ± 8.332	167.9 ± 7.689	133.1 ± 13.22	140.7 ± 10.87	194.5 ± 17.76	152.8 ± 9.041	166.0 ± 13.97	125.1 ± 8.272**
Day 174	166.8 ± 8.731	170.3 ± 14.85	130.0 ± 14.97	130.3 ± 15.23	234.5 ± 25.83	152.0 ± 10.17*	168.8 ± 10.93	104.3 ± 8.901** (194.5 ± 22.50)
Day 362	186.0 ± 13.27	195.5 ± 15.78	158.5 ± 11.45	170.7 ± 35.75	239.3 ± 21.09	185.8 ± 6.223	231.3 ± 44.73	144.7 ± 15.98 (211.5 ± 2.500)
Albumin (g/dL)								
Day -14	3.580 ± 0.053	3.637 ± 0.071	3.500 ± 0.089	3.570 ± 0.063	3.710 ± 0.060	3.700 ± 0.167	3.775 ± 0.086	3.790 ± 0.055
Day 85	3.410 ± 0.069	3.462 ± 0.060	3.425 ± 0.041	3.250 ± 0.085	3.570 ± 0.047	3.575 ± 0.056	3.450 ± 0.130	3.322 ± 0.1223
Day 174	3.400 ± 0.041	3.425 ± 0.095	3.500 ± 0.091	2.925 ± 0.214	3.617 ± 0.060	3.525 ± 0.075	3.550 ± 0.029	3.300 ± 0.227 (3.650 ± 0.250)
Day 362	3.375 ± 0.048	3.550 ± 0.065	3.500 ± 0.108	2.900 ± 0.306	3.567 ± 0.080	3.600 ± 0.108	3.475 ± 0.025	3.467 ± 0.285 (3.900 ± 0.100)
LDh (U/L)								
Day -14	59.90 ± 7.981	51.88 ± 7.412	48.38 ± 3.575	61.40 ± 9.540	49.50 ± 39.59	60.25 ± 8.306	61.25 ± 7.703	59.00 ± 10.40
Day 85	44.60 ± 4.056	49.75 ± 9.794	50.50 ± 7.267	80.00 ± 33.69	33.70 ± 4.539	41.50 ± 4.671	42.13 ± 7.047	61.56 ± 15.88
Day 174	63.75 ± 7.674	75.25 ± 16.32	52.50 ± 4.992	116.3 ± 34.74	60.00 ± 9.947	57.75 ± 6.498	91.75 ± 48.32	109.5 ± 52.02
Day 362	60.75 ± 7.993	63.00 ± 11.63	58.75 ± 10.66	87.00 ± 25.01	45.67 ± 8.508	50.75 ± 6.799	45.75 ± 13.92	50.00 ± 18.45

(continued)

TABLE 7. (continued)

ameter/ erval	Dietary Level (ppm)							
	Males				Females			
	0	5	100	1500/750	0	5	100	1500/750
Calcium (mg/dL)								
Day -14	11.14 ± 0.113	11.28 ± 0.131	11.04 ± 0.089	11.21 ± 0.127	11.71 ± 0.147	11.74 ± 0.134	11.48 ± 0.132	11.48 ± 0.136
85	10.42 ± 0.084	10.58 ± 0.094	10.44 ± 0.073	10.25 ± 0.076	10.65 ± 0.069	10.45 ± 0.107	10.48 ± 0.103	10.11 ± 0.121**
174	10.28 ± 0.155	10.38 ± 0.214	10.38 ± 0.225	9.600 ± 0.297	10.40 ± 0.129	9.875 ± 0.118*	10.28 ± 0.149	9.725 ± 0.184** (10.55 ± 0.050)
Day 362	10.28 ± 0.063	10.28 ± 0.085	9.950 ± 0.233	9.400 ± 0.321*	10.25 ± 0.152	10.05 ± 0.171	9.900 ± 0.168	9.667 ± 0.176 (10.05 ± 0.250)

[a] Data represent all animals treated for 13 weeks (interim sacrifice) and 52 weeks (including recovery periods).

[b] Abbreviations are as follows:

TCHOL = Total cholesterol
LDH = Lactic acid dehydrogenase

[c] Numbers in parentheses represent data for recovery females. Data for recovery females were not analyzed statistically.

*Statistically different from control value, $p \leq 0.05$.

**Statistically different from control value, $p \leq 0.01$.

7. Sacrifice and Pathology: All animals that died and that were sacrificed (scheduled or unscheduled) were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen	X	Eyes (optic nerve)†
X	Jejunum†	XX	Thymus		
X	Ileum†				
X	Cecum†				
X	Colon†				
X	Rectum		Urogenital		Glandular
XX	Liver†	XX	Kidney	XX	Adrenals†
X	Gallbladder†	X	Urinary bladder†	X	Lacrimal gland
X	Pancreas†	XX	Testes	X	Mammary gland†
		XX	Epididymides	XX	Thyroids†
		X	Prostate	X	Parathyroids†
			Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
X	Trachea†	X	Uterus		
X	Lung†	X	Vagina		
					Other
				X	Bone (sternum and femur)†
				X	Skeletal muscle†
				X	Skin
				X	All gross lesions and masses

Results: A complete gross examination was performed on each dog sacrificed or found dead. Organ weights were not measured on any dog that was sacrificed moribund during treatment.

a. Organ Weights: Tables 8 and 9 present mean absolute organ and organ-to-body weight data. Nonsignificant increases in mean absolute spleen weight (105.9% increase in males, 134.3% in females) and spleen weight

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 8. Selected Organ Weights (mean ± S.E.) and Organ-to-Body Weight Ratios in Dogs Fed Diaminochlorotriazine for 13 Weeks

Dietary Level (ppm)	Organ Weights					
	Kidney		Spleen		Liver	
	Absolute (g)	Relative to Body (%)	Absolute (g)	Relative to Body (%)	Absolute (g)	Relative to Body (%)
			Males			
0	50.82 ± 3.646	0.549 ± 0.023	40.84 ± 8.383	0.432 ± 0.082	297.4 ± 9.502	3.290 ± 0.272
5	46.17 ± 4.823	0.484 ± 0.017	37.23 ± 4.871	0.401 ± 0.063	292.7 ± 25.33	3.079 ± 0.076
100	44.73 ± 2.806	0.495 ± 0.019	43.49 ± 12.64	0.471 ± 0.116	306.6 ± 12.07	3.414 ± 0.187
1500/750	48.64 ± 4.580	0.539 ± 0.015	84.12 ± 51.78	0.872 ± 0.465	303.8 ± 41.69	3.346 ± 0.052
			Females			
0	39.10 ± 2.420	0.431 ± 0.022	30.18 ± 3.107	0.338 ± 0.047	269.1 ± 14.15	2.972 ± 0.156
5	33.94 ± 1.336	0.446 ± 0.015	53.63 ± 9.775	0.703 ± 0.123	250.2 ± 10.16	3.289 ± 0.147
100	36.87 ± 1.221	0.465 ± 0.031	47.04 ± 11.47	0.570 ± 0.117	251.5 ± 11.88	3.153 ± 0.136
1500/750	38.66 ± 3.127	0.532 ± 0.048	70.71 ± 35.92	1.000 ± 0.496	361.1 ± 99.69	4.754 ± 0.837*

*Statistically different from control value, $p \leq 0.05$.

TABLE 9. Selected Organ Weights (mean ± S.E.) and Organ-to-Body Weight Ratios in Dogs Fed Diaminochlorotriazine for 52 Weeks

Dietary Level (ppm)	Organ Weights: Kidney		Spleen		Liver	
	Absolute (g)	Relative to Body (%)	Absolute (g)	Relative to Body (%)	Absolute (g)	Relative to Body (%)
			Males			
0	46.82 ± 5.110	0.415 ± 0.046	35.15 ± 3.545	0.311 ± 0.031	292.2 ± 10.86	2.596 ± 0.166
5	51.59 ± 5.115	0.480 ± 0.027	82.04 ± 14.60*	0.790 ± 0.172*	295.7 ± 9.268	2.785 ± 0.139
100	50.49 ± 6.658	0.533 ± 0.037	55.47 ± 12.84	0.578 ± 0.106	282.8 ± 14.69	3.064 ± 0.274
1500/750	51.76 ± 2.547	0.596 ± 0.024*	39.99 ± 10.27	0.467 ± 0.122	330.2 ± 65.18	3.733 ± 0.537*
			Females			
0	37.47 ± 1.484	0.446 ± 0.025	46.69 ± 7.394	0.565 ± 0.101	250.7 ± 9.484	2.985 ± 0.156
5	43.54 ± 2.108	0.056 ± 0.029	40.45 ± 4.853	0.423 ± 0.051	269.7 ± 7.144	2.824 ± 0.100
100	42.77 ± 2.357	0.466 ± 0.023	53.58 ± 10.09	0.583 ± 0.108	285.4 ± 7.039*	3.112 ± 0.057
1500/750	44.81 ± 1.688 (33.42 ± 3.210)	0.523 ± 0.017 (0.374 ± 0.023)	63.23 ± 27.36 (65.29 ± 36.09)	0.732 ± 0.313 (0.719 ± 0.380)	315.1 ± 40.27* (250.9 ± 1.195)	3.694 ± 0.517 (2.815 ± 0.109)

*Statistically different from control value, $p \leq 0.05$.

relative to body weight (101.9% increase in males, 195.9% increase in females) were seen in both sexes administered the high dose for 13 weeks. Nonsignificant increases in mean absolute (35.4% increase) and relative spleen weight (30% increase) were also seen in the high-dose females following 52 weeks of treatment. Mean absolute and relative spleen weights were also increased to a similar extent in the high-dose recovery females at 52 weeks. Mean absolute liver weight was significantly ($p \leq 0.05$) increased (26%) in the high-dose females (excluding the recovery females) treated for 52 weeks. Relative liver weights were significantly increased in the females (60%) receiving the high dose for 13 weeks and in males (44%) receiving the high dose for 52 weeks. Mean absolute liver weights were slightly but nonsignificantly increased in the high-dose males (13%) treated for 52 weeks and in the high-dose females (34%) treated for 13 weeks. A nonsignificant increase (24%) in relative liver weight was seen in females (excluding recovery females) administered the high dose for 52 weeks. The increases in the aforementioned liver, kidney, and spleen weights were regarded by the study authors to be treatment related. Other statistically significant differences from control values were considered by the study authors to be unrelated to treatment.

b. Gross Pathology: Table 10 summarizes the incidence of frequently observed gross lesions in dogs receiving the high dose for 13 or 52 weeks. Findings in the heart included enlargement, softness, thickened valves, lesions, distension, red/dark color, and thrombosis.

Secondary changes were noted in the liver, thoracic cavity, and abdominal cavity. Liver alterations consisted of enlargement, red/dark color, lesions, adhesions, mottling, and rough texture. Fluid accumulation was seen in the pericardium, thoracic cavity, and abdominal cavity. These gross pathological findings were absent in dogs receiving 0, 5, or 100 ppm for 13 or 52 weeks, and in the two high-dose recovery females.

c. Microscopic Pathology: Tables 11 and 12 summarize the incidence of frequently occurring nonneoplastic lesions in dogs. Treatment-related alterations were present in the hearts of male and female dogs treated with

TABLE 10. Representative Gross Findings in Dogs Fed 1500/750 ppm Diaminochlorotriazine for 13/52 Weeks[a,b]

Organ/Finding	Study Weeks: Males 13	Males 52	Females 13	Females 52
Heart				
-Soft	2	1	1	1
-Fluid accumulation (Pericardium)	2	2	2	2
Right atrium				
-Lesion	3	0	0	0
-Distended	1	0	0	0
-Enlarged	2	0	0	1
-Thrombosis	1	0	1	0
-Thickened	0	0	1	0
-Color red/dark	4	0	1	0
Left atrium -Enlarged, thrombosis, color dark	1	0	0	0
Papillary muscle -Lesion, color tan	0	0	1	0
Valves -Thickened	0	0	0	0
Enlarged	0	1	0	0
Liver				
-Enlarged	1	1	1	0
-Color red/dark	2	1	0	0
-Lesion	1	0	0	0
-Adhesions	0	1	0	1
-Mottled	0	1	0	0
-Texture rough	0	1	0	0
Thoracic cavity				
Fluid accumulation	1	0	0	2
Abdominal cavity				
Fluid accumulation	3	2	1	2

[a]Based on six males and four females (including two recovery females) treated for up to 13 weeks, and four males and six females treated for 52 weeks.

[b]Includes those sacrificed moribund and those sacrificed on schedule.

TABLE 11. Representative Nonneoplastic Neoplastic Findings in Dogs Fed Diaminochlorotrazine for 13 Weeks[a]

Organ/Finding	Dietary Level (ppm)								
	Males				Females				
	0	5	100	1500/750	0	5	100	1500/750	1500/750 (Rec)[b]
Heart	(6)	(4)	(4)	(6)	(4)	(4)	(4)	(4)	(2)
Inflammation	0	0	0	1	0	0	0	0	0
Chronic myocarditis	0	0	0	4*	0	0	0	2	0
Necrosis	0	0	0	1	0	0	0	0	0
Thrombosis	0	0	0	1	0	0	0	1	0
Bone marrow	(6)	(4)	(4)	(6)	(4)	(4)	(4)	(4)	(2)
Erythropoietic hyperplasia	0	0	0	0	0	0	0	2	0
Hyperplasia	2	0	2	5	0	0	0	0	2
Liver	(6)	(4)	(4)	(6)	(4)	(4)	(4)	(4)	(2)
Inflammation	0	0	0	1	0	0	0	0	0
Atrophy, centrilobular	0	0	0	2	0	0	0	0	0
Bile stasis	0	0	0	2	0	0	0	0	0
Fibrosis, centrilobular	0	0	0	2	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	1	0
Hemosiderosis	0	0	0	1	0	0	0	1	1
Passive congestion	0	0	0	3*	0	0	0	1	0
Testes	(6)	(4)	(4)	(6)					
Hypospermatogenesis	0	0	0	4**					
Hypospermia	0	0	0	4**					
Thymus	(4)	(4)	(4)	(6)	(4)	(4)	(4)	(4)	(2)
Atrophy	1	0	1	4*	0	0	0	1	0

[a]The number in parentheses indicates the number of animals with tissues examined.

[b]Two recovery high-dose females received treatment for 13 weeks followed by a 39-week recovery period.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

TABLE 12. Representative Nonneoplastic Findings in Dogs Fed Diaminochlorotriazine for 52 Weeks[a]

	Dietary Level (ppm)							
	Males				Females			
Organ/Finding	0	5	100	1500/750	0	5	100	1500/750
Bone marrow	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Erythropoietic Hyperplasia	0	0	0	0	0	0	0	2
Heart	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Chronic myocarditis	0	0	0	1	0	0	0	1
Edema	0	0	0	1	0	0	0	0
Subacute lymphocytic inflammation	0	0	0	1	0	0	0	0
Thrombosis	0	0	0	1	0	0	0	1
Liver	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Atrophy, centrilobular	0	0	0	2	0	0	0	0
Bile statis	0	0	0	1	0	0	0	0
Fibrosis, centrilobular	0	0	0	3	0	0	0	1
Hemorrhage	0	0	0	1	0	0	0	1
Hemosiderosis	0	0	0	2	0	0	0	1
Passive congestion	0	0	0	2	0	0	0	2
Testes	(4)	(4)	(4)	(4)				
Hypospermatogenesis	0	0	0	2				
Hypospermia	0	0	0	2				
Thymus	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Atrophy	2	0	0	3	0	0	0	1

[a]The number in parentheses indicates the number of animals with tissues examined.

diaminochlorotriazine for 13 or 52 weeks. Chronic myocarditis, particularly of the right atrium, was the primary cardiac lesion noted in the high-dose dogs (observed in four out of six males at 13 weeks, in two out of four females at 13 weeks, in one out of four males at 52 weeks and in one out of four females at 52 weeks); this effect reached statistical significance ($p \leq 0.05$) in males receiving the high dose for 13 weeks.

Other cardiac lesions included thrombosis, inflammation, necrosis, hemorrhage, and hemosiderosis; the right atrium was particularly affected. The heart lesions corresponded to the gross pathological alterations in this organ. The hearts of the two high-dose recovery females were normal. Several lesions secondary in origin to the cardiac changes were also noted in the livers, testes, bone marrow, and thymus of dogs following treatment with 1500/750 ppm diaminochlorotriazine for 13 or 52 weeks. Liver changes included passive congestion, centrilobular fibrosis/atrophy, bile stasis, necrosis, hemosiderosis, and inflammation. Hypospermatogenesis in the testes with associated hypospermia in the epididymides was noted in the high-dose males at 13 and 52 weeks of treatment. Other changes included thymic atrophy and hyperplasia of the bone marrow. A variety of other tissue findings occurred infrequently in the dogs; these were often without relationship to treatment.

D. STUDY AUTHORS' CONCLUSIONS:

Diaminochlorotriazine was administered via the diet to four groups of dogs (8 to 10/sex/group) at dose levels of 0, 5, 100, and 1500 ppm. The dogs received 0, 5, and 100 ppm for either 13 weeks (interim sacrifice) or 52 weeks (main study). High-dose males received 1500 ppm for weeks 1 to 6, and then 750 ppm at weeks 7, 8, and 14 to 52 with an intervening period of 0 ppm at weeks 9 to 13. The dose of 1500 ppm was lowered to 750 ppm because of toxicity at 1500 ppm. High-dose females received 1500 ppm (weeks 1 to 6) and 750 ppm (weeks 7 to 52). At week 13, dogs receiving 0 ppm (six males and four females), 5 ppm (four/sex), 100 ppm (four/sex), and 1500/750 ppm (two males and four females) were sacrificed, while two control female and two high-dose females were placed on untreated diet for a 39-week recovery period.

No treatment-related effects were seen in dogs receiving 0, 5, or 100 ppm. Treatment-related effects were observed in dogs receiving ≥750 ppm. Effects included moribundity (five males and two females), clinical signs of inactivity, inappetence, labored breathing, cachexia, hunched posture, abnormal gait,

abdominal distention, hypothermia, paleness, fecal changes, lethargy, recumbency, vocalization, and emaciation; all of these clinical findings were attributed to impaired cardiac function. Body weight loss and reduced weight gain and reduced food consumption were noted in dogs receiving 1500 ppm. The body weight loss persisted for several weeks when the dose was lowered to 750 ppm. Reductions in body weight gain and food consumption were seen throughout the entire treatment period in the high-dose males and over the first 13 weeks in females. Other findings in the high-dose animals included tremors; tachyarrhythmia; atrial fibrillation; anemia with reticulocytosis; decreased mean serum albumin, calcium, and cholesterol levels; elevated LDH values; increased mean spleen, liver, and kidney weights; and gross and microscopic cardiac lesions primarily in the right atrium. Changes secondary to the cardiac lesions included fluid accumulation and lesions in the liver, testes, bone marrow, and thymus. The two high-dose recovery females did not show any clinical, gross, or microscopic pathological signs of heart impairment.

The results indicated clinical, electrocardiographic, gross, and microscopic evidence of impaired heart function in dogs administered diets containing ≥750 ppm of diaminochlorotriazine, and a level of 100 ppm is the no-observed effect level.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete and adequate, and the data were well reported. Summary data were supported by individual animal data, and mean values that were validated agreed with the authors' values. Dose levels were selected on the basis of the results of a previous 4-week study that was not available for our review.

Signs of toxicity from diaminochlorotriazine became evident in dogs receiving 1500 ppm for 6 weeks; consequently, the dose was lowered to 750 ppm. The males were more sensitive than females to the dose level of 750 ppm, and as a result, the males were placed on untreated diets for weeks 9 to 13. Four male dogs were then placed again on a diet containing 750 ppm until termination at 52 weeks. In contrast to the males, females received 750 ppm until termination at 13 or 52 weeks, except for a group of two that were dosed for 13 weeks followed by a 39-week recovery period.

The primary treatment-related effects of diaminochlorotriazine were observed in the hearts of dogs receiving the high dose. Most frequently, the right atrium was affected. Gross pathological findings in the heart consisted of enlargement, softness, thickened valves, lesions, distension, red/dark

color, and thrombosis. Correlative histopathological cardiac effects included chronic myocarditis, thrombosis, inflammation, necrosis, hemorrhage, and hemosiderosis. A number of changes arising secondarily to cardiac impairment were noted in the high-dose animals. These included clinical changes, physical examination findings, moribundity, and gross and microscopic pathological alterations in the liver, testes, thymus, and bone marrow. Clinical signs included inactivity, inappetence, labored breathing, cachexia, hunched posture, abnormal gait, abdominal distention, hypothermia, paleness, fecal changes, lethargy, recumbency, vocalization, emaciation, and tremors. Physical examination findings revealed the presence of irregular, rapid heart rate, precordial thrill, pulse deficit, abdominal ascites, and emaciation. Fluid accumulation was seen in the pericardium and the thoracic and abdominal cavities. Liver alterations consisted of enlargement, red/dark color, lesions, adhesions, mottling, rough texture, passive congestion, centrilobular fibrosis/atrophy, bile stasis, necrosis, hemosiderosis, and inflammation. Hypospermatogenesis in the testes with associated hypospermia in the epididymides was noted in the high-dose males. Other changes included thymic atrophy and hyperplasia of the bone marrow.

Administration of the high dose of diaminochlorotriazine to dogs also affected body weight and food consumption. Mean body weights and body weight gains were decreased in males and females receiving 1500 ppm during the first 6 weeks of the study; the body weight loss persisted in the high-dose males even when the dogs were fed a diet containing 750 ppm. However, body weights in the males attained a generally constant level when the dogs were given untreated diet. Unlike the males, females did not exhibit much fluctuation from baseline values in body weights when the dose level of 1500 ppm was lowered to 750 ppm at week 7. Mean body weights in these females were lower than controls between weeks 7 and 13. The apparent mean body weight gain noted in the high-dose dogs at week 25 was considered by the study authors to be the result of passive congestion of internal organs and edema in body cavities of one dog that was sacrificed during week 26. Food consumption in the high-dose males was lower than that of controls during the first 8 weeks of treatment. Food consumption in these dogs returned to normal when they were provided untreated diets at weeks 9 to 13. However, food consumption in these dogs was lower than controls when they were given a diet containing 750 ppm. The food intake of the high-dose females (except recovery females) was lower than that of controls only during weeks 3 to 7 and at week 13. Increases in the mean spleen, liver, and kidney weights were noted in the high-dose dogs.

Assessment of hematology data indicated treatment-related anemia with an accompanying reticulocytosis in the high-dose

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animals. The effects included decreased erythrocyte counts, hematocrit, and hemoglobin concentration. Alterations in erythrocyte size or color and the presence of Howell-Jolly bodies or nucleated red blood cells were noted in several high-dose animals. Severe anemia with pronounced increase in reticulocyte count was noted in one of the two recovery high-dose females at week 13. However, by week 25, this particular dog recovered from the anemia and exhibited normal erythroid levels and reticulocyte count. This finding suggests that this effect of diaminochlorotriazine is reversible. Increased reticulocyte count was observed in both high-dose males and females; the reticulocyte count became normal by week 52. A treatment-related increase in platelet levels was noted in high-dose females (excluding the recovery females) at weeks 25 and 52. Treatment-related alterations in clinical chemistry parameters of high-dose animals (except recovery animals) consisted of decreases in albumin levels and decreases in serum calcium levels. Total cholesterol levels were significantly decreased in high-dose females (excluding recovery females) at weeks 13 and 25. The return of total cholesterol levels in the recovery females to levels near control values also suggests that this effect of diaminochlorotriazine may be reversible. Nonsignificant increases in lactic acid dehydrogenase activity were noted in both sexes receiving the high dose at weeks 13 and 25. However, the elevation in the activity of this enzyme was attributed to two high-dose animals (one male and one female) that were sacrificed moribund because of heart failure.

We agree with the study authors' conclusion that administration of diets containing ≥750 ppm to dogs is associated with the symptomatology of cardiac failure, and the no-observed-effect level (NOAEL) of diaminochlorotriazine is 100 ppm.