6-11-93

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

010331

JUN 11 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Diazinon (MG-8) Submission of a Chronic Dog Feeding Study and a Chronic Feeding Study in Rats in Compliance with EPA's May 1, 1987 Data Call-In

Tox Chem. No.:	342
Project No.:	2-1418
PC No.:	057801
DP Barcode:	D174740
Submission No.:	S411957

FROM: William B. Greear, M.P.H. William B Greear 6/11/93
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Larry Schnaubelt/Robert Richards, PM #72
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Marion P. Copley, D.V.M., Section Head
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

I. CONCLUSIONS:

The chronic feeding study in dogs (T/PR #90093 [MIN 882014] 6/14/91) **satisfies** the requirement for a Guideline Series 83-1b Chronic **Feeding** Study in Dogs. The chronic feeding study in rats (#882018, 6/4/91) satisfies the requirement for a Guideline Series 83-1a Chronic Feeding Study in Rats, however, it is requested that the sponsor address the following:

It was stated on p. 21 that "...not all ocular abnormalities were addressed by the Staff Ophthalmologist." This requires further clarification by the authors because it is known that certain organophosphate pesticides cause ocular effects.

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MRID 41942001, 41942002

II. REQUESTED ACTION:

Under a cover letter dated July 8, 1991, Carolyn Bussey of the Ciba-Geigy Corporation has submitted the following two studies in compliance with the requirements of the Data Call-In Notice.

- 83-1a Chronic Feeding Study in Rats
- 83-1b Chronic Feeding Study in Dogs

III. DISCUSSION:

The results of the reviews are listed below:

- Chronic Feeding Study in Dogs (T/PR #90093 [MIN 882014]; 6/14/91)

  NOEL (ChE) = 0.1 ppm (0.0032 mg/kg/d-M; 0.0037 mg/kg/d-F)
  LEL (ChE) = 0.5 ppm (0.015 mg/kg/d-M; 0.020 mg/kg/d-F)
  (based on decr. serum ChE)

  NOEL (systemic) = 0.5 ppm (0.015 mg/kg/d-m; 0.020 mg/kg/d-F
  LEL (systemic) = 150 ppm (4.5 mg/kg/day-F; 4.7 mg/kg/d-M)
  (based on decr. food consumption and incr. in serum amylase in M&F; and decr. body wt. and body wt. gain in M)

  In addition, decr. in RBC and brain ChE in M and F at 150 ppm and 300/225 ppm (7.7 mg/kg/day-M; 9.2 mg/kg/day-F; decr. in body wt. and body wt. gain in F at 300/225 ppm.

  Dose levels: 0.1, 0.5, 150 and 300/225 ppm
  M=0.0032, 0.015, 4.7 and 7.7 mg/kg/day
  F=0.0037, 0.020, 4.5 and 9.1 mg/kg/day
  Route: Oral (in diet)
  Strain: beagle
  Classification - Core Guideline

  The study satisfies the requirement for a Guideline Series 83-1 Chronic Feeding in Dogs.

- Chronic Feeding Study in Rats (#882018; 6114191)
  This study was conducted to establish a NOEL for ChE inhibition.
  NOEL (ChE) = 0.1 ppm (0.004, mg/kg/d-M; 0.005 mg/kg/d-F)
  LEL (ChE) = 1.5 ppm (0.06 mg/kg/d-M; 0.07 mg/kg/d-F) - Serum
  NOEL (systemic) = ≥ 250 ppm (HDT)
  In addition, RBC and brain ChE activity were decreased at 125 and 250 ppm.

  Dose levels: 0.1, 1.5, 125 and 250 ppm
  (M - 0.004, 0.06, 5 and 10 mg/kg/day
  F - 0.005, 0.07, 6 and 12 mg/kg/day)

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Route: Oral (in diet)
Strain: Sprague-Dawley (Crl:VAF/Plus CD [SD]SD B)

Classification - Core Minimum (may be upgraded with submission of requested data as specified below)

The Study satisfies the requirement for a Guideline Series 83-1 Chronic Feeding Study in Rats.

Deficiency - It was stated on p. 21 that "...not all ocular abnormalities were addressed by the Staff Ophthalmologist." This requires further clarification by the authors because it is known that certain organophosphate pesticides cause ocular effects.

Reviewed by: William B. Greear, M.P.H. William B. Greear 6/11/93
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion Copley 6/11/93
Review Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

Study Type: Guideline Series 83-1a
Chronic Toxicity
Study - Rat

Tox. Chem. No.: 341
PC No.: 017801
MRID No.: 419420-02

Test Material: Diazinon (MG-8)

Synonyms: 0,0-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate, Basudin, Sarolex, AG-500, Dazzel, Diazajet, Dizinon, ENT 19,507 Drawizon

Study Number: 882018

Sponsor: Ciba-Geigy Corporation

Testing Facility: Ciba-Geigy Research Department
Summit, NJ 07901

Title of Report: Diazinon (MG-8): One/Two Year Oral Toxicity Study in Rats

Author: F.R. Kirchner, G.C. McCormick, A.T. Arthur

Report Issued: June 4, 1991

Conclusions: Dose Levels: 0, 0.1, 1.5, 125 or 250 ppm (M - 0.004, 0.06, 5 & 10 mg/kg/day; F - 0.005, 0.07, 6 & 12 mg/kg/d)
NOEL (ChE) = 0.1 ppm
LEL (ChE) = 1.5 ppm (based on a decrease in serum ChE activity)
NOEL (systemic) = ≥ 250 ppm (HDT)
In addition, RBC and brain ChE activity were decreased at 125 and 250 ppm.

Classification: Chronic Study - Core Minimum Data (may be upgraded with submission of the requested data specified below)

It was stated on p. 21 that "... not all ocular abnormalities were addressed by the Staff Ophthalmologist." This requires further clarification by the authors because it is known that certain organophosphate pesticides cause ocular effects.

Acceptability: This chronic study satisfies the requirement for a Guideline Series 83-1 Chronic Toxicity Study.

A. Materials:

1. Test Compound - Diazinon (MG-8); Description: not reported, Batch No. FL872049; Purity: 87.7%, Contaminants, not reported.

2. Test Animals - Species: rat; Strain: Sprague-Dawley (Crl: VAF/Plus CD [SD] Br); Age: 6 weeks; Weight: males - 158.3 to 241.1 g; females - 126.4 to 216.5 g; Source: Charles River Laboratories, Kingston, NY.

B. Study Design:

1. Animal Assignment - Animals were randomly assigned to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 98 Week		Interim Sac. 52 Week		Interim Sac. 52 Week & 4 Recovery	
		Male	Female	Male	Female	Male	Female
Control	0	20	20	10	10	10	10
Vehicle Control*	0	20	20	10	10	10	10
Low	0.1	20	20	10	10	0	0
Mid	1.5	20	20	10	10	0	0
Mid-High	125	20	20	10	10	0	0
High	250	20	20	10	10	10	10

* - "EOS" identified only as a "component of the diazinon formulation"

The animals were acclimated to laboratory conditions for approximately 3 weeks prior to dosing. During the study, the animals were individually housed in a single room with temperature of 73±5°F, relative humidity of 50±20% and a 12 hour light cycle. Gross necropsy and serologic determinations were conducted on 10 males and 10 females prior to initiation of the study.

2. Diet Preparation - An amount of the test substance was dissolved in acetone and premixed with a portion of the basal diet (powdered Certified Purina Rodent Chow #5002). The acetone was evaporated and the resulting premix was added to an amount of feed and mixed in a twin-shell mixer to provide the proper dietary concentration. The diets were adjusted for purity of the technical. The diets were stored at room temperature. The frequency of preparation of the test diets was based on the "available stability data." Samples of test diets were analyzed for concentration at study initiation and random samples were analyzed during the study. Thirteen separate dietary analyses were made each year for concentration. Homogeneity data were obtained during week 3. Dietary analyses were conducted prior to use.

Results - Chemical analyses indicated that all admixtures were stable for 41 to 45 days at room temperature over the concentration range of 0.1 to 3500 ppm. Admixtures were analyzed and indicated that they were from 85% to 120% of target concentrations. Diazinon was uniformly distributed in all test

admixtures. All control feed and vehicle control admixture samples analyzed contained less than 0.4 ppm diazinon. The concentration of ESO in the diazinon test sheets and in the ESO-vehicle control diet was not verified by analysis due to the lack of an assay methodology.

3. Animals received food and water ad libitum.

4. Statistics - Body weight, food consumption, water intake, selected clinical laboratory data and organ weights were analyzed.

C. Methods and Results:

1. Observations - Animals were observed at least daily for clinical signs of toxicity and mortality.

Results - No clinical signs of toxicity could be attributed to administration of the test material. Survival was comparable among the control and the treated groups (see Table 1). At termination (week 97) survival in males in the control, vehicle control, 0.1 ppm, 1.5 ppm, 125 ppm and 250 ppm groups was 60, 45, 30, 50, 35, and 58%, respectively. Female survival in the control, vehicle control, 0.1 ppm, 1.5 ppm, 125 ppm and 250 ppm groups was 70, 58, 40, 44, 68 and 58%, respectively (see Table 1). Due to increased mortality, particularly in the lower dose groups, it was decided to terminate the study at 97 weeks. EPA concurred.

Table 1.* Survival for Main Group and Percent Survival at Termination (97-Weeks)

	Dose Levels (mg/kg/day)					
	Control	Vehicle Control	0.1	1.5	125	250
Males	12/30(60)	9/20(45)	6/20(50)	10/20(50)	7/20(35)	11/19(58)
Females	10/20(70)	11/19(58)	8/20(40)	8/18(44)	13/19(68)	11/19(58)

*- Data were abstracted from Study No.882018 p. 68

2. Body Weight - There was no adverse effect on body weights in treated animals. Body weight increases were noted in the treated group when compared to controls. The increases were in all male treated groups and in females in the 250 mg/kg/day.

3. Food Consumption and Compound Intake - determined weekly for week 2 to week 13, and monthly thereafter.

Results - No adverse effect on food consumption was noted in the treated animals. Food consumption was increased in the treated and ESO-control groups. Mean compound intake was 0.004, 0.06, 5 or 10 mg/kg/day for males in the 0, 0.1, 1.5, 125 or 250 ppm groups and 0.005, 0.07, 6 and 12 mg/kg day for females int the 0, 0.1, 1.5, 125 or 250 ppm groups.

4. Water Intake - determined at week 2 and during weeks 15, 24, 49-50, 55 (recovery rats only), 77-78 and 97 (selected groups).

Results - Unremarkable.

5. Ophthalmoscopic Examinations - determined using a Fisch Ophthalmoscope at week 2 and during weeks 51, 97 (0.1 ppm group only) and 98 (all remaining groups). Mydriacil, a mydriatic, was used prior to each examination as to facilitate funduscopy.

Results - Unremarkable, however it was stated on p. 21 that "... not all ocular abnormalities were addressed by the Staff Ophthalmologist."

[This will require further clarification because it is known that certain organophosphate pesticides cause ocular effects.]

6. Blood was collected from the right orbital sinus on days 88, 181, 356, 390, 552 and 684. All surviving animals were used for the hematology analysis at each time point except for day 390 (10 rats/sex from the two control groups were examined). Ten rats/sex/group[received clinical chemistry evaluation evaluations at all time points.

a. Hematology

X	Hematocrit (HCT)		Total plasma protein (TP)
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)		Mean corpuscular volume MCV)
X	Platelet count	X	Clotting time
X	Erythrocyte morphology	X	Heinz body*
X	Reticulocyte count		

* - Determined on at least 50% of the animals used for clinical examinations in the control and 250 ppm groups.

In addition, blood samples were obtained from the abdominal aorta and blood smears were made from all animals that were sacrificed early during the dosing or recovery period.

Results - Unremarkable.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase	X	Total protein
X	RBC cholinesterase	X	Triglycerides
X	Serum cholinesterase		Thyroxine (T_4)
X	Brain cholinesterase		Triiodothyronine (T_3)
X	Lactic acid	X	Albumin/Globulin ratio
X	Serum alanine aminotransferase (SGPT)		
X	Serum aspartate aminotransferase (SGOT)		
X	Gamma glutamyltransferase		

Brain cholinesterase was determined at necropsy during weeks 53-54, 57 and 98-99. After weighing approximately one-half of the brain was analyzed for ChE activity.

Results - There was a dose-related decrease in serum ChE activity in males and females in the 1.5, 125 and 250 ppm groups beginning at the first measurement interval of 88 days and persisting until terminal sacrifice on day 684 (see Table 2). For males in the 1.5 ppm group, mean serum ChE values were decreased from 12 to 51% from day 88 to day 684. Significant differences were noted on day 88 (28%) and 684 (51%). In the 125 ppm group, male serum ChE was significantly decreased on days 88 (79%), 181 (66%), 552 (86%) and 684 (89%). In the 250 ppm group, male serum ChE was significantly decreased at days 88 (87%), 181 (81%), 356 (89%), 552 (92%) and 684 (94%). For females in the 1.5 ppm group, mean serum ChE values were decreased 30 to 58% from days 88 to 684. Significant decreases were noted on days 88 (58%), 181 (54%) and 552 (45%). For females in the 125 ppm group, significant decreases were noted on days 88 (96%), 181 (94%), 552 (94%) and 684 (94%). For females in the 250 ppm group, significant decreases were noted on days 88 (97%), 181 (97%), 390 (23%) [this is the 4 week recovery group], 552 (96%) and 684 (94%). There was also a dose-related decrease in RBC ChE activity in males and females in the 125 and 250 ppm groups beginning on day 88 and persisting until day 684 (termination) (see Table 3). Males in the 125 ppm group had significant decreases in RBC ChE on days 88 (16.3%), 181 (15.8%), 356 (15.5%), 552 (28.2%) and 684 (20.2%). Males in the 250 ppm group had significant decreases in RBC ChE on days 88 (18.3%), 181 (14.2%), 356 (12.7%), 552 (25.4%) and 684 (20.9%). Females in the 125 ppm group had significant decreases in RBC Che on days 88 (24%), 181 (24%), 356 (22%), 552 (25%) and 684 (26%). Females in the 250 ppm group had significant decreases in RBC ChE on days 88 (27%) 181 (21%), 356 (21%), 390 (7% [this is the 45 week recovery group], 552 (29%) and 684 (26%). Brain ChE activity was significantly decreased in females in the 125 ppm group at day 684 (24%) and in the 250 ppm group at day 684 (43%). 4/Brain ChE activity was significantly decreased in males in the 125 ppm group at days 370 (25%) and 684 (29%). Brain ChE activity was also significantly decreased in females in the 250 ppm group at days 370 (29%) and 684 (38%).

Table 2.*** Mean Serum Cholinesterase (ChE) Activity (Mu/mL) and Percent (%) Decrease when Compared to Controls

Day	0 to 2	Dose Level (ppm) 0.1	1.5	125	250
			MALES		
88	422.2/428.8	392.9 (6.9)	302.4* (28.4)	90.7** (78.5)	54.0**/** (87.2)/(87.4)
181	519.8/516.3	369.2 (29.0)	457.5 (12.0)	178.1** (65.7)	100.1**/** (80.7)/(80.6)
356	596.7/609.1	383.5 (35.7)	513.7 (13.9)	422.4 (29.2)	70.1**/** (88.3)/(89.2)
390	604.5/698.1	ND	ND	ND	640.9/ (+)/(8.2)
552	850.9/959.4	845.3 (0.7)	588.0 (39.1)	118.1** (86.1)	71.5*/** (91.6)/(92.5)
684	1206/1464	693.5 (42.5)	591.6* (50.9)	136.8** (88.6)	88.4**/** (92.7)/(94.0)
			FEMALES		
88	2765/2453	2045 (26.0)	1156** (57.8)	107.8** (96.1)	71.7**/** (97.4)/(97.1)
181	3061/2883	2301 (24.8)	1396** (54.4)	179** (94.2)	1060**/** (96.5)/(96.8)
356	2612/2741	2177 (16.7)	1259** (51.4)	115.7** (95.6)	82.7**/** (96.8)/(97.0)
390	2186/2553	ND	ND	ND	1961/* (10.3)/(23.2)
552	2873/2613	2393 (16.7)	1559** (45.3)	162.9** (94.3)	127.6**/** (95.7)/(95.1)
684	2442/1987	2056 (15.8)	1719 (29.6)	152.5** (93.8)	112.5**/** (95.4)/(94.3

1 - control group 1
2 - control group 2
* - .01 ≤p ≤0.5
** - p ≤.01
*** - Data were abstracted from Study 882018 pp. 563-839.
ND - not determined
+ - greater activity than controls

Table 3.*** Mean Erythrocyte (RBC) Cholinesterase (ChE) Activity (MU/mL) and Percent (%) Decrease when Compared to Controls

Day	0[1]/0[2]	Dose Level (ppm) 0.1	1.5	125	250
			MALES		
88	3011/2920	3000 (0.4)	3030 (+)	2520** (16.3)	2425**/** (19.5)/(17.0)
181	3430/3367	3400 (0.9)	3510 (2.3)	2889** (15.8)	2944**/** (14.2)/(14.2)
356	3030/3155	3211 (+)	3230* (+)	2560** (15.5)	2700**/** (10.9)/(14.4)
390	3400/3467	ND	ND	ND	3370/ (0.9)/(2.8)
552	3611/3389	3560 (1.4)	3638 (+)	2590** (28.2)	2610**/** (27.7)/(23.0)
684	2964/2943	3183 (+)	2830 (4.5)	2350** (20.7)	2336**/** (21.2)/(20.6)
			FEMALES		
88	3420/3467	3480 (+)	3289 (3.8)	2590** (24.3)	2522**/** (26.3)/(27.3)
181	3478/3400	3450 (0.8)	3300 (5.1)	2660** (23.5)	2720**/** (21.8)/(19.6)
356	3275/3335	3450 (+)	3270 (0.2)	2556** (22.0)	2620**/** (20.0)/(21.4)
390	3567/3600	ND	ND	ND	3330**/** (6.6)/(7.5)
552	3522/3467	3340 (5.2)	3310 (6.0)	2547** (25.0)	2490**/** (29.3)/(28.2)
684	3369/3391	3275 (2.8)	3278 (2.7)	2508** (25.6)	2527**/** (25.0)/(25.5)

1 - control group 1
2 - control group 2
* - $.01 \leq p \leq 0.5$
** - $p \leq .01$
*** - Data were abstracted from Study No. 882018 pp. 563-839.
ND - not determined
+ - greater activity than controls

Table 4.*** Mean Brain Cholinesterase (ChE) Activity (nMU/mL) and Percent (%) Decrease when Compared to Controls

Day	0	0[2]	Dose Level (ppm) 0.1	1.5	125	250
			MALES			
370	2818/2652		2717 (3.6)	2835 (+)	2770 (1.7)	2537/ (10.0)/(4.3)
393	2370/2636		ND	ND	ND	2489/ (+)/(5.6)
684	2808/2910		2733 (2.7)	2765 (1.5)	2134** (24.0)	1618**/** (42.4)/(44.4)
			FEMALES			
370	2510/2463		2636 (+)	2673 (+)	1877** (24.8)	1502**/** (40.2)/(39.0)
393	2682/2841		ND	ND	ND	1961/** (25.9)/(32.8)
684	2824/1987		2860 (+)	2981 (+)	2016** (28.6)	1457**/** (48.4)/(26.7)

[1] - control group 1
[2] - control group 2
* - $.01 \leq p \leq .05$
** - $p \leq .01$
*** - Data were abstracted from Study No. 882018 pp. 563-839.
ND - not determined
+ - greater activity than controls

7. Urinalysis - Determined for 10 rats/sex (baseline animals only) at week-1 and in all surviving rats in the chronic study subgroup at days 81, 189, 350, 545 and 679. The CHECKED (X) parameters were determined.

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

Results - Unremarkable.

8. Sacrifice and Pathology - All animals that died and that were sacrificed at the interim and final sacrifices were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed for all interim sacrificed and all terminally sacrificed animals.

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

a. Organ Weight - Unremarkable.

b. Gross Pathology - Unremarkable.

c. Microscopic Pathology

(1) Non-neoplastic - Unremarkable. (There were lesions in the high-dose groups that were significantly different from controls. However, either a dose-response relationship was not apparent or the lesion was 0 in the controls which tended to produce significant values when there was a low incidence in the high dose group. For example, the incidence of stomach ulcers in the female high dose group was 2/9 compared to 0/8 in the controls.

(2) Neoplastic - Unremarkable.

D. Discussion:

Administration of the test material produced decreased RBC and brain ChE activity at 125 and 250 ppm in both sexes with exception to male RBC ChE activity measured at the end of the recovery period (day 390). Decreased

serum ChE activity was produced in males and females at 1.5, 125 an 250 ppm. However, at the end of the recovery period (day 390), there were no significant differences among the control and treated groups. There were no other treatment related effects. No treatment related effects were produced with special reference to the ocular tissues of the eyes according to the opnthalmoscopic report (see pp. 1872-1875 and by examination of the histopathology report (see pp. 1898-2099).

[Sufficiently high doses were administered to achieve a toxic effect level as indicated by the decrease in serum, RBC and brain ChE levels.]

E. Deficiencies:

It was stated on p. 21 that "... not all ocular abnormalities were addressed by the Staff Ophthalmologist." This will require further clarification because it is known that certain organophosphate pesticides cause ocular effects.

012092

Reviewed By: William B. Greear, M.P.H. William B Greear 12/9/92
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M.
Review Section IV, Toxicology Branch I (H7509C) Marion Copley 12/21/92

DATA EVALUATION REPORT

Study Type: Guideline Series 83-1
Chronic Feeding - Dog

TOX Chem. No.: 342
PC No.: 57801
MRIDNo.: 419420-01

Test Material: Diazinon (MG-8)

Synonyms: Alfa-tox, Sarolex, Basudin, Spectracide, AG-500

Study Number: Toxicology/Pathology Report 90093 (MIN 882014)

Sponsor: Ciba-Geigy Corporation

Testing Facility: Ciba-Geigy Corporation
Division of Toxicology/Pathology
Summit, NJ 07901

Title of Report: Diazinon MG-8 52-Week Oral Toxicity Study in Dogs

Authors: M.W. Rudzki, G.C. McCormick, A.T. Arthur

Report Issued: June 14, 1991

Conclusions:

NOEL (ChE) = 0.1 ppm (0.0032 mg/kg/d-M; 0.0037 mg/kg/d-F)
LEL (ChE) = 0.5 ppm (0.015 mg/kg/d-M; 0.020 mg/kg/d-F)
(based on decr. serum ChE)

NOEL (systemic) = 0.5 ppm
LEL (systemic) = 150 ppm (4.5 mg/kg/day-F; 4.7mg/kg/d-M)
(based on decr. food consumption and incr. in serum amylase in M&F; and decr. body wt. and body wt. gain in M)

In addition, decr. in RBC and brain ChE in M and F at 150 ppm and 300/225 ppm (7.7 mg/kg/day-M; 9.2 mg/kg/day-F) decr. in body wt. and body wt. gain in F at 300/225 ppm.

Core Classification: Guideline

Study Acceptability: The study satisfies the requirements for a Guideline Series 83-1 Chronic Toxicity Study in Dogs.

A. Materials:

1. Test Compound - Diazinon MG-8 (FL 872049); Description: a light tan to brown liquid; Exp. No.: G 24480; Purity: 87.7 percent; Contaminants: not reported.

2. Test Animals - Species: Dog; Strain: Beagle; Age: 5 months; Weight: males - 5.9 to 9.1 kg; females - 4.6 to 6.6 kg; Source: Marshall Farms, North Rose, NY.

B. Study Design:

1. Animal Assignment - The animals were randomly assigned to the following groups:

Test Group	Dose in Diet (ppm)	52-Week Main Study Number of Animals Male	Female
1	0	4	4
2	0.1	4	4
3	0.5	4	4
4	150	4	4
5	300/225*	4	4

*300 ppm dose decreased to 225 ppm after 14 weeks of treatment due to lack of body wt. gain.

The animals were housed in two animal rooms according to the facility's SOP. The rooms were maintained in an environment with room temperature of 69 ± 5 °F, relative humidity of 50 ± 20 percent with a 12-hour on/12-hour off light cycle. The animals were given a preliminary examination by the attending veterinarian upon receipt and allowed to acclimate to laboratory conditions for 5 to 6 weeks prior to treatment. The study was initiated on August 15, 1998. Necropsies were conducted on August 29, 30, and 31, 1989.

2. Diet Preparation - Approximately 400 g portions of the test feed diets (dietary level of test material adjusted for purity) or control diets were offered for a 3-hour period daily. Dosage selection was based on the results of a 13-week study in which the NOEL was 0.1 ppm and significant inhibition of serum, RBC and brain ChE, and reduced body weight gain were observed at 300 ppm. Diazinon (MG-8, FL 872049) was initially analyzed for purity. Analysis of dietary mixtures containing diazinon at levels from 0.1 to 440 ppm for stability were measured when held over a 27-day period at room temperature. The homogeneity of the test diets was made at the top,

middle, and bottom levels. In addition, the test diets were analyzed for concentration at weeks 1, 2, 8, 11, 16, 17, 18, 24, 28, 31, 36, 40, 42, and 48 weeks.

Results - The purity of the test material was determined to be 87.7 percent. Feed containing 0.1, 0.439, and 439 ppm diazinon contained 91 to 103 percent of the target levels over a 27-day period. Homogeneity of test diets averaged 91 to 113 percent. Analysis of test diets over a 48-week period indicated they contained 86 to 113 percent of the target concentrations, except for the 0.1 ppm diet that contained 87 to 121 percent of the target concentration.

3. Animals received food, 400 g diet per day, and water ad libitum.

4. Statistics - Body weight, food consumption, clinical laboratory (for urinalysis and specific gravity only), urine volume, physical/auditory and organ weight data were analyzed for each sex using the Statistical Analysis System (SAS) Version 5 and SOGT Supplementary Library. Tests for homogeneity of variances were performed to check for deviations from normal. Then Dunnett's test was performed to compare each of the treatment groups with the control group. If significant model deviations were detected, then appropriate "ad hoc" analyses were performed. Nonparametric tests were conducted on non-normally distributed data.

5. Quality assurance was conducted on 11 intervals. The Quality Assurance Statement was signed by Lynn R. Miko dated June 14, 1991.

C. Methods and Results:

1. Observations - Animals were observed daily for mortality and clinical signs of toxicity. Physical/auditory examinations were conducted in weeks 4, 12, 26, 39, and 51. Ophthalmoscopic examinations were conducted in weeks 3, 25, and 53.

Results - One male (#18) in the 300/225 ppm group was sacrificed on test day 2. One female (#29) in the 0.5 ppm group was found dead on test day 12. Both deaths were attributed to gastrointestinal infections and the animals were replaced on days 4 and 15. Clinical signs of toxicity were limited to 1 male (#18) in the 300/225 ppm group which showed signs of dehydration and emaciation.

2. Body Weight - Recorded at -3 weeks and -2 weeks prior to dosing and immediately prior to the first dose, weekly during the first 13 weeks, and then monthly thereafter.

Results - During the first 3 months, males and females in the 300 ppm group exhibited decreases in body wt. gain of 101 and 76 percent, respectively. There was also a decrease of 64 percent in males in the 150 ppm group. Throughout the test period, males in the 150 and 300/225 ppm groups consistently exhibited decreases in weight gain when compared with controls. At termination, males in the 150 and 300/225 ppm groups had decreases of 42 and 27 percent in body wt. gain compared with controls. Female body wt. gain was variable at 300/225 ppm; however, it appears that there were decreases in body wt. gain at several intervals during the study (see Table 1 and Figures 1 and 2).

Table 1: Mean Cumulative Body Wt. Gain (kg) and Percent (%) Loss(-) Relative to Controls at Selected Intervals

Group (ppm)	Interval (Days) 0-91	0-196	0-280	0-364
Male				
0	2.83	3.98	4.45	4.68
0.1	2.98 (5.3)	4.23 (6.3)	4.73 (0.28)	4.93 (5.3)
0.5	2.63 (-2.6)	3.68 (-7.5)	3.98 (-10.6)	4.45 (4.9)
150	1.03 (-63.6)	1.68 (-57.8)	2.28 (-48.8)	2.70 (-42.3)
300/225	-0.03 (-101.1)	1.73 (-56.5)	2.80 (-37.1)	3.40 (-27.4)
Female				
0	1.60	2.73	2.65	3.08
0.1	1.43 (10.6)	2.15 (-21.2)	2.35 (-11.3)	2.33 (-24.4)
0.5	1.53 (-4.4)	2.28 (-16.5)	2.45 (-7.5)	2.33 (-24.4)
150	1.68 (5.0)	2.53 (-7.3)	2.90 (-9.4)	3.13 (1.6)
300/225	0.38 (-76.3)	1.58 (-42.1)	1.93 (-27.2)	2.50 (-18.9)

3. Food Consumption and Compound Intake - Recorded once prior to dosing at -1 week , weekly up to 16 weeks, and then monthly, thereafter.

Results - Food consumption was quite variable during the study; however, a trend was apparent in that males and females in the 150 and 300/225 ppm groups had decreases in food consumption up to ≈ 26 percent at certain time intervals (see Table 2 and Figures 3 and 4). Compound intake was 0.0032, 0.015, 4.7, and 7.7 mg/kg/day for males in the 0.1, 0.5, 150, and 300/225 ppm groups, respectively. Compound intake was 0.0037, 0.020, 4.5, and 9.1 mg/kg/day for females in the 0.1, 0.5, 150, and 300/225 ppm groups, respectively.

Table 2: Mean Food Consumption (g/animal/week), and Percent (%) Loss(-) Relative to Controls at Selected Intervals

Group (ppm)	Interval (Days) 7	91	196	280	364
Male					
0	1953	2554	2546	2508	2168
0.1	2359 (21.8)	2465 (-3.5)	2519 (-1.1)	2439 (-2.8)	1872 (-13.7)
0.5	1932 (-1.1)	2241 (-12.3)	2329 (-8.5)	2209 (-11.9)	1930 (-11.0)
150	1769 (-9.4)	1875 (-26.6)	2086 (-18.1)	2162 (-13.8)	1785 (-17.7)
300/225	1917 (-1.8)	1889 (-26.0)	2381 (-6.5)	2440 (-2.7)	1904 (-12.2)
Female					
0	1476	2200	2442	1901	1731
0.1	1402 (-5.0)	1939 (-11.9)	1868 (-23.5)	2030 (17.3)	1371 (-20.8)
0.5	1390 (-5.8)	2097 (-4.7)	2166 (-11.3)	1655 (-12.9)	1568 (-9.4)
150	1755 (19.0)	1737 (-21.0)	1792 (-26.6)	1807 (-4.9)	1544 (-10.8)
300/225	1271 (-13.9)	1656 (-24.7)	1846 (-24.4)	1840 (-3.2)	1326 (-23.4)

4. Ophthalmoscopic examinations were made once prior to dosing in Week 4 and in Weeks 12, 26, 39, and 51.

Results - Unremarkable.

5. Blood was collected at Weeks -4, 13, 26, 39, and 52. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)		Total plasma protein (TP)
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)		Mean corpuscular HGB conc. (MCHC)
X	Platelet count		Mean corpuscular volume
	Reticulocyte count	X	Erythrocyte morphology
X	Prothrombin time	X	Clotting time
		X	Heinz body

Reticulocyte counts and Heinz body determinations were conducted on all animals prior to treatment and on the controls and animals in the 300/225 ppm groups during the study.

Results - Unremarkable.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium	X	Albumin (A)
X	Chloride	X	Blood creatinine

	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Cholesterol
X	Potassium	X	Globulins (G)
X	Sodium	X	Glucose
	Enzymes:	X	A/G Ratio
X	Alkaline phosphatase	X	Total bilirubin
X	Cholinesterase		Direct bilirubin
X	Creatinine phosphokinase	X	Triglycerides
X	Lactic acid dehydrogenase	X	Total protein
X	Serum alanine aminotransferase (SGPT)	X	Amylase
X	Serum aspartase aminotransferase (SGOT)		
X	Gamma glutamyl transpeptidase (GGT)		

Results - Serum ChE was decreased in males in the 0.5, 150, and 300/225 ppm groups by approximately 22, 77, and 75 percent, respectively, throughout the dosing period. Statistical significance was present at all measurement periods at 150 and 300/225 ppm, and in the 0.5 ppm group at 176 days. Serum ChE was significantly decreased in females in the 0.5, 150, and 300/225 ppm groups at all intervals by approximately 28, 76, and 81 percent, respectively. Serum ChE was significantly decreased by 28.4 percent in the female 0.1 ppm group at Day 268. This is not considered to be of biological significance because decreases of less than 20% occurred at all other times and there were no significant differences between it's ChE values and the control group at the other intervals (see Table 3). RBC ChE was significantly decreased by approximately 26 and 23 percent, respectively, in males in the 150 and 300/225 ppm groups throughout the study. Females in the 150 and 300/225 ppm groups were significantly decreased by approximately 30 and 32 percent, respectively (see Table 4). Brain ChE was decreased in males in the 150 and 300/225 ppm groups by 15 and 25 percent, respectively. Brain ChE was also decreased by 26 and 35 percent, respectively, in females in the 150 and 300/225 ppm groups (see Table 5). Serum amylase was increased in males in the 150 and 300/225 ppm groups by 28 to 59 percent and 14 to 172 percent, respectively, during the study. Serum amylase was also increased in females in the 150 and 300/225 ppm groups by 24 to 38 percent and 24 to 174 percent, respectively, during the study (see Table 6).

Table 3: Serum Cholinesterase Levels (Mu/mL) and Percent (%) Decrease(-) Compared with Controls

Group (ppm)	Day -26	85	176	268	359
Males					
0	1716	1876	1978	1405	1935
0.1	1980 (5.4)	2012 (7.2)	2084 (5.4)	1648 (18.0)	2101 (8.6)
0.5	1756 (2.3)	1464 (-22.0)	1502* (-24.1)	1333 (-5.1)	1496 (-22.7)
150	1801 (5.0)	369** (-80.3)	445** (-77.5)	359** (-74.4)	426** (-78.0)
300/225	1969 (14.7)	424** (-77.4)	586** (-70.4)	484** (-65.6)	508* (-73.7)
Female					
0	2132	2252	2446	2569	2351
0.1	1851 (-13.2)	1841* (-18.3)	2232 (-8.7)	1839* (-28.4)	2130 (-9.4)
0.5	1912 (-10.3)	1533** (-31.9)	1551** (-40.1)	1710** (-33.4)	1909 (-18.8)
150	1913 (-10.3)	428** (-81.0)	615** (-74.9)	358** (-86.1)	526** (-77.6)
300/225	2001 (-6.1)	337** (-85.0)	506** (-79.3)	322** (-87.5)	532** (-78.2)

*.01 < p ≤ 0.05, 2-tailed Dunnett's T
**p ≤ 0.01, 2-tailed Dunnett's T

Table 4: Erythrocyte Cholinesterase Levels (Mu/mL) and Percent (%) Decrease(-) Compared with Controls

Group (ppm)	Day -26	85	176	268	359
Males					
0	2775	2625	2925	2525	2625
0.1	3000 (8.1)	2925 (11.4)	3250 (11.1)	2650 (5.0)	2775 (5.7)
0.5	2800 (-0.9)	2675 (1.9)	2800 (-4.3)	2225* (-11.8)	2300 (-12.4)
150	2750 (-0.9)	1950** (-25.7)	2200** (-24.8)	1675** (-33.7)	1900** (-27.6)
300/225	2750 (-0.9)	2075** (-21.0)	2150** (-26.5)	1925** (-23.8)	1900** (-27.6)
Female					
0	3050	2850	3125	2750	2650
0.1	2800 (-8.2)	2800 (-1.8)	3150 (0.1)	2700 (-1.8)	2575 (-2.8)
0.5	2925 (-4.1)	3000 (5.3)	3075 (-0.2)	2575* (-6.4)	2675 (-0.9)
150	2700 (-11.5)	2100** (-26.3)	2125** (-32.0)	1875** (-31.8)	1775** (-33.0)
300/225	3075 (-0.8)	1950** (-30.2)	2225** (-28.9)	1775** (-35.5)	1750** (-34.0)

*.01 < p ≤ 0.05, 2-tailed Dunnett's T
**p ≤ 0.01, 2-tailed Dunnett's T

Table 5: Brain Cholinesterase Levels (Mu/mL) and Percent (%) Decrease(-) Compared with Controls at Termination

	Group (ppm) 0	0.1	0.5	150	300/225
Sex					
Males	1995	2188 (9.7)	1873 (-6.1)	1695 (-15.0)	1500 (-24.8)
Females	2148	2238 (4.2)	2078 (-3.3)	1600* (-25.5)	1403** (-34.7)

*.01 < p ≤ 0.05, 2-tailed Dunnett's T
** p ≤ 0.01, 2-tailed Dunnett's T

Table 6: Serum Amylase Levels (Mu/mL) and Percent (%) Increase (+) Compared with Controls

Group (ppm)	Day -26	85	176	268	359
Males					
0	510	687	708	704	706
0.1	529 (3.7)	750 (9.2)	803 (13.4)	761 (8.1)	821 (16.3)
0.5	562 (10.2)	754 (9.8)	779 (10.0)	741 (5.3)	723 (2.4)
150	548 (7.5)	1090 (58.7)	988 (39.5)	906 (28.7)	910* (27.5)
300/225	527 (2.0)	1870 (172)	813 (14.8)	806 (14.5)	802 (13.6)
Females					
0	501	559	517	537	532
0.1	487 (-2.8)	537 (-3.9)	531 (2.7)	515 (-4.1)	494 (-7.1)
0.5	524 (4.6)	642 (14.8)	596 (15.3)	593 (10.4)	554 (4.1)
150	542 (8.2)	768 (37.4)	702 (35.8)	666 (24.0)	693 (30.3)
300/225	525 (4.8)	859 (53.7)	725 (40.2)	1469 (174)	662 (24.4)

*.01 < p ≤ 0.05 of 2-tailed Dunnett's T

6. Urinalysis - Urine was collected at Weeks -4, 13, 27, 39, and 52. The CHECKED (X) parameters were determined:

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

Results - Unremarkable.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were

collected for histological examination. The (XX) organs in addition were weighed.

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

a. Organ Weight - There were statistically significant decreases in the absolute weight of the lungs in females in the 150 ppm (72.89 g) and 300/225 ppm (64.90 g) when compared with controls (89.61 g). The relative weight of the lungs to brain weight was significantly decreased in females in the 0.1 (107.3), 0.5 (100.7), 150 (101.2), and 300/225 (88.6) ppm groups when compared with controls (124.7). These decreases were in a dose-response relationship. Although statistical significance was not achieved in comparisons of lungs/body weight ratios, a dose-response relationship was apparent. The authors state that the weight of the lungs of one high-dose female (38F) was below historical controls. But the weight of the lungs of two control females (22F and 24F) was greater than historical control values. Taking this information into consideration, together with the fact that there were no histological lesions associated with the decreased lung weights and lung weights were not decreased in males, this finding is considered to be of dubious biological significance. The absolute and relative (to body) mandibular salivary gland weights of females in the 150 and 300/225 ppm groups were significantly decreased; however, a dose-response relationship was not evident. In addition, the relative weight (to body) of the mandibular salivary gland was not significantly decreased in the 300/225 ppm group. The authors indicate that individual salivary gland

and salivary gland/body weight values were within the historical control range. No histological correlates were present, and males exhibited no similar decreases. The decrease salivary gland weight in females is of questionable biological significance.

b. Gross Pathology - Unremarkable.

c. Microscopic Pathology - Unremarkable.

D. Discussion

During the first 3 months, body wt. gain decreased in males and females in the 300/225 ppm group and in males in the 150 ppm group. Body wts. of males in the 150 and 300/225 ppm groups and females in the 300/225 ppm groups were decreased when compared with controls. A trend was apparent towards decreased food consumption in males and females in the 150 and 300/225 ppm groups. Serum ChE was decreased in males and females in the 0.5, 150, and 300/225 ppm groups. RBC and brain ChE were decreased in males and females in the 150 and 300/225 ppm groups. Serum amylase levels were increased in males and females in the 150 and 300/225 ppm groups.

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