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

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FEB 21 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No. 100-524. Evaluation of 21-Day Inhalation Toxicity Study in Rats, 90-Day Oral Feeding Study in Rats, 90-Day Oral Feeding Study in Dogs, and three preliminary studies, all with Diazinon MG-8 (Purity: 87.7%)

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TO: George T. LaRocca, PM Team (15)
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THRU: Edwin R. Budd, Section Head
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Record Nos.: 231259/231260/231261 Tox. Chem. No.: 342
T.B. Project Nos.: 8-1196/8-1198/8-1197

Toxicology Branch I/IRS has completed an evaluation of the following studies:

Study/Lab/Study No./ Date/MRID (Accession) No.	NOELs	Core Grade
Subchronic inhalation (21 days)-rat; Hazleton Laboratories America; No. 483-267; 7/28/88. 408150-02	< 0.1mg/m^3 (LDT), male and females; based on inhibition of brain, RBC and plasma AChE levels.	Supplementary (NOEL not determined)

Subchronic oral (6 weeks)-rat; Ciba-Geigy Corporation; No. 88076 (MIN 872348); 7/20/88. 408150-03	0.5 ppm (0.04 mg/kg*, males and 0.05 mg/kg*, females), based on inhibition of serum AChE activity.	Supplementary (Preliminary study)
Subchronic oral (90 days)-rat; Ciba-Geigy Corporation; No. 88083 (MIN 882011); 8/4/88. 408150-03	Males: 0.5 ppm (0.03 mg/kg*), for serum AChE activity and 5 ppm (0.3 mg/kg*) for RBC and brain AChE activities. Females: 0.5 ppm (0.04 mg/kg*) for serum and RBC AChE activities, and 5 ppm (0.4 mg/kg*) for brain AChE activity.	Guideline
Subchronic oral (4 weeks)-dog; Ciba-Geigy Corporation; No. 88077 (MIN 872349); 8/1/88. 408150-04	Males: 0.5 ppm (0.02 mg/kg*) Females: < 0.5 ppm (0.02 mg/kg*;LDT), each based on inhibition of serum AChE activity.	Supplementary (Preliminary study)
Subchronic oral (90 days)-dog; Ciba-Geigy Corporation; No. 88072 (MIN 882012); 8/4/88. 408150-04	0.1 ppm (0.0034 mg/kg*, males and 0.0037 mg/kg*, females), based on inhibition of serum AChE activity.	Supplementary**

AChE: Acetylcholinesterase

*Reported values, based on analytical data.

**This classification will be upgraded to Core-Guideline upon receipt and acceptance by Tox. Branch of the requested data (correction of errors and clarification of ambiguities detailed in the review).

Dose levels used in the 90-day oral feeding studies were based on the results of the preliminary studies. Exposure levels used in the 21-day inhalation toxicity study were based on the results of two range-finding inhalation toxicity studies. Because these studies were of short duration (3 days and 5 days), they were not evaluated separately but were included in the review of the 21-day inhalation toxicity study.

The major finding of concern in all of the above studies was the inhibition of AChE activities (or levels) in serum, RBC and brain. Of the three types of biological samples used in the assays for AChE activities (or levels), serum was most sensitive.

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Secondary Reviewer: Edwin R. Budd, Section Head
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DATA EVALUATION REPORT

Study Type: 82-4. Repeated Dose Inhalation (21 Days) - Rat

Tox. Chem. No.: 342

MRID No.: 408150-02

Test Material: Diazinon (MG-8); Purity (content of a.i.): 87.7%; Lot No.: FL-872049; brown liquid, stable at room temperature.

Study Number(s): 483-267

Sponsor: Ciba-Geigy Corporation, Greensboro, NC

Testing Facility: Hazleton Laboratories America, Inc., Rockville, MD

Title of Report: A 21-Day Inhalation Toxicity Study with Diazinon (MG-8) in the Rat

Author(s): James B. Terrill

Report Issued: July 28, 1988

Conclusions:

NOEL was not determined. Inhibition of brain, erythrocyte and plasma cholinesterase levels was observed at 0.1 mg/m^3, the lowest concentration of Diazinon (MG-8) tested.

Classification: Core-Supplementary (NOEL not determined)

Male and female Sprague-Dawley rats were exposed (whole body) to respirable aerosols of Diazinon (MG-8) for 3 weeks (6 hours/day, 7 days/week). The exposure concentrations (target) were 0, 0.1, 1.0, 10 and 100 mg/m^3 and corresponded closely to the analytical concentrations (0, 0.086, 1.12, 11.1 and 99.3 mg/m^3, respectively.

With the exception of cholinesterase inhibition in brain, erythrocytes (RBC) and plasma, Diazinon (MG-8) had no effect on any of the parameters examined. At the lowest exposure concentration tested (0.1 mg/m^3), brain, RBC and plasma cholinesterase levels in the males were inhibited 4.4, 17.5* and 30*%, respectively. The corresponding inhibitions for the female

rats were 5.7, 4.0 and 56.1*%, respectively. (* $p \leq 0.05$).

Experimental Procedures

Groups of 15 male and 15 female Sprague-Dawley rats were exposed (whole body) to respirable aerosols of Diazinon (MG-8) for three weeks (6 hours/day, for a total of 21 or 22 consecutive days). The calculated exposure levels (target concentrations), expressed as an active ingredient, were 0, 0.1, 1.0, 10 and 100 mg/m^3 for Groups 1, 2, 3, 4 and 5, respectively. The mass median aerodynamic diameter (MMAD) of the aerosols ranged from 1.5 to 3.52 um and the geometric standard deviation (GSD) from 1.6 to 2.9. The volume of each of the exposure chambers used was 2000 liters. The chamber temperature and relative humidity were 19.7 to 24.6 ^{o}C and 40.1 to 56.5 %, respectively.

The chamber atmosphere of Groups 2, 3, 4 and 5 was sampled at least six times during each exposure. The level of Diazinon (MG-8) was determined by both gravimetric and gas chromatography (GC, the primary method) assay. Group 1 samples were taken at 2 and 5 hours of exposure. All samples were drawn from the breathing zone of the animals.

The rats were 1) obtained from Charles River Laboratories, Raleigh, North Carolina; 2) acclimated for approximately two weeks; 3) randomly assigned to groups on a weight basis; 4) housed individually; and 5) given unrestricted amounts of food (Purina Laboratory Rodent Chow #5002) and tap water. A controlled diurnal cycle (12 hours light/12 hours dark) was maintained. At the start of the study, the rats were approximately 42 days old and weighed 186-280 g (males) and 130-160 g (females).

Toxicity of Diazinon (MG-8) was evaluated by 1) daily cageside observations for toxic signs and mortality; 2) weekly physical examination for gross signs of toxic and pharmacological effects; 3) weekly determinations of body weight and food consumption; 4) laboratory studies (hematology, clinical chemistry and urinalysis) performed before the initiation and at the termination of the study on 10 animals/sex/group; 5) ophthalmologic examination prior to the start of the study and at termination; 6) gross necropsy and histopathological examinations; and 7) organ weight and organ/terminal body weight ratio determinations. The following parameters were measured in hematology, clinical chemistry and urinalysis:

Hematology

Erythrocyte Count	Activated Thromboplastin Time
Leukocyte Count	Differential Leukocyte Count
Platelet Count	Cell Morphology
Hemoglobin	Heinz Bodies

Hematocrit
Prothrombin
Reticulocyte Count

Clinical Chemistry

Plasma Cholinesterase
RBC Cholinesterase
Brain Cholinesterase
Sodium
Potassium
Chloride
Calcium
Phosphorous
Blood Urea Nitrogen
Creatinine
Total Protein
Globulin
Glucose
Total Bilirubin
Alkaline Phosphatase
Lactic Dehydrogenase
Aspartate Aminotransferase
Alanine Aminotransferase
Cholesterol
Albumin/Globulin Ratio
Triglycerides
Albumin

Urinalysis

Volume (overnight)
Specific gravity
pH
Occult blood
Protein
Glucose
Urobilinogen
Bilirubin
Ketones
Microscopic inspection of sediment

Plasma and RBC cholinesterase levels were determined on unfasted animals. At the termination of the study, blood samples for these measurements were obtained immediately after the last exposure to Diazinon (MG-8). All of the remaining determinations were performed on blood or urine samples obtained the following day, just before sacrifice, after the animals were fasted overnight. Procedures used in clinical laboratory studies were referenced.

Necropsy was performed on each animal and included examination of the following:

The external surface
All orifices
Cranial cavity
Carcass
The cervical tissues and organs
External surface of the brain
The thoracic, abdominal and pelvic cavities and their viscera

The external surface of the spinal cord and cut surfaces of the brain and spinal cord were examined grossly at the time of tissue trimming.

The following organs were weighed from all animals on the study:

lungs	testes with epididymides (males)
liver	adrenals
brain	ovaries (females)
heart	spleen
kidneys	

Half of the brain from all animals was frozen as soon as possible after excision and was used from determination of the brain cholinesterase level; the remaining half was saved for histopathological examination.

Histopathological examination of tissues was performed by Experimental Pathology Laboratories, Herndon, VA. The following tissues were examined from all rats in all groups:

gross lesions and masses	spinal cord (cervical, thoracic, lumbar)
nasopharyngeal tissue	kidneys
brain	adrenals
pituitary	pancreas
thyroid and parathyroids	testes with epididymides
thymus	ovaries
lungs (perfused)	uterus
trachea	spleen
heart	aorta
bone marrow (femur)	esophagus
salivary glands (mandibular)	stomach
liver	exorbital lacrimal gland
sternum with marrow	seminal vesicles
urinary bladder	duodenum, jejunum, ileum
eyes	colon, cecum, rectum
mammary gland	tracheobronchiolar lymph node
mandibular lymph node	prostate
sciatic nerve with biceps femoris	mesenteric lymph node

Statistical analyses were performed on the following data: body weights, food consumption, hematology, clinical chemistry, urinalysis, and organ weights (see Attachment I for procedures).

Exposure levels used in the 21-day inhalation toxicity study were based on the results of two range-finding inhalation toxicity studies, conducted as follows:

In the first study, 5 Sprague-Dawley rats/sex/group were exposed to respirable aerosols (MMAD: 2.67-3.49 um) of Diazinon (MG-8) in whole body inhalation exposure chambers for 6 hours/day for 3 consecutive days. Target chamber concentrations of 50, 500, 1500 or 3000 mg/m^3 were used. Clinical condition and body weight changes were used to evaluate treatment effects. A two-

day recovery period followed the last day of treatment and the animals were then sacrificed. Necropsy was not performed and all tissues were discarded.

In the second study, groups of 5 male and 5 female Sprague-Dawley rats were exposed (whole body) to respirable aerosols (MMAD: 2.62-3.23 um) of Diazinon (MG-8) for 5 consecutive days (6 hours/day). The exposure levels (target concentrations) were 0, 40, 125, 500 or 2000 mg/m^3. Clinical condition, body weights and plasma, RBC and brain cholinesterase levels were evaluated. Body weights and cholinesterase data were analyzed statistically as in the 21-day inhalation study. Necropsy was not performed and all tissues other than brain were discarded.

Results: Range-Finding Studies

The target concentrations of Diazinon (MG-8) in the first study were 50, 500, 1500 or 3000 mg/m^3 and in the second study, 0, 40, 125, 500 or 2000 mg/m^3.— Gas chromatographic analyses of Diazinon (MG-8) in the exposure chambers showed good agreement between the target and measured concentrations.

There were no deaths in both studies. Toxic signs observed in treated animals from all groups in both studies included squinted eyes, salivation, languid behavior, increased secretory responses, rough haircoat, respiratory distress, tremors and other signs of poor condition, especially at the higher chamber concentrations. These findings followed a dose-related pattern with respect to incidence, time of onset and time to recovery (2-day recovery period was allowed only in the first study).

Body weights were not statistically analyzed in the first study, but dose-related weight losses were observed in all treated groups of both sexes. In the second study, statistically significant ($p \leq 0.05$) weight losses occurred in males and females exposed to 125, 500 or 2000 mg of Diazinon (MG-8)/m^3. Animals in the 40 mg/m^3 group gained less weight then did the controls, but the differences were statistically insignificant.

Brain, plasma and erythrocyte (RBC) cholinesterase levels were inhibited in all groups as follows:

Cholinesterase Inhibition, Expressed as Percent Decrease Compared to Control Value (Animals Exposed to Diazinon- Free Air)

Diazinon (MG-8) (mg/m^3)	RBC	Plasma	Brain
		Males	
40	62	85	24
125	69	92	45
500	77	92	78
2000	77	92	91
		Females	
40	67	87	56
125	67	89	82
500	73	91	89
2000	80	91	92

Each of these inhibitions was statistically significant ($p \leq 0.05$). Cholinesterase levels were reported as umoles of enzyme/mL of RBC or plasma, or per gram of brain tissue.

Based on the results from both studies, exposure levels for a 21-day inhalation study were selected to be 0.1, 1.0, 10 and 100 mg/m^3 Diazinon (MG-8). The 100 mg/m^3 level was regarded as a maximum tolerated dose (MTD) for the rat.

Results: 21-Day Inhalation Study

Exposure Levels

The calculated (target) and daily mean analytical concentrations of Diazinon (MG-8) in the inhalation chambers were as follows:

Group	Target (mg/m^3)	Gas Chromatography (GC)* (mg/m^3)
1	0	None detected
2	0.1	0.086 ± 0.007
3	1.0	1.12 ± 0.18
4	10	11.1 ± 0.88
5	100	99.3 ± 5.86

*Regarded as primary method; gravimetric determinations were also performed.

According to these data, the GC results were in close agreement with the target concentrations. The mean daily gravimetric

concentrations were similar to those obtained by GC.

Mortality

There were no deaths during the study.

Clinical Signs

One female in Group 5 had a crust on her nose and a sore on the front paw at test week 2. No other signs were observed during the study.

Body Weights

Diazinon (MG-8) had no effect on body weight gains. During the 3-week exposure period, male rats in Groups 1, 2, 3, 4 and 5 gained 152, 150, 146, and 150 g, respectively. The corresponding weight gains for the female rats were 80, 80, 85, 89 and 80 g, respectively.

Food Consumption

There was no difference in food consumption (g/week/rat) between the treated animals and their controls.

Ophthalmology

No treatment-related lesions were observed. The treatment-unrelated lesion, hyphema, was observed in two males, one from Group 1 and another from Group 5.

Hematology, Clinical Chemistry and Urinalysis

Except for the inhibition of plasma, erythrocyte and brain cholinesterase levels, the clinical laboratory studies were unremarkable.

Cholinesterase Levels in Brain, Erythrocytes and Plasma

Compared with the controls, statistically significant, exposure-related inhibition of plasma cholinesterase levels was observed in all treated groups of both sexes. Erythrocyte cholinesterase levels were also significantly decreased in all treated male groups and in female Groups 3, 4 and 5. Brain cholinesterase levels were significantly depressed in Groups 3, 4 and 5 of both sexes. These data are summarized below.

Cholinesterase Inhibition, Expressed as Percent Decrease Compared to Control Value (Group 1 Animals)[a]

Group	Diazinon (MG-8) (mg/m^3)	RBC	Plasma	Brain
		Males		
2	0.1	17.5*	30*	4.4
3	1.0	52.6*	50*	12.9*
4	10	75.4*	60*	37.1*
5	100	91.2*	80*	62.2*
		Females		
2	0.1	4.0	56.1*	5.7
3	1.0	44.7*	70.7*	15.1*
4	10	75.0*	75.6*	44.3*
5	100	92.9*	87.8*	79.8*

* $p \leq 0.05$

[a]See Attachment II for data on which these calculations were based. Cholinesterase levels were reported as umoles of enzyme/mL of RBC or plasma, or per gram of brain tissue.

It is not clear which procedure was used for the determination of cholinesterase in which tissue. The same six references/ procedures were listed under determination of cholinesterase in brain, erythrocytes and plasma (see Attachment III).

Gross Pathology

Treatment-related findings were not observed. Treatment-unrelated findings (generally single incidences) included dark areas in the adrenal cortex, lungs, stomach and cecum; dilated renal pelvis; enlargement of mesenteric lymph node; adhesion in thoracic cavity (one Group 2 female); and cyst in the kidney (one Group 5 female).

Organ Weights

Diazinon (MG-8) had no effect on absolute weights of organs when treated rats were compared with the controls.

The mean relative weight (organ to terminal body weight ratio) of lungs was increased for Group 2 (8.4%) and Group 5 (8.6%) females compared to control females. The mean relative kidney weight was increased for Group 5 females (8.8%) compared to control females. The mean relative liver weight was decreased for Group 2 (9.4%) and Group 3 (8.8%) males compared to control males, but was increased for Group 5 females (15%) compared to control females.

These changes in relative organ weights, although statistically significant ($p \leq 0.05$), were small, dose-unrelated and did not appear to be biologically significant.

Histopathology

No treatment-related microscopic lesions were reported by Experimental Pathology Laboratories (EPL), Inc. in male or female rats exposed to Diazinon (MG-8) at concentrations up to 100 mg/m^3. According to EPL, "Spontaneous disease lesions and incidental findings were for the most part minimal in severity and were the usual type commonly seen in Sprague-Dawley rats of this age. None of the spontaneous lesions seen in this study were of a severity or type that would have prevented the detection of compound-related changes."

The most common incidental lesions were: 1) periocular hemorrhage and plasmacytosis and congestion in the mandibular lymph node (all due to orbital sinus bleeding techniques); 2) presence of peribronchial and perivascular mononuclear cells in the lungs; and 3) focal accumulation of mononuclear and inflammatory cells and macrophages in the liver. The less frequently noted incidental lesions were focal pneumonitis, nonsuppurative myocarditis and mild nephropathy.

Comments

The 21-day inhalation toxicity study was well-planned, conducted in an acceptable manner (in most instances), and well-reported. However, because a NOEL was not determined and the study, therefore, does not meet the regulatory requirement, it was classified as Supplementary. As far as the execution of the study is concerned, a nose-only exposure method would obviously have been preferable. With whole-body exposure, there is always a possibility of ingestion during grooming. It should be noted that one of the treatment-related clinical signs, reported in the range-finding inhalation studies, was "compound on the fur."

Although the no observable effect level (NOEL) was less than 0.1 mg/m^3 in this study, the testing laboratory regarded this concentration of Diazinon (MG-8), the lowest tested, as a no observable adverse effect level (NOAEL). According to the testing laboratory, 1) the inhibition of brain cholinesterase at the 0.1 mg/m^3 exposure level was insignificant; 2) the inhibition of RBC cholinesterase was marginal and was restricted to males; 3) the inhibition of plasma cholinesterase was not correlated with any other meaningful biological changes; and 4) a reduction in plasma cholinesterase per se is not currently considered an adverse effect.

Toxicology Branch/HED disagrees with point 4) of the above characterization of the NOAEL. Furthermore, brain cholinesterase

inhibition of 4.4 or 5.7 percent, although not statistically 007041
significant, is regarded as biologically significant.

Quality Assurance Statement, dated August 10, 1988, was submitted with this report.

Attachment I

Statistical Analyses

Absolute body weights, body weight gains (start to Week 3), terminal body weights, food consumption, clinical pathology data (except cell morphology), urine volumes and organ weight data for the treated groups were compared statistically to the data of the control animals. Statistical analyses were performed as diagrammed in Figure 3.

If variances of untransformed data were heterogenous, analyses were performed on transformed data to achieve variance homogeneity. When the series of transformations were not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed routinely at 5% two-tailed probability level.

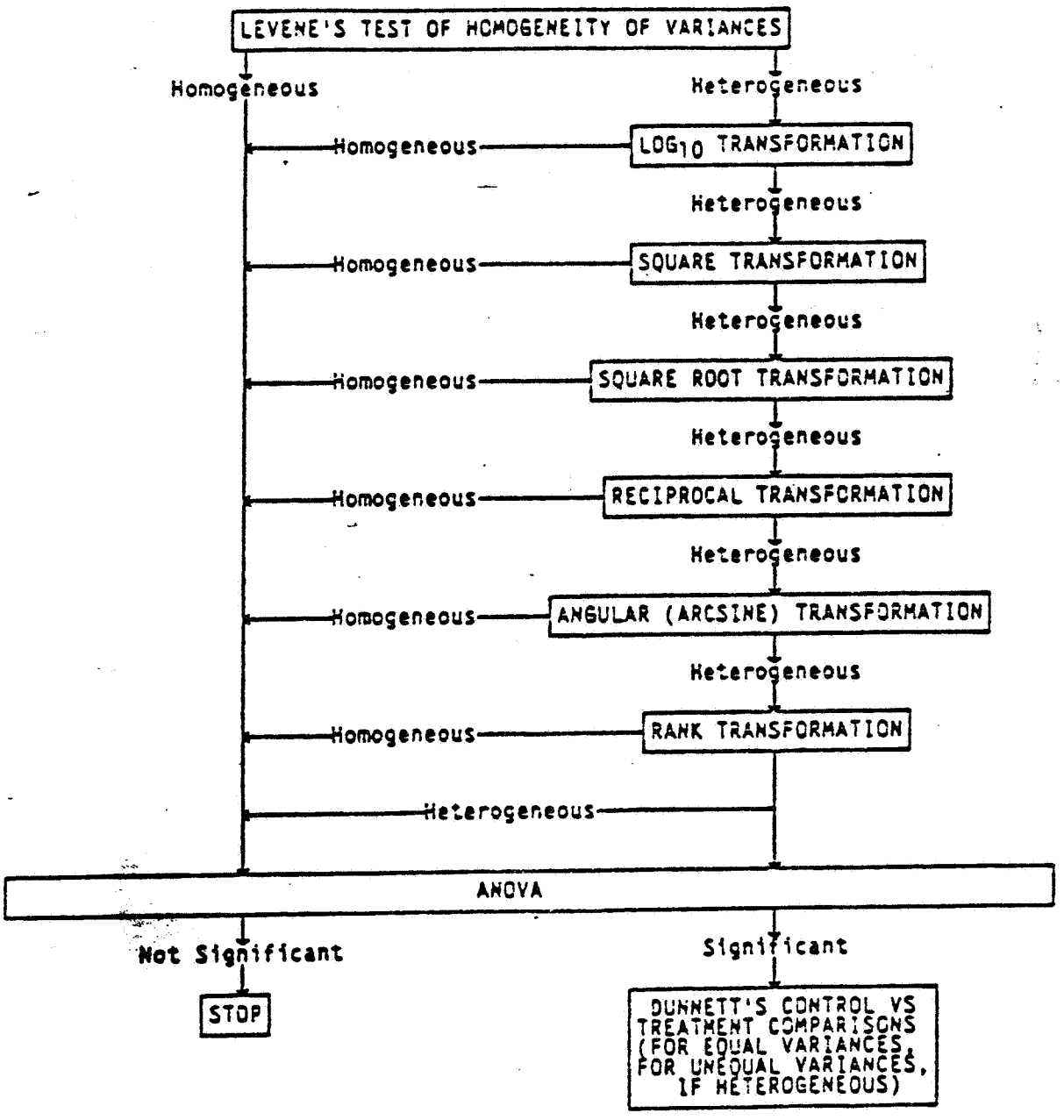
Statistical significance will be designated throughout the text of this report by the term "significant".

Asterisk (*) on mean tables indicates "significantly different from controls, $p \leq .05$".

Statistical procedures employed in the analysis of particle size data using the Andersen (Model 2000) Cascade Impactor were performed by a computerized particle size distribution analysis (PSDA) program, developed for Hazleton Laboratories America, Inc. by BeauLogics of Chesterfield, Missouri.

FIGURE 3

FLOWCHART OF ANOVA AND RELATED METHODS



Attachment II

TABLE 10 - CONTINUED
MEAN CLINICAL CHEMISTRY VALUES
21-DAY INHALATION TOXICITY STUDY WITH DIAZANON (MG-8) IN THE RAT

GROUP AND DOSAGE LEVEL		PL-CHE UMOL/ML WEEK -1	PL-CHE UMOL/ML WEEK 4[a]	RBC-CHE UMOL/ML WEEK -1	RBC-CHE UMOL/ML WEEK 4[b]	BR-CHE UMOL/G WEEK -1	BR-CHE UMOL/G WEEK 4[b]
							MALE
1	MEAN	2.2	2.0	6.4	5.7	60.6	52.9
0 MG/M3	S.D.	.28	.39	.45	.68	4.02	4.09
	N	10	10	9	10	10	10
2	MEAN		1.4 *		4.7*		50.6
0.1 MG/M3	S.D.		.30		.74		3.65
	N		10		10		10
3	MEAN		1.0*		2.7*		46.1*
1 MG/M3	S.D.		.09		.78		3.24
	N		10		10		10
4	MEAN		.8*		1.4*		33.3*
10 MG/M3	S.D.		.09		.34		2.46
	N		10		10		10
5	MEAN		.4*		.5*		20.0*
100 MG/M3	S.D.		.05		.06		1.94
	N		10		10		10
							FEMALE
1	MEAN	4.4	4.1	6.7	5.6	58.8	53.1
0 MG/M3	S.D.	1.13	.59	.63	.61	2.75	5.30
	N	10	10	10	10	10	10
2	MEAN		1.8*		5.4		50.1
0.1 MG/M3	S.D.		.43		.60		6.58
	N		10		10		10
3	MEAN		1.2*		3.1*		45.1*
1 MG/M3	S.D.		.22		.64		4.63
	N		10		10		10
4	MEAN		1.0*		1.4*		29.6*
10 MG/M3	S.D.		.13		.25		3.98
	N		10		10		10
5	MEAN		.5*		.4*		10.7*
100 MG/M3	S.D.		.08		.12		1.03
	N		10		10		10

[a]Analyses performed on rank-transformed data for the males and females.
[b]Analyses performed on Log10-transformed data for the males and females.

Attachment III

Brain Cholinesterase (BR-CHE) - AutoAnalyzer●

Erythrocyte Cholinesterase (RBC-CHE) - AutoAnalyzer●

Plasma Cholinesterase (PL-CHE) - AutoAnalyzer●

General operating instruction manual (1966). Section C-R. Technicon Instruments Corporation, Chaucey, NY.

LEVINE, J. B., SCHEIDT, R. A., AND NELSON, V. A. (1966). An automated micro determination of serum cholinesterase. Technicon Symposium 1965 Automation in Analytical Chemistry. Mediad Inc., New York, NY, pp. 582-585.

MERSMANN, H. J., AND SANGUINETTI, M. C. (1974). Automated determination of plasma and erythrocyte cholinesterase in various species. Am. J. Vet. Res. 35, 579.

TIETZ, N. (1976). Fundamentals of Clinical Chemistry, 2d ed. W. B. Saunders Co., Philadelphia, PA, p. 47.

WARD, F. P., AND HESS, T. H. (1969, May). An automated method with a micro-adaptation for cholinesterase assays in the dog. Department of the Army, Edgewood Arsenal, EATR 4279.

WARD, F. P., AND HESS, T. H. (1971, March). Automated cholinesterase measurement: canine erythrocytes and plasma. Am. J. Vet. Res. 32, 499-503.

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Secondary reviewer: Edwin R. Budd, Section Head
Section I, Tox. Branch I/IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 82-1. Subchronic Oral Toxicity (Rat): 6-Week Preliminary Study

TOX. CHEM. NO.: 342
MRID NO.: 408150-03

TEST MATERIAL: Diazinon (MG-8); Purity (content of a.i.): 87.7%; Lot No.: FL 872049

STUDY NUMBER(S): 88076 (MIN 872348)

SPONSOR: Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC

TESTING FACILITY: Division of Toxicology/Pathology, Ciba-Geigy Corporation, Summit, NJ

TITLE OF REPORT: Diazinon (MG-8): Pilot 6-Week Oral Feeding Study in Rats

AUTHOR(S): T. B. Barnes, J. R. Hazelette and A. T. Arthur

REPORT ISSUED: July 20, 1988

CONCLUSIONS:

NOEL: 0.5 ppm (0.04 mg/kg*, males and 0.05 mg/kg*, females)

LEL: 2 ppm (0.2 mg/kg*, males and females); inhibition of serum AChE activity.

*Reported values based on analytical data

CLASSIFICATION: Core-Supplementary (Preliminary study)

The purpose of this study was to determine the preliminary subchronic toxicity profile of Diazinon (MG-8) in order to project dose levels for a subsequent 90-day oral toxicity study.

Groups of 10 male and 10 female Sprague-Dawley rats received diets containing Diazinon MG-8 (0, 0.5, 2, 100 or 1000/2000/4000 ppm) for 6 consecutive weeks. Due to a lack of a significant effect on body weight, the high dose was increased from the initial 1000 ppm to 2000 ppm on day 15 and from 2000 ppm to 4000

ppm on day 36. Soft feces and decreased body weight gain and food consumption were the only treatment-related effects (other than AChE inhibition) observed in the high-dose male and female groups. Dose-related inhibition of serum acetylcholinesterase (AChE) activity occurred in all male and female groups, but the inhibitions in the low-dose group (0.5 ppm) were very small (4-9%) and statistically insignificant. Erythrocyte (RBC) and brain AChE activities were inhibited in the 100 ppm and 1000/2000/4000 ppm groups of both sexes. Histopathology was not performed and there was no mortality.

EXPERIMENTAL PROCEDURES

Dosing was initiated on October 12, 1987, and terminated on November 22, 1987. Necropsy took place on November 23, 1987.

Sprague-Dawley rats, 10/sex/group, received Diazinon (MG-8) in Powdered Certified Purina Rodent Chow #5002 at target concentrations of 0, 0.5, 2, 100 or 1000/2000/4000 ppm for 42 consecutive days. Due to a lack of a significant body weight effect, the high dose was increased from 1000 ppm to 2000 ppm on day 15 and from 2000 ppm to 4000 ppm on day 36. Food and water were provided ad libitum. Diets were prepared weekly, stored at room temperature and analyzed for homogeneity and Diazinon (MG-8) concentration. Dietary levels of Diazinon (MG-8) were adjusted for purity. Diazinon (MG-8) was stored at 2-8° C.

The rats were: 1) obtained from Charles River Laboratories, Kingston, NY; 2) acclimated for 2 weeks; 3) approximately 6 weeks old and weighing 180-185 g (males) and 142-146 g (females) at the initiation of the study; 4) assigned randomly to groups; 5) housed individually; and 6) identified with Monel ear tags.

The following parameters were examined for all rats on the study:

1. Observation for toxic signs and mortality: once or twice daily.

2. Body weight: on day 7 before dosing, just prior to dosing (day 0), weekly thereafter, and at the termination of the study.

3. Food consumptions: just prior to dosing (day 0) and weekly thereafter.

4. Physical/auditory examinations: on day 14 before dosing and on treatment day 29.

5. Acetylcholinesterase activity: in serum and RBC, on day 5 before dosing and on treatment days 14 and 24; in brain, at the termination of the study (day 42).

6. Necropsy

7. Absolute and relative (percent of terminal body weight) brain weight.

Parameters 2, 3, 5 and 7 were analyzed statistically by two-tailed Dunnett t test and Bartlett test for variability.

Histopathology was not performed.

RESULTS

Homogeneity and Concentration of Diazinon (MG-8) in Diets

Diets were checked for homogeneity during test weeks 1 and 6 by determining the concentration of Diazinon (MG-8) in the top, middle and bottom of the batches of feed. Analyses of multiple samples of test formulations indicated that homogeneity ranged from 92.2 to 103.2%, which was regarded as acceptable.

The mean analytical concentrations of Diazinon (MG-8) in diets administered to Groups 2 (0.5 ppm), 3 (2 ppm), 4 (100 ppm) and 5 (1000/2000/4000 ppm) were 92, 95, 99 and 103%, respectively, of the target concentrations.

Stability of Diazinon (MG-8) in diets was not reported.

Compound Intake

The mean daily intake of Diazinon (MG-8), calculated each week, was a follows:

Group	Diazinon MG-8 in Diets (ppm)*	Diazinon MG-8 (mg/kg b.w./day)** Males	Females
1	0	--	--
2	0.5	0.04	0.05
3	2.0	0.2	0.2
4	100	8.4	9.4
5	1000	105.1	114.9
	2000	152.7	194.2
	4000	236.6	284.9

*Target concentrations
**Based on analytical data

According to these data, females in Groups 4 and 5 ingested more Diazinon (MG-8) per kilogram of body weight than the males.

Clinical Signs and Mortality

There was no mortality. Soft feces (the only treatment-related clinical sign) were observed in most males and females from Groups 5 throughout most of the study. Other clinical signs noted during the treatment period included dehydration, lesions (not identified), nasal/ocular discharge and ocular abnormality (not identified). Due to a lack of a dose-response and/or isolated occurrences, these latter clinical signs were considered to be incidental in nature.

Physical/Auditory Examinations

Treatment-related changes were not observed.

Body Weight

Compared with the controls (Group 1), decreased body weight gains were observed in Group 5 males and females throughout the study. Although these decreases in weight gains were statistically significant ($0.01 < p \leq 0.05$), they were small (4-19% for males and 6-9% for the females) during the initial 35 days, when the rats received 1000 ppm (days 1-14) and 2000 ppm (days 15-35) of Diazinon (MG-8). At the termination of the study and after the rats received 4000 ppm of Diazinon (MG-8) for the last seven days, males and females in Group 5 gained, respectively, 36 and 18% less weight than did the controls, when the final body weights were compared with the initial body weights. The decreased weight gains of 36 and 18% were statistically significant at the $p \leq 0.01$ level.

Food Consumption

A statistically significant ($0.1 < p \leq 0.05$ or $p \leq 0.01$) reduction in mean food consumption occurred in Group 5 males on days 21 (10%) and 42 (22%), and in Group 5 females on day 42 (21%). This reduced food intake coincided with an increased dietary concentration of Diazinon (MG-8) in Group 5 from 1000 to 2000 ppm beginning on day 15 and again from 2000 to 4000 ppm on day 36. The food consumption of males and females in Groups 2, 3 and 4 was comparable to that of controls.

Acetylcholinesterase Activity

Compared with the controls, mean serum acetylcholinesterase (AChE) activity was significantly inhibited in the male Groups 4 and 5, and in the female Groups 3, 4 and 5 during both sampling intervals. Mean erythrocyte (RBC) AChE activity was

significantly decreased in the male and female Groups 4 and 5, also at both sampling intervals. Mean brain AChE activity was significantly reduced in the male Group 5 and in the female Groups 4 and 5. These data are summarized below.

Inhibition of AChE Activity

Group	Diazinon MG-8 (ppm)	Dosing Day	RBC Percent Inhibition	Serum Percent Inhibition	Brain Percent Inhibition
			Male Rats		
2	0.5	14	0	9.4	--
		24	0	4.2	--
		42	--	--	0
3	2	14	0	18.4	--
		24	0	17.9	--
		42	--	--	0
4	100	14	9.8**	76.4**	--
		24	20.7**	78.8**	--
		42	--	--	8
5	1000/2000/4000[a]	14	14.6**	92.4**	--
		24	20.3**	93.9**	--
		42	--	--	57.7**
			Female Rats		
2	0.5	14	0	5.2	--
		24	2.9	6.5	--
		42	--	--	0
3	2	14	0.6	31.6**	--
		24	8.8	35.8**	--
		42	--	--	0
4	100	14	16.1**	89.5**	--
		24	21.1**	88.9**	--
		42	--	--	24.1*
5	1000/2000/4000[a]	14	15.2**	93.3**	--
		24	24.5**	97.4**	--
		42	--	--	60.9**

*$0.01 < p \leq 0.05$ **$p \leq 0.01$

[a]In Group 5, Diazinon (MG-8) was fed as follows: 1000 ppm during days 1-14; 2000 ppm during days 15-35; and 4000 ppm during days 36-42.

The following procedure was referenced for the determination of

AChE activity:

Boehringer Mannheim Diagnostics, Inc.
BMD Reagent Set Cholinesterase. Boehringer Mannheim Diagnostics, Inc., Houston, TX, 1981 (Cholinesterase).

Brain Weight

Diazinon (MG-8), at all levels tested, had no effect on the mean absolute weights of the brain when the treated animals were compared with the controls. Statistically significant ($p \leq 0.01$) increases in the relative brain weights, observed in the Group 5 males (21.6%) and females (15%) were due to decreased body weights at the termination of the study in this group.

COMMENTS

1. There is an error on page 11 of the submitted report which should be corrected. The mean daily dose of Diazinon (MG-8) for Group 3 females was reported as 0.02 mg/kg, whereas 0.2 mg/kg is the correct value. Similarly, dose range for this group should be 0.2-0.2 mg/kg/day and not 0.02-0.02 mg/kg/day as reported.

2. There is an ambiguity on page 10 of the submitted report which requires clarification. The following was reported regarding cholinesterase determination: "Prior to initiation of treatment (test days -6, -5 and -4) and on test days 15, 16, 25 and 26, blood samples were obtained under ether anesthesia from the retro-orbital plexus for analysis of serum cholinesterase and RBC cholinesterase." However, serum and RBC acetylcholinesterase activities were reported for the pretest day 5 and the treatment days 14 and 24.

NOEL: 0.5 ppm (0.04 mg/kg*, males and 0.05 mg/kg*, females)

LEL: 2.0 ppm (0.2 mg/kg*), males and females; inhibition of serum AChE activity.

Classification of Study: Core-Supplementary (It is a preliminary study).

007041

Reviewed by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 12/7/89
Section I, Tox. Branch I/IRS (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section I, Tox. Branch I/IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 82-1. Subchronic Oral Toxicity (Rat): 90-Day Study

TOX. CHEM. NO.: 342
MRID NO.: 408150-03

TEST MATERIAL: Diazinon (MG-8); Purity (content of a.i.): 87.7%; Lot No.: FL 872049

STUDY NUMBER(S): 88083 (MIN 882011)

SPONSOR: Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC.

TESTING FACILITY: Division of Toxicology/Pathology, Ciba-Geigy Corporation, Summit, NJ.

TITLE OF REPORT: Diazinon (MG-8): 90-Day Oral Toxicity Study in Rats

AUTHOR(S): A. R. Singh

REPORT ISSUED: August 4, 1988

CONCLUSIONS:

The purpose of this study was to determine a 13-week toxicity profile of Diazinon (MG-8) in order to project dose levels for a subsequent chronic toxicity study in the rat.

Groups of 15 male and 15 female Sprague-Dawley rats received diets containing Diazinon MG-8 (0, 0.5, 5, 250 or 2500 ppm) for 13 consecutive weeks. According to the testing laboratory, these dose levels were equivalent to 0, 0.03, 0.3, 15 and 168 mg/kg of body weight for males in Groups 1, 2, 3, 4, and 5, respectively. The corresponding values for the females were 0, 0.04, 0.4, 19 and 212 mg/kg, respectively.

Treatment-related findings included inhibition of acetylcholinesterase (AChE) activities in serum (male and female Groups 3, 4 and 5), RBC (male Groups 4 and 5 and female Groups 3, 4 and 5) and brain (male and female Groups 4 and 5). The following treatment-related findings were observed only in the Group 5 rats: A) hypersensitivity to sound and touch, hyperactivity and aggressiveness (males and females); B) decreased body weight gains (males and females); C) decreased food consumption during dosing week 1 (males and females) and dosing week 2 (females); D) decreased hemoglobin and hematocrit, and increased WBC and reticulocytes (females); E) increased absolute and relative (to body weight) liver weight (females);

and F) hepatocellular hypertrophy (females). One control female died because of mechanical injuries, but there were no treatment-related deaths.

Based on decreases in body weight gains, a MTD for males and females was about 2500 ppm. Based on inhibition of AChE activities, the NOELs were as follows:

Male rats: 0.5 ppm (0.03 mg/kg)*; serum
5ppm (0.3 mg/kg)*; RBC and brain

Female rats: 0.5 ppm (0.04 mg/kg)*; serum and RBC
5 ppm (0.4 mg/kg)*; brain

*Reported values, based on analytical data.

CLASSIFICATION OF STUDY: Core-Guideline

EXPERIMENTAL PROCEDURES:

Dosing was initiated on January 8, 1988, and terminated on April 12, 1988. Necropsies were conducted on April 11 to 13, 1988.

Sprague-Dawley rats, 15/sex/group, received Diazinon (MG-8) in powdered Certified Purina Rodent Chow #5002 at target concentrations of 0, 0.5, 5, 250 or 2500 ppm for 13 consecutive weeks. Feed and tap water through an automatic delivery system were provided ad libitum. Diets were prepared weekly, stored at room temperature and analyzed for homogeneity and Diazinon (MG-8) concentration. Diazinon (MG-8) was added to diets by dissolving it in acetone, premixing with an appropriate amount of feed, evaporating acetone overnight and diluting the resulting premix with untreated feed to obtain the desired dose levels. The control diets were prepared in the same manner using acetone alone. The dose levels used were based on the results of a preliminary study (No. 88076/MIN 872348; see separate review) and the dietary levels were adjusted for purity of Diazinon (MG-8). Diazinon (MG-8) was stored at 2-8° C.

The rats were: 1) obtained from Charles River Laboratories, Kingston, NY; 2) acclimated for 3 weeks; 3) approximately 6 weeks old and weighing 181-248 g (males) and 139-195 g (females) at the initiation of dosing; 4) assigned randomly to groups; 5) housed individually at 73 ± 5° F, relative humidity of 50 ± 20%, and 12-hour light/dark cycle; and 6) identified with metal Monel ear tags.

The following parameters were examined for all rats on the study unless indicated otherwise:

1. Observation for toxic signs and mortality: once or twice daily.
2. Body weight: during predosing weeks 3 and 2, prior to dosing on day 1, weekly thereafter, and prior to sacrifice.
3. Food consumption: during predosing weeks 2 to 1 and weekly thereafter.
4. Physical/auditory examinations: during predosing week 3 and at the termination of the study.
5. Ophthalmoscopic examinations: during predosing week 3 and dosing week 12.
6. Water consumption and urine volume: during predosing week 1 and dosing week 12. Urine volumes and water consumption were collected over a 24-hour period. During collection, animals were maintained in metabolism cages and water was delivered via water bottles.
7. Clinical laboratory tests: hematology and biochemistry, during dosing week 13, and urinalysis (on 10 rats/sex/group) during dosing weeks 12 and 13. Clinical laboratory tests were also performed during week 1 before the initiation of dosing. The following determinations were performed:

Hematology

Differential Count (WBC)	Prothrombin Time
Heinz Bodies	RBC Count
Hematocrit	RBC Morphology
Hemoglobin	Reticulocyte Count
Platelets	WBC Count

Reticulocyte counts and Heinz Body determinations were performed on 10 animals in Groups 1 (control) and 5 (2500 ppm of Diazinon MG-8).

Serum Chemistry

Albumin	Globulin
A/G Ratio	Glucose
Alkaline Phosphatase	LDH
BUN	Phosphorous
Calcium	Potassium
Chloride	SGOT
Cholesterol	SGPT
Acetylcholinesterase (RBC, Serum, Brain)	Sodium
	Total Bilirubin
Creatinine	Total Protein
Gamma G-T	Triglycerides

Brain acetylcholinesterase activity was evaluated on samples collected at terminal necropsy.

Urinalysis

Bilirubin	Occult Blood
Glucose	pH
Ketones	Protein
Microscopic	Specific Gravity
Urobilinogen	Volume (24 hours)*

*Samples used for urine volume determinations were collected separately from samples used for the preceding measurements (parameter 6 above).

Procedures used for the clinical laboratory tests were referenced. The following reference was cited for the determination of acetylcholinesterase activities:

Boehringer Mannheim Diagnostics, Inc.
BMD Reagent Set Cholinesterase. Boehringer Mannheim Diagnostics, Inc., Houston, TX, 1981.

8. Necropsy: all animals were subjected to a full necropsy including a detailed examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. All surviving animals were fasted at least 12 hours prior to scheduled necropsy.
9. Organ weights: absolute and relative (organ/terminal body weight and organ/brain weight ratios). The following organs were weighed:

Adrenal glands	Pituitary
Brain (including brain stem)	Prostate
Epididymides	Salivary glands
Heart	Spleen
Kidneys	Testes
Liver	Thymus
Lungs	Thyroid with parathyroid glands
Ovaries	Uterus

After weighing, approximately one-half of the brain from each animal at terminal necropsy was used for the analysis of brain acetylcholinesterase activity and the remaining half for histopathological examination. The brain was maintained on dry-ice until it was submitted for acetylcholinesterase determination.

10. Histopathology: The following tissues from all rats were examined:

Adrenal glands
All gross lesions including tissue masses
Aorta
Brain (cerebral cortex, cerebellar cortex and medulla/pons)
Esophagus
Eyes with optic nerves
Female genital organs (ovaries, uterus, vagina)
Female mammary gland
Femur with marrow and joint
Heart
Kidneys
Lacrimal glands (exorbital)
Large intestine (cecum, colon, rectum)
Liver
Lungs
Lymph nodes (mesenteric, submaxillary)
Male genital organs (epididymides, prostate, seminal vesicles, testes)
Pancreas
Peripheral (sciatic) nerve
Pituitary
Salivary (submaxillary) glands
Skeletal (thigh) muscle
Skin (abdominal mammary region)
Small intestine (duodenum, jejunum, ileum)
Spinal cord (cervical, lumbar, thoracic)
Spleen
Sternum with marrow
Stomach (cardia, fundus, pylorus)
Thymic region
Thyroid with parathyroid glands
Tongue
Trachea
Urinary bladder

STATISTICAL EVALUATIONS

Body weight, food consumption, clinical laboratory, water intake and organ weight data were analyzed separately for each sex using two-tailed Dunnett t test and Bartlett test for variability. Histopathology data were analyzed separately for each sex using Fisher's exact test. (For details regarding statistical analysis, see Attachment I).

RESULTS

Homogeneity, Concentration and Stability of Diazinon (MG-8) in Diets

Diets were checked for homogeneity during test week 1 by determining the concentration of Diazinon (MG-8) in the top, middle and bottom of the batches of feed. Analyses of multiple samples of test formulations indicated that homogeneity ranged from 91.4 to 103.6%, which was regarded as acceptable.

All diets were analyzed for concentration of Diazinon (MG-8) during test weeks 1, 5, 9, and 13. The mean analytical concentrations of Diazinon (MG-8) in diets administered to Groups 2 (0.5 ppm) through 5 (2500 ppm) ranged from 91 to 109% of the target concentrations. The concentrations of Diazinon (MG-8) in the control samples (Group 1) was less than 0.02 ppm in all cases.

Diazinon (MG-8) was stable in diets for at least 20 days at room temperature.

Compound Intake

The mean daily intake of Diazinon (MG-8), calculated each week, was as follows:

Group	Diazinon MG-8 in Diets (ppm)*	Diazinon MG-8 (mg/kg b.w./day)**	
		Males	Females
2	0.5	0.03	0.04
3	5	0.3	0.4
4	250	15	19
5	2500	168	212

*Target concentrations **Based on analytical data

According to these data, female rats in each group (and especially those in Group 5) ingested more Diazinon (MG-8) per kilogram of body weight than did the males.

Mortality

There were no treatment-related mortalities during the dosing period; however, one female from the control group was sacrificed for humane reasons on day 89 due to a mechanical injury.

Clinical Signs

Treatment-related clinical signs were observed only in Group 5 and included 1) soft feces in 10 males and 15 females

during the entire study; 2) hypersensitivity to touch and sound in 12 males and 15 females during test weeks 3-14; and 3) aggressiveness in 3 males during test weeks 4-9. Other clinical signs (alopecia, hunched posture and ocular or nasal discharge) were either single incidences or occurred in a dose-unrelated manner, and were regarded as incidental or spontaneous in nature.

Physical/Auditory Examination

These examinations included observations for abnormal discharges or exudates from body orifices, character of haircoat, posture, and hearing tests (reaction to a sudden handclap).

The only treat-related finding noted at the termination of dosing was hyperactivity in 3 males and 3 females from Group 5.

Ophthalmoscopic Examinations

Treat-related ocular changes were not observed. Corneal opacity in one Group 4 female, the only finding noted during test week 12, was not attributed to treatment.

Body Weight

Compared with the controls (Group 1), decreased body weight gains were observed in Group 5 males and females throughout the study. The decreased body weight gains were statistically significant during the initial six or seven weeks and statistically insignificant thereafter, until the termination of the study. These data are summarized below.

Dosing Week	Percent Decreases in Mean Body Weight Gains for Group 5 (2500 ppm) Rats[a]	
	Males	Females
1	15.5**	4.0** (Weight loss)
2	18.6**	22.4**
3	18.1**	22.1**
4	16.8**	18.9**
5	14.9**	17.6**
6	14.7*	16.2**
7	12.7	14.7**
10	9.4	10.0
13	5.6	7.8

[a]Calculated as follows: Reported weekly percent body weight gain for the control group minus reported weekly percent body weight gain for the Group 5 rats.

*$0.01 < p \leq 0.05$ **$p \leq 0.01$

Body weight gains of the male and female rats in Groups 2, 3 and 4 were comparable to those of the controls(Attachment II).

Food Consumption

Compared with the controls, a statistically significant ($p \leq 0.01$) reduction in mean food consumption occurred only in Group 5, in males during dosing week 1 (17.2%) and in females during dosing weeks 1 and 2 (30.5 and 13.0%, respectively). At other times, the mean food consumption of the rats in Group 5, reported as grams of feed/week/rat, was similar to or greater than that in the control group. The mean food consumption of the rats in Groups 2, 3 and 4 was similar to that of controls.

Water Consumption

Diazinon (MG-8), at all levels tested, had no effect on mean water consumption determined on dosing day 84.

Hematology

Treatment-related statistically significant changes were observed only in Group 5 females and included reductions in mean hemoglobin (14%**) and hematocrit (39.3%** vs. 42.5% in controls), and increases in WBC (35%*) and reticulocytes (2.41% vs. 0.71% in controls).

*$0.01 < p \leq 0.05$ ** $p \leq 0.01$

Clinical Biochemistry

Compared with the controls, statistically significant changes were observed mostly in the Group 5 rats as follows: 1) an increase in SGPT activity (19.5%*) in the males and a decrease in SGPT activity (46.1%**) in the females; 2) a decrease in serum cholesterol (18.1%*) in the males; and 3) a decrease in sodium (1.1%**) and chloride (3.4%*) and an increase in inorganic phosphorous (31%**), all in the females. Statistically significant decreases in SGPT activity were also observed in the females from Group 2 (38.5%*) and Group 4 (35.8%*). However, considering the small magnitude of changes in some parameters, lack of a dose-response relationship and/or the finding that most of the individual values were within the concurrent control range, it appears that the above changes in biochemical parameters were treatment-unrelated.

*$0.01 < p \leq 0.05$ **$p \leq 0.01$

Acetylcholinesterase Activity

Compared with the controls, serum mean acetylcholinesterase (AChE) activity was significantly inhibited in male and female Groups 3, 4 and 5. Erythrocyte (RBC) mean AChE activity was significantly decreased in male Groups 4 and 5, and female Groups 3, 4 and 5. Statistically significant inhibitions in brain mean AChE activity was observed in male Group 5 and female Groups 4 and 5. These data are summarized below.

Inhibition of AChE Activity on Dosing Day 87[a]

Group	Diazinon (MG-8) (ppm)	RBC	Serum	Brain
		Percent Inhibition in Male Rats		
2	0.5	0	0	0
3	5	4.5	26.3**	0
4	250	27.1**	88.4**	3.6
5	2500	26.4**	96.6**	49.1**
		Percent Inhibition in Female Rats		
2	0.5	3.8	12.2	0
3	5	16.8**	78.1**	0
4	250	41.2**	97.0**	40.8**
5	2500	41.5**	98.5**	57.0**

[a]RBC and serum AChE activities were determined only once during the course of this study. Brain AChE activity was evaluated on samples collected at terminal necropsy.

**$p \leq 0.01$

All AChE activities were reported only as mu/mL.

Urinalysis

Treatment-related changes in urinalysis parameters were not observed. Specific gravities and urine volumes were reported as both individual and group mean values, and were statistically analyzed. Other parameters examined were reported only as individual values and were not statistically analyzed.

Necropsy

Gross findings were reported only as individual data. Treatment-related abnormalities were not observed.

Organ Weights

A statistically significant, treatment-related increase

in the mean absolute and relative (to body weight) liver weight was observed in females at the 2500 ppm dose level. Relative to control values, the mean absolute liver weight was increased by 17.9% ($0.01 < p \leq 0.05$) and the mean relative liver weight was increased by 25.4% ($p \leq 0.01$). This effect correlated with microscopic evidence of hepatocellular hypertrophy in the majority of females at this dose level.

A statistically significant ($0.01 < p \leq 0.05$) increase (13%) in the mean relative (to body weight) kidney weight in females at the 2500 ppm dose level was considered incidental, based on the lack of a dose-response relationship and the absence of a similar change in the mean absolute kidney weights.

Histopathology

Treatment-related hepatocellular hypertrophy was observed in 3/15 (20%) females at the 250 ppm dose level and in 13/15 (86.7%; $p < 0.001$) females at the 2500 ppm dose level. This change in hepatocellular morphology, which correlated with an increase in the mean absolute and relative liver weights in the 2500 ppm females, was regarded by the Ciba-Geigy pathologists as an adaptive physiological response. However, this adaptive physiological response was not observed in the 2500 ppm males. No other apparent treatment-related microscopic changes were noted. Neoplastic changes were not observed.

COMMENTS

This study is well planned and generally well reported. However, there is one error in the clinical biochemistry results. It was reported on page 25 of the submission that serum potassium was elevated in the 2500 ppm females. Yet, data on page 130 clearly indicate that inorganic phosphorous, and not potassium, was elevated in that group.

Ciba-Geigy (testing laboratory) considered the maximum tolerated dose (MTD) to be between 250 and 2500 ppm, based on decreased body weight gains in both sexes. The no-observable-effect level (NOEL), according to Ciba-Geigy, was 0.5 ppm, based on reductions in AChE activities. Specifically, the NOELs were as follows:

Male rats: 0.5 ppm (Serum)
5 ppm (RBC and brain)

Female rats: 0.5 ppm (Serum and RBC)
5ppm (Brain)

Classification of Study: Core-Guideline

Quality Assurance Statement, showing eight inspections/audits between December 30, 1987, and August 3, 1988, signed on August 4, 1988, was included in the report.

007041

Reviewed by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 12/01/88
Section I, Tox. Branch I/IRS (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section I, Tox. Branch I/IRS (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 82-1. Subchronic Oral Toxicity (Dog): 4-Week Preliminary Study

TOX. CHEM. NO.: 342

MRID NO.: 408150-04

TEST MATERIAL: Diazinon (MG-8);
Purity (content of a.i.): 87.7%
Lot No.: FL-872049

STUDY NUMBER(S): 88077 (MIN 872349)

SPONSOR: Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC.

TESTING FACILITY: Division of Toxicology/Pathology, Ciba-Geigy Corporation, Summit, NJ.

TITLE OF REPORT: Diazinon (MG-8): Pilot 4-Week Oral Feeding Study in Dogs

AUTHOR(S): A. R. Singh, G. C. McCormick and A. T. Arthur

REPORT ISSUED: August 1, 1988

CONCLUSIONS:

NOEL: 0.5 ppm (0.02 mg/kg*; LTD); males

NOEL: < 0.5 ppm; females (Inhibition of serum AChE activity)

*Reported value based on analytical data

CLASSIFICATION: Core-Supplementary (Preliminary study)

The purpose of this study was to determine the preliminary subchronic toxicity profile of Diazinon (MG-8) in order to project dose levels for a subsequent 90-day oral toxicity study.

Groups of 4 male and 4 female beagle dogs received diets (400 g/day) containing Diazinon (MG-8) (0, 0.5, 2, 20 or 500 ppm) for four consecutive weeks. Emesis, weight loss and decreased food consumption were observed in the 500 ppm male and female groups. Dose-related significant inhibition of serum

acetylcholinesterase (AChE) activity occurred in all female groups and in all but the 0.5 ppm male groups. Erythrocyte and brain AChE activities were inhibited significantly only in the 500 ppm male and female groups. Neurological symptoms, generally associated with brain AChE inhibition, were not observed. Nothing remarkable was noted at necropsy. Histopathology was not performed.

EXPERIMENTAL PROCEDURES

Dosing was initiated on October 5, 1987, and terminated on November 3, 1987.

Beagle dogs (4/sex/group) received Diazinon (MG-8) in Certified Powdered Laboratory Purina Canine Diet #5007 at target concentrations of 0, 0.5, 2, 20 or 500 ppm for 4 consecutive weeks. Each animal received approximately 400 g of feed for about 3 hours each morning. Water was provided ad libitum through an automatic watering system. Diets were prepared weekly, stored at room temperature and analyzed for homogeneity and Diazinon (MG-8) concentration. Dietary levels of Diazinon (MG-8) were adjusted for purity. Diazinon (MG-8) was stored at 2-8° C.

The dogs were: 1) obtained from Marshall Farms, North Rose, NY; 2) acclimated for 2 weeks; 3) approximately 6 months old and weighing 6.7-8.1 kg (males) and 6.4-7.9 kg (females) at the initiation of dosing; 4) housed individually; and 5) identified by numbers tattooed on the ears. Nothing was said about assignment of animals to groups.

The following parameters were examined for all dogs on the study:

1. Observations for toxic signs: twice daily and once on weekends.
2. Body weight: on day 8 before dosing, just prior to dosing on day 1, weekly thereafter, and at the termination of the study (test days 30 or 31).
3. Food consumption: determined weekly.
4. Acetylcholinesterase activity: in serum and RBC, two weeks before dosing and on dosing days 14 and 25; in brain, at the termination of the study.
5. Necropsy
6. Absolute and relative (percent of terminal body weight) brain weight.

Parameters 2, 3, 4 and 6 were analyzed statistically by two-tailed Dunnett t test and Bartlett test for variability.

Histopathology was not performed.

RESULTS

Homogeneity, Concentration and Stability of Diazinon (MG-8) in Diets

Diets were checked for homogeneity during the first test week by determining the concentration of Diazinon (MG-8) in the top, middle and bottom of the batches of feed. Analyses of multiple samples of test formulations indicated that homogeneity ranged from 90.6 to 98.6%, which was regarded as acceptable.

The mean analytical concentrations of Diazinon (MG-8) in diets administered to Groups 2 (0.5 ppm), 3 (2 ppm), 4 (20 ppm), and 5 (500 ppm) were 90, 97, 95 and 99%, respectively, of the target concentrations.

Diazinon (MG-8) was stable in diets for 27 days at room temperature.

Compound Intake

The mean daily intake of Diazinon (MG-8), calculated each week, was as follows:

	Diazinon (MG-8) (mg/kg b.w./day)*	
Diazinon (MG-8) in Diets (ppm)**	Males	Females
0.5	0.020	0.023
2.0	0.073	0.082
20	0.80	0.875
500	14.68	15.99

According to these data, males and females in each group ingested similar amounts of the test material.

*Based on analytical data
**Target concentrations

Clinical Signs

Treatment-related signs were observed only in the 500 ppm groups and included emesis (in 3 males and 4 females) and cachexia/emaciation in one female. There was no mortality.

Body Weight

Weight losses were observed at 500 ppm in both sexes throughout the study. On treatment days 21 and 28, males in this group weighed 4.0 and 6.2%, respectively, less than they did at the initiation of treatment. The corresponding weight losses for the females were 6.6 and 8.4%, respectively. All of these weight

losses, except 6.6%, were statistically significant ($0.01 < p \leq 0.05$).

Food Consumption

Decreased food consumption was observed at 500 ppm in both sexes throughout the study. During treatment weeks 2, 3, and 4, males consumed 17.7, 33.8** and 36.4**% less food, respectively, than did the controls. The corresponding decreases in food consumption for the females were 26.4*, 41.1** and 39.2**%, respectively. (* $0.01 < p \leq 0.05$; ** $p \leq 0.01$)

Acetylcholinesterase Activity

Compared with the controls, serum acetylcholinesterase (AChE) activity was significantly inhibited in the males in Groups 3, 4 and 5, and in all female groups. Erythrocyte and brain AChE activities were significantly inhibited only in Group 5. These data are summarized below.

Inhibition of AChE Activity

Group	Diazinon (MG-8) (ppm)	Dosing Day	RBC Percent Inhibition	Serum	Brain
					in Male Dogs
2	0.5	14	0	8.8	
		25	0.8	8.4	0
3	2	14	10.6	40.9**	
		25	11.3*	38.5**	2.7
4	20	14	9.7	69.5**	
		25	6.5	64.6**	1.3
5	500	14	38.8**	80.8**	
		25	29.8**	80.5**	44.4**
					in Female Dogs
2	0.5	14	5.3	29.6**	
		25	7.6	28.3**	4.1
3	2	14	1.8	47.7**	
		25	8.4	48.7**	3.8
4	20	14	0	69.8**	
		25	10.9	67.3**	9.9
5	500	14	25.7**	80.0**	
		25	37.8**	81.5**	50.4**

* $0.01 < p \leq 0.05$ ** $p \leq 0.01$

It was not reported what procedure was used for the determination of AChE activity and AChE activity was reported only as mu/mL. Although reported for dosing day 25, brain AChE activity was determined only at the termination of the study.

Necropsy

There were no treatment-related findings.

Brain Weight

There were no statistically significant differences in the mean absolute or relative (percent of terminal body weight) weights of the brain when the Diazinon (MG-8)-treated dogs were compared with the controls.

Comments

Although brain AChE activity was inhibited 44% in the high-dose (500 ppm) males and 50% in the high-dose females, the animals had no neurological symptoms.

Based on the results of this study, the maximum tolerated dose (MTD) was considered by the testing laboratory to be between 20 and 500 ppm. Toxicology Branch I/IRS agrees with this conclusion.

Based on the results of this study, "the essential no-observable-effect level (NOEL)," according to the testing laboratory, was 0.5 ppm. The term "essential NOEL" was somewhat clarified in the attachment entitled TOXICOLOGICAL ASSESSMENT OF DIAZINON; C. Breckenridge; Ciba-Geigy Corporation; August 29, 1988. (MRID 408150-01). The following statement was made on page 10 of the attachment: "The no observable effect level was less than 0.5 ppm although the changes noted at 0.5 ppm, were not considered biologically significant, adverse effects of treatment."

Toxicology Branch I/IRS concluded that a NOEL for the male dogs was 0.5 ppm, but it was < 0.5 ppm for the female dogs (because of significant inhibition of serum AChE activity at this level).

Classification of study: Core-Supplementary (It was a preliminary study)

007041

Reviewed by: Krystyna K. Locke, Toxicologist Krystyna K. Locke 2/7/89
Section I, Tox. Branch I/IRS (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section I, Tox. Branch I/IRS (TS-769C)

Budd 2/15/89

DATA EVALUATION REPORT

STUDY TYPE: 82-1. Subchronic Oral Toxicity (Dog): 90-Day Study

TOX. CHEM. NO.: 342

MRID NO.: 408150-04

TEST MATERIAL: Diazinon (MG-8); Purity (content of a.i.): 87.7%; Lot No.: FL:872049

STUDY NUMBER(S): 88072 (MIN 882012)

SPONSOR: Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC

TESTING FACILITY: Division of Toxicology/Pathology, Ciba-Geigy Corporation, Summit, NJ

TITLE OF REPORT: Diazinon (MG-8): 90-Day Oral Toxicity Study in Dogs

AUTHOR(S): T. B. Barnes

REPORT ISSUED: August 4, 1988

CONCLUSIONS:

NOEL: 0.1 ppm (0.0034 mg/kg* for males and 0.0037 mg/kg*for females), based on the inhibition of acetyl-cholinesterase (AChE) activity in serum.

LEL: 0.5 ppm (0.020 mg/kg* for males and 0.021 mg/kg* for females); inhibition of serum AChE activity in both sexes.

*Reported values based on analytical data

CLASSIFICATION: Core-Supplementary, because of errors and ambiguities detailed in the review under COMMENTS. This classification will be upgraded to Core-Guideline upon receipt and acceptance by Toxicology Branch of the requested data.

Groups of 4 male and 4 female beagle dogs received diets (400 g/day) containing Diazinon (MG-8) (0, 0.1, 0.5, 150 or 300 ppm)

for 13 consecutive weeks (92-94 days). According to the testing laboratory, these dose levels were equivalent to 0, 0.0034, 0.02, 5.9 and 10.9 mg/kg of body weight for males in Groups 1, 2, 3, 4 and 5, respectively. The corresponding values for the females were 0, 0.0037, 0.021, 5.6 and 11.6 mg/kg, respectively.

Treatment-related effects included inhibition of serum AChE activities in Groups 3, 4, and 5, and of erythrocyte (RBC) and brain AChE activities in Groups 4 and 5; emesis/diarrhea, decreased weight gain, mean total protein and calcium levels in Groups 4 and 5; and pancreatic acinar atrophy in one Group 5 male. There were no deaths during the study. Neurological symptoms, generally associated with brain AChE inhibition, were not observed.

EXPERIMENTAL PROCEDURES

Dosing was initiated on January 26, 1988, and the study terminated on April 29, 1988. Necropsies were performed on April 27, 28 and 29, 1988.

Beagle dogs (4/sex/group) received Diazinon (MG-8) in Certified Powdered Laboratory Purina Canine Diet #5007 at target concentrations of 0, 0.1, 0.5, 150 or 300 ppm for 13 consecutive weeks. Each animal received approximately 400 g of feed for about 3 hours each morning. Water was provided ad libitum through an automatic watering system. Diets were prepared weekly, stored at room temperature and analyzed for homogeneity and Diazinon (MG-8) concentration. The dose levels used were based on the results of a preliminary study (No. 88077/MIN 872349; see separate review) and the dietary levels were adjusted for purity. Diazinon (MG-8) was stored a 2-8° C.

The dogs were: 1) obtained from Laboratory Research Enterprises, Inc., Kalamazoo, MI; 2) acclimated for 6 weeks; 3) approximately 6 months old and weighing 6.5-9.9 kg (males) and 6.1-9.3 kg (females); 4) assigned randomly to groups through a computerized system based on body weight; 5) housed individually at 69±5° F, relative humidity of 50±20%, and 12-hour light/dark cycle; and 6) identified by ear tattoos.

The following parameters were examined for all dogs on the study:

1. Daily observations for toxic signs and mortality.
2. Body weight: at predose week 2, prior to dosing during week 1, and weekly thereafter.
3. Food consumption: determined weekly.
4. Physical/auditory examinations: at predose week 3 and at the termination of the study.
5. Ophthalmoscopic examinations: at predose week 3 and at

the termination of the study.

6. Hematology, clinical biochemistry and urinalysis: on fasted animals at predose week 2, during test weeks 5 and 9, and at the termination of the study.

The following determinations were performed:

HEMATOLOGY

Clotting time
Differential Count (WBC)
Heinz Bodies
Hematocrit
Hemoglobin
Platelets
Prothrombin Time
RBC Count
RBC Morphology
Reticulocyte Count
WBC Count

Reticulocyte counts and Heinz body determinations were performed for all dogs during the pretreatment period and on control and high-dose (300 ppm) dogs during the treatment period.

SERUM CHEMISTRY

Albumin
A/G Ratio
Alkaline Phosphatase
BUN
Calcium
Chloride
Cholesterol
Acetylcholinesterase (RBC, Serum, Brain)
CPK
Creatinine
Gamma G-T
Globulin
Glucose
LDH
Phosphorous
Potassium
SGOT
SGPT
Sodium
Total Bilirubin
Total Protein
Triglycerides

Brain acetylcholinesterase activity was determined only at the termination of the study.

URINALYSIS

Bilirubin
Clarity
Color
Glucose
Ketones
Microscopic
Occult Blood
pH
Protein
Specific Gravity
Urobilinogen

Urine was collected by catheterization or by collection with a plastic liner. At necropsy, urine samples were obtained with a syringe by needle puncture of the urinary bladder when necessary.

Procedures used for the clinical laboratory tests were

referenced. The following reference was cited for the determination of acetylcholinesterase activities: Boehringer Mannheim Diagnostics, Inc. BMD Reagent Set Cholinesterase. Boehringer Mannheim Diagnostics, Inc., Houston, TX, 1981.

7. Necropsy: All animals were subjected to a full necropsy including a detailed examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. All animals were fasted at least 12 hours prior to scheduled necropsy.

8. Organ weights: Absolute and relative (organ/terminal body weight and organ/brain weight ratios) weights were determined. The following organs were weighed:

adrenal glands
brain (including brain stem)
epididymides
heart
kidneys
liver
lungs
ovaries
uterus
pituitary
prostate
salivary (mandibular) glands
spleen
testes
thymus
thyroid with parathyroid glands

After weighing at terminal necropsy, approximately one-half of the brain from each animal was utilized for the analysis of brain acetylcholinesterase activity and the remaining half for histopathological examination.

9. Histopathology. The following tissues were examined:

adrenal gland
all gross lesions including tissue masses
aorta
brain (cerebral cortex, cerebellar cortex, medulla/pons)
esophagus
eyes with optic nerves
female genital organs (ovaries, uterus, vagina)
female mammary gland
femur with marrow and articular surface
gallbladder
heart
kidneys
lacrimal glands
large intestine (cecum, colon, rectum)
liver
lung
lymph nodes (medial retropharyngeal, mesenteric)

male genital organs (epididymides, prostate, testes)
pancreas
peripheral (sciatic) nerve
pituitary
salivary (mandibular) glands
skeletal (thigh) muscle
skin (abdominal mammary region)
small intestine (duodenum, jejunum, ileum)
spinal cord (cervical, lumbar, thoracic)
spleen
sternum with marrow
stomach
thymic region
thyroid with parathyroid glands
tongue
trachea
urinary bladder

STATISTICAL EVALUATIONS

Body weight, food consumption, clinical laboratory and organ weight data were analyzed separately for each sex as detailed in Attachment I. Histopathology data were analyzed separately for each sex using Fisher's exact test.

RESULTS

Homogeneity, Concentration and Stability of Diazinon (MB-8) in Diets

Diets were checked for homogeneity during test weeks 1, 3 and 5 by determining the concentration of Diazinon (MG-8) in the top, middle and bottom of the batches of feed. Analyses of multiple samples of test formulations indicated that homogeneity, in most cases, ranged from 95.0 to 98.7%, which was regarded as acceptable.

The mean analytical concentrations of Diazinon (MG-8) in diets administered to Groups 2 (0.1 ppm) through 5 (300 ppm) ranged from 94 to 107% of the target concentrations. Only in one instance (Group 2, week 1) the analytical concentration of Diazinon (MG-8) was 129% of the target concentration or outside of the limits specified in the protocol. Diets were analyzed for Diazinon (MG-8) concentrations during test weeks 1, 5, 9 and 13, and, for Group 2, also during week 3.

Diazinon (MG-8) was stable in diets for 27 days at room temperature.

Compound Intake

The mean daily intake of Diazinon (MG-8), calculated each week,

was as follows:

Diazinon (MG-8) in Diets (ppm)*	Diazinon (MG-8) (mg/kg b.w./day)** Males	Females
0.1	0.0034	0.0037
0.5	0.020	0.021
150	5.9	5.6
300	10.9	11.6

*Target concentrations **Based on analytical data

According to these data, males and females in each group ingested similar amounts of the test material.

Clinical Signs

Toxic signs were observed occasionally in one or two high-dose (300 ppm) males and one mid-dose (150 ppm) female and included emesis, emesis with food, bloody feces and/or diarrhea. There was no mortality.

Physical/Auditory Examinations

These included auscultation of the thorax, palpation of the abdomen and determination of heart rate and rectal temperature. There were no adverse effects attributable to Diazinon (MG-8).

Ophthalmoscopic Examinations

Treatment-related ocular changes were not observed. The ocular changes observed ("cherry eye" in one control female and one 0.5 ppm male, and corneal opacity in one 150 ppm female) were considered incidental changes frequently observed in dogs.

Body Weight

Treatment-related reduction in mean percent body weight gain was noted throughout most of the treatment period in Group 5 (300 ppm) males and Group 4 (150 ppm) and Group 5 (300 ppm) females. These differences in mean percent weight gain were not statistically significant, although at the termination of the study Group 5 males and Group 4 and 5 females weighed, respectively, 33, 34, and 45% less than did the controls. Dogs in Groups 2 (0.1 ppm), 3 (0.5 ppm) and 4 (150 ppm; males only) gained more weight than did the controls during the course of the study.

Food Consumption

These data were reported as weekly consumption/animal and as group mean consumption (grams/week) for 13 weeks, for each sex.

Food consumption varied considerably within each group including the controls. According to the testing laboratory, slight

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reductions in mean food consumption values were noted in both sexes of Group 5 (300 ppm) during the treatment period. Actually, compared with the controls, males consumed less food (10-17%) only during weeks 2, 3, 4, 5 and 7. Compared with the controls, females consumed less food (4-16%) only during weeks 2, 3, 4, 5, 6, 8, 10 and 12. During the remaining weeks, dogs in Group 5 consumed more food than did those in Group 2 (0.1 ppm) or Group 3 (0.5 ppm). All of the differences in food consumption were statistically insignificant and were considered by the testing laboratory to represent normal variation within this species.

Hematology

Treatment-related changes in hematological parameters were not observed in any group during the study.

Clinical Biochemistry

Statistically significant findings that appeared to be treatment-related included:

1. Decreased mean total protein levels in Group 4 (1.3* or 1.4%**) and Group 5 (8-15%**) males on days 29, 56 and 86.

2. Decreased mean calcium levels in Group 4 females (4.8 or 5.0%* and Group 5 males (4.8* or 6.2%**) and females (5.1%*) on days 29 and/or 56.

*$0.01 < p \leq 0.05$ **$p \leq 0.01$

Acetylcholinesterase Activity

Compared with the controls, serum acetylcholinesterase (AChE) activity was significantly inhibited in male Groups 3, 4 and 5, and in female Groups 4 and 5. Erythrocyte and brain AChE activities were significantly inhibited in male and female Groups 4 and 5. These data are summarized on the next page.

Inhibition of AChE Activity

Group	Diazinon (MG-8) (ppm)	Dosing Day	RBC	Serum	Brain[a]
			Percent Inhibition in Male Dogs		
2	0.1	29	1.6	21.1	
		56	5.7	19.9	
		86	0	17.8	4.1
3	0.5	29	4.0	28.8*	
		56	8.2	26.6	
		86	17.8	30.2*	0
4	150	29	26.2**	80.4**	
		56	25.4**	77.7**	
		86	24.6**	80.4**	30.7**
5	300	29	33.3**	80.2**	
		56	27.9**	74.9**	
		86	31.4**	83.4**	42.2**
			Percent Inhibition in Female Dogs		
2	0.1	29	0	0	
		56	0	0	
		86	0	0	0
3	0.5	29	7.3	16.8	
		56	0	15.8	
		86	4.1	14.6	0
4	150	29	30.9**	78.8**	
		56	30.9**	76.9**	
		86	30.9**	81.4**	29.9**
5	300	29	33.3**	79.5**	
		56	37.4**	78.8**	
		86	30.9**	83.4**	45.1**

[a]Brain AChE activity was determined on dosing day 92.

*$0.01 < p \leq 0.05$ **$p \leq 0.01$

All AChE activities were reported only as mu/mL.

Urinalysis

Treatment-related changes in urinalysis parameters were not observed in any group during the study.

Organ Weights

No treatment-related changes in absolute and relative organ weights were observed.

Necropsy

Gross pathology findings were reported in the pathology report concerned with individual data and were not attributed to treatment with Diazinon (MG-8). According to the Ciba-Geigy pathologists who examined the tissues (Donald N. McMartin, D.V.M., Ph.D. and William O. Iverson, D.V.M., both Diplomates of the American College of Veterinary Pathologists), the gross lesions observed were spontaneous and/or incidental, and of the types frequently encountered in beagle dogs. These lesions are summarized below.

Incidence of Gross Lesions[a]

Diazinon (MG-8) (ppm)	0		0.1		0.5		150		300	
	M	F	M	F	M	F	M	F	M	F
Right thyroid smaller than normal	1									1*
Dark, red or tan lesions in the lungs	1*							2		2*
Multiple red lesions in urinary bladder	1*								1	1*
Thickened urinary bladder full of red fluid										1*
Enlarged prostate					1					
Red and enlarged iliac, popliteal or sublumbar lymph nodes					1				1	

[a]Number of dogs with lesions. All dogs (4/sex/group) were examined.
*All of these lesions were observed in the same dog. In the case of the high-dose (300 ppm) females, one dog had lung lesions only and the second dog had lung and other lesions.

Histopathology

The only lesion attributed to treatment was atrophy of the pancreatic acini observed in one Group 5 male dog. According to the testing laboratory, Diazinon has been reported to produce pancreatic acinar lesions in dogs at high doses (Frick et al., 1987)*. All urinary bladder lesions were considered to be secondary to catheterization. Other lesions observed were regarded as spontaneous and/or incidental, and of the type frequently encountered in beagle dogs. These lesions are summarized below.

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*Frick, T. W., Dalo, S., O'Leary, J. F., Runge, W., Borner, J. W., et al. Effects of Insecticide, Diazinon, on pancreas of dog, cat and guinea pig. J. Environ. Path. Toxicol. Oncol. 7:1-12, 1987.

Incidence of Microscopic Lesions in Male and Female Dogs Treated with Diazinon (MG-8) for 13 Weeks[a]

Diazinon (MG-8) (ppm)	0		0.1		0.5		150		300	
	M	F	M	F	M	F	M	F	M	F
Adrenals:										
Vacuolation			1*							
Epididymis:										
Hypospermia									1*	
Inflammation	1*									
Lungs:										
Purulent Inflammation	1**		1*					2		2
Granulomatous Inflammation	1*						1	1		1**
Foamy Macrophages									1*	

Lymph nodes:										
Congestion									1*	
Erythrophago-cytosis	1**									
Hyperplasia					1*					
Pancreas: Acinar atrophy									1**	
Pituitary: Cysts										1*
Prostate: Hyperplasia			—		1*					
Skin: Lymphocytic inflammation	2*									
Spinal cord: Hemorrhage		1								
Stomach: Lymphocytic inflammation							1			
Testes: Atrophy of seminiferous tubules									1*	
Thyroid: Hyperplasia		1				1	1		1**	1#
Tongue: Lymphocytic inflammation										1#
Urinary Bladder: Purulent inflammation					1*				1**	1**
Hemorrhage	1**									1**
Ulcer									1**	

[a]Number of organs or tissues with lesions. With one exception (pancreas), tissues from all dogs (4/sex/group) were examined. In the case of the mid-dose females, pancreas from only 3 dogs were examined; there were no abnormalities. No reason was given for not examining pancreas in the fourth dog.

Lesions marked with *, **, or # occurred in the same dog. In the case of the high-dose (300 ppm) females, one dog had purulent lung inflammation and lesions marked with *, and another dog had purulent lung inflammation and lesions marked with **.

COMMENTS

This study is well planned but there are errors or ambiguities in the reported results. These errors and ambiguities, detailed below, require corrections or clarifications as applicable.

1. Although the study number and the animal ID numbers are correct, and the findings reported in the individual pathology data agree with those discussed in other sections of the submission, individual histopathology data (pages 485-493) were reported for dose levels of 20, 100, 500 or 1500 ppm. Since the dose levels of Diazinon (MG-8) used in this study were 0.1, 0.5, 150 or 300 ppm, correctly identified pages 485-488 and 490-493 are required.

2. In the mortality records (pages 324 and 325), an observation period of 14 days is reported for each dog. Since the acclimation period was about 6 weeks, the study was initiated on January 26, 1988, and the necropsies were performed on April 27, 28 and 29, this ambiguity (14-day observation period) requires an explanation.

3. According to the clinical biochemistry data, brain acetylcholinesterase activity for all dogs was determined on day 92 (pages 205 and 260). Yet, 50 or 75 percent of the dogs in each group were dosed for 93 or 94 days (pages 324 and 325). A clearer presentation of these data seems in order.

4. A properly identified table is required on page 504. The currently available data (TABLE 6.3) is entitled Results of concentration analyses of Diazinon MG-8 in rodent feed. Yet, the study number and dose levels are those used for the 90-day oral feeding dog study.

5. On page 21, the terms "acetylcholinesterase levels" and "acetylcholinesterase activity" are used interchangeably which is incorrect. Acetylcholinesterase activity was determined in this study.

NOEL = 0.1 ppm (0.0034 mg/kg*, males; and 0.0037 mg/kg*, females), based on the inhibition of acetylcholinesterase (AChE) activity in serum.

Although serum AChE activity was inhibited 18-20% in male dogs at this dose level, these inhibitions were statistically insignificant and none occurred in the female dogs.

LEL = 0.5 ppm (0.020* and 0.021 mg/kg*, respectively, in males and females); inhibition of serum AChE activity in both sexes.

Although serum AChE activity was not inhibited significantly in the female dogs at this dose level, it was inhibited significantly in the male dogs at this dose level, and the next dose level used (150 ppm) produced severe inhibitions of serum, RBC and brain AChE activity in both sexes. For this reason, 0.5 ppm also seems a more realistic LEL than 150 ppm for the females.

*Values reported by the testing laboratory

CLASSIFICATION OF STUDY: Core-Supplementary. This classification will be updated to Core-Guideline upon receipt and acceptance by Toxicology Branch of the data discussed under COMMENTS.

Quality Assurance Statement, showing seven inspections/audits between January 25, 1988, and July 27, 1988, signed on August 2, 1988, was included in the report.

REFERENCE DOSES (RFDs) FOR ORAL EXPOSURE

Chemical: Diazinon

CAS #: 333-41-5
Caswell #: 342

Carcinogenicity: No evidence of carcinogenicity in available rat and mouse studies.

Systemic Toxicity: See below.

Preparation Date: 9/18/86

Endpoint	Experimental Doses	UF	MF	RfD
Davies and Holub (1980)	0.1 ppm (0.009 mg/kg/day) NOEL*	100	—	0.00009 mg/kg/day
Subchronic Rat Feeding Study				
inhibition of plasma ChE	0.5 ppm LEL			

* Actual Dose Tested

Endpoint and Experimental Doses:

Davies, D.B. and B.J. Holub
Toxicological Evaluation of Dietary Diazinon in the Rat
Arch. Environm. Contam. Toxicol. 9, 637-650 (1980)

Female Wistar rats were fed a semipurified diet containing either no pesticide or 0.1 to 15 ppm diazinon for up to 92 days. At specified times animals were bled from the orbital sinus to facilitate measurement of plasma and erythrocyte cholinesterase activity using a highly sensitive radiometric assay. Feeding diazinon at the levels employed produced no visible toxic manifestations. Feeding trials up to 90 days revealed that rats were highly sensitive to diazinon after 31 to 35 days exposure, as judged by reduction in plasma and erythrocyte cholinesterase activities. The NOEL is based on plasma ChE inhibition noted for up to 35 days of feeding. Other data in this reference indicates that the depression of plasma ChE is not further inhibited by continued dosing (up to 90 days).

.....

Uncertainty Factors (UFs):

The endpoint of concern is ChE inhibition and a 100-fold uncertainty factor is customarily used for subchronic ChE studies.

.....

Modifying Factors (MFs):

None

.....

Additional Comments:

Data Considered for Establishing the RfD

1) Subchronic Feeding - Rat NOEL = 0.1 ppm (0.009 mg/kg/day; actual calculated dose), LEL = 0.5 ppm (inhibition of plasma ChE); core grade supplementary

2) Subchronic Feeding - Rat NOEL < 2 ppm (0.1 mg/kg/day) for inhibition of plasma ChE (20% inhibition in females), LEL = 4 ppm (0.2 mg/kg/day); core grade supplementary

3) Chronic Feeding - Rat NOEL < 10 ppm (0.5 mg/kg/day)(plasma and RBC ChE inhibition); at 100 ppm (brain ChE inhibition); at 1000 ppm (hematocrit depression (males)); core grade supplementary

4) Chronic Feeding - Dog NOEL < 4.6 mg/kg/day (ChE inhibition) (Test material was Diazinon 25% formulation); core grade supplementary

5) Subchronic (Capsule) - Dog NOEL = 0.02 mg/kg/day, LEL = 0.04 mg/kg/day (plasma ChE inhiibition up to 35%) (Test material was Diazinon 25%); core grade supplementary

6) Teratology - Rat Maternal toxicity = 20 mg/kg, Maternal toxicity LEL = 100 mg/kg (decreased food consumption and body weight gain); Fetotoxic NOEL = 100 mg/kg (HDT); Teratogenic NOEL > 100 mg/kg (HDT); core grade guideline

7) Teratology - Rabbit Teratogenic NOEL = 100 mg/kg (HDT); Maternal toxicity = 25 mg/kg, Maternal toxicity LEL = 100 mg/kg (lethality); Fetotoxic NOEL = 100 mg/kg (HDT); core grade minimum

8) 3-Generation Reproduction - Rat NOEL = 8 ppm (HDT) for 2 generations and 4 ppm for 3 generations; core grade supplementary (ChE not measured)

9) Chronic Dosing - Monkeys NOEL = 0.05 mg/kg/day, LEL = 0.5 mg/kg/day (plasma and RBC ChE inhibition) (Test material was Diazinon 50WP); core grade supplementary

D

Data Gap(s)

1) Chronic Rat Feeding Study
2) Chronic Dog Feeding Study
3) Rat Reproduction Study

Other Data Considered

1) Oncogenicity - Mouse Not up to and including 200 ppm (30 mg/kg/day)(HDT); core grade minimum

2) Oncogenicity - Rat Not oncogenic up to 800 ppm (HDT); core grade supplementary

Confidence in the RfD:

Study: Low Data Base: Low RfD: Low

The critical study is of sufficient quality but is given a low confidence rating, since insufficient toxicological parameters were studied. Since the data base is deficient in subchronic and chronic exposure studies and available studies usually only investigate ChE inhibition without evaluation of other toxicity endpoints, the RfD is given a low confidence rating.

Documentation of RfD and Review:

Registration Standard, 1986
Registration Files

Agency RfD Review:

First Review: 10/28/86
Second Review: 11/26/86
Verification Date: 11/26/86

U.S. EPA Contact:

Primary: John Doherty FTS 557-7395

Secondary: Reto Engler FTS 557-7491