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

007482

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dicofol (Kelthane): Review of One-Year Dietary Toxicity Study in Beagle Dogs

Caswell No.: 93 HED Project No.: 9-1239
MRID/Ac. NO.: 409971-01 EPA Record No.: 243075
EPA ID No.: 010501-5

TO: Thomas Luminello, Jr., PM(50)
Registration Division (H7508C)

FROM: Whang Phang, Ph.D. 8/29/89
Pharmacologist
HFAS / Tox. Branch II / HED (H7509C)

THROUGH: K. Clark Swentzel, Acting Section Head 8/30/89
and
Marcia van Gemert, Ph.D. 9/5/89
Branch Chief
HFAS / Tox. Branch II / HED (H7509C)

In response to the Registration Standard for the dicofol requirements, the registrant, Rohm and Haas Co., submitted a chronic dog study (one-year). This study has been evaluated by Dynamac Corp. and approved by Toxicology Branch II. The Data Evaluation Report is attached, and the conclusion of the review is summarized below:

Groups of beagle dogs (6/sex/dose) received dicofol at dietary levels of 0, 5, 30, or 180 ppm for 1 year. Additional high dose Groups (6/sex) received the compound for 14 weeks. Subsequently they were placed on the control diet, and these dogs were designated as a recovery group. In 180 ppm males, increases in the serum alkaline phosphatase level and in the liver weight were found. Histopathologic findings also indicated dose-related hypertrophy of the hepatocytes in 180 ppm male and female dogs. Based upon these findings, the NOEL was established as 30 ppm; LEL, 180 ppm. The study was classified as minimum.

Tox Chem No. 12 (Dicofol; Kelthane) File Last Updated _____ Current Date 5/28/89

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
One-year Dog Feeding Tegeris Labs. Inc. Study No.: 87RC-027 12/14/88	Dicofol 93.3% pure	409971-01	NOEL = 30 ppm LEL = 180 ppm (increased ALP; Liver weight, + hypertrophy of hepatocytes) Doses tested: 5, 30, + 180 ppm		Minimum.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 189-A
TASK No.: 1-89A
July 14, 1989

DATA EVALUATION RECORD

DICOFOL

One-Year Dietary Toxicity Study in Beagle Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Pienta for

Date: July 14, 1989

EPA No.: 68D80056
DYNAMAC No.: 189-A
TASK No.: 1-89A
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DATA EVALUATION RECORD

DICOFOL

One-Year Dietary Toxicity Study in Beagle Dogs

REVIEWED BY:

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DATA EVALUATION RECORD

GUIDELINE §83-1

STUDY TYPE: Chronic dietary toxicity in dogs.

MRID NUMBER: 409971-01.

TEST MATERIAL: Dicofol.

SYNONYM(S): Kelthane; 1,1-bis(chlorophenyl)-2,2,2-trichloroethanol.

STUDY NUMBER(S): 87RC-027.

SPONSOR: Rohm and Haas Company, 727 Norristown Road, Springhouse, PA.

TESTING FACILITY: Tegeris Laboratories, Inc., 9705 N. Washington Boulevard, Laurel, MD.

TITLE OF REPORT: Dicofol (Kelthane Technical Miticide): One-Year Dietary Toxicity Study in Beagle Dogs.

AUTHOR(S): Tegeris, A.S.

REPORT ISSUED: December 14, 1988.

CONCLUSIONS:

Dicofol was fed to beagle dogs at dietary levels of 0, 5, 30, or 180 ppm for 1 year to determine toxicological responses. Six animals of each sex were allocated to each group except at the high-dose level where an additional six males and six females were fed compound-free control diets after week 14 and designated as a recovery group. There were no apparent dose-related effects observed on clinical signs, body weight, food consumption, ophthalmology, hematology, urinalysis, body temperatures, and heart rates; evaluation of electrocardiogram examinations revealed no evidence of cardiotoxicity. One male in the 180-ppm recovery group died during study week 28 and an additional male of the same group was sacrificed during week 42 when observed in a moribund state. Clinical chemistry evaluation disclosed significantly ($p < 0.05$) increased serum alkaline phosphatase (ALP) levels in males receiving 180 ppm when compared with controls; several females of the same group had increased ALP levels but means were not significantly different from controls. In the plasma cortisol assays, injections of adrenal cortical trophic hormone (ACTH) induced markedly reduced cortisol responses in males and females receiving 180 ppm. Other clinical chemistry parameters were considered to be within the normal range. Males receiving 180 ppm were noted with increased liver weights when compared to controls. Gross pathological examinations did not reveal any apparent tissue alterations; histopathologic findings were characterized by dose-related hypertrophy of the hepatocytes in males and females receiving 180 ppm.

On the basis of these results, it was concluded that the NOEL is 30 ppm and LOEL is 180 ppm for dicofol fed orally to dogs for 52 weeks.

Classification: CORE Minimum. The registrant should provide a rationale for the selection of dose levels.

A. MATERIALS:

1. Test Compound: Dicofol; description: dark brown viscous liquid; batch No.: RS-4503, TD#85-211; purity: 93.3%.

2. Test Animals: Species: dogs; strain: beagle; age: 6 months; weight: males--5.9-8.8 g, females--5.1-8.4 g; source: Hazleton Research Animals, Inc., Cumberland, MD.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimatized to laboratory conditions for 7 weeks, their health was monitored, and

animals free of toxic signs were assigned to the following groups using a computer randomization method:

Test group		Dose in diet (ppm)	Number of Animals Males	Females
1	Control	0	6	6
2	Low (LDT)	5	6	6
3	Mid (MDT)	30	6	6
4	High (HDT)	180	6	6
4A	High (HDT)[a] (Recovery group)	180	6	6

[a]High-dose recovery group (4A): Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

The animals were housed in stainless steel cages in two rooms in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. All animals were identified by an ear tattoo number and were also assigned unique identification numbers.

2. Diet Preparation: Animal diets containing the test compound were prepared weekly. At each preparation, basal diet and test compound were weighed, uniformly mixed, blended, and stored at an ambient temperature. All samples were analyzed weekly for the first 4 weeks and once monthly thereafter for stability, homogeneity, and concentration.

   Results: Table 1 presents analysis of dosed feeds for homogeneity of dicofol. Diets were reported to be homogenous and demonstrated to be stable during the period each diet was used. The test compound was administered at a constant concentration in the diet. The mean concentrations in diets during the first 26 weeks were 4.76, 29.54, and 177.03 ppm at dietary levels of 5, 30 and 180 ppm, respectively; from weeks 28 to 52, the mean concentrations were 4.54, 29.02, and 181.69 ppm at dietary levels of 5, 30, and 180 ppm, respectively. The mean concentration during the first 14 weeks for the 180-ppm recovery group was 183.27 ppm. All diets were within the target range of nominal concentrations.

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

4. Statistics: All test data for males and females consisting of body weight, food consumption, clinical chemistry and hematology parameters, cortisol analyses, heart rate, and body temperature were analyzed by one-way analysis of

TABLE 1. Analysis of Dosed Feeds for Homogeneity of Dicofol[a]

Theoretical Concentration (ppm)	Actual Sample Concentrations			Recovery	
	1	2	3	Mean (±S.D.)	%
5	5.05	4.85	4.96	4.95 ± 0.10	99.1
30	27.42	26.18	26.50	26.70 ± 0.64	89.0
180	173.78	177.42	165.98	172.39 ± 5.84	95.8

[a]Data extracted from study No. 87RC-027, Table No. T-4-2.1.

variance (ANOVA). Significant differences were examined further by Dunnett's t-test at the 95% confidence level ($p = 0.05$) to determine the means of the treated groups that were statistically different from the control group.

5. Quality Assurance: A quality assurance statement was signed and dated December 16, 1988.

C. METHODS AND RESULTS:

1. Observations: All animals were observed twice daily for mortality and clinical signs including toxicity, behavioral effects, physical changes, appetite, and excretory status. A detailed physical examination of each dog was conducted weekly.

Results: The most common findings were alopecia, skin cuts or abrasions, and interdigital cyst-like masses; incidences were similar in dosed and control groups. Abrasions and interdigital masses are commonly observed in caged laboratory dogs and alopecia observed was at an extensive rate (70-80% of animals in several groups) due to mange infestation. Skin scrapings from several dogs confirmed two males and four females that were positive for ectoparasites; five males and one female were found negative. During the study, some affected dogs were observed with regressed alopecia while in others it progressed to more severe skin damages.

There were two mortalities in the recovery group of males that had received the high dose. One male of the high-dose recovery group was found dead during study week 28 and an additional male of the same group was sacrificed during week 42 when observed to be in a moribund state. The dog that died was on a control diet starting at week 15 and appeared in a normal physical state; this was considered to be a random death occurrence without any relationship to treatment. The other male dog was observed with a generalized alopecia and a severe weight loss (3.1 kg) between weeks 28 and 40, which caused debility and necessitated sacrifice.

2. Body Weight: Individual body weights were recorded prior to study initiation, at study initiation, and every week thereafter through termination week 52.

Results: Table 2 summarizes mean body weight data at selected intervals for dogs fed dicofol for 1 year. Administration of dicofol did not adversely affect the body weights of males and females. Mean body weights of control and treated males and females were similar throughout the study, although males receiving 30 and 180 ppm of test compound were observed with slightly but consistently lower

TABLE 2. Mean Body Weights at Selected Intervals for Dogs Fed Dicofol for 1 Year[a]

Dietary Level (ppm)	Mean Body Weights (KG/Dog ± S.D.) Selected Study Weeks						
	-1	1	7	15	25	39	52
				Males			
0	7.22 ± 0.61	7.85 ± 0.76	9.43 ± 1.12	10.43 ± 1.25	10.87 ± 1.38	10.68 ± 1.40	10.72 ± 1.69
5	7.20 ± 0.98	7.80 ± 1.18	9.22 ± 1.27	10.02 ± 1.30	10.42 ± 1.36	10.42 ± 1.62	10.68 ± 1.80
30	7.27 ± 0.71	7.77 ± 0.69	8.97 ± 0.54	9.62 ± 0.66	9.92 ± 0.44	9.80 ± 0.27	9.68 ± 0.16
180	7.23 ± 0.57	7.62 ± 0.74	8.83 ± 0.87	9.53 ± 0.90	10.18 ± 1.00	10.13 ± 1.07	10.40 ± 0.96
180[b]	7.22 ± 1.01	7.65 ± 1.01	9.15 ± 1.13	10.17 ± 1.19	10.72 ± 1.59	10.42 ± 2.56	11.38 ± 2.69
				Females			
0	6.93 ± 0.79	7.17 ± 0.71	7.88 ± 0.68	8.37 ± 0.40	8.75 ± 0.40	8.72 ± 0.41	8.55 ± 0.50
5	6.93 ± 1.01	7.25 ± 1.03	8.03 ± 1.27	8.35 ± 1.30	8.63 ± 1.07	8.65 ± 1.32	8.62 ± 1.25
30	6.92 ± 0.96	7.38 ± 0.95	8.08 ± 1.08	8.63 ± 1.26	9.02 ± 0.94	9.07 ± 0.85	8.95 ± 1.19
180	6.75 ± 0.58	7.17 ± 0.69	7.97 ± 0.62	8.62 ± 0.74	9.06 ± 0.77	9.07 ± 0.89	9.05 ± 1.00
180[b]	6.92 ± 1.13	7.32 ± 1.23	8.22 ± 1.39	8.83 ± 1.50	9.27 ± 1.53	9.48 ± 1.66	9.35 ± 1.70

[a]Data extracted from study No. 87RC-027, Table Nos. T-4.4.1 and T-4.4.2, and Appendix Nos. A-4.4.1 and A-4.4.2.

[b]Recovery group = Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

mean body weights; these differences were not significant when compared with the control groups.

3. Food Consumption and Compound Intake: Food consumption values of individual animals were determined every day. Four hundred grams of feed was offered to each dog for 1 hour every day and unconsumed food was calculated. The feed efficiency was calculated on a weekly basis.

   Results: Table 3 presents mean food consumption at selected intervals for dogs fed dicofol for 1 year. Mean food consumption of animals of controls and treated groups at dietary levels of 5- and 30 ppm were essentially similar; males receiving 180 ppm were noted with consistently higher mean food consumption values than the controls or any other dose groups throughout the study. Significantly ($p < 0.05$) increased food consumptions were noted in males receiving 180 ppm at weeks 30 and 45 when compared with controls; these increases were considered random occurrences and further were not considered of toxicologic importance by the study author. The calculated mean compound intakes for the entire study were 0.12, 0.82, and 5.71 mg/kg/day for males receiving dietary levels of 5, 30, or 180 ppm and 0.13, 0.85 and 5.42 mg/kg/day for females receiving the same dietary levels of dicofol. In the 180 ppm recovery group, mean compound intakes during the first 14 weeks were 6.46 and 6.25 mg/kg/day for males and females, respectively, which compares favorably to those of males and females receiving 180-ppm during this same time period.

4. Ophthalmological and Electrocardiogram Examinations, Heart Rates, and Body Temperatures: Ophthalmological examinations were conducted on each animal prior to the start of the study and, except for the high-dose recovery group, at week 26 and prior to termination. Electrocardiograms, heart rates and body temperatures were obtained on each animal (except for the high-dose recovery group) during the pretest period, at weeks 1, 4, 13, and 26, and prior to termination.

   Results: Ophthalmological findings did not reveal any indication of compound-related ocular abnormalities.

   Evaluation of electrocardiogram tracings revealed no evidence of direct cardiotoxicity of the test article. Results of mean heart rates calculated for each animal from the individual electrocardiogram tracings revealed no effects of the test article. Mean body temperatures of control and treated animals were all within the acceptable range.

TABLE 3. Mean Food Consumption Data at Selected Intervals for Dogs Fed Dicofol for 1 Year[a]

Dietary Level (ppm)	Mean Food Consumption (G/Dog/Day ± S.D.) Selected Study Weeks						
	-1	1	7	15	30	45	52
				Males			
0	274.31 ± 47.58	274.70 ± 36.13	280.68 ± 29.75	264.80 ± 27.25	233.80 ± 27.03	270.48 ± 38.99	259.43 ± 55.13
5	269.81 ± 43.66	260.11 ± 81.49	272.96 ± 40.84	245.44 ± 27.42	212.10 ± 36.60	275.27 ± 23.51	248.32 ± 51.55
30	258.21 ± 17.05	268.70 ± 28.94	297.72 ± 19.32	267.79 ± 25.90	252.01 ± 55.23	275.78 ± 27.69	263.74 ± 33.85
180	285.48 ± 29.61	293.27 ± 38.01	301.45 ± 35.98	298.99 ± 58.87	317.97 ± 60.48*	321.61 ± 22.52*	303.40 ± 58.66
180[b]	280.86 ± 56.42	283.08 ± 41.04	306.68 ± 57.50	317.65 ± 56.16	284.75 ± 84.95	282.15 ± 30.45	263.51 ± 70.98
				Females			
0	266.00 ± 16.17	237.81 ± 14.69	249.74 ± 33.49	249.21 ± 8.97	253.56 ± 25.07	266.23 ± 42.05	239.25 ± 37.92
5	268.24 ± 19.02	234.46 ± 43.59	261.18 ± 35.56	239.39 ± 47.17	248.17 ± 34.88	254.51 ± 29.58	232.93 ± 40.85
30	239.69 ± 21.10	250.76 ± 23.94	246.12 ± 26.94	263.69 ± 14.65	257.21 ± 29.46	256.92 ± 26.95	237.99 ± 60.82
180	269.21 ± 26.01	255.67 ± 25.07	262.17 ± 21.65	272.59 ± 28.60	262.41 ± 47.27	278.40 ± 29.55	287.73 ± 56.69
180[b]	250.64 ± 38.71	277.40 ± 65.52	272.15 ± 38.94	282.88 ± 29.04	262.60 ± 68.01	263.55 ± 43.65	244.87 ± 44.45

[a]Data extracted from study No. 87RC-027, Table Nos. T-4.4.1 and T-4.4.2, and Appendix Nos. A-4.4.1 and A-4.4.2.

[b]Recovery group = Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

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

5. Hematology, Clinical Chemistry, and Plasma Cortisol Analyses: Blood was collected from all fasted animals (except animals of the high-dose recovery group) twice during the pretest period, at weeks 13, 26, and 39, and prior to termination for determination of hematology and clinical chemistry parameters.

Plasma cortisol analyses were conducted after intramuscular injection of 20 units of adrenal cortical trophic hormone (ACTH) to all animals. Blood samples for analysis were obtained from each animal prior to ACTH injections and at 30 and 90 minutes following the ACTH injections. The ACTH challenge test was conducted prior to study initiation, during weeks 12 and 25, and prior to termination. Animals of the high-dose recovery group were included in the terminal ACTH challenge test.

a. Hematology: The CHECKED (X) parameters were examined:

X	Hematocrit (HCT)[+]	X	Leukocyte differential count
X	Hemoglobin (HGB)[+]	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)[+]	X	Mean corpuscular HGB concentration (MCHC)
	Erythrocyte count (RBC)[+]	X	Mean corpuscular volume (MCV)
X	Platelet count[+]		Coagulation:thromboplastin time (PT)
	Reticulocyte count (RETIC)		
X	Red cell morphology		

Results: Tables 4 and 5 summarize selected hematology data at specific study weeks for male and female dogs, respectively, fed dicofol for 1 year.

Pretest values for hematology were essentially similar in all groups of males and females. At the second pretest sampling, males in the low-dose group had significantly ($p < 0.05$) increased mean values of red blood cell (RBC) counts, hemoglobin (HGB), and hematocrit (HCT) when compared to controls. Mean RBC counts in males receiving 180 ppm at weeks 13, 26, 39, and 52 (prior to termination) were significantly ($p < 0.05$) decreased when compared with controls; males receiving the same dose were also observed with significant ($p < 0.05$) increases when compared to controls in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at week 26. At termination (week 52), mean RBC counts of 30-ppm males were significantly ($p < 0.05$) lower than controls and mean platelet counts of 180-ppm males were significantly ($p < 0.05$) elevated when compared with controls. Other hematologic parameters were not observed with significant differences between control and dosed males.

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 4. Selected Hematology Data (Mean ±S.D.) at Specific Intervals for Male Dogs Fed Dicofol for 1 Year[a,b]

Dietary Level (ppm)	Selected Weeks on Study											
	RBC (10^6/mm^3)			MCV (μ^3)			MCH ($\mu\mu$g)			PLT (10^3/mm^3)		
	13	26	52	13	26	52	13	26	52	13	26	52
0	7.00±0.48	7.29±0.33	7.40±0.34	67.33±2.50	64.67±0.82	65.83±2.78	21.87±0.65	22.05±0.75	23.05±1.07	272.00±40.68	249.00±67.56	255.67±42.17
5	7.11±0.20	7.27±0.35	7.30±0.33	68.33±1.63	66.17±1.47	67.33±3.20	22.30±0.64	22.67±0.59	23.45±0.89	316.33±66.75	305.50±54.24	297.50±48.51
30	6.75±0.41	6.79±0.38	6.67±0.57*	69.00±2.28	67.17±2.71	68.00±2.00	22.18±0.63	22.68±0.96	23.62±0.71	318.00±77.85	294.50±54.74	299.83±62.20
180	6.36±0.21*	6.46±0.30*	6.66±0.50*	70.50±1.87	67.83±1.72*	69.83±4.07	22.90±0.90	23.83±0.96*	24.53±1.73	338.00±63.15	316.83±75.42	350.83±51.26*
180[c]	6.86±0.38	---	---	70.33±1.63	---	---	22.52±0.78	---	---	345.33±87.89	---	---

[a]Data extracted from Study No. 87RC-027, Table No. T-4.8.1 and Appendix No. A-4-8.1

[b]Abbreviations are as follows:

RBC = Erythrocyte count and morphology
MCV = Mean corpuscular volume
MCH = Mean corpuscular hemoglobin
PLT = Platelet count
S.D. = Standard deviation

[c]Recovery group: Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

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

TABLE 5. Selected Hematology Data (Mean ±S.D.) at Specific Intervals for Female Dogs Fed Dicofol for 1 Year[a,b]

Dietary Level (ppm)	Selected Weeks on Study											
	RBC (10^6/mm^3)			WBC (10^3/mm^3)			HGB (g/dL)			MCH (µµg)		
	13	26	39	13	26	39	13	26	39	13	26	39
0	7.21±0.27	7.38±0.28	7.34±0.58	10.80±2.24	12.67±1.46	10.17±2.48	16.38±0.71	17.15±0.78	16.72±1.09	22.73±0.69	23.23±0.57	22.77±0.70
5	7.28±0.33	7.30±0.72	7.46±0.73	10.57±1.54	11.03±1.26	11.82±2.31	16.52±0.65	16.78±1.75	16.72±1.41	22.70±0.60	22.98±0.56	22.45±0.61
30	7.33±0.42	7.20±0.45	7.44±0.60	11.08±2.39	9.83±1.38*	10.27±2.35	16.85±0.53	16.72±0.53	16.95±1.15	23.00±0.73	23.27±0.75	22.80±0.70
180	6.99±0.38	6.94±0.61	6.69±0.60	12.47±1.44	13.23±2.53	11.20±0.91	16.10±0.86	16.40±0.98	15.97±1.23	23.03±0.55	23.67±0.70	23.88±0.69*
180[c]	6.65±0.27*	---	---	14.07±2.60*	---	---	15.28±0.86*	---	---	22.98±0.42	---	---

[a]Data extracted from study No. 87RC.27, Table No. T-4.8.2 and Appendix No. A-4.8.2.

[b]Abbreviations are as follows:

RBC = Erythrocyte count and morphology
WBC = Total leukocyte count
HGB = Hemoglobin
MCH = Mean corpuscular hemoglobin
S.D. = Standard deviation

[c]Recovery group: Dosed at 180 ppm for week 1-14 and 0 ppm thereafter.

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

At the second pretest sampling, the mean corpuscular hemoglobin concentration (MCHC) values of females in group 4A (high-dose recovery group) were significantly ($p < 0.05$) lower than the controls; at week 13, females receiving the same dose were observed with significant ($p < 0.05$) differences when compared with control groups in hematological parameters, including increased total leukocyte (WBC) counts, increased monocyte counts (WBC differential count), decreased RBC counts, and decreased HGB levels. WBC counts were significantly ($p < 0.05$) decreased in females receiving 30 ppm when compared with controls at week 39, MCH values in females at 180 ppm were significantly ($p < 0.05$) increased when compared with control. There were no other significant differences observed prior to termination between control and dosed females.

The study author did not consider any of the changes in hematology parameters to be compound related since mean values were within the normally expected ranges and the changes were not consistent.

b. Clinical Chemistry: The CHECKED (X) parameters were examined:

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

	Enzymes
X	Alkaline phosphatase (ALP)
	Cholinesterase
X	Creatinine phosphokinase+
X	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (SGPT)+
X	Serum aspartate aminotransferase (SGOT)+
X	Gamma glutamyltransferase (GGT)

	Other
X	Albumin+
X	Albumin/globulin ratio
X	Blood creatinine+
X	Blood urea nitrogen+
X	Cholesterol+
X	Globulins
X	Glucose+
X	Total bilirubin+
	Direct bilirubin
X	Total protein+
X	Triglycerides
X	Plasma cortisol analysis (ACTH challenge test)

Results: Table 6 summarizes mean alkaline phosphatase (ALP) data for dogs at selected study weeks and Tables 7 and 8 present selected clinical chemistry data at specific study weeks for male and female dogs, respectively.

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 6. Alkaline Phosphatase (ALP) Data (Mean ± S.D.) at Selected Intervals for Dogs Fed Dicofol for 1 Year[a]

Dietary Level (ppm)	Selected Weeks on Study — ALP (IU/L)				
	Pretest 1	13	26	39	52
			Males		
0	103.83 ± 18.57	51.67 ± 7.58	35.83 ± 7.63	29.67 ± 7.39	35.50 ± 6.29
5	98.33 ± 15.59	47.17 ± 8.66	36.33 ± 12.86	29.50 ± 12.33	33.67 ± 12.75
30	81.17 ± 15.89	43.17 ± 8.64	31.50 ± 6.06	23.67 ± 4.80	29.17 ± 6.37
180	107.50 ± 9.59	104.00 ± 32.82*	113.70 ± 53.05*	97.67 ± 39.35*	130.67 ± 36.76*
180[b]	97.00 ± 20.71	85.17 ± 18.76*	---	---	---
			Females		
0	103.50 ± 40.68	70.33 ± 37.21	67.33 ± 38.67	50.33 ± 32.74	63.67 ± 37.03
5	94.83 ± 27.79	52.83 ± 13.21	43.00 ± 11.88	35.33 ± 11.11	42.83 ± 12.58
30	90.50 ± 11.78	54.67 ± 13.82	46.33 ± 12.19	36.00 ± 18.84	51.33 ± 26.23
180	80.67 ± 14.21	113.33 ± 76.82	120.83 ± 76.48	100.33 ± 72.54	148.67 ± 106.16
180[b]	91.50 ± 16.43	97.50 ± 40.40	---	---	---

[a]Data extracted from Study No. 87RC-027, Table No. T-4.6.1 and T-4.6.2 and Appendix Nos. A-4.6.1 and A-4.6.2.

[b]Recovery group: Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

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

TABLE 7. Selected Clinical Chemistry Data (Mean ± S.D.) at Specific Intervals for Male Dogs Fed Dicofol for 1 Year[a,b]

Dietary Level (ppm)	Selected Weeks on Study							
	CHOL (mg/dL)				ALB (g/dL)			
	13	26	39	52	13	26	39	52
0	141.37±20.47	137.27±23.92	137.92±18.03	134.37±21.21	3.52±0.17	3.68±0.24	3.52±0.15	3.73±0.08
5	157.15±27.16	158.82±31.65	149.70±31.64	141.27±26.57	3.55±0.16	3.62±0.25	3.48±0.18	3.70±0.13
30	140.37±21.23	138.92±22.14	127.42±30.08	120.60±20.39	3.50±0.21	3.58±0.28	3.48±0.23	3.62±0.32
180	168.83±17.94	187.03±18.54*	176.23±23.63*	167.08±17.42*	3.30±0.17	3.28±0.21*	3.12±0.28*	3.23±0.23*
180[c]	165.27±21.62	---	---	---	3.37±0.12	---	---	---

[a]Data extracted from study No. 87RC-027, Table No. T-4.6.1 and Appendix No. A.4.6.1.

[b]Abbreviations are as follows:

CHOL = Cholesterol
ALB = Albumin
S.D. = Standard deviation.

[c]Recovery group: Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

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

TABLE 8. Selected Clinical Chemistry Data (Mean ±S.D.) at Specific Intervals for Female Dogs Fed Dicofol for 1 Year[a,b]

Dietary Level (ppm)	Selected Weeks on Study											
	CHOL (mg/dL)			LDH (IU/L)			TBR (mg/dL)			ALB (g/dL)		
	13	39	52	13	39	52	13	39	52	13	39	52
0	155.4±22.93	185.10±39.41	160.85±31.10	29.67± 5.96	51.00±20.03	31.67±7.63	0.25±0.09	0.26±0.06	0.34±0.09	3.55±0.19	3.55±0.22	3.73±0.18
5	130.10±14.24	140.7±36.93	140.30±26.46	31.33± 7.94	98.67±46.56	37.83±6.88	0.23±0.05	0.27±0.07	0.29±0.09	3.58±0.10	3.47±0.21	3.52±0.18
30	124.63±16.63*	124.15±14.02*	133.80±31.79	34.00± 4.05	59.00±20.14	43.50±26.73	0.18±0.04	0.17±0.03	0.29±0.11	3.62±0.16	3.62±0.23	3.70±0.22
180	141.68±14.09	173.97±42.64	146.18±16.25	37.00± 2.76	110.00±50.41*	42.83±21.23	0.17±0.04	0.22±0.08	0.25±0.11	3.33±0.12*	3.20±0.13*	3.35±0.26*
180[c]	151.85±26.82	---	---	32.00±11.65	---	---	0.15±0.08*	---	---	3.38±0.10	---	---

[a]Data extracted from Study No. 87RC-027, Table No. T-4.6.2 and Appendix No. A-4.6.2.

[b]Abbreviations are as follows:

CHOL = Cholesterol
LDH = Lactic dehydrogenase
TBR = Total bilirubin
ALB = Albumin
S.D. = Standard deviation.

[c]Recovery group: Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

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

At the second pretest sampling, males receiving 30 ppm were observed with significantly ($p < 0.05$) decreased serum alkaline phosphatase (ALP) level when compared with control but this decrease was within the expected range of the study laboratory. At week 13, males receiving 180 ppm and the 180-ppm recovery group were observed with significantly ($p < 0.05$) increased ALP level when compared with control values. At weeks 26, 39, and 52 (termination week), consistently significant ($p < 0.05$) increases were noted in males receiving 180 ppm in mean values of ALP and cholesterol when compared with the control groups; mean albumin level was significantly ($p < 0.05$) decreased at the same intervals for the same dose group. Other clinical chemistry parameters did not reveal any significant difference between control and dosed males.

High-dose females showed elevated mean ALP levels throughout the study even though the increase was not statistically significant. In females, significant ($p < 0.05$) differences in comparison with controls in clinical chemistry parameters at week 13 included decreased mean cholesterol values at 30 ppm, decreased total bilirubin (TBR) at 180 ppm/recovery group, and decreased mean albumin (ALB) at 180 ppm. At week 39, the significant ($p < 0.05$) differences included decreased cholesterol at 30 ppm and increased lactic acid dehydrogenase (LDH) and decreased albumin at 180 ppm. At week 52, the only significant ($p < 0.05$) difference when compared to controls was a decreased albumin value in 180-ppm females. Other clinical chemistry parameters were not significantly different between control and dosed females.

Elevated mean ALP values and decreased mean albumin levels in high-dose males and females were considered to be compound related. The response of elevated mean cholesterol values in high-dose male was not observed in females; the author considered increased LDH and decreased TBR in females random occurrences incidental to compound treatment and not toxicologically significant.

ACTH Challenge Test--Results: Table 9 summarizes mean 90-minute plasma cortisol levels calculated after subtracting prechallenge baseline values. Dosing with dicofol caused an inhibition of ACTH-stimulated cortisol release. There were significant ($p < 0.05$) inhibitory effects 30 and 90 minutes following injection of ACTH in both high-dose males and females. The decreases were significant at 12, 25, and 52 weeks in males and at 12 and 52 weeks in females. Similar results (lesser magnitude) were seen 30 minutes after ACTH challenge. The author presented statistical analysis of mean values both before and after subtraction of baseline values and found no difference between statistical values in Table 6. There were no significant

TABLE 9. Mean 90-Minute Cortisol Levels for Dogs Fed Dicofol[a]

Dietary Level (ppm)	90-Minute Cortisol Levels (µg/100 mL ± S.D.) Weeks of Study			
	Pretest	12	25	52
		Males		
0	8.90 ± 1.19	10.23 ± 3.93	8.65 ± 2.94	7.77 ± 1.93
5	8.77 ± 2.32	10.15 ± 3.81	9.35 ± 2.76	8.38 ± 1.13
30	8.95 ± 1.56	7.12 ± 2.05	7.27 ± 2.65	6.88 ± 1.48
180	8.68 ± 1.43	4.52 ± 1.85*	2.58 ± 1.29*	4.17 ± 1.85*
180[b]	8.27 ± 0.95	4.35 ± 1.60*	6.10 ± 2.00	8.45 ± 1.07
		Females		
0	9.80 ± 2.18	12.83 ± 4.31	6.22 ± 1.44	9.40 ± 2.36
5	9.32 ± 2.42	11.85 ± 4.39	7.93 ± 3.41	8.52 ± 2.48
30	9.55 ± 2.07	9.07 ± 2.69	7.57 ± 1.72	7.23 ± 2.50
180	8.67 ± 1.79	6.15 ± 3.95*	4.15 ± 1.00	4.47 ± 1.28*
180[b]	8.95 ± 1.95	5.83 ± 3.75*	6.42 ± 1.40	8.35 ± 2.01

[a]Data extracted from Study No. 87RC-027, Table Nos. T-4.7.1 and T-4.7.2, and Appendix Nos. A-4.7.1 and A-4.7.2.

[b]Recovery group: Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

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

effects at dose levels of 5 or 30 ppm. There was inhibition in the high-dose recovery group at week 12 but after dosing was discontinued there was a reversal of the inhibitory response. It was completely reversed in females on recovery diets by week 25 and in males by week 52. There was no effect of dosing on the baseline values of plasma cortisol. There was some variability in these values but no pattern of variability. Mean baseline cortisol values ranged from 0.82 to 1.75 μg/100 mL in male groups and 0.93 to 2.6 μg/100 mL in female groups.

6. Urinalysis: Urinalyses were performed on all males and females during the pretest period and, except for the high-dose recovery group, at 6 months and prior to termination. At 6 months, urinalyses were performed only on control and high-dose animals but were subsequently repeated within 2 weeks on dogs in all dose groups. The CHECKED (X) parameters were examined:

X	Appearance[+]	X	Glucose[+]
	Volume[+]	X	Ketones
X	Specific Gravity[+]	X	Bilirubin[+]
X	pH	X	Blood[+]
X	Sediment (microscopic)[+]		Nitrate
X	Protein[+]	X	Urobilinogen

Results: Table 10 presents the results of selected urinalysis parameters in dogs fed Dicofol for 1 year. Evaluation of urinalysis parameters revealed no consistent dose-related changes that could be attributed to the test article. All values were within the expected normal range and distribution except the number of RBC per high-power field (hpf) in treated males.

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

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 10. Results of Selected Urinalysis Parameters for Dogs Fed Dicofol for 1 Year[a,b]

Parameter	Dietary Level (ppm)							
	Males				Females			
	0	5	30	180	0	5	30	180
Number examined	6	6	6	6	6	6	6	6
Protein								
Negative	0	2	1	1	2	1	0	3
Trace	1	1	0	2	1	1	4	0
1+	3	1	2	0	1	1	1	0
2+	1	2	2	2	2	1	0	0
3+	0	0	0	0	0	1	1	2
4+	1	0	1	1	0	1	0	1
Ocult Blood								
Negative	6	5	6	5	5	5	6	6
1+	0	0	0	1	0	0	0	0
2+	0	1	0	0	1	0	0	0
3+	0	0	0	0	0	1	0	0
WBC/hpf								
0	0	0	0	1	1	0	1	1
1-5	4	1	3	2	5	3	5	5
6-10	0	0	0	1	0	1	0	0
>10-TNTC	2	5	3	2	0	2	0	0
RBC/hpf								
0	4	2	1	0	2	1	3	4
1-5	2	4	5	5	4	4	3	2
6-10	0	0	0	1	0	0	0	0
>10-TNTC	0	0	0	0	0	1	0	0

[a]Data extracted from Study No. 87RC-027, Table Nos. T-4.9.1 and T-4.9.2, and Appendix Nos. A-4.9.1 and A-4.9.2.

[b]Abbreviations:
WBC = Total leukocyte count
RBC = Red blood cells
HPF = High-power field
TNTC = Too numerous to count

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

Results:

a. Organ Weights: Table 11 presents mean liver weights for dogs fed dicofol for 1 year. The mean absolute liver weights of males and females receiving 180 ppm were higher than the controls but the differences were not significant. Significant ($p < 0.05$) increases compared to the controls in liver-to-body weight and liver-to-brain weight ratios were noted in males receiving 180 ppm; these increases suggested a toxicological effect of the compound on hepatic function. There were no other apparent differences in mean absolute or relative liver weights and other organ weights of control and treated dogs of either sex.

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 11. Mean (± S.D.) Liver Weight Summary For Dogs Fed Dicofol for 1 Year[a,b,c]

Dietary Level (ppm)	Liver Weight (g)	Liver/Body Weight Ratio (%)	Liver/Brain Weight Ratio (%)
		Males	
0	288.33 ± 48.24	27.24 ± 2.72	3.58 ± 0.57
5	292.50 ± 56.10	27.26 ± 2.90	3.90 ± 0.85
30	281.67 ± 42.27	29.87 ± 4.49	3.52 ± 0.47
180	340.00 ± 29.16	33.29 ± 3.49*	4.63 ± 0.41*
180[d]	258.75 ± 38.81	23.72 ± 2.37	3.25 ± 0.40
		Females	
0	245.00 ± 22.58	29.15 ± 2.38	3.38 ± 0.30
5	225.83 ± 39.68	27.18 ± 4.09	3.13 ± 0.56
30	247.50 ± 15.41	28.33 ± 3.30	3.34 ± 0.24
180	286.67 ± 36.00	32.47 ± 3.80	3.75 ± 0.46
180[d]	260.83 ± 70.74	28.46 ± 5.38	3.48 ± 0.73

[a]Data extracted from study No. 87RC-027, Table Nos. T-4.14.1 and T-4.14.2, and Appendix Nos. A-4.14.1 and A-4.14.2.

[b]Abbreviation is as follows:
S.D. = Standard deviation.

[c]Based on six animals/sex/group with the exception of four males in the 180-ppm recovery group.

[d]Recovery group: Dosed at 180 ppm for weeks 1-14 and 0 ppm thereafter.

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

b. Gross Pathology: A variety of gross pathology findings occurred sporadically without any relationship to treatment. The male dog found dead during week 28 in the 180-ppm recovery group was observed with hemorrhages in the ventricles and the thickened left ventricle; hemorrhages were also present in the submucosa of esophagus. The other male of the same group sacrificed during week 42 was thin and emaciated with general alopecia.

c. Microscopic Pathology:

1) Nonneoplastic: Table 12 summarizes selected nonneoplastic lesions of liver in dogs administered dicofol for 1 year. Treatment-related alterations in liver morphology were characterized primarily by minimal to mild hypertrophy of hepatocytes observed in males and females receiving 180 ppm. Congestion of multiple organs and hemorrhages involving the heart and esophagus were observed in the dog found dead at week 28, which were not dose-related; histopathologic examination of male dog sacrificed at week 42 revealed no definitive lesions that could be attributed to the test chemical. Other histopathological findings in animals of control as well as treated groups were considered to be incidental or as spontaneous common lesions with no apparent dose response.

2) Neoplastic: No neoplasms were observed in dogs fed dicofol for up to 1 year.

D. STUDY AUTHOR'S CONCLUSIONS:

The study author concluded that decreases of albumin and elevation of serum ALP levels in some but not all males and females at 180 ppm may be attributed to a possible compound-related effect. ACTH injections inhibited markedly reduced plasma cortisol responses in males and females at 180 ppm with no effect at 5- or 30-ppm dose levels. Necropsy and gross examination of tissues revealed no compound-related lesions; liver weights of 180-ppm males were increased. Histopathological findings of tissues revealed minimal to mild hypertrophy of hepatocyte and considered to be related to decreased albumin and elevated serum ALP values.

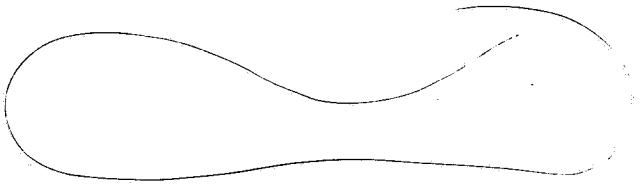
It was concluded that the LOEL is 180 ppm and the NOEL is 30 ppm for dogs when dicofol was fed for 1 year.

TABLE 12. Selected Nonneoplastic Lesions of the Liver of Dogs Fed Dicofol for 1 Year[a]

Organ/Diagnosis	Dietary Level (ppm)							
	Males				Females			
	0	5	30	180	0	5	30	180
Liver	(6)[b]	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Hypertrophy, hepatocytes	0	0	0	5	0	0	0	5
Pigment, hepatocytes	1	2	3	5	4	3	4	3
Pigment, Kupffer cells	0	2	3	0	1	3	3	3
Chronic inflammation	1	0	2	0	0	1	0	1

[a]Data extracted from study No. 87RC-027, table No. T-4.15.3 and Appendix No. A-4.15.3.

[b]Numbers in parentheses are the number of animals with tissues examined microscopically.



E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

Dicofol was fed to beagle dogs for 1 year at dietary levels of 0, 5, 30, and 180 ppm to assess its toxicity. There were four groups designated as control, low-, mid-, and high-dose groups, each consisting of six males and six females. An additional high-dose group was designated as a recovery group where six male and six female dogs were fed compound-free control diets after week 14.

The study design was complete and conduct of the study was adequate. All in-life parameters were completely reported; results obtained during the study were summarized and supported by individual animal data. No rationale for the dosage selection was provided.

We agree with the study author that there were no dose-related effects on clinical signs, body weight, food consumption, ophthalmology, hematology, urinalysis, body temperatures, heart rates, and electrocardiogram examinations.

One male of the 180-ppm recovery group was found dead during study week 28 and a second male from the same group was sacrificed moribund during week 42 and necropsy was performed; cause of natural death moribundity was not attributed to the test compound.

Statistically significant decreases noted for albumin in males at weeks 26, 39, and 52 (3.12-3.28 g/dL) and for females at weeks 13, 39, and 52 (3.20-3.35 g/dL) were within the normal historical control reference range (2.5-3.7 g/dL) provided by the Hazleton Laboratories America, Inc., and we do not consider these decreases attributed to the toxic response. Significant ($p < 0.05$) increases when compared with controls were noted in the serum enzyme activities of ALP in males at 180 ppm at weeks 13, 26, 39, and 52; females at all samplings and at the same dose level were also observed with increased ALP values. These increased ALP values in male and female dogs receiving 180 ppm of dicofol appear to be compound related. Mean plasma cortisol responses following injections of ACTH were markedly reduced in males and females at 180 ppm, which inhibited a compound-related effect.

We further agree with the study author that decreased albumin and elevated ALP in males receiving 180 ppm correlated with increased liver weights in males at 180 ppm and compound-related minimal to mild hypertrophy of hepatocytes in male and female dogs receiving 180 ppm of dicofol.

Based on the results of the study, we agree with the study author that the LOEL is 180 ppm and the NOEL is 30 ppm for dogs when dicofol is fed for 1 year.