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

NOV -7 1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Dyfonate. Review of Chronic/Oncogenicity Feeding Study in Rats.

Tox. Chem. No. 454B
Project No. 1-1027

TO: Lois Rossi
Special Review and
Reregistration Division (H7508C)

FROM: Pamela M. Hurley, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (H7509C)
Pamela M. Hurley 10/1/91

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C)
Roger L. Gardner 11/4/91

Background and Request:

A chronic/oncogenicity feeding study on dyfonate in rats was submitted by ICI Americas, Inc. as a generic data submission in support of reregistration (FIFRA '88). The Toxicology Branch (TB-I) was asked to review and comment on the study.

Toxicology Branch Response:

The Toxicology Branch (TB-I) has reviewed the chronic/oncogenicity feeding study. The study adequately satisfies the regulatory requirements for a chronic/oncogenicity feeding study in rats. It is classified as Core Minimum data. The following statement is a summary of the study.

Dyfonate was not oncogenic when administered in the diet to Sprague-Dawley CD rats for 24 months at dietary levels of 0, 4, 16, or 24 ppm and groups of 20/sex at 120 ppm for 12 months. The LOEL was 60 ppm and the NOEL was 15 ppm, based on decreased body weights and body weight gains and on cholinesterase inhibition (brain, serum and erythrocyte).

SOP# 2000: **ATTACHMENT D**

Reviewer Assessment of Contractor Performance

TASK No. 3-67
DYN. No. 367A

Chemical Name: Dyfonate

Study Type	Accession Number/MRID No.	Final DER Completion Date	EPA Reviewer Evaluation	Hours Spent Performing	
				Secondary Reviews	On-Site Visits
Chronic - Rats	406179-01	--		00878	

Reviewer Signature: _____

Section Head Initials: _____

EPA No.: 68D80056
DYNAMAC No.: 367-A
TASK No.: 3-67A
September 4, 1991

DATA EVALUATION RECORD

DYFONATE

Chronic Oncogenicity Study in Rats

APPROVED BY:

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

Signature: William L. McLellan for

Date: Sept 4, 1991

EPA No.: 68D80056
DYNAMAC No.: 367-A
TASK No.: 3-67A
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DATA EVALUATION RECORD

DYFONATE

Chronic Oncogenicity Study in Rats

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L McLellan
Date: Sept 4, 1991

Charles O. Shore, M.S.
Independent Reviewer
Dynamac Corporation

Signature: Robert J. Weir for
Date: Sept 4, 1991

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
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Signature: Nicolas P. Hajjar
Date: September 4, 1991

Esther Saito, Ph.D.
EPA Reviewer, Section Head
Science Administration Section
(H-7509C)

Signature: Roger Hardan for
Date: 11-4-91

DATA EVALUATION RECORD

GUIDELINE §83-2

STUDY TYPE: Chronic toxicity/oncogenicity feeding study in rats.

MRID NUMBER: 406179-01.

TEST MATERIAL: Dyfonate.

SYNONYMS: O-Ethyl-s-phenylethylphosphodithionate; phosphorodithioic acid, ethyl-, O-ethyl s-phenyl ester; fonofos.

STUDY NUMBER: T:11997.

SPONSOR: ICI Americas, Inc.

TESTING FACILITY: ICI Americas, Inc.; Environmental Health Center; 400 Farmington Avenue, Farmington, CT 06032.

TITLE OF REPORT: Rat Chronic Toxicity and Oncogenicity Study with Dyfonate T11997.

AUTHORS: Pavkov, K.L.; Taylor, D.O.N.

REPORT ISSUED: 05/02/88.

CONCLUSIONS:

Dyfonate was fed for 2 years to groups of 50 rats/sex at dietary levels of 0, 4, 16, or 60 ppm and groups of 20/sex at 120 ppm for 12 months. The mean compound intake (averaged across sexes) was approximately 0.17, 0.65, 2.6, and 6.6 mg/kg/day at 4, 15, 60, or 120 ppm. No carcinogenic response was observed. Survival was not affected by dosing. Mean body weights and weight gains were significantly ($p < 0.05$ or < 0.01) depressed in females but not males receiving 120 ppm; over 52 weeks, weight gain was 21% lower in these females than in controls. Serum cholinesterase activity was significantly ($p < 0.01$) depressed in both sexes receiving 120 ppm; depression compared to controls at 3, 6, and 12 months was 38, 38, and 33% in males and 59, 56, and 54% in females. Erythrocyte cholinesterase activity was not affected in males or females receiving 120 ppm, but brain cholinesterase was depressed 35% in females ($p < 0.01$). At 60 ppm, serum cholinesterase activity was moderately depressed in males but more markedly depressed in females; erythrocyte cholinesterase activity was depressed only at 18 and 24 months in both sexes receiving 60 ppm ($p < 0.01$). Brain cholinesterase was not affected in males but was depressed 35% and 14% at 12 months in females receiving 120 and 60 ppm, respectively. No effects of dosing were observed on clinical laboratory findings (other than cholinesterase), or on organ weight, gross necropsy, or neoplastic findings. The LOEL is 60 ppm and the NOEL is 15 ppm, based on body weight data and cholinesterase activity depression.

Classification: CORE Minimum.

A. MATERIALS:

1. Test Compound: Dyfonate; description: not provided; lot No.: EHC 0586-32/WRC 4921-28-27; purity: 94% by weight.

2. Test Animals: Species: rat; strain: Sprague-Dawley (CD); age: approximately 6 weeks at initiation of exposure; weight: ranged from 132.2 g to 133.2 g (males) and 123.3 g to 123.7 g (females); source: Charles River Breeding Laboratories, Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimatized to laboratory conditions and were held in quarantine between 12 and 14 days. Healthy animals were assigned to the following test groups such that all study groups of the same sex had similar mean body weights:

Test group	Dose in diet (ppm)	Main study (24 months) Males	Main study (24 months) Females	Interim sacrifice (12 months) Males	Interim sacrifice (12 months) Females
Control	0	50	50	10	10
Low (LDT)	4	50	50	10	10
Mid (MDT)	15	50	50	10	10
High (HDT)	60	50	50	10	10
Satellite Toxicity	120	-	-	20	20

Dose levels were selected on the basis of the results of a 5-week rangefinding study in rats. Mortality was observed at 150 and at 300 ppm (it was not stated as to which sex mortality was observed). The range-finding study was not available.

Animals were individually housed in a temperature- and humidity-controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: Diets were prepared at approximately biweekly intervals. Stock mixtures of diets (5 kg) of 400 and 3000 ppm were prepared using a Hobart mixer. Aliquots of these stocks were diluted with feed to give the approximate concentration and mixed in a 3 cubic foot Twinshell tumbler for 25 to 30 minutes. Prepared diets were stored at room temperature in closed containers and offered to animals weekly. Stability of test compound in diets was measured before study initiation. Homogeneity and concentration were checked monthly.

Results: It was reported that test compound was stable in diets and that homogeneity of diets was acceptable. Table 1 summarizes data on concentrations of test compound in diets.

TABLE 1. Concentration Analysis for Dyfonate in Test Diets Fed to Rats for up to 2 Years[a]

Nominal Level (ppm)	n (months)	Summary Mean ± RSD[a]	Coefficient of Variation[b] (%)	Percent of Nominal[b]
4	25	3.70 ± 6.28	9.87%	92.5
15	25	14.20 ± 3.92	6.34%	94.7
60	25	57.67 ± 4.06	4.99%	96.1
120	13	116.46 ± 2.85	4.89%	97.1

[a]The relative standard deviations (RSDs) were calculated by the study authors based on three to six samples at each level for each monthly analysis. The values presented are the average of 13 or 25 monthly RSDs.

[b]Calculated by the reviewers.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights, food consumption, hematology data, clinical chemistry data, and organ weights were analyzed by one-way analysis of variance (ANOVA) and Dunnett's Test or other appropriate tests. Incidence and frequency data were compared using Mantel-Haenzel and other tests available under SAS software.

5. Quality Assurance: A quality assurance statement was signed but not dated.

C. METHODS AND RESULTS:

1. Observations: All animals were inspected at least twice daily for signs of morbidity and mortality. A weekly detailed physical examination of individual rats was conducted for physical changes, and rats were palpated for tissue masses.

Results: Table 2 summarizes mortality and survival data. No effect of dosing on survival was observed. The satellite groups receiving 120 ppm for 12 months also had survival comparable to controls; 18 of 20 males and 17 of 20 females survived until the scheduled sacrifice (53 weeks). The median time to death was 642, 600, 604, and

TABLE 2. Cumulative Mortality and Percent Survival in Rats Fed Dyfonate for 104 Weeks[a]

Dose Group (ppm)	Mortality (percent survival) at Week: 46	54	62	70	86	Termination
			Males			
0	2 (96)	2 (96)	3 (94)	5 (90)	11 (78)	35 (30)
4	3 (94)	3 (94)	4 (92)	8 (84)	21 (58)	38 (24)
15	0 (100)	0 (100)	0 (100)	5 (90)	16 (68)	35 (30)
60	3 (94)	5 (90)	7 (86)	10 (80)	19 (62)	37 (26)
			Females			
0	2 (96)	4 (92)	7 (86)	12 (76)	25 (50)	33 (34)
4	3 (94)	3 (94)	5 (90)	8 (84)	22 (56)	36 (28)
15	3 (94)	3 (94)	6 (88)	8 (84)	22 (56)	35 (30)
60	1 (98)	2 (96)	6 (88)	12 (76)	23 (54)	39 (22)

[a]Data were extracted from study No. T:11997, Tables 1, D1, and D2. Mortality and percent survival were based on 50 rats/sex/dose of the main group. A total of 10 rats/sex/dose survived until their scheduled interim sacrifice dates (12 months) and are not included in this table.

563 days for males fed 0, 4, 15, or 60 ppm, respectively, and 523, 590, 580, or 577 days for females at the same dietary levels.

Clinical signs were those usually found in rats of the strain and age on study. No concentration-response changes were seen, although a few findings were increased in some dosed groups: exophthalmus in males receiving 4 and 15 ppm (17 and 19 compared to 9/60 for controls), hair loss in males receiving 15 ppm, head tilt in 10 females receiving 60 ppm compared to 6/60 for controls, and ataxia (10 females each at 4 and 60 ppm, compared to 5/60 in controls); increased incidence of trunk and limb hair thinning and hair loss in 60-ppm females was also observed (28/60 in treated females vs. 14-15/60 in controls). Loose stools in 13/60 females at 60 ppm compared to 4/60 in controls may have been related to dosing; however, the finding was present in 47 to 60% of males in all groups. No increases in clinically observed or palpable masses or their distribution was observed in dosed males or females.

2. Body Weight: Body weights were recorded weekly for the first 13 weeks and at least every 4 weeks thereafter. Body weights were also recorded immediately prior to scheduled sacrifices at 12 and 24 months.

   Results: Table 3 summarizes mean body weight data at selected intervals during the study. Mean body weights in females receiving 120 ppm were significantly lower than in controls at weeks 1 and 2 ($p < 0.01$ or 0.05) and from weeks 5 to 52 ($p < 0.01$). The mean weight gain over 52 weeks (348 g) for females receiving 120 ppm was 32% lower than in control females. No similar effect was seen in males. There were no significant differences in either body weights or body weight gain at any other dose level in either sex.

3. Food Consumption and Compound Intake: Consumption was determined, and mean daily diet consumption was calculated each week for the first 13 weeks of the study and at least every 4 weeks thereafter.

   Results: No significant effects of dosing on food consumption were observed. At some intervals, food consumption in females receiving 120 ppm was 1 or 2 g/day less than in controls. The mean compound intake was 0.15, 0.61, 2.4, and 5.8 mg/kg/day for males receiving 4, 15, 60, and 120 ppm, and 0.19, 0.70, 2.8, and 7.4 mg/kg/day for females in the same groups.

TABLE 3. Mean Body Weights at Selected Intervals for Rats Fed Dyfonate for up to 104 Weeks[a]

Dose Group (ppm)	Mean Body Weight (g ± S.D.) at Study Weeks: 0	12	28	50	78	102
			Males			
0	180 ± 11.4	569 ± 44.1	678 ± 69.9	764 ± 82.7	808 ± 101.5	711 ± 124.5
4	189 ± 11.6**	569 ± 46.5	674 ± 64.9	765 ± 78.2	796 ± 120.8	785 ± 165.1
15	191 ± 10.5**	587 ± 41.5	695 ± 61.3	783 ± 86.7	792 ± 104.4	716 ± 133.9
60	180 ± 10.7	563 ± 45.9	683 ± 76.9	786 ± 87.3	782 ± 115.2	730 ± 111.6
120	180 ± 6.9	554 ± 38.1	652 ± 71.4	762 ± 64.8		
			Females			
0	149 ± 8.6	303 ± 32.6	355 ± 43.2	441 ± 54.6	489 ± 88.4	495 ± 119.2
4	153 ± 8.8*	307 ± 35.3	371 ± 45.8	435 ± 59.8	501 ± 82.6	467 ± 106.6
15	153 ± 8.2*	308 ± 29.8	369 ± 36.9	445 ± 49.6	474 ± 76.5	492 ± 112.7
60	147 ± 7.9	313 ± 33.4	371 ± 48.1	442 ± 60.0	493 ± 107.3	512 ± 120.5
120	149 ± 5.5	279 ± 42.1*	309 ± 56.8**	348 ± 81.3**		

[a]Data were extracted from study No. T:11997, Tables 6 and 7.

*Significantly different from controls using Dunnett's test, $p < 0.05$.

**Significantly different from controls using Dunnett's test, $p < 0.01$.

4. Ophthalmological Examinations: Ophthalmological examinations were performed on all animals prior to dosing and on all animals scheduled for necropsy at 12 months. Because of adverse findings in 120-ppm females at 12 months, all female controls were examined on study day 384. At 24 months, examinations were performed on rats receiving 60 ppm and on control rats.

   Results: No treatment-related ophthalmologic lesions were seen in males or females receiving 4, 15, or 60 ppm Dyfonate. Females receiving 120 ppm had an increased incidence of shredded iris and/or remnants (9/19, $p < 0.01$) and miotic pupil (7/19, $p < 0.01$) at 12 months. The incidence in iris defects in control females at day 388 was low (2 females of 50). The incidence of cataracts in surviving 60-ppm males at 24 months was 8/16 compared to 4/18 for controls. However, these were not considered to be either biologically or statistically significant effects.

5. Hematology and Clinical Chemistry: Blood was collected from predesignated rats of each sex at each dose level at 3, 6, 12, 18, and 24 months for hematology and clinical analysis. The blood samples at the 12- and 24-month scheduled necropsies were taken from the abdominal aorta, while those taken at the other intervals were from the right intraorbital plexus. Hematology parameters were examined for approximately 20 rats/sex/group at 3 and 6 and 18 months (20/sex for the satellite group) or 10/sex/group at 12 months (20/sex for the satellite group) and on all survivors at 24 months. Differential white cell counts were performed on the 120-ppm and control groups at 3, 6, and 12 months, and on the 60-ppm and control groups at 18 and 24 months. Clinical chemistry parameters were determined for 10 rats/sex/group at all intervals. The CHECKED (X) parameters were examined:

   a. Hematology:

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

†Recommended by Subdivision F (November 1984) Guidelines.

[a]Included if hematocrit levels indicated possible anemia.

[b]Evaluated for control and high-dose rats; if there were significant findings at the high dose, differential counts were performed on 10/sex in the intermediate dose groups.

Results: There were no biologically important effects of dosing on hematology parameters. At the 6-month interval, the RBC count and the hemoglobin level was slightly decreased ($p < 0.05$) in males receiving 60 and 120 ppm, hemoglobin and hematocrit values were decreased ($p < 0.01$) in females receiving 120 ppm and hemoglobin values were decreased ($p < 0.05$) in females receiving 60 ppm.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†	X	Albumin/globulin ratio
	Magnesium†	X	Blood creatinine†
X	Phosphorus† (inorganic)	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol†
X	Sodium†	X	Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)†	X	Direct bilirubin
X	Serum cholinesterase	X	Total protein†
X	Erythrocyte cholinesterase	X	Triglycerides
X	Brain cholinesterase[a]		
	Creatine phosphokinase†		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
X	Gamma glutamyltranspeptidase (GGT)		

Results: Cholinesterase activity data are presented in Tables 4 and 5. It was noted that the methodology for RBC cholinesterase was modified after 12 months and the values are not comparable to those at previous intervals. In males, serum cholinesterase activity was depressed 38, 38, and 33% at 3, 6, and 12 months in the group receiving 120 ppm and 21, 13, 19, 48, and 36% at 3, 6, 12, 18, and 24 months in the group receiving 60 ppm; only the decrease at 18 months was significant ($p < 0.05$). Red cell cholinesterase activity (RBC ChE) was depressed 57 and 45% at 18 and 24 months in the group receiving 60 ppm ($p < 0.01$). At 24 months, RBC ChE was 73 and 74% of the control activity ($p < 0.05$) in the 4- and 15-ppm groups; this is probably not of toxicologic importance (see Reviewers' Discussion and Interpretation of Results, Section E). Brain cholinesterase was not affected in males. In females, serum cholinesterase activity was depressed 59, 56, and 56% at 3, 6, and 12 months in the 120-ppm group; it was depressed 45, 45, 55, 37, and 37% at 3, 6, 12, 18, or 24 months in the

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 4. Mean Cholinesterase Activity (IU/L ± S.D.) in Male Rats Fed Dyfonate for 104 Weeks[a]

Dietary Level (ppm)	Mean Cholinesterase Activity at Month: 3	6	12	18	24
			Serum Cholinesterase (IU/L)		
0	545 ± 94	609 ± 124	657 ± 148	1271 ± 647	1126 ± 739
4	569 ± 181	655 ± 296	803 ± 223	1258 ± 477	1431 ± 776
15	544 ± 130	645 ± 222	724 ± 148	1234 ± 725	1006 ± 361
60	428 ± 70	530 ± 132	530 ± 124	667 ± 189*	717 ± 344
120	339 ± 54**	377 ± 67*	440 ± 94**	--	--
			Erythrocyte Cholinesterase (IU/L)		
0	5542 ± 679	5224 ± 403	5532 ± 570	1631 ± 203	1854 ± 253
4	5595 ± 363	5228 ± 239	5676 ± 309	1400 ± 187	1368 ± 507*
15	5555 ± 268	5138 ± 405	5670 ± 342	1600 ± 199	1376 ± 493*
60	5255 ± 275	5116 ± 224	6042 ± 256*	698 ± 83**	1014 ± 345**
120	5236 ± 650	5278 ± 188	6064 ± 326**	--	--
			Brain Cholinesterase (IU/g tissue)		
0			1.41 ± 0.16		1.40 ± 0.19
4			1.48 ± 0.10		1.56 ± 0.24
15			1.53 ± 0.10		1.54 ± 0.19
60			1.54 ± 0.10		1.39 ± 0.26
120			1.34 ± 0.07		--

*Significantly decreased from control value, $p < 0.05$.

**Significantly decreased from control value, $p < 0.01$.

TABLE 5. Mean Cholinesterase Activity (IU/L ± S.D.) in Female Rats Fed Dyfonate for 2 Years

Dietary Level (ppm)	Mean Cholinesterase Activity at Month: 3	6	12	18	24
			Serum Cholinesterase		
0	2933 ± 806	2919 ± 812	2295 ± 755	2169 ± 628	1773 ± 384
4	3007 ± 986	2877 ± 1062	2222 ± 870	2327 ± 690	1786 ± 562
15	2749 ± 728	2868 ± 929	2193 ± 695	2690 ± 1008	2146 ± 670
60	1627 ± 394**	1612 ± 431**	1036 ± 515*	1363 ± 374*	1117 ± 535*
120	1203 ± 279**	1295 ± 727**	1002 ± 373**		
			Erythrocyte Cholinesterase		
0	4794 ± 944	5309 ± 339	5570 ± 324	1450 ± 331	1654 ± 275
4	5588 ± 407*	5208 ± 207	5686 ± 466	1551 ± 317	1726 ± 232
15	5486 ± 414*	5304 ± 171	5644 ± 410	1560 ± 221	1708 ± 251
60	5604 ± 676	5154 ± 172	5566 ± 521	786 ± 166**	866 ± 178**
120	5094 ± 279	5362 ± 327	5404 ± 229	--	--
			Brain Cholinesterase		
0			1.33 ± 0.08		1.40 ± 0.13
4			1.43 ± 0.12		1.53 ± 0.21
15			1.57 ± 0.16		1.60 ± 0.16
60			1.15 ± 0.12**		1.21 ± 0.19
120			0.86 ± 0.11**		

[a]Data were extracted from study No. T:11997, Tables 20 and 21.

*Significantly decreased from control value, $p < 0.05$.

**Significantly decreased from control value, $p < 0.01$.

60-ppm group. Erythrocyte cholinesterase was depressed 46 and 48% at 18 and 24 months in the 60-ppm group but not affected at earlier intervals; activity was not depressed at 3, 6, or 12 months in the females receiving 120 ppm. Brain cholinesterase was depressed 35% compared to control in the females receiving 120 ppm (12 months) and 14 and 14% at 12 and 24 months in the group receiving 60 ppm.

No treatment-related effects on other clinical chemistry parameters were observed at levels up to 60 ppm (12, 18, or 24 months). In females receiving 120 ppm, but not males, the glucose level was decreased (113 mg/dL) compared to control (168 mg/dL), blood urea nitrogen was increased (22 mg/dL vs. 14 mg/dL), and alkaline phosphatase was increased (30 IU/L vs. 15 IU/L). Similar effects were not seen in males.

6. Urinalysis: Urine samples were collected from animals allowed access to water overnight. Samples were taken from 10 predesignated rats/sex/group of each sex at each dose level at 3, 6, 12, 18, and 24 months. The CHECKED (X) parameters were examined:

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

Results: No toxicologically important effects of dosing on urinary parameters were observed. Changes in protein and volume occurred sporadically.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed for 10 animals/sex/group at 12 and 24 months, and 20/sex for rats receiving 120 ppm (12 months):

†Recommended by Subdivision F (November 1984) Guidelines.

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

All tissues in the control and 120-ppm groups for animals sacrificed at 12 months, all tissues in the control and 60-ppm groups at the terminal sacrifice, and all tissues in all groups for animals that died or were sacrificed moribund were examined histologically. Liver, lungs, and kidneys and other target organs were examined histologically in other groups. All gross lesions and masses were examined.

Results:

a. Organ Weights: No effects on organ weight data were considered of toxicologic importance. Absolute organ weights and weights relative to body weight or brain weight did not differ from control at doses of 4, 15, or 60 ppm at either 12 or 24 months. In females receiving 120 ppm (12 months), the mean weights of

†Recommended by Subdivision F (November 1984) Guidelines.

brain, kidney, and ovary relative to body weight were increased compared to controls but organ-to-brain weight ratios of adrenals, kidney, and liver were slightly decreased compared to control. The increased organ-to-body weight ratios were caused by decreased body weights and not considered a primary effect of dosing. No effects of dosing on organ weights for either sex were observed at the 24-month sacrifice.

b. Gross Pathology:

c. Microscopic Pathology:

1) Nonneoplastic: There were no significant increases in nonneoplastic findings in dosed rats as compared to controls. Table 6 presents selected data for frequent findings. Although a few findings are somewhat increased in rats receiving 60 ppm compared to controls, there is no apparent dose-related trend and the increases are probably random.

2) Neoplastic: Table 7 summarizes data on neoplasms. No increase at any site was caused by dosing. The incidences of neoplasms in dosed as well as control groups were all within the normal historical range. It is interesting to note that the 4-ppm females had a significantly greater number of pituitary carcinomas than adenomas, but the total number of animals with pituitary neoplasms is not different from controls.

D. STUDY AUTHORS' CONCLUSIONS:

Dyfonate had no effect on survival. It produced toxicity in female rats at 120 ppm, expressed as a 21% decrease in mean body weight compared to control. No body weight effect was seen in males at any dose. No effects on food consumption were observed. Serum cholinesterase was inhibited at 120 ppm in both sexes. At 60 ppm, inhibition was statistically significant in females throughout the entire study but in males only at 18 months. Red cell cholinesterase activity was inhibited at 60 ppm in both sexes at 18 and 24 months after the analytical methodology was changed to improve sensitivity. Brain cholinesterase activity was not altered in male rats but was inhibited in females at 60 and 120 ppm at 12 but not at 24 months. An increase in iridial lesions and miotic pupil in 120-ppm female rats at 12 months, and in loose stools in the 60-ppm female group, may have been related to cholinesterase

TABLE 6. Frequent Nonneoplastic Findings in Rats Fed Dyfonate for 2 Years[a]

Organ/Finding	Dietary Level (ppm): Males 0	Males 4	Males 15	Males 60	Females 0	Females 4	Females 15	Females 60
Kidney	(58)	(57)	(60)	(59)	(59)	(59)	(60)	(59)
Progressive nephropathy	48	29	35	44	10	4	5	15
Urothelial degeneration/ calcification	0	1	0	0	32	28	32	38
Liver	(60)	(57)	(60)	(57)	(60)	(59)	(60)	(59)
Spongiosis hepatica	9	7	8	14	2	1	0	4
Bile duct hyperplasia/ fibrosis	46	36	39	41	35	35	38	32
Mammary gland	(40)	(33)	(33)	(36)	(60)	(50)	(53)	(57)
Cystic hyperplasia	6	7	9	11	17	19	23	21
Ovaries					(60)	(43)	(39)	(48)
Stomal cyst					11	9	11	14
Uterus					(59)	(40)	(39)	(48)
Endometrial cyst					1	0	0	4
Hyperplastic cyst					3	2	2	5
Pituitary gland	(57)	(43)	(39)	(48)	(59)	(50)	(48)	(55)
Hyperplasia/hypertrophy (pars distalis)	2	2	0	5	3	2	1	4
Bone marrow	(59)	(37)	(35)	(50)	(60)	(36)	(35)	(49)
Myeloid hyperplasia	14	10	15	19	15	12	8	12

[a]Data for rats receiving 120 ppm for 52 weeks are not included. Data were extracted from study No. T: 11997, Tables 26E, 27E.

TABLE 7. Neoplastic Findings in Rats Fed Dyfonate for 2 Years[a]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	4	15	60	120	0	4	15	60	120
Mammary gland	(40)	(33)	(33)	(36)	(16)	(60)	(50)	(53)	(57)	(20)
Benign[b]	1	2	2	2	0	28	28	28	28	0
Malignant, primary[c]	0	1	0	0	0	13	14	12	9	0
Thyroid gland	(53)	(25)	(33)	(43)	(18)	(57)	(29)	(30)	(39)	(18)
Benign[d]	4	2	4	6	0	2	1	2	4	0
Malignant[e]	0	0	2	4	0	0	0	0	1	0
Liver	(60)	(57)	(60)	(57)	(20)	(60)	(59)	(59)	(59)	(20)
Hepatocellular adenoma	3	4	7	5	0	6	7	4	10	0
Hepatocellular carcinoma	0	1	1	1	0	0	0	0	0	0
Kidney	(58)	(57)	(60)	(59)	(19)	(59)	(59)	(60)	(59)	(18)
Carcinoma	1	2	0	3	0	0	0	0	0	0
Adrenals	(58)	(41)	(37)	(47)	(20)	(59)	(43)	(46)	(49)	(20)
Cortical adenoma	5	8	7	8	0	17	23	20	20	1
Pheochromocytoma	10[f]	9[f]	8[f]	9[f]	0	7	1	2	5	0
Carcinoma	0	0	0	0	0	0	1	1	0	0
Brain	(59)	(41)	(37)	(48)	(20)	(59)	(43)	(41)	(49)	(20)
Carcinoma	2	0	0	1	0	2	3	3	4	0
Pituitary gland	(57)	(43)	(39)	(48)	(20)	(59)	(50)	(47)	(55)	(18)
Adenoma	28	18	27	20	4	32	9	21	27	6
Carcinoma	6	12	5	11	0	14	33	21	23	0

[a]Data were extracted from study No. T: 11997, Tables 28E, 29E.

[b]Adenoma; adenoma-cyst; fibroadenoma; fibroadenoma-cystic.

[c]Adenocarcinoma; cystadenocarcinoma.

[d]Adenoma, adenoma-cyst, parafollicular cell adenoma.

[e]Adenocarcinoma-parafollicular; adenocarcinoma-sclerosing carcinoma (1); carcinoma.

[f]One pheochromocytoma in each of the groups of males receiving 0, 4, 15, or 60 ppm was malignant.

inhibition resulting from treatment. There were no gross necropsy observations, changes in organ weights, or changes in neoplastic findings that could be attributed to administration of Dyfonate. The NOEL for the 2-year study was 15 ppm, 0.6 and 0.7 mg/kg/day for males and females, respectively. Dyfonate is not considered to be oncogenic at any dose level.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The formats for data presentation, particularly for summary histologic findings, were not standard and were rather difficult to follow. Sections of the copy reviewed also had table pages that were of poor quality and unreadable data, and had to be verified by accessing individual pathology sheets. Data on homogeneity of diets were inadequate for complete evaluation. In general, the summary data on mean values that were checked reflected the individual animal data, and outlier values in the individual data were not a problem. The method for cholinesterase activity was not provided or referenced, and the values changed markedly for erythrocyte activity when the methodology was changed for the 18- and 24-month assays. It is possible that effects on RBC cholinesterase were actually present but not detected at the 3-, 6-, and 12-month intervals of analysis.

The reviewers assess that the rats (particularly males) could have tolerated a slightly higher dose between 60 and 120 ppm. No effects on weight gain were observed in males at 120 ppm for 12 months when intake was 5.8 mg/kg/day, whereas in females at 120 ppm, intake was 7.4 mg/kg/day and an effect on weight gain was observed. Also no effect on brain cholinesterase was seen in males at levels up to 120 ppm, and at 60 ppm no effect on serum cholinesterase activity was observed except at months 18 and 24; furthermore, the decrease at month 24 was not statistically significant. The effect on brain cholinesterase activity was marginal at 60 ppm.

We agree with the study authors that there was no oncogenic effect and that the NOEL is 15 ppm.