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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 Oncogenicity Study in Mice.

Tox. Chem. No. 454B
Project No. 1-1028

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

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

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

Background and Request:

An oncogenicity study on dyfonate in mice was submitted by Stauffer Chemical Company 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 oncogenicity study. The study adequately satisfies the regulatory requirements for an oncogenicity study in mice. It is classified as Core Guideline data. The following statement is a summary of the study.

Dyfonate was not oncogenic when administered in the diet to CD-1 mice for 18 months at dietary levels of 0, 5, 25, or 100 ppm (males: 1, 3, and 12 mg/kg/day; females: 1, 4, and 15 mg/kg/day). An MTD was approached. Gross pathological changes in the duodenum (raised foci, masses and thickening, hyperplasia and hypertrophy) were noted in the high-dose males. Brain cholinesterase activity was reduced in males and erythrocyte and serum cholinesterase activities were reduced in both sexes. The LOEL was 25 ppm and the NOEL was 5 ppm based on inhibition of cholinesterase activity.

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EPA No.: 68D80056
DYNAMAC No.: 367-B
TASK No.: 3-67B
August 28, 1991

DATA EVALUATION RECORD

FONOFOS

Oncogenicity Feeding Study in Mice

APPROVED BY:

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

Signature: Robert J. Weir

Date: August 28, 1991

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DYNAMAC No.: 367-B
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DATA EVALUATION RECORD

FONOFOS

Oncogenicity Feeding Study in Mice

REVIEWED BY:

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

Signature: John J. Liccione
Date: August 28, 1991

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Date: _____

DATA EVALUATION RECORD

GUIDELINE § 83-2

STUDY TYPE: Oncogenicity feeding study in mice.

MRID NUMBER: 401501-21.

TEST MATERIAL: Fonofos; 0-ethyl-S-phenylethylphosphonodithioate.

SYNONYM: Dyfonate.

STUDY NUMBER: T-11995.

SPONSOR: Stauffer Chemical Company.

TESTING FACILITY: Stauffer Chemical Company, Environmental Health Center, Farmington, CT.

TITLE OF REPORT: 18-Month Dietary Oncogenicity Study with Dyfonate Technical in Mice.

AUTHORS: Sprague, G.L., and Zwicker, G.M.

REPORT ISSUED: March 12, 1987.

CONCLUSIONS:

Dyfonate was not oncogenic when administered in the diet to CD-1 mice for 18 months at dietary levels of 0, 5, 25, or 100 ppm (males: 1, 3, and 12 mg/kg/day; females: 1, 4, and 15 mg/kg/day). Gross pathological changes in the duodenum (raised foci, masses and thickening) were noted in the high-dose males. Gross findings in the duodenum correlated with histological effects (hyperplasia and hypertrophy). The biological significance of pathological changes in the duodenum is unknown. Slight reductions in body weights and body weight gains were noted in the high-dose males during the first 13 weeks of treatment. Food intake in the high-dose males was slightly less than controls during the first 25 weeks of the study. The activity of serum cholinesterase was reduced in the mid-dose males at 12 months and in high-dose males and females at 12 and 18 months. Brain cholinesterase activity was depressed in the high-dose males at 12 and 18 months and in the mid- and high-dose males at 18 months. Inhibition of erythrocyte activity was noted in the high-dose males and females at 18 months. There were no significant effects on organ weights, hematology, ophthalmology, clinical signs, and mortality.

Based on inhibition of cholinesterase activity, the LOEL is 25 ppm, and the NOEL is 5 ppm.

Classification: Core Guideline. This study satisfies the requirements for an oncogenicity study (Guideline 83-2) in the mouse.

A. MATERIALS:

1. Test Compound: Dyfonate technical; description: liquid; lot No.: WRC 4921-28-27; EHC-0536-32; purity: 94.0% (wt).

2. Test Animals: Species: mouse; strain: CD-1; age: approximately 6 weeks at study initiation; weight: males--16.8 to 27.6 g, females--14.8 to 22.3 g; source: Charles River Breeding Laboratories, Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: Mice were acclimated to laboratory conditions for approximately 12 to 15 days prior to treatment. They were randomly assigned to the following test groups on the basis of body weight:

Test Group		Dose in Diet (ppm)	Main Study (18 months) Males	Main Study (18 months) Females	Interim Sacrifice (12 months) Males	Interim Sacrifice (12 months) Females
1	Control	0	50	50	10	10
2	Low	5	50	50	10	10
3	Mid	25	50	50	10	10
4	High	100	50	50	10	10

Dose levels were selected on the basis of the results of a 5-week range-finding study. This study was not available for review. In the 5-week study, a loss in body weight (12-15% below control) was observed in male mice receiving 150 ppm for 2 weeks; mortality occurred in males receiving 300 ppm. Cholinesterase inhibition was noted at dietary levels as low as 32 ppm.

In the present study, mice were housed singly in cages in a room with temperature and humidity controls set at 19-24°C and 40-60%, respectively, and with a 12-hour light/dark cycle.

2. Diet Preparation: The test substance was thoroughly mixed with approximately 4.5 kg of feed. This premix was then adjusted to the required concentration by adding the appropriate amount of feed, and was blended for 5 minutes using an intensifier bar and 15 minutes without it. Diets containing the test substance were prepared at monthly intervals. The stability of the test substance (2 ppm) in the diet was determined prior to initiation of the study for a period of 47 days. The homogeneity and concentration of the test substance in the diet were analyzed at approximately monthly intervals. Homogeneity was determined by comparing dyfonate concentrations for multiple samples (up to nine) from each diet analyzed. The mean, standard deviation, and relative standard deviation were calculated, and the latter was used as a measure of homogeneity.

Results: Stability data indicated that the test substance was stable in the diet over a period of 33 days. Forty-seven days after preparation, approximately 80% of the

original test substance was detectable. Table 1 summarizes data on nominal and analyzed dietary levels of the test substance. Mean concentrations in the diets at dose levels of 5, 25, and 100 ppm were 93.0 ± 9.2%, 93.8 ± 7.9%, and 94.7 ± 6.2% of target (20 intervals of analysis), respectively. The average relative standard deviations over the entire study were 5.6, 3.8, and 2.9% for the 5-, 25- and 100-ppm nominal dietary levels.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Chow No. 5002) and water ad libitum.

4. Statistics: Mean body weights, food consumption, hematology, and organ weights were analyzed for significance by Dunnett's test. Nonparametric data were evaluated by the Mann-Whitney U-test. Incidences were compared using the Mantel-Haenzel and other tests available under SAS software.

5. Quality Assurance: A quality assurance statement was signed and dated March 9, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for moribundity and mortality. Each animal was palpated for masses during weekly physical examination.

Results: Cumulative mortality and survival data are summarized in Table 2. The mortality of the animals was not affected by the administration of dyfonate. No clinical signs attributable to treatment were observed during the study period.

2. Body Weight: Body weights were recorded weekly for the first 13 weeks and monthly thereafter.

Results: Table 3 summarizes data on mean body weights at selected intervals. Mean body weights were moderately decreased in males receiving 100 ppm during the first 13 weeks of the study; the decreases reached statistical significance ($p < 0.05$) at most of the weekly intervals during the first 13 weeks. Mean body weights were approximately 9% lower than controls in males receiving 100 ppm during the first week. Body weight gains in control males and high-dose males were 2.0 g and -1.0 g, respectively, during the first week. From weeks 1 to 13, mean body weights were approximately 3-6% lower than control in the high-dose males. Body weight gains in

TABLE 1. Dietary Levels of Dyfonate

Nominal Level (ppm)	Analyzed Level ppm (mean ± S.D.)	Range
5	4.65 ± 0.32	(4.2 - 5.6)
25	23.47 ± 1.35	(21.7 - 26.8)
100	94.68 ± 4.34	(87.4 - 105.0)

TABLE 2. Cumulative Mortality and Percent Survival in Mice Fed Dyfonate for 79 Weeks[a]

Dietary Level (ppm)	Mortality (Percent Survival)[b] at Week:				
	44	52	60	68	80
			Males		
0	5 (90)	6 (88)	9 (82)	19 (62)	32 (36)
5	7 (86)	12 (76)	16 (68)	22 (56)	31 (38)
25	2 (96)	3 (94)	9 (82)	21 (58)	32 (36)
100	1 (98)	5 (90)	14 (72)	22 (56)	26 (48)
			Females		
0	2 (96)	6 (88)	8 (84)	15 (70)	27 (46)
5	3 (94)	6 (88)	12 (76)	16 (68)	20 (60)
25	4 (92)	7 (86)	11 (78)	16 (68)	21 (58)
100	3 (94)	4 (92)	7 (86)	14 (72)	23 (54)

[a]Data were extracted from study No. T-11995, Table 1.

[b]Mortality and percent survival were based on 50 mice/sex/dose of the main group. An additional 10 mice/sex/dose survived until their scheduled sacrifice at week 55 and are not included in this table.

TABLE 3. Mean Body Weights at Selected Intervals for Mice Fed Dyfonate for 79 Weeks[a]

Dietary Level (ppm)	Mean Body Weight (g ± S.D.) at Selected Study Weeks: 0	13	25	52	77
			Males		
0	30 ± 1.6	39 ± 3.1	42 ± 4.4	43 ± 5.5	44 ± 6.5
5	30 ± 1.6	39 ± 3.7	42 ± 5.3	45 ± 5.6	43 ± 5.4
25	30 ± 1.5	41 ± 3.7*	44 ± 5.5*	45 ± 5.9	43 ± 5.6
100	30 ± 1.6	37 ± 3.8	41 ± 6.1	43 ± 7.2	42 ± 7.8
			Females		
0	22 ± 1.1	30 ± 2.8	32 ± 2.8	33 ± 3.0	35 ± 4.0
5	23 ± 1.2	29 ± 2.4	32 ± 3.5	34 ± 3.1	34 ± 3.6
25	23 ± 1.3	30 ± 2.5	33 ± 2.9	34 ± 3.0	35 ± 3.1
100	23 ± 1.1	30 ± 2.7	33 ± 3.6	35 ± 4.4	36 ± 4.1

[a]Data were extracted from study No. T-11995, Table D1.

*Significantly different from control values at $p < 0.05$.

control and high-dose males were 9.0 g and 7.0 g, respectively, between weeks 1 and 13. Between weeks 1 and 25, mean body weights of the mid-dose males were slightly (3-8%) higher than in controls; the increases in body weight reached statistical significance ($p < 0.05$) at various intervals during this time period. The study authors considered the increase in body weights of the mid-dose males to be possibly treatment-related. The increase in body weights is of doubtful biological significance. No treatment-related differences in either body weights or body weight gains were observed in females.

3. Food Consumption and Compound Intake: Food consumption was determined weekly during the first 13 weeks and monthly thereafter. Compound intake (mg/kg/day) was calculated based on nominal dyfonate concentration and body weights.

   Results: Table 4 summarizes data on food consumption. The mean food consumption of the high-dose males was slightly less than that of controls during the first 25 weeks. The slight decrease in food consumption reached statistical significance ($p < 0.05$) at various intervals during this time period. Compound intakes in males receiving 5, 25, or 100 ppm were 1, 3, and 12 mg/kg/day, respectively. In females receiving the same doses, the compound intakes were 1, 4, and 15 mg/kg/day, respectively.

4. Ophthalmological Examinations: Ophthalmological examinations were performed on 10 control and 10 high-dose males and females prior to interim sacrifice, and on 10 mice of each sex from each treatment group prior to study termination.

   Results: There were no abnormalities of the eyes that were considered by the study author to have been induced by treatment with dyfonate.

5. Hematology and Clinical Chemistry: Blood was collected from the abdominal aorta at 12 and 18 months for hematology and clinical analysis from 10 mice/sex/dose. Both fasted and unfasted mice were used at study termination to evaluate the effect of fasting on the extent of cholinesterase inhibition. The CHECKED (X) parameters were examined:

TABLE 4. Mean Food Consumption at Selected Intervals for Mice Fed Dyfonate for 79 Weeks*

Dietary Level (ppm)	Mean Food Consumption (g/day) at Selected Study Weeks: 1	13	25	52	77
			Males		
0	5.3 ± 1.3	4.8 ± 0.8	4.6 ± 0.8	4.3 ± 0.6	4.8 ± 0.6
5	5.6 ± 1.3	4.8 ± 0.7	4.4 ± 0.9	4.5 ± 0.6	5.1 ± 0.7
25	5.0 ± 0.8	4.7 ± 0.7	4.6 ± 0.8	4.5 ± 0.9*	4.1 ± 0.9*
100	4.5 ± 1.2	4.3 ± 0.5*	4.2 ± 0.5*	4.4 ± 0.8	4.8 ± 0.7
			Females		
0	5.5 ± 1.4	5.6 ± 1.2	4.7 ± 1.3	4.4 ± 1.1	4.6 ± 0.8
5	5.7 ± 1.8	5.9 ± 1.8	4.9 ± 1.3	4.8 ± 1.3	4.8 ± 1.0
25	4.9 ± 1.5	5.6 ± 1.4	4.8 ± 1.4	4.5 ± 1.3	4.8 ± 1.4
100	4.4 ± 1.0*	4.7 ± 1.3*	3.9 ± 0.9*	4.4 ± 1.0	4.2 ± 0.7

*Data were extracted from study No. T-11995.

*Significantly different from control values at $p < 0.05$.

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count
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)		
	Red cell morphology		

Results: No effects of dosing on hematology parameters were seen.

b. Clinical Chemistry:

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

Results: Table 5 summarizes data on the inhibition of the activities of serum, erythrocyte, and brain cholinesterase. Serum cholinesterase activity was reduced by 80% in the high-dose males, 46% in the mid-dose males, and 68% in the high-dose females after 12 months of treatment. Brain cholinesterase activity was reduced by 59% in the high-dose

†Recommended by Subdivision F (November 1984) Guidelines.

[a]Erythrocyte cholinesterase was determined at termination only due to methodological problems encountered at interim sacrifice.

TABLE 5. Cholinesterase Activity in Mice Fed Dyfonate for 12 and 18 Months[a]

Dietary Level (ppm)	Cholinesterase Activity (Mean ± S.D.) at Sampling Intervals:				
	Serum Cholinesterase (IU/L)		RBC Cholinesterase (IU/10^{15} RBC)	Brain Cholinesterase (IU/G Tissue)[b]	
	12 months	18 months	18 months	12 months	18 months
			Males		
0	9693 ± 3280	9404 ± 3027	1854 ± 437	1.71 ± 0.18	1.63 ± 0.13
5	8509 ± 4559	9508 ± 5309	2500 ± 1210	-	1.58 ± 0.14
25	5172* ± 745	6573 ± 1470	1416 ± 518	-	1.57 ± 0.16
100	1917* ± 403	3282* ± 1603	954* ± 426	0.72* ± 0.13	1.01* ± 0.13
			Females		
0	9992 ± 1101	10425 ± 2673	1372 ± 505	1.83 ± 0.15	1.71 ± 0.18
5	10007 ± 1590	9470 ± 2809	1412 ± 569	-	1.56 ± 0.13
25	6813 ± 1207	8560 ± 1455	918 ± 387	-	1.46* ± 0.16
100	3215* ± 725	4513* ± 1721	502* ± 197	0.80* ± 0.09	0.98* ± 0.15

[a]Data were extracted from study No. T-11995, Table F1.

[b]Brain cholinesterase activity was not measured in the low- and mid-dose animals.

*Significantly different from control value at $p < 0.05$.

males at 12 months of treatment. Serum cholinesterase activity was reduced by 64% in the high-dose males and by 57% in the high-dose females following 18 months of treatment. Erythrocyte cholinesterase activity was reduced in the high-dose males and females by 49% and 63%, respectively, following 18 months of treatment. Brain cholinesterase activity was reduced by 38% in the high-dose males, 43% in the high-dose females, and 15% in the mid-dose females.

6. Urinalysis: Urinalysis was not performed.

7. Sacrifice and Pathology: All 0- and 100-ppm mice at interim and terminal sacrifice; target organs from 5- and 25-ppm groups; all animals that died during study; and all gross lesions or masses and lungs, livers, and kidneys from all animals were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue	X Aorta†	XX Brain
X Salivary glands†	X Heart†	X Peripheral nerve (sciatic nerve)†
X Esophagus†	X Bone marrow† (sternum)	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
XX Liver†	XX Kidneys†	XX Adrenals†
X 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 Vagina	
	X Cervix	Other
	X Coagulating glands	X Bone (tibia/femur and joint)†
		X Skeletal muscle (thigh)†
		X Skin
		X All gross lesions and masses

†Recommended by Subdivision F (November 1984) Guidelines.

Weights were recorded from organs obtained from 10 mice/dose group sacrificed at interim and from up to 27 mice/dose group sacrificed at termination.

Results:

a. Organ Weights: There were no organ weight changes that could be considered a result of treatment.

b. Gross Pathology: Male mice receiving 100 ppm for 18 months showed an increased incidence of findings involving the duodenal mucosa. These changes included raised foci (4/60 control males, 1/60 low-dose males, 5/60 mid-dose males, and 12/60 high-dose males), and duodenal thickening (0/60 control males, 3/60 low-dose males, 3/60 mid-dose males, and 5/60 high-dose males). The study authors indicated that the increase in gross findings in the duodenum was significant ($p < 0.05$) only in the high-dose males, although statistical analyses of the data were not presented in any of the summary or individual tables of necropsy findings. Other gross pathological findings noted at gross necropsy either occurred with similar incidence in the treated and control animals or occurred sporadically. The authors stated that no treatment-related effects were found in females; however, the numbers of raised foci for the duodenal mucosa were 2/60, 5/60, 10/60, and 14/60 for the controls, low-, mid-, and high-dose animals, respectively. In addition, the incidences of duodenal thickening were 0/60, 2/60, 3/60, and 4/60 for these same groups.

c. Microscopic Pathology:

1) Nonneoplastic: Table 6 summarizes the incidence of frequently occurring nonneoplastic lesions in mice. The most frequent nonneoplastic finding noted in this study was amyloidosis, which occurred with similar incidence in control and all treated animals at 12 months and 18 months. The incidence of amyloidosis varied among the different organs. Minimal to moderate hyperplasia/hypertrophy of the duodenum occurred with a higher incidence in the high-dose males; this lesion in males was regarded by the study authors to be treatment-related. Duodenal hypertrophy/hyperplasia was seen in 14 of 30 high-dose males with gross duodenal lesions. The duodenal hyperplasia/hypertrophy was characterized by increased number and size of crypt columnar epithelial cells resulting in a lengthening of crypts and shortening of villi.

TABLE 6. Selected Nonneoplastic Lesions in Mice Fed Dyfonate for 18 Months[a,b]

	Dietary Level (ppm)							
	Males				Females			
Organ/Finding	0	5	25	100	0	5	25	100
Heart	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
Amyloidosis	35	29	35	23	24	26	31	16
Inflammation, chronic	6	7	7	6	5	3	8	6
Liver	(59)	(58)	(58)	(60)	(59)	(60)	(60)	(60)
Amyloidosis	37	27	32	31	25	27	27	27
Inflammation, lymphocytic	9	3	10	12	8	9	15	10
Stomach	(54)	(27)	(30)	(58)	(56)	(24)	(22)	(57)
Amyloidosis	21	15	19	24	21	10	9	17
Hyperplasia/hypertrophy	2	0	2	5	6	2	1	10
Duodenum	(52)	(27)	(51)	(59)	(52)	(23)	(28)	(55)
Amyloidodsis	37	21	29	35	26	19	20	31
Hyperplasia/hypertrophy	5	0	7	26	19	6	9	21
Inflammation, subacute	0	0	0	3	0	0	0	0
Kidneys	(59)	(59)	(59)	(60)	(59)	(60)	(60)	(59)
Amyloidosis	30	37	36	36	28	33	32	33
Glomerulonephropathy	19	20	22	24	21	33	32	33

[a]Data extracted from study No. T-11995, Tables 5 and 6.

[b]The numbers in parentheses indicate the number of animals with tissues examined (includes animals in main groups and satellite groups).

These changes resulted in the elevation or thickening of the duodenal mucosa. Mild to moderate submucosal amyloid was present in 19 of 30 high-dose males with proliferative mucosa. Four high-dose males displayed mild to moderately severe inflammation of the duodenum in addition to proliferative mucosa and amyloid deposition. Theincidence of duodenal lesions in females of all treated groups was similar to controls. A variety of other tissue findings occurred infrequently in mice of both sexes; these were without relationship to treatment.

2) Neoplastic: No neoplastic lesions attributable to treatment with any dietary level of dyfonate were found in any tissue of any mouse.

D. STUDY AUTHORS' CONCLUSIONS:

Dyfonate was administered via the diet to groups of CD-1 mice (50/sex/main group) at dose levels of 0, 5, 25, or 100 ppm fonofos for 18 months. In addition, groups of CD-1 mice (10/sex/satellite group) received the same dose levels for 12 months. Dose levels, based on nominal fonofos concentrations, were 1, 3, and 12 mg/kg/day for males and 1, 4, and 15 mg/kg/day for females. Reduced mean body weights (3-9% below control level) were observed in the high-dose males during the first 2-3 months. There were no adverse effects of fonofos on organ weights, clinical observations, hematology parameters, or survival. Inhibition of serum, erythrocyte, and brain cholinesterase (35-80% inhibition depending on the enzyme and time measured) was noted in the high-dose males and females. Inhibition of serum cholinesterase activity was noted in mid-dose males at 12 months; the mid dose approached the threshold level for statistically and biologically significant inhibition of serum cholinesterase. The increased incidence of gross pathological changes in the duodenal mucosa, consisting of raised foci, thickening, and masses, was noted in the high-dose males sacrificed between 12 and 18 months. Duodenal mucosal hypertrophy and hyperplasia were observed histopathologically in the high-dose males. The most frequent histopathological finding noted in all dose groups was amyloidosis, a common finding for CD-1 mice. There were no treatment-related effects on neoplasms.

In conclusion, no evidence of an oncogenic effect was seen in mice treated with fonofos in the diet for 18 months. The high dose (100 ppm) resulted in minimal toxicity in male mice, including reduced body weights early in the study, mucosal hypertrophy and hyperplasia in the duodenum, and cholinesterase inhibition. Cholinesterase inhibition was also noted in mid-dose males. No signs of toxicity were seen in mice treated with 5 ppm fonofos. Since no oncogenic effects resulted at the highest dose level, the no-observed effect level for oncogenicity in this study was 100 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete and adequate, and the data were reported in a satisfactory manner. Summary data were supported by individual animal data. A tabular correlation of gross and histopathological findings was presented. Dose levels were selected on the basis of the results of a 5-week range-finding study that was not available for our review.

We agree with the study authors' assessment that dyfonate was not oncogenic under the conditions of the study.

The reviewers agree with the study authors' assessment that the highest dietary level in the study (100 ppm) was associated with minimal toxicity, including reduced body weights early in the study, mucosal hypertrophy and hyperplasia in the duodenum, and cholinesterase inhibition. Also, an MTD was approached.

Gross pathological and histopathological changes were limited to the duodenum of the high-dose males. Gross changes in the duodenum of high-dose males consisted of raised foci, masses, and thickening. The study authors indicated that this effect was significant ($p < 0.05$) in the high-dose males, although statistical analyses were not presented in individual or summary tables of gross effects. Gross findings in the duodenum correlated with histopathological changes. Histopathological effects in the high-dose males included minimal to moderate hyperplasia and hypertrophy of the duodenal mucosa. The biological significance of the pathological changes in the duodenum is unknown. Amyloidosis, a lesion common in aging mice of the CD-1 strain, was present in a variety of organs, and with a similar incidence in control and treated mice.

Mean body weights were approximately 3-9% lower than controls in the high-dose males during the first 13 weeks of the study. Food intake in these males was also slightly less than control levels during the first 25 weeks of treatment.

Assessment of clinical chemistry data indicated treatment-related inhibition of serum, erythrocyte, and brain cholinesterase activity. The activity of serum cholinesterase was significantly ($p < 0.05$) reduced by greater than 40% in the mid- and high-dose males and high-dose females at 12 months, and in the high-dose males and females at 18 months. The activity of brain cholinesterase was significantly ($p < 0.05$) reduced by 59% in the high-dose males at 12 months, while the activity of this enzyme was significantly ($p < 0.05$) reduced by approximately 40% in the high-dose males and females and by 15% in the mid-dose males at 18 months of treatment. The activity of erythrocyte cholinesterase was reduced by greater than 40% in the high-dose males and females at 18 months of treatment.

There was no significant effect of dosing on organ weight, hematology, ophthalmology, or mortality.

The LOEL for systemic toxicity is 25 ppm based on cholinesterase inhibition, and the NOEL is 5 ppm.