CASWELL 454
FILE

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

008806

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Dyfonate. Review of Chronic Feeding Study in Dogs.

Tox. Chem. No. 454B
Project No. 1-1724

TO: Joanne Edwards, PM Team #72
Special Review and
Reregistration Division (H7508C)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 10/24/91
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C) Roger L. Gardner 11-1-91 KB 11/5/91

Background and Request:

A chronic feeding study on dyfonate in dogs was submitted by ICI Agricultural Products as a generic data submission in support of reregistration. 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 feeding study. The study does not adequately satisfy the regulatory requirement for a chronic feeding study in dogs. It is classified as Core Supplementary data. The following statements summarize the study and specify the deficiencies.

Dyfonate was tested in a chronic feeding study in dogs at 0, 16(8.0), 60 and 240 ppm for 2 years. The cholinesterase NOEL is 8.0 ppm and the LOEL is 16.0 ppm. The systemic NOEL is 16.0(8.0) ppm and the LOEL is 60 ppm [at 240 ppm: deaths, clinical signs, decrease in body weight, increase in serum alkaline phosphatase, possible liver effects (organ weights and histopathology) and acute tissue congestion; at 60 ppm: a few clinical signs, liver weight increases and some possible body weight decreases, however, there were no major systemic effects].

This study is classified as Core Supplementary. The quality of the study was not sufficient for regulatory purposes. The following list discusses all the difficulties with the study:

1. Fonofos arrived in 4 different batches. There was insufficient detail on the differences between the batches (purity, etc.).

2. Some of the dogs were of borderline age at the start of the study (some were over 9 months in age).

3. The source of the dogs was from 2 places. There was insufficient detail on how these were randomized and distributed.

4. There was an unusual feeding pattern. The animals were fed 6 days per week. On Saturday, they were given double the amount of diet and then were not fed on Sundays. Since dogs will generally eat all of what they are given at one time, they were receiving double the dose of the test chemical on Saturday and nothing on Sunday.

5. There was no information on the frequency of diet preparation, storage, stability of the test chemical in the diet, homogeneity of mixing or concentration analyses. It is not known what the dogs were actually receiving.

6. In the high dose group, two dogs were replaced, one on day 1 and one 6 weeks into the study. From the data, it appears that the replacement dog at 6 weeks was not kept on the diet an extra 6 weeks at the other end of the study. It appears that this animal was terminated at 96 weeks. Since there are so few dogs in a chronic feeding study, the fact that this dog was terminated early for no reason other than assumed convenience, then this could have had a significant effect on the outcome of the study.

7. No electrolytes were measured for the clinical chemistry analyses.

8. The microscopic examinations were incomplete. For a nonrodent study, organs from <u>all animals in all dose groups</u> are required to be examined. Only selected organs were examined in selected dose groups.

9. Statistical calculations were not conducted. Without these, one can only "eyeball" the data and guess as to which might be significant.

10. The individual animal data for the microscopic examinations consisted of one scanty table which had no legend. The table was unreadable without the legend.

Reviewed By: Pamela Hurley, Ph.D. Pamela M. Hurley 10/24/91
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner Roger L. Gardner 11/1/91
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DATA EVALUATION REPORT 009806

STUDY TYPE: Chronic feeding - nonrodent (dog) 83-1

TOX. CHEM. NO.: 454B

ACCESSION NUMBER/MRID NO.: 00822-33

TEST MATERIAL: Dyfonate

SYNONYMS: Fonofos

STUDY NUMBER(S): T-2153?

SPONSOR: ICI Agricultural Products, Wilmington, Delaware

TESTING FACILITY: Woodard Research Corporation, Herndon, VA

TITLE OF REPORT: Dyfonate (N-2790) - Safety Evaluation by Dietary Administration to Dogs for 106 Weeks

AUTHOR(S): Woodard, M.W., Donoso, J., Gray, J.P., Banerjee, B.N., Woodard, G.

REPORT ISSUED: 1/10/69

CONCLUSION: Dyfonate was tested in a chronic feeding study in dogs at 0, 16(8.0), 60 and 240 ppm for 2 years. The cholinesterase NOEL is 8.0 ppm and the LOEL is 16.0 ppm. The systemic NOEL is 16.0(8.0) ppm and the LOEL is 60 ppm [at 240 ppm: deaths, clinical signs, decrease in body weight, increase in serum alkaline phosphatase, possible liver effects (organ weights and histopathology) and acute tissue congestion; at 60 ppm: a few clinical signs, liver weight increases and some possible body weight decreases, however, there were no major systemic effects].

Classification: Supplementary (see discussion)

Testing Guideline Satisfied: None

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: o-ethyl s-phenyl ethylphosphonodithioate

Description: clear liquid
Batch #(s), Other #(s): OD-173-RAS-66, OD-173-RAS-71, HMP 26:4-12-66 and HMP 26
Purity: 99.5%, 99.8-99.9% pure
Source: Stauffer Chemical Company
Vehicle (if applicable): acetone (evaporated off during mixing of diet)
Positive Control(s) (if applicable): N/A

2. Test Animals and/or Other Test System (if applicable):

Species and Strain (sexes): Male and female purebred beagle dogs

Age: 4-8 months
Weight(s): 9.3-10.2 kg (M - mean); 7.8-8.3 kg (F - mean)
Source(s): Richard E. Saunders Corporation, Richmond, VA, and Dublin Laboratories, Dublin, VA

3. Procedure:

a. Dietary Preparation (if applicable): Calculated amounts of Dyfonate were dissolved in 100 ml acetone and mixed with a small amount of diet (dog meal) in order to permit evaporation of acetone. The mixtures were then added to a measured amount of diet in order to obtain the final concentrations.

Frequency of preparation: Not given.

Storage conditions: Not given.

Stability Analyses: Not given.

Homogeneity Analyses: Not given.

Concentration Analyses: Not given.

b. Basis For Selection of Dose Levels: Not given.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 106 weeks male	female
Contr.	0	4	4
1	16 (8.0)[1]	4	4
2	60	4	4
3	240	4[2]	4[3]

[1] Reduced to 8.0 ppm at week 14
[2] One dog died on the first day of the study and was replaced.
[3] One dog died during week 6 of the study and was replaced.

d. Unusual Procedures Feeding Study: Each dog in the treated groups was fed 200 grams of the treated diet, plus 45 grams of canned beef (12 grams dry weight). Double portions were fed on Saturday with no food offered on Sunday until the 85th week of the study. Thereafter single portions were fed daily 7 days per week. Control dogs were similarly fed the untreated diet.

e. Clinical Observations and Mortality: Daily for clinical signs, changes in behavior and food consumption. Frequent intervals for more detailed examinations (body temperature, heart rate, respiration rate, appearance of visible mucous membranes, condition of skin and hair coat, locomotor activity). Blood pressures were taken at weeks 0, 7, 14, 20, 27, 43, 56, 82, 91 and 104 weeks. Electrocardiograms were taken at these same time intervals. Heart rates were measured from the electrocardiographic tracings.

f. Body Weight Determinations: weekly.

g. Food and/or Water Consumption: Changes in food consumption were noted daily.

h. Ophthalmological Examinations (if applicable): Initially and at weeks 7, 13, 23, 57, 94 and 105 weeks. The examinations included observations of intra-ocular tension (by finger pressure technique), pupillary reflex, and condition of the conjunctivae, eyelids, cornea, aqueous humor, iris, lens, vitreous humor, fundus, and sclera. A Keeler Pantoscope was used to assist in these examinations. A 1.0% cyclogel solution was used as the mydriatic.

i. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): All animals at 0, 6, 13, 19, 26, 39, 53, 78, 91 and 104 weeks.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*		Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*		Mean corpuscular HGB conc.
x	Thrombocyte count		(MCHC)
x	Leukocyte count (WBC)*		Mean corpuscular volume (MCV)
	Erythrocyte count (RBC)*		Reticulocytes
	Platelet count*	x	Sedimentation rate
	Total plasma protein (TP)	x	Coagulation time
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
	Calcium*		Albumin*
	Chloride*		Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
	Phosphorus*		Cholesterol*
	Potassium*		Globulins
	Sodium*	x	Glucose*
	Enzymes:		Total bilirubin*
x	Alkaline phosphatase		Total protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*		A/G Ratio
	Lactic acid dehydrogenase	x	Prothrombin time
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
	Gamma-glutamyl transpeptidase (GGTP)		
x	Plasma cholinesterase - method of Frawley		
x	Red blood cell cholinesterase - method of Frawley		
x	Brain cholinesterase at termination - method of Frawley		

3) Urinalysis:

Collection times for urine (including # of animals): All dogs at 0, 6, 19, 26, 39, 52, 78, 91 and 104 weeks.

The following CHECKED (X) parameters were examined:

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

j. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: All animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All dogs.

k. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: All animals.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: Tissues on all dogs were saved, however, only some were examined. These will be noted in a second table below the following table.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

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

	No. of Dogs Examined Microscopically							
	Control		16 (8.0) ppm		60 ppm		240 ppm	
	M	F	M	F	M	F	M	F
Esophagus	4	4					4(1)	3(2)
Liver	4	4	4	4	4	4	4	3(2)
Trachea	4	4					4(1)	3(2)
Kidney	4	4	4	4	3	4	4(1)	3(2)
Heart	4	4	4	4	3	4	4(1)	3(2)
Spleen	4	4	1	1	1		4(1)	3(2)
Lung	4	4	4	4	4	4	4(1)	3(2)
Adrenal	4	4	4	4			4(1)	3(2)
Thyroid	4	4	4	4			4(1)	3(2)
Pituitary	4	4		3	3	3	4(1)	3(2)
Lymph Node	4	3	4				4(1)	3(2)
Salivary Gland	4	4					4(1)	3(2)
Thymus	4	3					4	3
Gonads	4	4	4	4	4	4	4(1)	3(2)
Uterus/Prostate	4	4					4(1)	3(2)
Stomach	4	4	4	4	4	4	4(1)	3(2)
Small Intestine	4	4					4(1)	3(2)
Pancreas	4	4	4	4	4	4	4(1)	3(2)
Colon	4	4					4(1)	3(2)
Cecum	4	4					4	3(2)
Brain	4	4	4	4	4	4	4(1)	3(2)
Spinal Cord	4	4					4(1)	3(2)
Bone Marrow	4	4	4	4	4	4	4(1)	3(2)
Skeletal Muscle	4	4					4(1)	3(2)
Mammary Gland	4	2					3(1)	3(1)
Skin	4	2					3(1)	3(1)
Eye	4	4					4(1)	3(2)
Gall Bladder	4	4			1		3(1)	3(2)
Nerve	4	4			4	4	4(1)	3(2)
Urinary Bladder	4	4	4	4			4	3(2)

( ) = animals that died.

1. Statistical Analyses: The report did not mention any statistical analyses.

B. RESULTS:

1. Dietary Preparation: Since dietary analyses were not conducted, there were no results.

2. Clinical Observations and Mortality: Three dogs died at the highest dose level. Two were replaced, one at day one and one at six weeks. It was not mentioned whether or not the 6 week replacement dog was continued on the project for the full 106 weeks; however, judging from the individual animal data, it appears that this dog was not continued for 106 weeks since measurements stopped at 96 weeks. One male died on day 1 of the

study. Gross necropsy revealed severe enteritis with ulceration of the duodenum and loss of blood and fluid from the intestine. The pathologist believed that these effects were due to an acute infection, possible viral in origin. One female died at week 6. This dog had lost weight and there was evidence of hemorrhage from the rectum. Gross necropsy revealed depressed, light-colored areas on the spleen and hemorrhage of the G.I. tract. The third dog (female) died at week 76, following inanition and emaciation. Microscopic examination of the tissues for all 3 dogs showed marked acute congestion, especially in the small intestine. There was evidence of recent mucosal hemorrhage.

Most of the clinical signs of toxicity were observed in the high dose animals. The report stated that emesis and diarrhea were observed in all high dose dogs on day 1. Soft stools, sometimes containing mucus, were observed occasionally throughout the study. Alopecia was also observed in high dose dogs. The skin became dry and flaky and the hair coat had an oily appearance prior to hair loss. Hair loss occurred around the eyes, on both the dorsal and ventral surfaces of the body and on the rear limbs. In addition, increased lacrimal, nasal, and salivary secretions were frequently observed at the high dose level. Conjunctival hyperemia was also observed. Gingival hyperemia persisted throughout the study and at times the gums appeared inflamed. Nervous behavior was evident. Generalized tremors were noted for 2 dogs at the high dose level, but infrequently. Food consumption was decreased the first week of the study. Food consumption was decreased prior to death of several dogs.

At the mid- and low dose levels, pharmacologic signs were limited to conjunctival hyperemia and lacrimation with slight exception. One dog at the low dose level experienced severe muscular fasiculations during the 5th month of the study. Recovery was complete within 24 hours. This same dog experienced a slight hair loss and gingival hyperemia.

The measurements of heart rates, respiration rates, blood pressures and body temperatures appeared to be comparable to controls in all treated groups. Heart rates measured from the electrocardiogram tracings indicated a trend towards reduced rates in the high dose animals. A cursory examination of the electocardiogram tracings did not indicate any remarkable differences between treated and control groups except for the one dog which died on week 6.

3. Body Weight Determinations: Although body weight gains were not calulated from the body weights, the authors stated that it appeared that the body weight gain for the high dose dogs was generally less than the control dogs. They stated that this may have been due to the fact that one male dog in the high dose group lost 4.0 kg and the high dose female dog which died at week 76 lost 3.7 kg. Otherwise, the authors stated that mean body weights appeared to be comparable between all groups. When examining the mean data, however, it should be noted that in males, the mean body weights in all of the treated groups were somewhat decreased when compared to controls. By week 4, the mean body weights of the mid- and low-dose groups were 89% of the control values. During the study, these values decreased to as low as 82% of the control values. The mean body weights in the high dose groups decreased to as low as 71% of the control values by week 104. When examining the individual animal data, it appears that in both the low and mid-dose groups, there was one animal that did not gain much weight. This one animal in each group probably affected the mean values in both cases. In females, there did not appear to be any general trends towards reduced body weights in any dose group, including the high dose group. At week 75, the mean body weight for the high dose group was 87% of the control value. This was one week before the high dose female dog mentioned above died. The following tables summarize some of the mean body weights reported.

Mean Body Weight in Kg - Males

Interval Week	Control	16 (8.0) ppm	60 ppm	240 ppm
0	10.2	9.6 (94)	9.3 (91)	9.7 (95)
4	10.9	9.7 (89)	9.7 (89)	9.4 (86)
8	11.3	9.8 (87)	9.8 (87)	9.1 (81)
13	12.1	10.2 (84)	10.0 (83)	9.3 (77)
26	12.3	10.8 (88)	10.2 (83)	9.3 (76)
39	12.3	10.6 (86)	10.5 (85)	9.2 (75)
52	12.6	10.3 (82)	10.7 (85)	9.1 (72)
75	13.7	11.3 (82)	11.6 (85)	9.7 (71)
106	13.7	11.6 (85)	13.7 (100)	9.7 (71)

( ) = % of control value

Mean Body Weight in Kg - Females

Interval Week	Control	16 (8.0) ppm	60 ppm	240 ppm
0	7.8	8.0 (103)	8.2 (105)	8.3 (106)
4	8.4	8.3 (98)	8.8 (105)	7.6 (90)
8	8.3	8.2 (99)	8.7 (105)	8.5 (102)
13	9.2	8.4 (91)	8.8 (95)	8.9 (96)
26	8.7	8.4 (97)	9.2 (106)	9.1 (104)
39	8.9	8.4 (94)	9.6 (109)	9.3 (104)
52	9.2	8.8 (96)	9.4 (102)	9.6 (104)
75	10.5	9.4 (89)	10.2 (97)	9.1 (87)
106	11.1	9.2 (83)	11.1 (100)	11.1 (100)

( ) = % of control value.

4. Food and/or Water Consumption: Food consumption values were not recorded. See the section on clinical signs for a statement on food consumption.

5. Ophthalmological Examinations: No consistent treatment-related changes were observed. The report stated, "A bilateral grayish, and sometimes green, spotting or mottling of the tapetum lucidum was observed for seven dogs. The mottling was observed for four dogs at the 240 ppm level, one dog at the 60 ppm level, and for two dogs at the 16(8.0) ppm level." Some of the dogs were litter mates which came from litters sired by males known to have sired other litters with this same eye finding. No eye abnormalities were observed in dogs from the high dose group when microscopically examined.

6. Hematology: The high dose female which died at week 6 exhibited an elevated leucocyte count prior to death (from 11.1 1K/CMM to 21.2 1K/CMM for the first 4 weeks). The high dose male which lost 4.0 kg during the study exhibited a reduction in hemoglobin and hematocrit values (from 16.7 gm% to 12.4 gm% and from 53 to 39 for hemoglobin and hematocrit, respectively) by 104 weeks. No other treatment-related changes were seen in hemotology.

7. Clinical Chemistry:

Biochemical Determinations: Serum alkaline phosphatase (SAP) values were slightly increased during the first year for high dose animals. During the second year, these values became progressively higher with the exception of 1 male and 1 female whose values remained only slightly elevated. Serum glutamic pyruvic transaminase (SGPT) values were high prior to death for the 2 high dose females which died prior to termination. No other consistent treatment-related changes were observed. The following table summarizes the SAP and SGPT values.

SAP and SGPT Values at Selected Time Points for Individual Dogs in Control and High Dose Groups in King-Armstrong Units/100 ml

Dog No. and Sex	Level PPM	Interval in Weeks 0	6	26	53	78	104	0	6	26	53	78	104
		SAP						SGPT					
4791M	0	7.0	5.5	4.4	2.9	3.1	2.9	17	26	16	27	29	36
4899M	0	7.5	5.7	2.9	1.8	2.2	0.7	17	26	12	23	44	39
4901M	0	14.1	10.3	4.8	2.9	5.1	3.5	15	27	17	30	39	33
4924M	0	7.7	6.6	3.7	3.1	4.4	3.5	15	25	17	19	39	36
Mean		9.1	7.0	4.0	2.7	3.7	2.7	16	26	16	25	38	36
4852F	0	6.4	6.4	3.5	8.4	5.7	5.5	21	20	16	23	29	31
4895F	0	9.3	6.8	4.6	9.3	7.5	8.4	24	43[4]	17	21	27	31
4910F	0	7.3	5.1	2.4	3.3	5.3	5.5	35[4]	17	11	27	36	37
4923F	0	7.3	5.7	2.2	4.2	5.1	4.4	49[4]	37[4]	17,	15	40	36
Mean		7.6	6.0	3.2	6.3	5.9	6.0	32	29	15	22	33	34
4700M	240	4.2	15.0	15.6	15.9	20.4	>19.1	25	47	26	15	38	32
4720M	240	8.1	15.4	18.0	15.2	17.4	>18.9	37	47[4]	23	13	31	41
4906M	240	9.9	DEAD	-	-	-	-	12	DEAD	-	-	-	-
4915M	240	8.6	13.4	20.9	26.4	>19.6	>18.9	21	17	15	25	30	33
4942M	240	5.9	6.8	7.9	7.9	8.1	9.3	15	19	14	26	31	38
Mean		7.3	12.7	15.6	16.4	>16.4	>16.6	22	33	20	20	33	36
4652F	240	2.6	15.8[2]	DEAD	-	-	-	30	>130[2]	DEAD	-	-	-
4779F	240	4.6	8.6	10.3	14.8	108.0[3]	DEAD	49[4]	55[4]	19	15	>140[3]	DEAD
4836F	240	7.9	10.1	14.3	14.3	13.2	12.5	25	23	16	11	36	35
4898F	240	12.8	21.8	19.4	23.8	20.0	>19.1	17	26	17	13	38	37
4947F[1]	240	5.7	14.5	24.8	23.5	>20.4	>19.4	11	19	9	19	41	39
Mean		6.7	13.8	17.2	19.1	>17.9	>17.0	26	31	15	15	39	37

[1] Measured at intervals 0, 5, 31, 53, 83, 96
[2] 4-week value, not included in the mean.
[3] Terminal value, not included in the mean.
[4] Hemolyzed

Cholinesterase Measurements: At the high dose level, plasma cholinesterase was reduced approximately one-half. At the mid-dose level, it was reduced by one-fourth to one-half. There were no effects at the lowest dose level. Erythrocyte cholinesterase was almost completely inhibited at the highest dose level. At the mid-dose level, the activity was reduced approximately two-thirds, and at the lowest dose level, activity was reduced by about one-third at the 6, 13 and 19 week intervals. This was the reason why the dose level was reduced from 16 ppm to 8 ppm at week 14. Reduction of the dose level resulted in the cholinesterase values returning to values comparable to the control values. Brain cholinesterase showed no inhibition at any level. Mean cholinesterase values at selected intervals are given in the following tables.

Mean Plasma Cholinesterase in Δ pH per Hour

	Control		16(8.0)ppm		60 ppm		240 ppm	
Week	Male	Female	Male	Female	Male	Female	Male	Female
0	1.09	1.11	1.15	1.10	1.07	1.15	1.08	1.04
6	0.98	1.03	0.94	0.96	0.54	0.79	0.27	0.33
13	0.98	1.04	0.85	0.82	0.48	0.59	0.36	0.44
19	0.79	0.97	0.79	0.89	0.48	0.65	0.43	0.60
26	1.17	1.17	1.11	1.14	0.87	1.01	0.58	0.72
39	1.07	1.13	1.14	1.13	0.84	1.09	0.42	0.54
53	0.76	1.05	1.01	0.98	0.56	0.71	0.44	0.73
78	1.07	1.12	1.02	1.04	0.55	0.90	0.45	0.32
91	1.10	1.10	1.02	1.12	0.58	0.70	0.42	0.45
104	1.01	1.09	0.99	1.05	0.44	0.57	0.49	0.44

Mean Erythrocyte Cholinesterase in Δ pH per Hour

	Control		16(8.0)ppm		60 ppm		240 ppm	
Week	Male	Female	Male	Female	Male	Female	Male	Female
0	0.83	0.87	0.80	0.88	0.85	0.82	0.84	0.88
6	0.60	0.76	0.54	0.54	0.24	0.20	0.00	0.00
13	0.57	0.80	0.35	0.49	0.16	0.20	0.06	0.06
19	0.48	0.75	0.33	0.62	0.22	0.22	0.12	0.08
26	0.80	0.97	0.62	0.87	0.40	0.39	0.10	0.08
39	0.70	0.96	0.70	0.75	0.33	0.34	0.16	0.07
53	0.48	0.87	0.58	0.73	0.28	0.18	0.11	0.22
78	0.54	0.65	0.63	0.62	0.15	0.34	0.00	0.07
91	0.51	0.60	0.42	0.62	0.24	0.16	0.21	0.49
104	1.01	0.54	0.43	0.55	0.38	0.49	0.46	0.48

Mean Brain Cholinesterase in Δ pH per Hour

Control		16(8.0)ppm		60 ppm		240 ppm	
Male	Female	Male	Female	Male	Female	Male	Female
0.72	0.75	0.70	0.76	0.70	0.73	0.72	0.73

8. Urinalysis: One male dog in the low dose group at the 91 and 104 week intervals exhibited a moderate presence of albumin in the urine. No other differences were observed between the treated groups and the controls at any time interval.

9. Gross Pathology: There was no statement in the report on gross pathology. However, there was a summary table. The table covered one control animal, 2 high dose males, 3 high dose females, 3 mid-dose females and 1 male and female from the low dose group. There did not appear to be anything remarkable in the table. The gross pathology descriptions of the animals that died are given in the mortality and clinical signs section.

10. Organ Weights: Slightly increased liver weights were observed for some dogs in both the mid- and high dose groups. Prostate weights were reduced for males in the high dose group. The following table summarizes the values for these two organs in the controls and the high dose groups.

Mean Absolute and Relative Liver and Prostate Weights for Dogs Fed Dyfonate for Two Years

Level ppm	Dog #	Liver (g) Absolute	Liver (g) Relative	Prostate (g) Absolute	Prostate (g) Relative
			Males		
0	4791M	348.0	3.35	11.40	0.1096
0	4899M	352.0	2.17	6.68	0.0412
0	4901M	355.0	2.65	7.63	0.0569
0	4924M	356.0	2.52	14.40	0.1021
0	Mean	352.8	2.67	10.03	0.0775
240	4700M	296.0	3.61	1.90	0.0232
240	4720M	441.0	4.85	2.70	0.0297
240	4906M	351.0	3.58	1.07	0.0109
240	4915M	400.0	3.51	0.81	0.0071
240	4942M	401.0	3.93	5.70	0.0559
240	Mean	377.8	3.90	2.44	0.0254
			Females		
0	4852F	280.0	2.75	-	-
0	4895F	364.0	3.25	-	-
0	4910F	218.0	2.40	-	-
0	4923F	300.0	2.33	-	-
0	Mean	290.5	2.68	-	-
240	4652F	205.0	4.27	-	-
240	4779F	166.0	3.39	-	-
240	4836F	478.0	3.82	-	-
240	4898F	343.0	3.90	-	-
240	4947F	373.0	3.81	-	-
240	Mean	313.0	3.84	-	-

11. Histopathology: There were no treatment-related changes in the mid- and low dose groups when compared to controls. In the three high dose dogs which died, there was marked acute congestion of the tissues, especially seen in the small intestine. Of the 7 high dose dogs which were sacrificed at the end of the study, 6 showed marked increase in basophilic granulation of the myofibril of the inner layer of the muscularis in the small intestine. There were relatively slight changes in the liver. These included increased numbers of binucleated hepatocytes, increased hepatic cell pigmentation and some homogeneity and eosinoplilia of hepatocellular cytoplasms. In one of the dogs that died there was also the same evidence of hepatic changes. No other differences between the treated and control groups were noted. This information could not be verified because the

histopathology table had no legend and the abbreviations that were used were not understandable.

12. Quality Assurance Measures: There were none noted; however, this study was probably conducted prior to requirements for Good Laboratory Practices.

C. DISCUSSION: This study is classified as Core Supplementary. The quality of the study was not sufficient for regulatory purposes. The following list discusses all the difficulties with the study:

1. Fonofos arrived in 4 different batches. There was insufficient detail on the differences between the batches (purity, etc.).

2. Some of the dogs were of borderline age at the start of the study (some were over 9 months in age).

3. The source of the dogs was from 2 places. There was insufficient detail on how these were randomized and distributed.

4. There was an unusual feeding pattern. The animals were fed 6 days per week. On Saturday, they were given double the amount of diet and then were not fed on Sundays. Since dogs will generally eat all of what they are given at one time, they were receiving double the dose of the test chemical on Saturday and nothing on Sunday.

5. There was no information on the frequency of diet preparation, storage, stability of the test chemical in the diet, homogeneity of mixing or concentration analyses. It is not known what the dogs were actually receiving.

6. In the high dose group, two dogs were replaced, one on day 1 and one 6 weeks into the study. From the data, it appears that the replacement dog at 6 weeks was not kept on the diet an extra 6 weeks at the other end of the study. It appears that this animal was terminated at 96 weeks. Since there are so few dogs in a chronic feeding study, the fact that this dog was terminated early for no reason other than assumed convenience, then this could have had a significant effect on the outcome of the study.

7. No electrolytes were measured for the clinical chemistry analyses.

8. The microscopic examinations were incomplete. For a nonrodent study, organs from all animals in all dose groups are required to be examined. Only selected organs were examined in selected dose groups.

9. Statistical calculations were not conducted. Without these, one can only "eyeball" the data and guess as to which might be significant.

10. The individual animal data for the microscopic examinations consisted of one scanty table which had no legend. The table was unreadable without the legend.

The cholinesterase NOEL is 8.0 ppm and the LOEL is 16.0 ppm. The systemic NOEL is 16.0(8.0) ppm and the LOEL is 60 ppm [at 240 ppm: deaths, clinical signs, decrease in body weight, increase in serum alkaline phosphatase, possible liver effects (organ weights and histopathology) and acute tissue congestion; at 60 ppm: a few clinical signs, liver weight increases and some possible body weight decreases, however, there were no major systemic effects].