UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 011881
WASHINGTON, D.C. 20460

CASWELL FILE

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OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

March 15, 1996

SUBJECT: 041701. Fonofos. Review of Chronic Dog Study (83-1(b)) for Reregistration

PC Code 041701
DP Barcode D223012
Case No. 818850
MRID No. 43914601

Tox. Chem. No. 454B
Reregistration Case No. 0105
ID No. 041701

TO: Judith Loranger, CRM Team # 73
Reregistration Branch
Special Review and
Reregistration Division (7508W)

FROM: Pamela M. Hurley, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)

Pamela M. Hurley 3/15/96

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C)

Ron Gardner 3/15/96

KB 3/20/96

Background and Request:

In response to the fonofos data call-in, Zeneca Ag Products has submitted a chronic oral study conducted with technical fonofos in dogs (83-1b). The Toxicology Branch (TB-I) has been asked to review the study and determine whether or not it satisfies regulatory requirements for a chronic oral study in nonrodents.

Toxicology Branch Response:

TB-I has reviewed the chronic oral conducted with fonofos in dogs and has determined that it is acceptable for regulatory purposes. The study satisfies the regulatory requirements for a chronic oral study conducted with technical fonofos in a nonrodent species (83-1b). The following list is a list of the toxicology data requirements that have been satisfied for reregistration. Only one requirement remains: a multigeneration reproduction study in the rat (83-4).

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	Required	Satisfied
Technical Product		
Acute oral LD_{50}	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
Acute delayed neurotoxicity (hen)	Yes	Yes
Acute neurotoxicity screening (mammalian)	Yes	Yes
90-day subchronic oral		
rodent	Yes	Yes (comment 1)
nonrodent	Yes	Yes (comment 2)
90-day delayed neurotoxicity (hen)	Yes	Yes (comment 3)
Subchronic neurotoxicity screening (mammalian)	Yes	Yes
6-month dog (ocular effects)	Yes	No (comment 4)
Chronic feeding		
rodent	Yes	Yes
nonrodent	Yes	Yes
Oncogenicity		
rat	Yes	Yes
mouse	Yes	Yes
Teratology		
rabbit	Yes	Yes
mouse	Yes	Yes
2-Generation reproduction	Yes	No (comment 5)
Gene mutation	Yes	Yes
Chromosome aberration	Yes	Yes
Other genotoxic effects	Yes	Yes
Metabolism	Yes	Yes

Comments

1. An acceptable chronic/oncogenicity feeding study in the rat is available. Therefore, the requirement for a 90-day oral study in the rat has been satisfied.

2. An acceptable chronic oral study in a nonrodent species is available. Therefore, the requirement for a 90-day oral study in the dog has been satisfied.

3. A 90-day delayed neurotoxicity study is available in the hen. This study is classified as Core Minimum because it is based on the old neurotoxicity testing guidelines. The Office of Pesticide Programs (OPP) is in the process of finalizing new guidelines for neurotoxicity testing. The 90-day hen study published in OPP's previous guidelines is missing the assays for acetylcholinesterase (AchE) and neuropathy target esterase (NTE). This assay is included in the new guidelines for hen studies. Therefore, the 90-day hen study will apply to the previously published OPP neurotoxicity testing guidelines. TB-I notes that the Registrant has added the AchE and NTE assays in the acute delayed neurotoxicity study in the hen.

4. This study has been requested in the FIFRA '88 requirements. The Agency has approved a deferral for ocular effects testing until further guidance can be provided.

5. This study has been rereviewed and has been reassessed as Core Supplementary. It does not satisfy the regulatory requirements for a reproduction study.

The following paragraph summarizes the results of the chronic oral study in dogs.

In a chronic toxicity study (**MRID 43914601**) fonofos (94.6% a.i.) was administered to groups of 4 beagle dogs/sex/dose by capsule at dose levels of 0, 0.2, 0.4 or 1.75 mg/kg/day in corn oil for a period of at least one year. Observations and measurements included the usual required parameters plus cholinesterase activities (ChE). Additional (satellite) groups of 2 males and 2 females were dosed with either 0 or 1.75 mg fonofos/kg/day for 4 weeks. For these dogs, clinical observations were recorded as well as bodyweights, food consumption and ChE activity (including brain).

At 0.2 mg/kg/day, minimal sporadic plasma cholinesterase inhibition was observed in both sexes (7-13%; 20% only once at 52 weeks in females). At 1.0 mg/kg/day, there were increases in alkaline phosphatase levels (130-194% of control values) and inhibition of erythrocyte (51% in males, 53% in females) and plasma cholinesterase (50% in both sexes) activities. At 1.75 mg/kg/day, there were clinical signs of toxicity in one animal, decreases in serum albumin and total protein levels, increases in alkaline phosphatase levels (up to 217%), inhibition of erythrocyte (62% in males, 63% in females), plasma (57% in males, 58% in females) and possibly brain (20% in females) cholinesterase activities and increases in absolute liver weights in males (18.5%). One female dosed with 2.0 mg/kg/day for 3 days developed clinical signs and intussusception of the terminal ileum, possibly due to uncontrolled peristaltic movement

following substantial depression of cholinesterase activity. The NOEL is 0.2 mg/kg/day. The NOEL is considered to be a borderline NOEL/LOEL because there was minimal plasma cholinesterase inhibition at 0.2 mg/kg/day which was generally weak and was not consistent. The LOEL, 1.0 mg/kg/day is based on plasma and erythrocyte cholinesterase inhibition and increases in alkaline phosphatase levels at 1.0 mg/kg/day and above, and clinical signs of toxicity, decreases in selected blood chemistry values, increases in liver weights and histologic changes in the ileum at 1.75 mg/kg/day.

EPA Toxicologist: Pamela M. Hurley Pamela M. Hurley, Date 3/15/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 2/16/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity - capsule - dog; OPPTS 870.4100 [§83-1(b)]

DP BARCODE: D223012
P.C. CODE: 041701
TEST MATERIAL (PURITY): Fonofos (94.6%)
SUBMISSION CODE: S500417
TOX. CHEM. NO.: 454B
REREG. CASE NO.: 0105

SYNONYMS: Dyfonate

CITATION: Hodge, M. (1995) Fonofos: 1 year oral toxicity study in dogs. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report Number CTL/P/4499, Study Number PDO944, December 14, 1995. MRID 43914601. Unpublished.

SPONSOR: Zeneca AG Products, Wilmington, Delaware

EXECUTIVE SUMMARY:

In a chronic toxicity study (**MRID 43914601**) fonofos (94.6% a.i.) was administered to groups of 4 beagle dogs/sex/dose by capsule at dose levels of 0, 0.2, 0.4 or 1.75 mg/kg/day in corn oil for a period of at least one year. Observations and measurements included the usual required parameters plus cholinesterase activities (ChE). Additional (satellite) groups of 2 males and 2 females were dosed with either 0 or 1.75 mg fonofos/kg/day for 4 weeks. For these dogs, clinical observations were recorded as well as bodyweights, food consumption and ChE activity (including brain).

At 0.2 mg/kg/day, minimal sporadic plasma cholinesterase inhibition was observed in both sexes (7-13%; 20% only once at 52 weeks in females). At 1.0 mg/kg/day, there were increases in alkaline phosphatase levels (130-194% of control values) and inhibition of erythrocyte (51% in males, 53% in females) and plasma cholinesterase (50% in both sexes) activities. At 1.75 mg/kg/day, there were clinical signs of toxicity in one animal, decreases in serum albumin and total protein levels, increases in alkaline phosphatase levels (up to 217%), inhibition of erythrocyte (62% in males, 63% in females), plasma (57% in males, 58% in females) and possibly brain (20% in females) cholinesterase activities and increases in absolute liver weights in males (18.5%). One female dosed with 2.0 mg/kg/day for 3 days developed clinical signs and intussusception of the terminal ileum, possibly due to uncontrolled peristaltic movement--following substantial depression of cholinesterase activity. **The**

NOEL is 0.2 mg/kg/day. The NOEL is considered to be a borderline NOEL/LOEL because there was minimal plasma cholinesterase inhibition at 0.2 mg/kg/day which was generally weak and was not consistent. The LOEL, 1.0 mg/kg/day is based on plasma and erythrocyte cholinesterase inhibition and increases in alkaline phosphatase levels at 1.0 mg/kg/day and above, and clinical signs of toxicity, decreases in selected blood chemistry values, increases in liver weights and histologic changes in the ileum at 1.75 mg/kg/day.

This chronic toxicity study in the dog is acceptable and satisfies the guideline requirement for a chronic oral study (83-1b) in dogs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: fonofos
   Description: yellow liquid
   Lot/Batch#: P3/D7534/27; Y02743/020
   Purity: 94.6% a.i.
   Stability of compound: stable (tested at the end of the study)
   CAS #: 944-22-9

2. Vehicle: corn oil

3. Test animals: Species: male and female dogs
   Strain: beagle
   Age at study initiation: 24-28 weeks
   Source: Zeneca Pharmaceuticals, Alderley Park, Cheshire, UK
   Housing: indoor pens (365 x 115 cm floor area), heated floor and separate exercise area
   Diet: Laboratory Diet A (Special Diets Services Ltd, Stepfield, Witham, Essex, UK) ad libitum
   Water: potable ad libitum
   Environmental conditions: Temperature: 18-24°C
   Humidity: not given.
   Air changes: 10/hour
   Photoperiod: 12 hours light/dark

   Acclimation period: 6 to 8 weeks

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B. STUDY DESIGN:

1. In life dates - start: May 5, 1993 end: May 12, 1994

2. Animal assignment

Animals were assigned randomly to the test groups listed in table 1.

TABLE 1: STUDY DESIGN

Test Group	Dose to animal (mg/kg/day)	Main Study 12 months		Interim Sac. 1 month[b]	
		male	female	male	female
Control	0	4	4	2	2
Low (LDT)	0.2	4	4	-	-
Mid (MDT)	1.0	4	4	-	-
High (HDT)	1.75[a]	4	4	2	2

[a]Half of the animals were dosed for 2 days at 2.0 mg fonofos/kg/day but this dose was then reduced to 1.75 mg fonofos/kg/day.

[b]Additional (satellite) control and high dose groups were dosed for 4 weeks. For these dogs, only clinical observations were recorded as well as bodyweights, food consumption and ChE activity (including brain).

3. Dose selection rationale: The dose levels were selected on the results from previous dog studies conducted with this chemical (previous chronic dog study had a dose range of 0.2 to 6 mg/kg/day).

4. Dosing preparation and analysis

The dogs were dosed orally on a daily basis using gelatin capsules. Formulations of fonofos were prepared in corn oil; 1 mg/ml for group 2, 10 mg/ml for groups 3, 4 and 6. The concentrations of fonofos were adjusted to allow for its known purity and the amount of compound in each dose was based on the most recent bodyweight. Control animals received corn oil alone at a weight equivalent to that given to the high dose group. Homogeneity was not assessed because the formulations were considered to be true solutions. Concentration analyses were conducted on the first 2

batches and then at approximately 2 monthly intervals. The stability of fonofos in corn oil was assessed over 63 days, which exceeded the maximum period of use.

Results - Stability Analysis: Fonofos appeared to be stable over a period of 63 days (91-105.2% of initial value). The report stated that "an apparent decline in concentration after 28 days was clearly anomalous since subsequent values were close to the initial concentration."

Concentration Analysis: Individual values for the achieved concentrations were within 10% of the nominal values (90-103%) with the overall means within 3% of the nominal values (97%).

5. Statistics - Bodyweights were considered by analysis of covariance on initial bodyweight, separately for males and females. Hematology, clinical chemistry and plasma and erythrocyte cholinesterase values were considered by analysis of covariance on pre-experimental values. Urine clinical chemistry and brain cholinesterase were considered by analysis of variance at each time of sampling. Male and female data were analyzed together and the results examined to determine whether any differences between control and treated groups were consistent between sexes. The covariant adjustment was based on the separate sex pre-experimental group means. Organ weights were considered by analysis of variance and analysis of covariance on final bodyweight. The data from paired organs were examined for differential effects on left and right components. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean, using a two-sided Student's t-test based on the error mean square in the analysis.

C. METHODS:

1. Observations:

Animals were inspected twice daily for clinical signs of toxicity and mortality. They were given a thorough examination on a weekly basis. Gastro-intestinal findings were assessed daily and any abnormal findings recorded. In addition, all dogs were given a full clinical examination by a veterinarian prior to the

start of the study, during weeks 13, 26, 39 and prior to termination. The examination included cardiac and pulmonary auscultation and measurement of body temperature and pulse rate.

2. Body weight

Animals were weighed weekly before feeding throughout the pre-study period, on day 1 and at weekly intervals thereafter during the treatment period.

3. Food consumption

Food residues were recorded daily prior to giving the next meal. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.

4. Ophthalmoscopic examination

Eyes were examined prior to the initiation of the study, during weeks 13, 26 and 39 and prior to termination using indirect ophthalmoscopy.

5. Blood was collected from the jugular vein before feeding from all dogs in weeks -1, 4, 13, 26 and prior to termination for hematology and clinical analysis from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	ENZYMES	x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)*
	Cholinesterase (ChE)	x	Triglycerides
x	Creatine kinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)	x	Plasma cholinesterase[a]
x	Serum alanine amino-transferase (also SGPT)*	x	Erythrocyte cholinesterase[a]
x	Serum aspartate amino-transferase (also SGOT)*	x	Brain cholinesterase[a]
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines
[a]Blood samples for plasma and erythrocyte cholinesterase activities taken at weeks -3, -2, -1, days 2, 3, 8, 10 and 15, then in weeks 4, 6, 10, 13, 19, 26, 39 and prior to termination. At termination, the right half of the brain was taken for determination of cholinesterase activity. Blood cholinesterase activity was measured using the KONE Specific analyzer and brain cholinesterase activity was measured using the method of Ellman et al. 1961.

6. Urinalysis

Urine was collected from all animals pre-experimentally, at week 26 and in the week prior to termination. The following CHECKED (X) parameters were examined.

X		X	
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

* Required for chronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	x	Heart*	x	Periph.nerve*
x	Esophagus*	x	Bone marrow*	xx	Spinal cord (3
x	Stomach*	x	Lymph nodes*		levels)*
x	Duodenum*	xx	Spleen*	x	Pituitary*
x	Jejunum*	x	Thymus*	x	Eyes (optic n.)*
x	Ileum*				
x	Cecum*		UROGENITAL		
x	Colon*	xx	Kidneys*+		GLANDULAR
x	Rectum*	x	Urinary bladder*	xx	Adrenal gland*
xx	Liver*+	xx	Testes*+		Lacrimal gland
x	Gall bladder*	xx	Epididymides	x	Mammary gland*
x	Pancreas*	x	Prostate	x	Parathyroids*++
			Seminal vesicle	xx	Thyroids*++
	RESPIRATORY	x	Ovaries*+		
x	Trachea*	x	Uterus*		
x	Lung*	x	Cervix		OTHER
	Nose			x	Bone*
	Pharynx			x	Skeletal muscle*
	Larynx			x	Skin*
				x	All gross lesions and masses*

* Required for chronic studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight reguired for non-rodent studies.
++ Organ weight required for non-rodent studies.

II. RESULTS:

A. Observations

1. Toxicity - The report stated that the only compound-related findings occurred in the first 3 days of dosing. Two females from the high dose group (2.0 mg/kg/day) had a clinical reaction on day 3 which included tremors, diarrhea and reduced coordination. The affected animals were treated with 100µg atropine/kg approximately 1 hour after onset of the signs with an additional 100-120µg atropine/kg 6 hours later. Dosing was temporarily suspended for these two animals and the 2.0 mg fonofos/kg/day dose level was reduced to 1.75 mg/kg/day for both sexes from day 4 on.

Since the dogs were placed in replicate groups which were put on test one week apart, not all of the animals in the high dose group were dosed with 2.0 mg/kg/day fonofos during the first 4 days. Due to the delayed start for half of the animals, these animals received 1.75 mg/kg/day from the beginning of the study. The affected animals appeared to recover well but by day 6, one was moribund. This female was replaced. One male in the high dose group dosed at 1.75 mg/kg/day from the beginning also showed an adverse reaction on day 3 and was again treated with 100 µg atropine/kg approximately 1 hour after the onset of the symptoms, with temporary suspension of dosing. No other compound-related clinical signs of toxicity were observed, including any gastro-intestinal signs.

2. Mortality - one female in the high dose group was moribund by day 6 and was therefore replaced with another female.

B. Bodyweight - In the treated female groups, there were statistically significant decreases in bodyweight when compared to the control group, particularly in the mid-dose group during the first 20 weeks of the study. These decreases were generally within 95% of the mean control values and were not consistently dose-related. The following table provides a summary of selected mean bodyweight values for females. No statistically significant differences were observed in males.

Selected Mean Bodyweight Values for Females Within 20 Weeks (kg)

Week		Dose Levels (mg/kg/day)			
		0	0.2	1.0	1.75
1	Mean	10.75	10.48	10.43	10.23
	S.D.	1.67	1.65	1.82	1.03
3	Mean	11.20	10.88	10.73	10.63
	S.D.	1.70	1.59	1.72	1.00
	Adjusted Mean[a]	10.92	10.87	10.77*	10.88
5	Mean	11.60	11.23	11.00	10.98
	S.D.	1.63	1.56	1.72	1.01
	Adjusted Mean	11.33	11.22	11.04**	11.24
10	Mean	12.63	11.95	11.75	11.60
	S.D.	1.62	1.48	1.64	1.07
	Adjusted Mean	12.34	11.94*	11.79**	11.93*

Selected Mean Bodyweight Values for Females Within 20 Weeks (kg)					
Week		Dose Levels (mg/kg/day)			
		0	0.2	1.0	1.75
15	Mean	13.25	12.70	12.43	12.15
	S.D.	1.40	1.43	1.50	1.00
	Adjusted Mean	13.00	12.69	12.46*	12.43*
20	Mean	13.50	12.90	12.53	12.23
	S.D.	1.24	1.28	1.45	1.11
	Adjusted Mean	13.25	12.89	12.56*	12.60

*p < 0.05; **p < 0.01
[a]adjusted for initial weight

C. Food consumption - Some individual animals from various treated groups occasionally left uneaten food, particularly one female in the high dose group; however no consistent pattern emerged.

D. Ophthalmoscopic examination - No treatment-related effects were observed when compared to the control groups.

E. Blood work

1. Hematology - No treatment-related differences were observed when the treated groups were compared to the control groups.

2. Clinical chemistry - Statistically significant decreases in mean plasma albumin were observed in mid- and high dose males at week 13 and in high dose males and females at week 26. By week 52 the values were not significantly less than controls. Mean plasma total protein was also statistically significantly less than the control values in high dose males and females at both weeks 13 and 26 and at week 4 in high dose females. At week 52 in females, this value was statistically significantly less than the control value for the low and mid-dose females but not for the high dose females. Plasma alkaline phosphatase was statistically significantly elevated in high dose males at weeks 4, 13, 26 and 52. Mid-dose male values were elevated at week 13. These values ranged from slightly elevated in the earlier stages of the study to approximately double the control values by the end of the study. In females, plasma alkaline phosphatase was elevated in both the mid- and high dose groups at weeks 26 and 52. In females again, plasma chloride was

slightly elevated in the high dose group at weeks 4, 26 and 52 and in the mid-dose group at weeks 26 and 52. Calcium levels were slightly decreased at weeks 13 and 26 but not at week 52. Other statistically significant differences between the treated and control groups were not considered to be biologically significant. The following table summarizes the values for the parameters listed above.

Selected Adjusted Mean Plasma Clinical Chemistry Values

Parameter	Dose Levels (mg fonofos/kg/day)			
	0	0.2	1.0	1.75
Males				
Albumin				
Pre-experimental	3.03	3.18	3.13	3.05
Week 13	3.09	2.93	2.82*	2.76*
Week 26	3.10	2.91	2.93	2.73**
Week 52	2.77	2.49	2.87	2.52
Total protein				
Pre-experimental	5.33	5.35	5.38	5.30
Week 4	5.20	5.17	5.09	5.06
Week 13	5.33	5.20	5.19	4.96**
Week 26	5.41	5.34	5.21	5.04*
Week 52	4.78	4.60	4.50	4.13
Alkaline phosphatase				
Pre-experimental	218	237	223	228
Week 4	196	188	212	224*
Week 13	144	137	187*	211**
Week 26	111	122	142	230**
Week 52	74	69	92	151**
Females				
Albumin				
Pre-experimental	3.18	3.15	3.20	3.28
Week 26	3.29	3.25	3.13	2.90**
Week 52	2.81	2.82	2.63	3.02
Total protein				
Pre-experimental	5.58	5.40	5.48	5.63
Week 4	5.43	5.41	5.49	5.13*
Week 13	5.46	5.40	5.35	5.06**
Week 26	5.57	5.55	5.51	5.06**
Week 52	4.48	3.72*	3.47**	4.20

Selected Adjusted Mean Plasma Clinical Chemistry Values				
Parameter	Dose Levels (mg fonofos/kg/day)			
	0	0.2	1.0	1.75
Alkaline phosphatase				
Pre-experimental	230	223	239	213
Week 4	199	200	206	219
Week 13	138	146	169	168
Week 26	95	124	144*	161**
Week 52	53	65	103*	115*
Chloride				
Pre-experimental	113.8	113.0	113.3	114.5
Week 4	113.5	115.5	113.9	116.3*
Week 13	111.0	112.2	112.1	111.6
Week 26	112.2	112.6	115.1*	115.0*
Week 52	111.7	113.2	114.4**	114.8**
Calcium				
Pre-experimental	11.4	11.1	11.5	11.8
Week 4	11.3	11.4	11.4	10.8
Week 13	11.5	11.2	11.1*	11.0*
Week 26	11.1	11.3	10.8*	10.5**
Week 52	9.7	10.1	10.1	9.9

* $p < 0.05$; ** $p < 0.01$

3. Erythrocyte, Plasma and Brain Cholinesterase

Main Study - In males, statistically significant decreases in erythrocyte cholinesterase activity were observed in the high dose group starting on day 3 and in the mid-dose group starting on day 8. These decreases were observed throughout the study. Only one value in the low dose group was significantly less than the control group. Statistically significant decreases in plasma cholinesterase were observed in both the mid- and high dose groups starting on day 2 and continuing throughout the study. A "plateau" of inhibition was reached by week 10 for both enzymes such that the maximum level of inhibition was approximately 43-49% for the mid-dose group and 52-60% for the high dose group. Spurious statistically significant decreases in plasma cholinesterase activity were also observed in the low dose group starting at week 4. In females, statistically significant decreases in erythrocyte cholinesterase activities were observed in the high dose group starting on day 2 and in the mid-dose group starting on day 8. Only one value in the low dose group was significantly less than the control group.

Statistically significant decreases in plasma cholinesterase were observed in both the mid- and high dose groups starting on day 2 and continuing throughout the study. Again, spurious statistically significant decreases in plasma cholinesterase activity were also observed in the low dose group, starting at week 10.

Brain cholinesterase values were not significantly different from the control group for any of the treated groups in either sex at week 52. The maximum inhibition was about 7% in the high dose females.

Satellite Study - In both sexes, statistically significant decreases in erythrocyte cholinesterase activities were observed on day 8 and at week 4 in the high dose group. Decreases in plasma cholinesterase activity were observed at all time points. Brain cholinesterase values were not significantly different from the control group for either sex at week 5. However, in the high dose group females, activity was 20% less than the control group.

The following tables summarize selected mean adjusted cholinesterase values from the main study and the satellite study (brain cholinesterase only).

Mean Adjusted Erythrocyte and Plasma Cholinesterase Values for Selected Time Periods (U/l)				
	Dose Levels (mg fonofos/kg/day)			
Week	0	0.2	1.0	1.75
Males				
Erythrocyte				
Pre-experimental	3043	3180	3118	3475
Day 10	3320	2922*	2448**	1358**
Week 4	2688	2541	1889**	1212**
13	2994	2661	1691**	1268**
26	3234	2642	1616**	1366**
52	2931	2499	1446**	1122**

Mean Adjusted Erythrocyte and Plasma Cholinesterase Values for Selected Time Periods (U/l)				
	Dose Levels (mg fonofos/kg/day)			
Week	0	0.2	1.0	1.75
Plasma				
Pre-experimental	1923	1912	2018	1903
4	1863	1751*	1144**	990**
10	2052	1809**	1072**	1008**
13	1842	1764	1084**	915**
26	2001	1758	1086**	952**
39	2079	1824**	1036**	923**
52	1771	1628	1009**	765**
Females				
Erythrocyte				
Pre-experimental	3358	2728	3460	2943
4	2648	2350	1606**	1142**
13	3146	2571	1481**	1159**
26	3482	2719*	1807**	1400**
52	2846	2320	1360**	1188**
Plasma				
Pre-experimental	2020	1913	1939	2051
4	1818	1827	1220**	966**
10	2037	1868*	1172**	955**
13	1916	1748**	1068**	903**
26	2188	2028	1094**	920**
52	2071	1660**	1034**	879**

*p < 0.05, **p < 0.01

Mean Brain Cholinesterase Activity (micromoles/l/min/g)				
Sex/Time	Dose Levels (mg fonofos/kg/day)			
	0	0.2	1.0	1.75
Males: Week 52	5.79	5.54	5.62	5.62
Females: Week 52	6.10	6.53	6.35	5.67
Males: Week 5	6.19	-	-	5.41
Females: Week 5	5.65	-	-	4.54

F. Urinalysis - No treatment-related differences were found between the treated and control groups.

G. Sacrifice and Pathology

1. Organ weight - Statistically significant increases in the absolute adrenal and liver weights were observed in high dose males. The relative weights were not significantly increased. No other significant differences in organ weights were observed in any of the treated groups when compared to controls. The following table summarizes the liver and adrenal weight data.

Intergroup Comparison of Adrenal and Liver Weights				
Organ	Dose Level (mg fonofos/kg/day)			
	0	0.2	1.0	1.75
Males				
Adrenal				
Absolute weight[a]	1.40	1.36	1.45	1.64*
Relative weight (%)	0.01	0.01	0.01	0.01
Liver				
Absolute weight[a]	443	428	460	525**
Relative weight (%)	2.86	2.71	3.02	3.37
Females				
Adrenal				
Absolute weight[a]	1.56	1.52	1.50	1.57
Relative weight (%)	0.01	0.01	0.01	0.01
Liver				
Absolute weight[a]	372	378	398	406
Relative weight (%)	2.71	2.78	2.96	3.02

[a]Adjusted for bodyweight; *p < 0.05; **p < 0.01

2. Gross pathology - The report stated that "female 429 which was killed on day 6 showed the presence of an intussusception of the terminal ileum. There were no compound-related findings in dogs killed at termination."

3. Microscopic pathology

a) Non-neoplastic - There were no compound-related microscopic lesions in the dogs that were killed at termination. The report stated that "in female 429 killed intercurrently there was infarction of the ileum due to the intussusception and a reduction in the cortical lymphocytes of the thymus." This was the only treatment-related microscopic finding observed in the study. The following table summarizes microscopic findings for selected organs taken at termination.

Microscopic Findings for Selected Organs Taken at Termination				
Organ	Dose Levels (mg/kg/day)			
	0	0.2	1.0	1.75
Males				
Liver (# examined) Mononuclear cell infiltration	4 1	4 0	4 0	4 0
Ileum (# examined) No abnormalities	4 4	4 4	4 4	4 4
Kidney (# examined) Pelvic lymphoid infiltration	4 0	4 0	4 2	4 1
Lung (# examined) Pneumonitis Granuloma	4 3 3	4 0 0	4 1 0	4 1 1
Thyroid (# examined) Follicular cyst Lymphocytic infiltration	4 0 0	4 0 1	4 1 0	4 0 1
Females				
Liver (# examined) Fibrous capsular adhesion	4 0	4 1	4 0	4 0
Ileum (# examined) No abnormalities	4 4	4 4	4 4	4 4

Kidney (# examined)	4	4	4	4
Tubular dilatation with eosinophilic casts	1	0	0	0
Interstitial mononuclear cell infiltration	0	1	1	0
Pelvic lymphoid infiltration	1	0	0	0
Lung (# examined)	4	4	4	4
Pneumonitis	0	1	0	1
Granuloma	0	1	0	0
Thyroid (# examined)	4	4	4	4
Lymphocytic infiltration	2	1	2	0
Squamous cyst	1	0	0	0

b) Neoplastic - No neoplastic lesions were observed.

III. **DISCUSSION**

This was a well conducted study. There are no deficiencies. Cholinesterase inhibition is the major toxicological endpoint for fonofos. In this study, erythrocyte and plasma cholinesterase inhibition were observed in the mid- and high dose groups of both sexes. Statistically significant inhibition of plasma cholinesterase was observed at the lowest dose as well in both sexes, although not at every time point (ranging from 7 to 13% in males and from 8 to 20% in females; the 20% was at week 52). There was no statistically significant inhibition of brain cholinesterase cholinesterase in either the main study or in the satellite group. However, brain cholinesterase was inhibited by 20% in the high dose females in the satellite group. Inhibition of brain cholinesterase was not observed in the previous dog study at dose levels up to 6 mg/kg/day. Cholinergic signs were observed in the high dose group in the first few days of the study. Based on these and on the death of one animal, the dose level was lowered from 2.0 to 1.75 mg/kg/day. One animal at 1.75 mg/kg/day also displayed some cholinergic signs in the first few days of the study. The animal that was sacrificed in moribund condition had an infarction of the ileum due to the intussusception and a reduction in the cortical lymphocytes of the thymus. This was thought to be treatment-related and was believed to have occurred because of uncontrolled peristaltic movement following substantial depression of cholinesterase activity. There were several other effects which were also seen in the previous dog study. These include elevated liver enzymes and liver weight. In the previous dog study, there were minimal liver effects (increased numbers of binucleated hepatocytes, increased hepatic cell pigmentation and some

homogeneity and eosinoplilia of hepatocellular cytoplasms). In addition, in the previous study there was an increase in basophilic granulation of the myofibril of the inner layer of the muscularis in the small intestine. These were observed at 6 mg/kg/day but not at 1.5 mg/kg/day, which is the dose that is closer to the highest dose tested in this study. The effects in the small intestine support the effect observed in the one dog which died in this study. Therefore, although the previous study had major problems and was not acceptable for regulatory purposes, the results support this study. In this study, there was a minimal cholinesterase effect at the lowest dose level. Therefore, the lowest dose level is close to a NOEL/LOEL.

Caswell No. 454B File Last Updated _____ Current Date _____
Chemical Name Fonofos
Shaughnessy No.: 041701

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
83-1b Chronic oral study in dog/Zeneca Central Tox. Labs/Study No. CTL/P/4499; 12/14/95	Technical (94.6% pure)	43914601	4 beagle dogs/sex/dose received by capsule: 0, 0.2, 0.4 or 1.75 mg/kg/day in corn oil for at least one year. Additional (satellite) groups of 2 males and 2 females dosed with either 0 or 1.75 mg fonofos/kg/day for 4 weeks. 0.2 mg/kg/day: minimal sporadic plasma ChE inhibition in both sexes (7-13%; 20% only once at 52 weeks in females). 1.0 mg/kg/day: ↑ in AP levels (130-194% of control values) & inhibition of erythrocyte (51% in males, 53% in females) & plasma ChE (50% in both sexes) activities. 1.75 mg/kg/day: clinical signs of toxicity in 1 animal, ↓ in serum albumin and total protein levels, ↑ AP levels (up to 217%), inhibition of erythrocyte (62% in males, 63% in females), plasma (57% in males, 58% in females) and possibly brain (20% in females) cholinesterase activities & ↑ in absolute liver weights in males (18.5%). One female dosed with 2.0 mg/kg/day for 3 days developed clinical signs and intussusception of the terminal ileum, possibly due to uncontrolled peristaltic movement following substantial depression of cholinesterase activity. **NOEL: 0.2 mg/kg/day. NOEL considered to be borderline NOEL/LOEL because there was minimal plasma cholinesterase inhibition at 0.2 mg/kg/day which was generally weak and was not consistent. LOEL: 1.0 mg/kg/day.**	N/A	Acceptable