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

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: 627. Fonofos (Dyfonate®). Review of Submitted Acute and Subchronic Mammalian Neurotoxicity Studies

PC Code: 041701
Tox. Chem. No. 454B
Project Nos. D191634, D192110
Submission No. S441345, S442309

TO: Judith Loranger, PM Team # 73
Special Review and
Reregistration Division (H7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 8/12/93
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Karl P. Baetcke, Chief
Toxicology Branch I
Health Effects Division (H7509C) Karl P. Baetcke 8/16/93

Background and Request:

Zeneca Ag Products has submitted acute and subchronic mammalian neurotoxicity studies conducted with Technical Fonofos in response to FIFRA '88 requirements. The Health Effects Division (HED) has been requested to review the two studies.

Health Effects Division Response:

HED has reviewed the two submitted studies and has determined that they do not meet the regulatory requirements for acute and subchronic mammalian neurotoxicity studies at this time. Both studies are classified as Core Supplementary. Both may be upgraded to Acceptable. The following paragraphs discuss the two studies and what is needed in order to upgrade them.

Acute Mammalian Neurotoxicity Study in Rats:

In this study, Fonofos was tested at the following dose levels: 0, 2, 4 or 7 mg/kg. The NOEL is 4 mg/kg and the LOEL is 7 mg/kg based on clinical signs of reduced foot withdrawal reflex, urinary incontinence, tip toe gait and upward curvature of the spine in 1 high dose female, which were totally reversible by 24 hours.

The Guidelines require positive control data. Submission of these data were previously recommended when TB-I reviewed the protocols for the acute and subchronic mammalian neurotoxicity studies that were to be conducted on fonofos (see memorandum from P. Hurley to J. Edwards, dated 4/16/92). Since these are new guidelines and since the Health Effects Division (HED) does not have any positive control data from the Zeneca Central Toxicology Laboratories conducted with the Alpk:APfSD strain, the data need to be submitted. If possible, it would be preferable if these data were from a cholinesterase inhibitor.

There is some concern over the interpretation of the clinical findings in the one female at 7 mg/kg. The Registrant has interpreted these findings to be systemic when the symptoms mimic neurological dysfunction. Also, a concern has been expressed over the fact that at a dose close to the LD_{50}, signs were only seen in one animal. Therefore, HED is requesting a submission of the results from the preliminary study used to select dose levels.

A question has been raised over the sciatic nerve fiber degeneration in both control and treated animals, especially in light of the results observed in the subchronic study. Although this is not a treatment-related effect in this study, it would be helpful for assessment of this study and for assessment of future studies from Zeneca's laboratories with the Alpk:APfSD strain for the Registrant to submit any available historical control data for these types of studies. These data would be used to resolve any questions on the background incidences of these types of lesions. **It is noted that the historical control data are not required, but if they are available, they would be very useful to HED for the reasons stated above.**

Subchronic Mammalian Neurotoxicity Study in Rats:

In this study, Fonofos was tested in a 90-day feeding study at the following dose levels: 0, 15, 50 or 125/150 ppm in the diet. The NOEL is 50 ppm and the LEL is 125/150 ppm based on clinical signs and several possible effects in the functional observational battery and in the motor activity observations in high dose females. Neuropathological findings were evident, but may be common to this strain of rat. Since HED does not have access to the historical control data on these animals and since these findings did appear in high dose animals and not in the controls and that there were some indications of possible effects in the high dose animals in motor activity and in the functional observational battery, HED has considered these lesions in defining the NOEL.

The NOEL for cholinesterase inhibition is either at or near 15 ppm (LDT) based on statistically significant decreases in

cholinesterase activity observed in both sexes for brain, erythrocyte and plasma at 50 ppm.

Again, positive control data need to be provided (see above comment).

The Registrant is requested to define "splay reflex".

The Registrant needs to be aware of the following: in the spring/summer of 1992, an additional requirement of red blood cell and brain acetylcholinesterase as well as plasma butyrylcholinesterase measurements were added to the acute and 90-day mammalian neurotoxicity testing guidelines for all organophosphates and anti-cholinesterase carbamates. HED is aware that the two mammalian neurotoxicity studies conducted on fonofos were started prior to this new requirement. However, there is a possibility that HED may still need these data, particularly for acute exposure in order to complete the neurotoxicity assessment for fonofos.

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Section I, Tox. Branch (H7509C)
Secondary Reviewer: William F. Sette, Ph.D. William F. Sette 8/9
Peer Review Section, Science Analysis Branch
Health Effects Division (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Mammalian Neurotoxicity - rat (81-8)

SHAUGHNESSY NO./TOX. CHEM. NO.: 041701/454B

ACCESSION NO./MRID NO.: 427778-01

DP BARCODE/SUBMISSION NO.: D191634/S441345

TEST MATERIAL: Fonofos

SYNONYMS: Dyfonate

STUDY NUMBER(S): AR5434

REPORT NUMBER: CTL/P/3946

SPONSOR: Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Fonofos: Acute Neurotoxicity Study in Rats

AUTHOR(S): J. M. Horner

REPORT ISSUED: 3/17/93

CONCLUSION: Fonofos was tested in an acute neurotoxicity screening battery in rats at the following dose levels: 0, 2, 4 or 7 mg/kg. The NOEL is 4 mg/kg and the LOEL is 7 mg/kg based on clinical signs of reduced foot withdrawal reflex, urinary incontinence, tip toe gait and upward curvature of the spine in 1 high dose female, which were totally reversible by 24 hours. No positive control data were provided.

Classification: Core Supplementary, upgradable upon receipt of additional data (see discussion).

Testing Guideline Satisfied: Not yet

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: o-ethyl s-phenyl ethylphosphonodithioate

Description: Amber yellow liquid
Batch #(s): P3/D7534/27; CTL Y02743/020
Purity: 94.6%
Source: ICI Agrochemicals
Vehicle (if applicable): Kraft Wesson Corn Oil
Positive Control(s): Not provided

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: 35 days old upon arrival; 42 days old at start of test.
Source(s): ZENECA Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

a. Preparation of Dose Levels: The test substance was weighed out for each dose level and an appropriate amount of the vehicle was added. Samples of each preparation were analyzed prior to the start of dosing in order to verify the concentrations desired. The chemical stability of fonofos was determined after a 4 day period.

b. Basis For Selection of Dose Levels: The dose levels were selected from the results of a preliminary study in which lethality was observed at 7.5 mg/kg.

c. Animal Assignment and Dose Levels: Rats were dosed one time at 1 ml/100 g body weight.

Test Group	Dose Administered mg/kg	male	female
Control	0	10	10
1	2	10	10
2	4	10	10
3	7	10	10

*Five animals/sex from each group were designated for terminal neuropathology.

d. Clinical Signs of Toxicity and Mortality: All rats were examined prior to the start of the study and daily during the study for clinical signs of toxicity and mortality.

e. Body Weight Determinations: Bodyweights were recorded immediately prior to dosing, 6 hours after dosing and on days 8 and 15.

f. Food and/or Water Consumption: Food consumption was measured and calculated on a weekly basis.

g. Functional Observational Battery: The report stated that "detailed clinical observations ...and quantitative assessments of landing foot splay, sensory perception (tail flick test) and muscle weakness (fore and hindlimb grip strength) were made in week -1, on day 1 (at 6 or 7 hours after dosing), and on days 8 and 15. The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour etc; reactivity to stimuli; changes in level of arousal; sensorimotor responses; [and] alterations in respiration. The observations were made by one observer who was 'blind' with respect to the animal's treatment, and recorded on a computer system by personnel not directly involved in the clinical observations. The observations were carried out in a room separate from that in which the animals were housed and animals were presented to the observer with no indication of the treatment group. The observations were coded and the degree of condition noted (slight, moderate or extreme) where appropriate. This included the recording of no abnormalities detected."

h. Motor Activity: An automated activity recording apparatus was used to measure locomotor activity. The animals were tested in week -1, on days 1 (6-7 hours after dosing), 8 and 15. The report stated that "each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices, and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor

activity was assessed in a separate room to minimize disturbances."

i. Neuropathology: Five animals/sex/group were anesthetized with halothane, exsanguinated and subjected to a full post mortem examination. The tissues listed below were removed and fixed in 10% neutral buffered formol saline. Also, five other animals/sex/group were deeply anesthetized with sodium pentobarbitone and killed by perfusion fixation with modified Karnovsky's fixative. The tissues listed below were removed and brain weight, length and width were recorded. The tissues from these latter groups were further microscopically examined. The neuropathological examination was performed on the control and highest dose groups only. All sections were examined by light microscopy. The brain and gastrocnemius muscle were embedded in paraffin wax, and 5 micrometer thick sections were cut and stained with H & E stain. Transverse sections of the vertebral column containing samples from the lumbar and cervical regions, with dorsal root ganglia and spinal roots attached, were decalcified, embedded in paraffin wax and 5 micrometer thick sections were also cut and stained with H & E. The remaining tissues were embedded in ARALDITE and semi-thin sections (1-2 micrometers) were cut and stained with toluidine blue. An initial examination of the brain was conducted on 1 male and 1 female from the 7 mg/kg group. The brain was examined in the transverse plane at 12 levels. On the basis of this examination, the remaining 4 animals/sex from this group and 5 rats/sex from the control group were examined in the transverse plane at the following 6 levels: 2, 5, 6, 7, 8 and 9. The spinal cord from the cervical region (C3-C6) and from the lumbar region (L1-L4) was also examined in the transverse plane. Spinal roots and the dorsal root ganglia were examined from the C3-C6 and L1-L4 levels and the gasserian ganglia were examined from the trigeminal nerve. Transverse and longitudinal sections of the sciatic nerve and transverse sections of the sural and tibial nerves were also examined. In addition, samples of the gastrocnemius muscle were examined in the transverse plane.

The following tissues were removed and examined microscopically:

|x| Brain
|x| Gasserian ganglia
|x| Vertebral column including spinal cord
|x| Dorsal root ganglea including spinal roots
|x| Gastrocnemius muscle
|x| Sciatic nerve
|x| Sural nerve
|x| Tibial nerve

j. Statistical Analyses: Day 1 bodyweights, functional observational battery data (day -1 measurements), brain measurements on method of kill and the replicate structure of the study design were analyzed by analysis of covariance. Motor activity measurements for each 5 minute period and overall (minutes 1-50), weekly food consumption and replicate structure of the study design were all analyzed by analysis of variance. Least squares means for each group were calculated. 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.

B. RESULTS:

1. Dosage Preparation: The concentration analyses revealed that the mean achieved concentrations were within 8% of the nominal concentrations (5%, 2.5% and 7.1% less than the nominal concentrations for the 0.2, 0.4 and 0.7 mg/ml concentrations, respectively). The 0.2 and 0.7 mg/ml formulations were stable for a period of 4 days, which covered the period of use during the study (111.1% and 104.6 % and the initial concentrations for the 0.2 mg/ml and the 0.7 mg/ml solutions, respectively).

2. Clinical Observations and Mortality: This is discussed under Functional Observational Battery.

3. Body Weight Determinations: No treatment-related effects were observed.

4. Food and/or Water Consumption: No treatment-related effects were observed.

5. Functional Observational Battery: One female in the 7 mg/kg group displayed reduced foot withdrawal reflex, shaking, signs of urinary incontinence, tip toe gait and upward curvature of the spine 6 hours after dosing. Recovery in this animal was observed by 24 hours. There were no treatment-related effects in males. There was one isolated finding of reduced splay reflex in one 2 mg/kg male, noted on day 15 and on day 8, there was a statistically significant decrease in the mean tail flick response for the 2 mg/kg/day male group. These are not considered to be biologically significant since there was no evidence of a dose-response. For the tail flick response, landing foot splay measurements and grip strength measurements, there was no evidence of a treatment-related effect.

6. Motor Activity: There was no indication of a treatment-related effect. There were a few spurious statistically significant differences observed between a mean from a particular group and the control mean, however, these were neither consistent nor dose-related. None of these statistically significant differences were observed at the highest dose group.

7. Neuropathology: There was no evidence of a treatment-related effect on brain weight, length or width. Only one value was statistically significantly less than controls, and this was the mean brain length of the 2 mg/kg female group. There were no macroscopic or microscopic findings that were considered to be related to treatment. The report stated that "minimal nerve fibre degeneration was noted for 2 male and 2 female control animals and 2 male and 1 female given 7 mg/kg fonofos. The degeneration was of a Wallerian type, and is an incidental feature of the peripheral nervous system in a number of strains of rat, including the Alderley Park strain. In the absence of a dose response, it is considered that this minimal degeneration is indicative of possible remodelling, rather than a very early onset of peripheral neuropathy which is known to be an age-related phenomenon in old rats." This is a plausible explanation. The following table summarizes the findings.

Intergroup Comparison of Microscopic Findings

	Dose Level of Fonofos (mg/kg)			
	Males		Females	
	0 mg/kg	7 mg/kg	0 mg/kg	7 mg/kg
Animals on Study	10	10	10	10
Animals Completed	5	5	5	5
Sciatic Nerve Examined	5	5	5	5
# Abnormalities Detected	3	3	3	4
Nerve fibre degeneration (total)	2	2	2	1

8. Quality Assurance Measures: Signed Good Laboratory Practice and Quality Assurance Statements were provided.

C. DISCUSSION: This study has been classified as Core Supplementary, upgradable to Acceptable. The Guidelines require positive control data. Submission of these data were previously recommended when TB-I reviewed the protocols for the acute and subchronic mammalian neurotoxicity studies that were to be conducted on fonofos (see memorandum from P. Hurley to J. Edwards, dated 4/16/92). Since these are new guidelines and since the Health Effects Division (HED) does not have any positive control data from the Zeneca Central Toxicology Laboratories conducted with the Alpk:APfSD strain, the data need to be submitted.

In addition, the report stated that "the clinical findings in [the] female at 7 mg/kg fonofos confirms previous findings that female rats are more sensitive to oral administration of fonofos than males. In a preliminary study it was established that females treated with a single oral dose of fonofos showed a steep dose-response curve over the range 5.0 - 7.5 mg/kg with no adverse effects at 5 mg/kg and lethality at 7.5 mg/kg. In addition, wide interanimal variability was noted in the clinical response to the compound. Therefore, a dose level of 7 mg/kg fonofos was the highest dose which was anticipated to show evidence of clinical toxicity without lethality. A screening battery revealed no treatment-related effects on neurological dysfunction. Therefore, the clinical changes which occurred at a perilethal dose level are considered to reflect systemic toxicity rather than neurotoxicity. This view is supported by a comprehensive histopathological evaluation of the nervous system of rats from the control and 7 mg/kg fonofos groups which revealed no evidence of treatment-

related change." There is a concern over the interpretation of the results and over the fact that at a dose close to the LD_{50}, signs were only seen in one animal. Therefore, HED is requesting a submission of the results from the preliminary study used to select dose levels.

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Secondary Reviewer: Karl P. Baetcke, Chief
Toxicology Branch I
Health Effects Division (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity - rat - **supplemental DER**

SHAUGHNESSY NO./TOX. CHEM. NO.: 041701/454B

ACCESSION NO./MRID NO.: 427926-01

DP BARCODE/SUBMISSION NO.: D192110/S442309

TEST MATERIAL: Fonofos

SYNONYMS: Dyfonate

STUDY NUMBER(S): PRO889

REPORT NUMBER: CTL/P/3879

SPONSOR: Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Fonofos: Subchronic Neurotoxicity Study in Rats

AUTHOR(S): J. M. Horner

REPORT ISSUED: 4/27/93

CONCLUSION: The RfD Committee reviewed this study on 8/12/93 and concluded the following from the study: fonofos was tested in a 90-day feeding neurotoxicity screening battery in the rat at the following dose levels: 0, 15, 50 or 125/150 ppm in the diet. The NOEL is 50 ppm and the LEL is 125/150 ppm based on clinical signs and several possible effects in the functional observational battery and in the motor activity observations in high dose females. Neuropathological findings were evident, but may be common to this strain of rat. These findings were included in the determination of the NOEL.

The NOEL for cholinesterase inhibition is either at or near 15 ppm (LDT) based on statistically significant decreases in cholinesterase activity

observed in both sexes for brain, erythrocyte and plasma at 50 ppm.

Classification: Core Supplementary, upgradable to acceptable upon receipt of positive control data.

Testing Guideline Satisfied: Not yet

DISCUSSION: This study has been classified as Core Supplementary, upgradable to Acceptable. The Guidelines require positive control data. Submission of these data were previously recommended when TB-I reviewed the protocols for the acute and subchronic mammalian neurotoxicity studies that were to be conducted on fonofos (see memorandum from P. Hurley to J. Edwards, dated 4/16/92). Since these are new guidelines and since the Health Effects Division (HED) does not have any positive control data from the Zeneca Central Toxicology Laboratories conducted with the Alpk:APfSD strain, the data need to be submitted.

In the study, treatment related clinical signs were observed in high dose females. These included: upward curvature of the spine, tiptoe gait, signs of urinary incontinence, pinched in sides, reduced splay reflex, splayed gait, eye bulging and shaking. In addition to these, there were several possible effects in the functional observational battery and in the motor activity observations. These included: statistically significant increases in the mean time to tail flick at week 14 in high dose males, in mean landing foot splay at week 9 in high dose females and in motor activity at various times in high dose females; a statistically significant decrease in mean forelimb grip strength at week 14 in high dose females; non-statistically significant increases in mean landing foot splay in high dose females at week 14; and non-statistically significant decreases in forelimb grip strength in high dose males at week 14 and in mean hindlimb grip strength in both sexes at week 14 (it is especially noted in the latter that while the control and lower dose groups all showed increases in this measure between weeks 9 and 13, high dose males and females showed decreases).

Neuropathological findings included a slight but statistically significant increase in mean brain weight in high dose females and 1 high dose female with a "collapsed brain, consistent with hydrocephalus". Microscopic findings included minimal nerve fibre degeneration in high dose males. The authors stated that these were either within the historical control range or were common in this particular strain of rat. Since HED does not have access to the historical control data on these animals and since these

lesions did appear in high dose group males and not in the controls and there were some indications of possible effects in the high dose animals in motor activity and in the functional observational battery, HED has considered these lesions in defining the NOEL. However, HED is not requesting additional microscopic examinations at the lower dose levels because the additional data would not add any significantly new information to the toxicology data base (particularly due to the fact that decreases in brain cholinesterase were already observed at lower dose levels).

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Secondary Reviewer: William F. Sette, Ph.D. William F Sette 8/9/93
Peer Review Section, Science Analysis Branch
Health Effects Division (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity - rat (82-7)

SHAUGHNESSY NO./TOX. CHEM. NO.: 041701/454B

ACCESSION NO./MRID NO.: 427926-01

DP BARCODE/SUBMISSION NO.: D192110/S442309

TEST MATERIAL: Fonofos

SYNONYMS: Dyfonate

STUDY NUMBER(S): PRO889

REPORT NUMBER: CTL/P/3879

SPONSOR: Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Fonofos: Subchronic Neurotoxicity Study in Rats

AUTHOR(S): J. M. Horner

REPORT ISSUED: 4/27/93

CONCLUSION: Fonofos was tested in a 90-day feeding neurotoxicity screening battery in the rat at the following dose levels: 0, 15, 50 or 125/150 ppm in the diet. In the study, with the exception of cholinesterase inhibition, all the effects were observed at 125/150 ppm. However, there were some questions with the microscopic examinations that need to be answered before a NOEL may be established (see discussion).

Treatment related clinical signs were observed in high dose females. These included: upward curvature of the spine, tiptoe gait, signs of urinary incontinence, pinched in sides, reduced splay reflex, splayed gait, eye bulging and shaking. In addition to these, there were several possible effects in the functional observational battery and in the motor activity observations.

Neuropathological findings included a slight but statistically significant increase in mean brain weight in high dose females and 1 high dose female with a "collapsed brain, consistent with hydrocephalus". Microscopic findings included minimal nerve fibre degeneration in high dose males. These may be common to this strain of rat, but no data were provided to support this statement.

The LOEL for cholinesterase inhibition is 15 ppm (LDT) based on statistically significant decreases in erythrocyte cholinesterase activity in both sexes and in plasma cholinesterase activity in females. At 50 ppm, statistically significant decreases in cholinesterase activity were observed in both sexes for all 3 parameters.

Classification: Core Supplementary (see discussion)

Testing Guideline Satisfied: None

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: o-ethyl s-phenyl ethylphosphonodithioate

Description: Yellow liquid
Batch #(s), Other #(s): P3/D7534/27; CTL Y02743/020
Purity: 94.6%
Source: ICI Agrochemicals
Vehicle: Mixed in diet
Positive Control(s): Not provided

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: 28 days old upon receipt.
Source(s): ZENECA Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

a. Dietary Preparation: The diets were prepared in 30 kg batches from premixes prepared by grinding a weighed amount of the test substance with 500 g of milled CT1 diet. The premixes were then added to additional CT1 diet and mixed thoroughly.

Frequency of preparation: Not stated.

Storage conditions: The first and second batches were stored frozen until required. The diet was removed from the freezer and allowed to thaw prior to use. After the results from the stability analyses were available, all subsequent batches were stored at room temperature.

Stability Analyses: Stability studies of the chemical in the diet were conducted on the 15 and 150 ppm dose levels for a period of at least 4 weeks at room temperature. In addition, the stability of the test chemical at the 15 ppm dose level was conducted at -20°C for 83 days.

Homogeneity Analyses: Homogeneity analyses were conducted on samples from the 15, 125 and 150 ppm dose levels.

Concentration Analyses: Samples from all dietary levels were taken at intervals and analyzed for concentration of the test material.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of results from a preliminary study in the same strain of rat.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 90 days male	female
Control	0	12	12
1	15	12	12
2	50	12	12
3	125/150**	12	12

*Six animals/sex in each group were designated for terminal neuropathology.
** The dose level was increased from 125 ppm to 150 ppm from the beginning of week 5, for both sexes.

d. Clinical Signs of Toxicity and Mortality: All rats were examined prior to the start of the study and cageside checks were conducted daily during the study for clinical signs of toxicity, behavior changes and mortality. At weekly intervals, each rat was removed from its cage and physically examined for changes in general health status.

e. Body Weight Determinations: Bodyweights were recorded weekly and at termination.

f. Food and/or Water Consumption: Food consumption was recorded and calculated weekly.

g. Functional Observational Battery: The report stated that "detailed clinical observations ...and quantitative assessments of landing foot splay, sensory perception (tail flick test) and muscle weakness (fore and hindlimb grip strength) were made in weeks -1, 5, 9 and 14. The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour etc; reactivity to stimuli; changes in level of arousal; sensorimotor responses; [and] alterations in respiration. The observations were made by one observer who was 'blind' with respect to the animal's treatment, and recorded on a computer system by personnel not directly involved in the clinical observations. The observations were carried out in a room separate from that in which the animals were housed and animals were presented to the observer with no indication of the treatment group. The observations were coded and the degree of condition noted (slight, moderate or extreme) where appropriate. This included the recording of no abnormalities detected."

h. Motor Activity: An automated activity recording apparatus was used to measure locomotor activity. The animals were tested in week -1, 5, 9 and 14 of the exposure period. The report stated that "each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices, and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimize disturbances."

i. Cholinesterase Activity: Blood samples were taken from the tail vein from all females at the beginning of weeks 4 and 7, and from males at the beginning of week 7, for determination of plasma and erythrocyte cholinesterase activity. At the beginning of week 13, blood samples were taken from 6 males and 6 females/group, also for-

determination of plasma and erythrocyte cholinesterase activity. These same animals were selected for determination of brain cholinesterase activity at termination. These animals were exsanguinated under terminal anesthesia with halothane Ph Eur vapor. The whole brain was removed rapidly, rinsed in ice-cold saline and stored on ice prior to measurement of brain cholinesterase activity. The method of Ellman et al (1961) was used for all assays.

j. Neuropathology:
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: Any rat requiring euthanasia was subject to a full post mortem examination. The tissues listed below were removed and fixed (except the brains which were submitted for cholinesterase activity). The brains were weighed and the length and width were recorded with calipers. These tissues were not microscopically examined.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: Up to six animals/sex/group were given full post mortem examinations. Again, the tissues listed below were removed and fixed (except the brains which were submitted for cholinesterase activity). The brains were weighed and the length and width were recorded with calipers. These tissues were not microscopically examined either. However, six other animals/sex/group were deeply anesthetized with sodium pentobarbitone and killed by perfusion fixation with modified Karnovsky's fixative. The tissues listed below were removed and brain weight, length and width were recorded. The tissues from these latter groups were further microscopically examined. The neuropathological examination was performed on the control and the 125/150 ppm groups only. All sections were examined by light microscopy. The brain and gastrocnemius muscle were embedded in paraffin wax, and 5 micrometer thick sections were cut and stained with H & E stain. Transverse sections of the vertebral column containing samples from the lumbar and cervical regions, with dorsal root ganglia and spinal roots attached, were decalcified, embedded in paraffin wax and 5

micrometer thick sections were also cut and stained with H & E. The remaining tissues were embedded in ARALDITE and semi-thin sections (1-2 micrometers) were cut and stained with toluidine blue. An initial examination of the brain was conducted on 1 male and 1 female from the 125/150 ppm dose group. The brain was examined in the transverse plane at 12 levels. On the basis of this examination, the remaining 5 animals/sex from this group and 6 rats/sex from the control group were examined in the transverse plane at the following 6 levels: 2, 5, 6, 7, 8 and 9. The spinal cord from the cervical region (C3-C6) and from the lumbar region (L1-L4) was also examined in the transverse plane. Spinal roots and the dorsal root ganglia were examined from the C3-C6 and L1-L4 levels and the gasserian ganglia were examined from the trigeminal nerve. Transverse and longitudinal sections of the sciatic nerve and transverse sections of the sural and tibial nerves were also examined. In addition, samples of the gastrocnemius muscle were examined in the transverse plane.

The following tissues were removed and examined microscopically:

x	Brain
x	Gasserian ganglia
x	Vertebral column including spinal cord
x	Dorsal root ganglea including spinal roots
x	Gastrocnemius muscle
x	Sciatic nerve
x	Sural nerve
x	Tibial nerve

k. Statistical Analyses: Day 1 bodyweights and the replicate structure of the study design were analyzed by analysis of covariance. Motor activity measurements, weekly food consumption, food utilization during the period weeks 1-4, 5-8, 9-13 and 1-13, tail flick response, landing foot splay, fore and hindlimb grip strength, cholinesterase activity, brain weight, length and width and replicate structure of the study design were all analyzed by analysis of variance. Least squares means for each group were calculated. 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.

B. RESULTS:

1. Dietary Preparation: The mean analyzed concentrations for the 15 ppm dose group were 15.5 (103.3%) and 13.4 (89.3 %) ppm. The mean analyzed concentrations for the 50 ppm dose group were 52.0 (104.0%) and 45.4 (90.4%) ppm. The mean analyzed concentrations for the 125/150 ppm dose group were 123 (98.4%), 144 (96.0%) and 156 (104.0%) ppm. One of the latter samples was considerably higher (185 ppm) and one was considerably lower (124 ppm).

   The homogeneity study indicated the following: at 15 ppm, the mean analyzed concentrations from the top, middle and bottom of the mixing chamber were 15.1, 16.2 and 14.8 ppm, respectively; at 125 ppm, the mean analyzed concentrations from the top, middle and bottom of the mixing chamber were 135, 127 and 122 ppm, respectively and at 150 ppm, the mean analyzed concentrations from the top, middle and bottom of the mixing chamber were 167, 148 and 101 ppm, respectively. Two of these latter values were either 20% high or 27% low.

   The chemical stability study indicated that the test chemical was stable in the diet at both freezer and room temperatures. At room temperature, the 15 ppm dose level remained stable after 28 days (104% of the initial concentration) and the 150 ppm dose level remained stable after 59 days (88.7% of initial concentration). In the freezer, the 15 ppm dose level remained stable after 83 days (97.9% of the initial concentration).

2. Clinical Observations and Mortality: One female in the 50 ppm dose group was killed for humane reasons during week 5, due to a twisted snout with associated malocclusion. This death was not considered to be treatment-related. Other clinical signs are described in the functional observational battery section.

3. Body Weight Determinations: There was a marginal reduction in mean body weight in high dose females during week 1. These animals recovered by the end of the second week. At week 5 following the increase in dose level at the highest dose, a further slight reduction in mean bodyweight in females was observed for week 7. The mean bodyweights continued to be lower until week 12. No treatment-related differences in mean bodyweights were observed when compared to controls for either the males in the high dose group or in either sex at any of the other dose levels. The

mean bodyweights in high dose females was only statistically significantly less than controls at week 7, week 8 and week 10. At no time were they less than 90% of the control values. Therefore, these differences did not appear to be biologically significant.

4. Food and/or Water Consumption: There was a slight reduction in food consumption in high dose females during week 1 (89%). No other reductions were observed at any other time, for any other dose level or sex. Food utilization calculations for both sexes at all dose levels indicated that there was no effect on food utilization at any time.

5. Functional Observational Battery: Treatment-related clinical signs were observed in high dose females. These included upward curvature of the spine, tiptoe gait, signs of urinary incontinence, reduced splay reflex, sides pinched in, splayed gait, eye bulging and shaking. These were observed from week 9 to the end of the study. There were no other treatment-related clinical signs in any of the males or in any of the other dose groups. The following tables summarize these data:

Clinical Observations Incidence in Females

Observation	Dietary Concentrations (ppm) 0	125/150
Signs of urinary incontinence		
# Observations	-	21
# Animals	-	7
Upward curvature of spine		
# Observations	-	9
# Animals	-	6
Splayed gait		
# Observations	-	3
# Animals	-	3
Tip toe gait		
# Observations	-	3
# Animals	-	2
Sides pinched in		
# Observations	-	3
# Animals	-	2
Reduced splay reflex		
# Observations	-	2
# Animals	-	2

Clinical Observations Incidence in Females

Observation	Dietary Concentrations (ppm) 0	125/150
Eye bulging		
# Observations	-	3
# Animals	-	1
Piloerection		
# Observations	-	2
# Animals	-	1
Shaking		
# Observations	-	1
# Animals	-	1

Frequency of Observations/Animal in High Dose Females

Observation	Frequency
Signs of urinary incontinence	2 animals 5 times each 1 animal 4 times 1 animal 3 times 1 animal 2 times 2 animals 1 time each
Upward curvature of spine	1 animal 3 times 1 animal 2 times 4 animals 1 time each
Splayed gait	3 animals 1 time each
Tip toe gait	1 animal 2 times 1 animal 1 time
Sides pinched in	1 animal 2 times 1 animal 1 time
Reduced splay reflex	2 animals 1 time each
Eye bulging	1 animal 3 times
Piloerection	1 animal 2 times
Shaking	1 animal 1 time

There was a statistically significant increase in the mean time to tail flick in high dose males at week 14 (2.9, 2.7, 3.5 and 4.9 [$p < 0.05$] for the controls, low, mid- and high dose groups, respectively). This was not considered by the authors to be related to treatment. However, the Health Effects Division (HED) considers

this to be a possible part of an overall neuropathological response (see discussion).

There was an increase in mean landing foot splay in the high dose females at week 9 (60.0, 57.6, 58.1 and 72.1 mm [$p < 0.05$] for the controls, low dose, mid-dose and high dose groups, respectively). At week 14, the mean landing foot splay in females was still greater than controls, but no longer statistically significantly different from controls. The authors did not consider these to be biologically significant. However, HED considers them to be possible evidence of neuropathology (see discussion).

In high dose females at week 14, there was a statistically significant decrease in mean forelimb grip strength. In high dose males, there was a also a non-statistically significant decrease in forelimb grip strength at week 14. In high dose males and females, non-statistically significant decreases in mean hind-limb grip strength were also observed at week 14. It is especially noted that at 9 weeks, all groups show comparable means. However, while the control and lower dose groups all showed increases in this measure between weeks 9 and 13, high dose males and females showed decreases. The authors did not consider the one mean statistically significant incidence to be biologically significant because there was no corresponding statistically significant change in hindlimb grip strength and because the decrease was not seen in earlier measurements. However, HED believes that these changes may be associated with possible neuropathology (see discussion).

6. Motor Activity: There were significant decreases in motor activity in high dose females at weeks 5, 9 and 14. These are summarized in the following table.

Intergroup Comparison of Motor Activity in Females

	Dietary Concentration of Fonofos (ppm) 0	125/150
	Week 5	
Minutes 6-10	70.9	53.4**
Minutes 11-15	69.1	54.1*
Minutes 21-25	58.9	38.6*
Minutes 26-30	56.8	36.8*
Minutes 31-35	57.3	32.8*
Minutes 41-45	49.4	26.0*
Overall (1-50)	580.4	416.3**
	Week 9	
Minutes 6-10	69.2	55.2*
Minutes 26-30	54.8	42.4
Minutes 46-50	49.5	36.1
Overall (1-50)	552.3	475.4
	Week 14	
Minutes 1-5	74.9	62.8**
Minutes 21-25	58.9	37.1*
Minutes 31-35	62.7	36.8*
Minutes 36-40	62.0	28.4**
Minutes 41-45	53.2	27.9*
Minutes 46-50	53.8	26.8*
Overall (1-50)	602.8	411.4*

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

7. Cholinesterase Activity: In males, there were statistically significant decreases in brain cholinesterase activity at 50 and at 125/150 ppm. The decrease at 50 ppm was 8% less than controls and the decrease at 125/150 ppm was 31% less than controls. Statistically significant decreases in erythrocyte cholinesterase activity were observed at all dose levels at week 7 and at the mid- and high dose levels at week 13. Statistically significant decreases in plasma cholinesterase activity were observed at 50 and at 125/150 ppm at both weeks 7 and 13.

In females, statistically significant decreases in brain cholinesterase activity were observed at 50 and at 125/150 ppm. Statistically significant decreases in erythrocyte cholinesterase activity at all treated levels at weeks 4, 7 and 13. Statistically significant decreases in plasma cholinesterase activity were

observed at 50 and 125/150 ppm at week 4 and at all dose levels at weeks 7 and 13. The following table summarizes the results.

Intergroup Comparison of Cholinesterase Activity

	Concentrations (ppm)			
	0	15	50	125/150
Males				
Brain cholinesterase (micromoles/min/g)				
Week 14	8.97	9.10 +1%	8.27* -8%	6.17** -31%
Erythrocyte cholinesterase (U/l)				
Week 7	1526	1395** -9%	1096** -28%	918** -30%
Week 13	1697	1767 +4%	1103** -35%	913** -46%
Plasma cholinesterase (U/l)				
Week 7	521	481 -8%	442** -15%	249** -52%
Week 13	577	544 -6%	474* -28%	201** -65%
Females				
Brain cholinesterase (micromoles/min/g)				
Week 14	9.07	8.50 -6%	5.98** -34%	1.93** -79%
Erythrocyte cholinesterase (U/l)				
Week 4	1516	1421* -6%	766** -49%	673** -56%
Week 7	1689	1458** -14%	970** -43%	845** -50%
Week 13	1778	1630* -8%	1105** -38%	842** -53%
Plasma cholinesterase (U/l)				
Week 4	1172	1112 -5%	569** -51%	183** -84%
Week 7	1270	1116* -12%	622** -51%	150** -88%
Week 13	1796	1567* -13%	864** -52%	202** -89%

*p < 0.05
**p < 0.01

8. Neuropathology: In high dose females, there was a statistically significant increase in mean brain weight (2.03 [$p < 0.01$] versus 1.93 g in the control group (5% greater). This was not due to an outlier in the individual animal data. The authors stated that the mean brain weight for the high dose females was within the normal range for animals of this strain and age, and therefore the increase was not treatment-related. However, no historical control data were provided to support this statement. There was also a statistically significant increase in brain weight in the mid-dose males (2.15 [$p<0.05$] versus 2.10 grams in the controls) but not in the high dose males. Thus, there was no indication of a dose-response. There were no treatment-related differences in any of the other brain parameters in any sex or dose group (length and width).

In the macroscopic examinations, 1 high dose female had a bulging eyeball and there were 1 and 3 females in the mid- and high dose groups, respectively which had hair loss. The number of females with distended uteri were 1,1,1 and 4 in the control, low, mid- and high dose groups, respectively. The authors did not consider any of these findings or any of the other incidental findings to be treatment-related. In addition to the above, 1 female in the high dose group had a collapsed brain (flaccid, fluid leakage). The authors stated that this was consistent with hydrocephalus, a known incidental finding in this strain of rat. No historical control data were provided to support this statement.

Microscopic findings included minimal nerve fibre degeneration in high dose males. The authors stated that these are a common finding in the Alderley Park strain of rat and is therefore considered to be incidental and unrelated to treatment. However, no historical control data were provided to support this statement. These results are summarized in the following table.

Intergroup Comparison of Microscopic Findings

	Dose Level of Fonofos (ppm)			
	Males		Females	
	0	150	0	150
Animals on study	12	12	12	12
Animals completed	6	6	6	6
Sciatic nerve Examined	6	6	6	6
# Abnormalities detected	6	4	5	5
Nerve fibre degeneration (total)	0	2	1	1
minimal	0	2	1	1

9. Quality Assurance Measures: Signed Quality Assurance and GLP statements were provided.

C. DISCUSSION: This study has been classified as Core Supplementary, possibly upgradable to Acceptable. The Guidelines require positive control data. Submission of these data were previously recommended when TB-I reviewed the protocols for the acute and subchronic mammalian neurotoxicity studies that were to be conducted on fonofos (see memorandum from P. Hurley to J. Edwards, dated 4/16/92). Since these are new guidelines and since the Health Effects Division (HED) does not have any positive control data from the Zeneca Central Toxicology Laboratories conducted with the Alpk:APfSD strain, the data need to be submitted.

In the study, with the exception of cholinesterase inhibition, all the effects were observed in the high dose group. However, there were some questions with the microscopic examinations that need to be answered before a NOEL may be established.

Treatment related clinical signs were observed in high dose females. These included: upward curvature of the spine, tiptoe gait, signs of urinary incontinence, pinched in sides, reduced splay reflex, splayed gait, eye bulging and shaking. In addition to these, there were several possible effects in the functional observational battery and in the motor activity observations. These included: statistically significant increases in the mean time to tail flick at week 14 in high dose males, in mean landing foot splay at week 9 in high dose females and in motor activity at various times in high dose females; a statistically significant decrease in mean forelimb grip strength at week 14 in high dose females; non-statistically significant increases in mean

landing foot splay in high dose females at week 14; and non-statistically significant decreases in forelimb grip strength in high dose males at week 14 and in mean hindlimb grip strength in both sexes at week 14 (it is especially noted in the latter that while the control and lower dose groups all showed increases in this measure between weeks 9 and 13, high dose males and females showed decreases).

Neuropathological findings included a slight but statistically significant increase in mean brain weight in high dose females and 1 high dose female with a "collapsed brain, consistent with hydrocephalus", both of which the authors stated were either within the historical control range or were common in this particular strain of rat. Microscopic findings included minimal nerve fibre degeneration in high dose males. Considering the fact that HED does not have access to the historical control data on these animals, that these lesions did appear in high dose group males and not in the controls and that there were some indications of possible effects in the high dose animals in motor activity and in the functional observational battery, HED is requesting a more thorough histopathological evaluation of the nervous tissue. This should include examination of samples from the intermediate and low dose groups, a coded subjective diagnosis performed for the lesions found in all dose groups and examination of immersion fixed tissues. In addition, in view of the other effects seen, other planes should be examined for all high dose subjects and any effects seen in the high dose groups, pursued by examination of the intermediate dose groups.

The LOEL for cholinesterase inhibition is 15 ppm (LDT) based on statistically significant decreases in erythrocyte cholinesterase activity in both sexes at 15 ppm (up to -14%) and in plasma cholinesterase activity in females at 15 ppm (up to -13%). At 50 ppm, statistically significant decreases in cholinesterase activity were observed in both sexes for all 3 parameters, brain (up to -34% in females), erythrocyte (up to -49% in females) and plasma (up to -52% in females).