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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: 627. Dyfonate®. Review of 90-Day Delayed Neurotoxicity Study in Hens

Tox. Chem. No. 454B
Project No. 2-1134

TO: Joanne Edwards, PM Team # 72
Special Review and
Reregistration Division (H7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 4/1/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner 4-8-92 KB 4/11/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

DP Barcode: D173440
Submission: S410122

Background and Request:

A 90-day delayed neurotoxicity study on Dyfonate® in hens 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 examined the Health Effects Division (HED) files and has found that this study has been previously reviewed (see HED files, Document # 7273). TB-I rereviewed the study because the Data Evaluation Report (DER) from the first review was very brief.

The submitted 90-day hen study generally follows the EPA Office of Pesticide Programs (OPP) testing guidelines for a 90-day neurotoxicity study in hens with several deviations that are not significant enough to invalidate the study for regulatory purposes. However, TB-I has determined that in order for the Agency to conduct an overall assessment of the potential for Fonofos to induce delayed neurotoxicity, it is desireable to have data from an acute delayed neurotoxicity study in the hen. This

study has already been required in the Registration Standard for Fonofos. Therefore, the 90-day neurotoxicity study in the hen is classified as Core Supplementary until submission of data from the acute delayed neurotoxicity study. At that time, TB-I will reconsider the 90-day study for upgrading to an acceptable study for regulatory purposes.

As the Registrant is aware, 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 reviewed in the attached Data Evaluation Report (DER) will apply to the previously published OPP neurotoxicity testing guidelines. TB-I notes that the Registrant has added the AchE and NTE assays in their protocols for the rat neurotoxicity screening battery for Fonofos (recently required by OPP). The following paragraph summarizes the 90-day hen study evaluated in the attached DER.

Technical Dyfonate^{R} was administered orally to adult hens for 90 days at 2, 4 and 8 mg/kg/day. Control groups were either untreated or given corn oil. The positive control group was administered tri-o-cresyl phosphate (TOCP). No evidence of delayed neurotoxicity was observed in any of the Dyfonate^{R}-treated hens, whereas the positive controls displayed marked evidence of delayed neurotoxicity in addition to progressive loss of body weight, inhibition of plasma cholinesterase, impaired egg production and death. The Dyfonate^{R}-treated animals exhibited significant weight loss in the high dose group, clinical signs of toxicity in the mid- and high dose groups (possibly the low dose group), inhibition of plasma cholinesterase in all dose groups and impaired egg production in all dose groups. The NOEL for inhibition of plasma cholinesterase is < 2.0 mg/kg/day (LDT) and the NOEL for other acute neurotoxic effects is < 2.0 mg/kg/day.

Tox. Chem No. 454B File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
90-day delayed neurotox. in hens/ Stauffer Chem. Co./# T-6237; 11/8/78; reissue: 3/24/87	Tech. Dyfonate Lot #CEL-3001; 93.5% pure	401501-20	Dose levels: 2, 4 & 8 mg/kg/day by gavage. Positive control: tri-o-cresyl phosphate (TOCP). No evidence of delayed neurotoxicity observed in any treated hens. Treated animals exhibited significant weight loss in high dose group, clinical signs of toxicity in mid- and high dose groups (possibly low dose group), inhibition of plasma cholinesterase in all groups & impaired egg production in all groups. NOEL for inhibition of plasma cholinesterase < 2.0 mg/kg/day (LDT). NOEL for other acute neurotoxic effects < 2.0 mg/kg/day. Need acute delayed neurotoxicity study for full assessment of study.	N/A	Supplementary (upgradable when acute study submitted. See Doc. # 7273 for previous DER. This is a rereview.

DRAFT

Reviewed By: Pamela Hurley, Ph.D. Pamela M. Hurley 4/1/92
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner
Section I, Tox. Branch (H7509C)

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DATA EVALUATION RECORD

STUDY TYPE: 90-Day Neurotoxicity in Adult Hens (82-5)

TOX. CHEM. NO.: 454B

ACCESSION NUMBER/MRID NO.: 401501-20

TEST MATERIAL: Dyfonate

SYNONYMS: Fonofos

STUDY NUMBER(S): T-6237

SPONSOR: Stauffer Chemical Company, Richmond, CA

TESTING FACILITY: Stauffer Chemical Company, Richmond Toxicology Laboratory, Richmond, California

TITLE OF REPORT: Neurotoxicity of 90-Day Oral Administration of Technical Dyfonate to Adult Hens

AUTHOR(S): J.L. Miller

REPORT ISSUED: 11/8/78; reformatted and reissued: 3/24/87

CONCLUSION: Technical Dyfonate® was adminstered orally to adult hens for 90 days at 2, 4 and 8 mg/kg/day. Control groups were either untreated or given corn oil. The positive control group was administered tri-o-cresyl phosphate (TOCP). No evidence of delayed neurotoxicity was observed in any of the Dyfonate®-treated hens, whereas the positive controls displayed marked evidence of delayed neurotoxicity in addition to progressive loss of body weight, inhibition of plasma cholinesterase, impaired egg production and death. The Dyfonate®-treated animals exhibited significant weight loss in the high dose group, clinical signs of toxicity in the mid- and high dose groups (possibly the low dose group), inhibition of plasma cholinesterase in all dose groups and impaired egg production in all dose groups. The NOEL for inhibition of plasma cholinesterase is < 2.0 mg/kg/day (LDT) and the NOEL for other acute neurotoxic effects is < 2.0 mg/kg/day.

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Classification: Core Supplementary (upgradable - see discussion)

A. MATERIALS AND METHODS:

1. Test Compound

Chemical Name: o-ethyl s-phenyl ethylphosphonodithioate

Description: Not given
Batch #(s), Other #(s): Lot #CEL-3001
Purity: 93.5%
Source: Not stated, assumed Sponsor
Vehicle: Corn oil
Positive Control: Tri-o-cresyl phosphate (TOCP)

2. Test Animals

Species and Strain (sexes): Adult white Leghorn hens
Age: Approximately 12 months
Weight(s): 1.3-2.1 kg
Source(s): Feather Hill Farms, Petaluma, CA

3. Procedure:

a. Dosage preparation: Each daily treatment was administered orally via gavage in a volume of 1 ml/kg bodyweight. Corn oil was the vehicle. No description was given as to how the doses were prepared.

Frequency of preparation: Not stated.

Storage conditions: Not stated.

Stability Analyses: Not conducted.

Homogeneity Analyses: Not conducted.

Concentration Analyses: Not conducted.

b. Basis For Selection of Dose Levels: Dose levels were selected on the basis of a range-finding study which was summarized in the report.

c. Animal Assignment and Dose Levels:

Range-Finding Study

Test Group		Dose Administered 1 ml/kg/day (mg/kg/day)[a]	# Animals
A	Corn Oil (18 doses)	0	6
B	TOCP (17 doses)	20	6
C	TOCP (17 doses)	100	6
D	High Dose (12 doses) followed by (7 doses)	0.1 10.8	6
E	Mid-Dose 1 (12 doses) followed by (7 doses)	0.3 5.4	6
F	Low-Dose (20 doses)	0.9	6
G	Mid-Dose 2 (21 doses)	2.7	6

Main Study

Test Group		Dose Administered 1 ml/kg/day for 90 days (mg/kg/day)[b]	# Animals
1	Untreated Control	0	10
2	Corn Oil Control	0	10
3	TOCP	60	10
4	High Dose	8	10
5	Mid-Dose	4	10
6	Low-Dose	2	10

[a] Via intubation 5 days/week.
[b] Via intubation, not specifically stated, but implied 7 days/week.

d. Clinical Observations and Mortality: For the range-finding study, each animal was observed twice daily during the week and once a day on weekends for clinical signs of toxicity. Signs of delayed neurotoxicity were scored on days 14 and 22. For the main study, each hen was observed daily for clinical signs of toxicity. Weekly measurements were made on number of eggs produced and behavioral signs of delayed neurotoxicity.

For the scoring of behavioral signs of delayed neurotoxicity, hens were placed in a walk-in hood equipped with a rubber floor mat. They were then scored for signs of delayed neurotoxicity according to a preset scoring system which includes parameters in increasing severity such as leg weakness (score 1); lack of leg coordination even though movement is free, loss of balance and

tendency to fall back on rump due to leg weakness (score 2); to inability to walk and hyperextension, ataxia, completely prostrate and moribund (score 3); and finally, death (score 4).

e. Body Weight Determinations: Body weights were measured weekly.

f. Clinical Pathology: Plasma cholinesterase activity was determined in both the range-finding study and in the main study. In addition, a preliminary study was conducted to determine the limit of detectability (LD), the stability of Dyfonate® in hen blood, and the appropriate solvent for use in spiking samples. For the range-finding study, blood samples (1 ml in 100µl 12% sodium citrate) were drawn from the wing veins of the treated hens before the first dose and at 2 hours (one-half of each group) and 24 hours (the other half of each group) after the last dose for both the determination of plasma cholinesterase activity and blood Dyfonate® levels.

For the determination of the limit of detectability (LD), acetone proved to be the solvent of choice for spiking samples. Dyfonate® was added to previously drawn blood samples in acetone (>25 ul/ml) at final levels ranging from 0.005 to 100 ppm in the sample. Recoveries of Dyfonate® from the blood ranged from 39-70%. The differences in percentage of recovery were not dose related. Extractions of 5 ml samples gave slightly higher recoveries than did 1 ml samples. Amounts of Dyfonate® oxone analogues were also checked, and these were in very low concentrations.

For the stability study of Dyfonate® in blood, 5 ml samples were taken from surviving hens in the 5.4 and 10.8 mg/kg/day group. The samples were extracted with hexane, the organic layer was dried and injected into a gas-liquid chromatograph. Amounts of Dyfonate® and its oxygen analogue were calculated.

For the main study, citrated blood samples were drawn in the same manor as in the range-finding study before initiation of treatment and 24 hours after the last (90th) treatment, only for determination of plasma cholinesterase activity.

Plasma cholinesterase activity was determined without delay from citrated plasma samples using a Clinicard® Analyzer (Model 368, Instrumentation Laboratory, Inc., Mt. Vernon, NY).

g. Histopathology:

CHECKED (X) tissues were preserved for histopathological examination within 15 minutes after termination. The (*) tissues were recommended by the Guidelines. Slides were stained with either hematoxylin and eosin and/or hematoxylin and eosin counterstained with Luxol Fast Blue.

X	
x	Medulla oblongata* - stated "through the middle of cerebellum and the underlying medulla".
x	Cerebellum
x	Spinal cord*
x	Upper cervical bulb* - just stated cervical region
x	Mid-thoracic*
x	Lumbo-sacral region*
	Peripheral nerves*
	Proximal region of tibial nerve and branches*
x	Proximal region of sciatic nerve* plus 2-3 mm. of main trunk.

h. Statistical Analyses: Mean body weights were statistically compared using the Students t-test.

B. RESULTS:

1. Clinical Observations and Mortality: For the range-finding study, no signs of delayed neurotoxicity were observed in hens given corn oil, the low dose of TCOP (20 mg/kg/day), or 0.1, 0.3, 0.9, and 2.7 mg/kg/day Dyfonate®. The report stated that "adverse clinical signs in these groups were limited to an occasional incidence of listlessness, droopy comb and/or slight diarrhea." After elevation of the two lower dose levels to 5.4 and 10.8 mg/kg/day, the following clinical signs of toxicity were observed: an increased incidence of droopy combs, listless behavior and greenish colored diarrhea (5.4 mg/kg/day); and ataxia, listlessness, labored breathing, wing drop and green diarrhea (10 mg/kg/day). At 10.8 mg/kg/day, transient paralysis, lasting 3-6 hours was noted in 2/6 hens. Two hens died; the deaths appeared to be related to severe cholinesterase inhibition. The report stated that "tolerance to the acute cholinergic effects of Dyfonate® apparently developed in the survivors after

the sixth dose as evidenced by the decrease in number and severity of symptoms." Hens given the high dose TCOP (100 mg/kg) showed behavioral signs of delayed neurotoxicity by day 14. By day 21, all these positive control hens exhibited marked leg paralysis and became moribund. They were terminated.

In the main study, a number of clinical signs were observed in the treated animals, including the positive controls. The only sign observed in both untreated and corn oil controls was occasional diarrhea. This sign was observed at a much higher frequency in the treated animals. The positive control animals appeared normal until 8 treatment days. At that time several hens began exhibiting diarrhea, sometimes colored green with bile pigments. On day 14 and thereafter, other signs appeared which included hock sitting, curled toes, ataxia, non-vocalization, listlessness, comb droop, wing droop, ptosis, cyanosis, dyspnea, salivation, loss of righting reflex and paralysis. All TOCP-treated hens became moribund after 19 to 31 days of treatment. The high dose Dyfonate®-treated hens (8 mg/kg/day) exhibited many of the same signs as the positive control hens except for cyanosis, curled toes and paralysis. Most of these signs occurred early in the treatment period (days 1-18), and became less frequent and severe during the latter stages of the treatment period. These same signs were also observed in the mid-dose hens, except generally were less severe and less frequent. There were 3 deaths in the high dose group but no deaths in the mid-dose group. Non-vocalization, listlessness, feather loss, comb droop and diarrhea were also observed in the low-dose group, but at much less frequency. The following table taken directly from the report summarizes the data on clinical signs.

Clinical Signs Observed During the 90-Day Study in Untreated Control Hens and Hens Given Daily Oral Doses of Corn Oil, Tri-o-cresyl Phosphate (TOCP) or Dyfonate®

	Treatment (mg/kg/day)					
Clinical Signs	Contr.	Corn Oil[a]	TOCP 60.0	Dyfon. 2.0	Dyfon. 4.0	Dyfon. 8.0
Behavioral						
Non-Vocal	0/10[b]	0/10	10/10	2/10	10/10	10/10
Listless	0/10	0/10	10/10	10/10	10/10	10/10
Appearance						
Feather Loss	0/10	0/10	0/10	1/10	6/10	5/10
Dry or Atrophied Comb	0/10	0/10	0/10	0/10	3/10	5/10
Comb Droop	0/10	0/10	10/10	3/10	10/10	10/10
Wing Droop	0/10	0/10	1/10	0/10	5/10	4/10
Ptosis	0/10	0/10	4/10	0/10	4/10	4/10
Cyanosis	0/10	0/10	10/10	0/10	0/10	0/10
Involuntary Function						
Dyspnea	0/10	0/10	2/10	0/10	0/10	3/10
Salivation	0/10	0/10	1/10	0/10	2/10	2/10
Diarrhea	3/10	1/10	10/10	5/10	7/10	10/10
Green Diarrhea	0/10	0/10	8/10	1/10	6/10	10/10
Voluntary Function						
Ataxia	0/10	0/10	10/10	0/10	4/10	7/10
Tremors	0/10	0/10	0/10	0/10	0/10	1/10
Hock Sitting	0/10	0/10	10/10	0/10	2/10	5/10

[a] 1 ml/kg/day
[b] Number of chickens with sign/number of chickens observed.

Clinical Signs Observed During the 90-Day Study in Untreated Control Hens and Hens Given Daily Oral Doses of Corn Oil, Tri-o-cresyl Phosphate (TOCP) or Dyfonate® (Cont.)

	Treatment (mg/kg/day)					
Clinical Signs	Contr.	Corn Oil[a]	TOCP 60.0	Dyfon. 2.0	Dyfon. 4.0	Dyfon. 8.0
Loss of Righting Reflex	0/10	0/10	10/10	0/10	2/10	4/10
Curled Toes	0/10	0/10	3/10	0/10	0/10	0/10
Paralysis	0/10	0/10	4/10	0/10	0/10	0/10
Dead or Moribund	0/10	0/10	10/10	0/10	0/10	3/10

[a] 1 ml/kg/day
[b] Number of chickens with sign/number of chickens observed.

Neither the control hens nor the hens treated with Dyfonate® displayed any signs of delayed neurotoxicity. The positive control hens exhibited motor impairment and paralysis which began in the second week and rapidly became more severe until the hens died or became moribund. At 8 mg/kg/day, Dyfonate® treated hens had a score of 0.1 on day 14 (10 hens). All the rest of the controls and Dyfonate® treated groups had scores of 0 at all time points. TOCP-treated hens had a score of 2 on day 14 (10 hens), 2 on day 21 (7 hens) and 3 on day 28 (1 hen). By day 35, there were no hens left to score.

Egg production was suppressed in all treated groups, particularly during the third week of treatment. There was a dose-response. The controls were producing between 5-6 eggs/hen/week. The positive controls were producing approximately 1 egg/hen/week, which went to 0 by week 4 after which all the hens died. At 2.0 mg/kg/day Dyfonate®, the hens started out producing a mean of 3 eggs/hen/week which increased to 5 eggs/hen/week by week 12. At 4.0 mg/kg/day, the hens started out producing a mean of 0 eggs/hen/week which increased to 2 eggs/hen/week by week 12, and at 8.0 mg/kg/day, the hens started out producing 0 eggs/hen/week which increased to 1 egg/hen/week by week 12.

2. Body Weight Determinations: For the range-finding study, the mean body weights of control hens and those given the lower dose levels (0.1, 0.3, 0.9, and 2.7 mg/kg/day) of Dyfonate® were well maintained throughout the study. Groups which received TCOP or the higher doses of Dyfonate® (when levels were raised to 5.4 and 10.8 mg/kg/day) exhibited marked weight loss which appeared to be dose-related.

For the main study, the untreated and corn oil controls maintained their mean body weights throughout the study. The positive control hens lost a significant amount of body weight (most pronounced on days 14-21) until their deaths. The Dyfonate-treated hens also lost a significant amount of body weight, particularly between days 7 and 18. There was a dose-related effect, however, the final body weights of hens in the 2 mg/kg/day group were not significantly different from controls by the end of the study. In addition, although the figure in the text plainly shows that there was a dose-response, only the final mean body weights of the highest dose group were less than 90% of the controls by the end of the study. The following table taken from the report summarizes the initial and final mean body weights of the controls and treated groups.

Final and Initial Mean Body Weights in Control and Treated Hens

Treatment (mg/kg/day)	Body Weights (Kg) on Day 0	90	% Change
None	1.707 ± 0.160[a] (10)	1.761 ± 0.166 (10)	+ 3.2
Corn Oil (1 ml/kg/day)	1.676 ± 0.130 (10)	1.734 ± 0.187 (10)	+ 3.5
TOCP (60)	1.600 ± 0.143 (9)	[b]	-
Dyfonate^{R} (2)	1.555 ± 0.067 (10)	1.644 ± 0.064 (10)	+ 5.7
Dyfonate^{R} (4)	1.708 ± 0.155 (10)	1.589 ± 0.104 (10)*	- 7.0
Dyfonate^{R} (8)	1.697 ± 0.187	1.465 ± 0.106 (5)*	-13.7

[a] Mean ± standard deviation (N)
[b] All hens were dead by day 31.
* $P < 0.05$ compared to untreated or corn oil-treated hens. The probability (P) set for significance was 0.05.

3. Plasma Cholinesterase Activity: In the range-finding study, plasma cholinesterase activity was inhibited at all dose levels at both 2 and 24 hours after the last dose of Dyfonate^{R}. A dose-related response was observed and the magnitude of the response decreased over time. The following table summarizes the response.

Plasma Cholinesterase Activity in Hens
Given Daily Oral Doses of Dyfonate[a,b]

Treatment	Time Blood Sampled After Last Dose (hrs)	Pretreatment ChE Activity (i.u.)	Treated ChE Activity (i.u.)
Dyfonate® (0.1 mg/kg/day increased to 10.8 mg/kg/day after 12th dose	2	86	Nil[c]
	24	100	18[c]
Dyfonate® (0.3 mg/kg/day increased to 5.4 mg/kg/day after 12th dose	2	116 ± 15	Nil
	24	124 ± 52	57[c]
Dyfonate® (2.7 mg/kg/day)	2	103 ± 3	48 ± 14
	24	123 ± 14	72 ± 13
Dyfonate® (0.9 mg/kg/day)	2	91 ± 6	68 ± 7
	24	121 ± 20	97 ± 23

[a] From table 4 in report.
[b] Mean ChE activity ± standard deviation of single determinations in 3 hens unless otherwise indicated. Values are in international units (i.u.).
[c] Mean of 2 hens.

In the main study, plasma cholinesterase activity in the positive control and in the treated groups was significantly decreased immediately prior to termination of the study. The report stated that the "inhibition® ranged from 64-77% in the hens given TOCP or Dyfonate® (8 or 4 mg/kg/day). Hens given 2 mg/kg/day of Dyfonate® showed 50% inhibition of plasma cholinesterase prior to termination." The following table taken from the report summarizes mean plasma cholinesterase values prior to treatment and after treatment.

Plasma Cholinesterase Activity in Control and Treated Hens Before and After Treatment

Treatment (mg/kg/day)	Plasma Cholinesterase Activity (AChE) International Units: Prior to Chronic Treatment	After Chronic Treatment[b]
Untreated	99 ± 21 (10)[a]	102 ± 19 (10)
Corn Oil (1 ml/kg/day)	84 ± 12 (10)	97 ± 21 (10)
TOCP (60)	84 ± 12 (10)	27 ± 12 (9)*
Dyfonate®		
8 mg/kg/day	84 ± 14 (10)	30 ± 7 (7)*
4 mg/kg/day	86 ± 15 (10)	20 ± 11 (10)*
2 mg/kg/day	84 ± 9 (10)	42 ± 5 (10)*

[a] Values are the mean ± standard deviation.
[b] Activity measured 24 hours after last dose and immediately prior to termination.
* $P < 0.05$ compared to controls (untreated or corn oil). The probability (P) set for significance was 0.05.

4. Determination of Concentrations of Dyfonate in the Blood: In the range finding study, hens that were dosed with 10.8 mg/kg/day had a Dyfonate® blood level at the lower limit of detectability (0.013 ppm) 2 hours after dose administration. After 24 hours, Dyfonate® was undetectable (<0.01 ppm). It was also undetectable at both 2 and 24 hours after administration of 5.4 mg/kg/day treatment. The report stated that "in view of the low levels of Dyfonate® noted in hens given 5.4 mg/kg/day, blood samples from hens treated with lower doses of Dyfonate® were not analyzed." The amount of the oxygen analogue was undetectable at both dose levels at all sample times.

5. Histopathology: No treatment-related changes were observed between Dyfonate®-treated and negative control groups. Several microscopic changes were seen in all groups, including untreated, vehicle and positive controls as well as the Dyfonate®-treated groups. These were lymphocytic perivascular cuffing and focal glial cell proliferation. The perivascular cuffs were usually only a few cells thick and the affected vessels were scattered throughout the cerebellum, medulla and spinal cord. The observed glial cell nodules were also

small and randomly scattered, except in a few hens in which the changes were more frequent and were rated as moderate in severity. The report also stated that in sciatic nerve sections, a few small lymphocytic foci were observed in the perineurium of 2 hens which appeared to be consistent with those associated with Marek's disease herpes virus. Several other microscopic lesions were observed that were randomly located in the medulla and spinal cord and in some cases adjacent to cuffed vessels or glial nodules. The authors stated that these may have been related to Marek's disease or other field and vaccine viruses common to commercial chickens.

The positive controls had the following specific changes that were either not seen or were distinctly more severe and more consistent than in the negative control groups: retrograde degeneration of axon filaments and some associated fragmentation of myelin sheaths in the medulla and all three levels of the spinal cord. These changes were localized to the specific white matter tracts (funiculi). There was also individual nerve fiber degeneration in the sciatic nerve and its branches. This consisted of swelling and fragmentation of the nerve fiber and globular degeneration of the myelin sheath and was substantially more severe in the tibial branches than in the main sciatic trunk.

The microscopic summary tables from the report are attached to this Data Evaluation Report.

6. Quality Assurance Measures: This study was conducted prior to the Good Laboratories Practice requirements. The authors stated that the study was carried out following appropriate EPA guidelines using accepted laboratory methods and standard data management procedures.

C. DISCUSSION: This study generally follows the EPA Office of Pesticide Programs (OPP) testing guidelines for a 90-day neurotoxicity study in hens. Deviations from the Guidelines include lack of detail on dosage preparation, lack of stability, homogeneity and concentration analyses on the test material and differences in the preparation of the tissues. In this study, the tissues were removed from the animal prior to fixation. The Guidelines suggest perfusing the tissues with fixative in situ prior to removal from the animal. This reduces damage to the tissues and reduces artifacts that may confound the reading of the prepared tissue slides. These deviations from the test Guidelines

are not significant enough to invalidate the study for regulatory purposes.

In the study, no evidence of delayed neurotoxicity was observed in any of the Dyfonate®-treated hens, whereas the positive controls displayed marked evidence of delayed neurotoxicity in addition to progressive loss of body weight, inhibition of plasma cholinesterase, impaired egg production and death. The Dyfonate®-treated animals exhibited significant weight loss in the high dose group, clinical signs of toxicity in the mid- and high dose groups (possibly the low dose group), inhibition of plasma cholinesterase in all dose groups and impaired egg production in all dose groups. The NOEL for inhibition of plasma cholinesterase is < 2.0 mg/kg/day (LDT) and the NOEL for other acute neurotoxic effects is < 2.0 mg/kg/day. It is noted that the microscopic examinations of neurological tissue were not perfectly clean, although the same effects were seen in the untreated controls. Some of these effects may have been related to Marek's disease or other field and vaccine viruses common to commercial chickens. Since the results from the microscopic examination were slightly unclear, it would be helpful for an overall assessment of delayed neurotoxicity to have data from an acute delayed-neurotoxicity study in the hen. The Toxicology Branch (TB-I) notes that this study has already been required in the Registration Standard, but TB-I has not yet received the data. Therefore, the 90-day neurotoxicity study in the hen is classified as Core Supplementary, upgradable when the data on the acute delayed neurotoxicity study are submitted.

TB-I notes that OPP is in the process of finalizing new guidelines for neurotoxicity testing. The 90-day hen study in OPP's previously published 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 reviewed in this Data Evaluation Report will apply to the previously published OPP neurotoxicity testing guidelines. TB-I notes that the Registrant has added the AchE and NTE assays in their protocols for the rat neurotoxicity screening battery for Fonofos (recently required by the Agency).

Fonofos tox review #9430

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