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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: **CHLORPYRIFOS:** Review of subchronic neurotoxicity study (§82-7) to support reregistration

EPA ID NO's: Caswell No.: 219AA
P.C. Code: 059101
D.P. Barcode: D195337
Submission No.: S448898

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 21 Apr 94
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TO: Joanne Edwards
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THRU: Susan L. Makris, M.S. Susan L. Makris 4/21/94
Toxicology Branch II, Acting Head, Section IV
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and

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Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: DowElanco

Chemical: Chlorpyrifos (Dursban)

Action Requested: Review Subchronic Neurotoxicity Screening Battery (§82-7) toxicology study in rat to support reregistration.

1. The following study was reviewed: Chlorpyrifos: 13-Week Neurotoxicity Study in Fischer 344 Rats (MRID No.: 429298-01).

RESULTS: Fischer 344 rats were exposed for 13 weeks to diets containing sufficient chlorpyrifos to yield doses of 0, 0.1, 1.0, 5.0 or 15 mg/kg/day. No significant treatment-related effects were noted during the study. NOEL for neurotoxicity = 15 mg/kg/day (high dose tested); the LOEL was not established.

CLASSIFICATION: Core - guideline.

2. Toxicology Issues

a. RfD: This study does not change the previously established RfD of 0.003 mg/kg/day with an uncertainty factor of 10. This RfD was established from a 28-day human exposure study, in which four males in each group were given daily doses, in tablet form, of 0.0, 0.1, 0.03 or 0.014 mg/kg/day for 42, 99, 20, or 27 days, respectively. The LOEL was established at 0.1 mg/kg/day, based on decreased plasma cholinesterase activity; the NOEL was established at 0.03 mg/kg/day.

b. Toxicology Data Gaps: The completion of this study satisfies the Neurotoxicity Screening Batteries recommended by the Health Effects Division RfD/Peer Review Committee on 9 September 1993 to support reregistration of chlorpyrifos. **A developmental neurotoxicity study (§83-6) still remains a data gap. This is, however, a new requirement to which the Registrant has not had time to respond.**

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 19 Apr 94
Section IV, Tox. Branch II (7509C)

Secondary Reviewer: Susan L. Makris, M.S. Susan L. Makris 4/19/94
Section IV, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Study - Rat (§82-7)

EPA ID NO's: MRID No.: 429298-01
Pesticide Chemical Code: 059101
Toxicology Chemical Code: 219AA
DP Barcode: D195337
Submission No: S448898

TEST MATERIAL: Chlorpyrifos

SYNONYMS: Dursban F
O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate

STUDY NUMBER: K-044793-094

SPONSOR: DowElanco, 9330 Zionville Rd, Indianapolis, IN

TESTING LAB: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI

REPORT TITLE: Chlorpyrifos: 13-Week Neurotoxicity Study in Fischer 344 Rats

AUTHORS: M.R. Shankar, D.M. Bond and J.W. Crissman

REPORT ISSUED: 16 September 1993

EXECUTIVE SUMMARY: In this subchronic neurotoxicity study, male and female Fischer 344 rats were treated for 13 weeks with diets containing sufficient chlorpyrifos to yield doses of 0, 0.1, 1.0, 5.0 or 15 mg/kg/day. During the study, body weights, clinical signs, FOB, motor activity and neuropathology were examined. FOB, performed at pre-study and Weeks 4, 8, 13, consisted of hand-held and open field observations and measurement of grip performance and landing foot splay.

The study indicated that treatment-related effects included decreased motor activity and an increased incidence of urine incontinence in females. Although a statistically significant depression in motor activity was present in high-dose animals at Week 4. The transitory nature of the effect suggests that the differences were not treatment-related. In addition, a low, and statistically non-significant, increase in the incidence of urine incontinence was observed in several 5 and 15 mg/kg/day females

during the clinical examinations and FOB evaluations. One high-dose female showed urine incontinence at Weeks 4, 8, and 13 and another, only at Weeks 4 and 8. None of the other animals showed urine incontinence in more than one FOB session. There was no clear dose- or time-relationships which would suggest that the incontinence was treatment-related. Body weights of treated animals were comparable to controls. Neuropathological examination did not reveal any differences which might be attributed to treatment. No neurotoxicity was noted at 15 mg/kg/day, a dose previously shown to markedly inhibit plasma (> 80%), RBC (>45%) and brain (> 62%) cholinesterase activities.

Based on the results of this study, the NOEL for neurotoxicity was established at 15 mg/kg/day (high dose tested); the LOEL was not established.

This study is classified as Core - Guideline and satisfies guideline requirements (§82-7) for a subchronic neurotoxicity screening battery in the rat.

I. MATERIALS

A. Test Compound: Chlorpyrifos, technical Description: whitish-tan granular crystals Batch #: MM-890115-616 Purity: 98.1% Contaminants: Not given

B. Test Animals: Species: Rat Strain: Fischer 344, Age: 7 weeks Weight (g): 137 - 161 (males), 122 - 142 (females) Source: Charles River Laboratories, Kingston, NY Housing: Individually in mesh-bottom cages Feed: Certified Purina Rodent Chow, *ad libitum* (#5002, Purina Mills, Inc., St. Louis, MO) Water: tap water, *ad libitum* Environment: Temperature, 21.4 ± 0.9°C; Humidity, 51.1 ± 1.2%; Light cycle, 12 hr light/12 hr dark

II. METHODS

A. Study Design

1. Dose selection: Doses were selected based upon the results of a previously submitted and reviewed subchronic toxicity study (MRID No.: 409528-01, HED Doc No: 07102). The review established the NOEL at 0.1 mg/kg/day and the LOEL at 1.0 mg/kg/day, based on decreased plasma and RBC cholinesterase activities. From these results, doses for the present study were set at 0.1, 1, 5 and 15 mg/kg/day.

2. Study design: Five groups of 10 animals/sex/group were randomly assigned to control and treatment groups. Test diets contained sufficient amounts of chlorpyrifos to achieve intakes of 0, 0.1, 1.0, 5.0 or 15.0 mg/kg/day.

Table 1: Animal Assignment to Study Groups

	Dietary Dose	Main Study	
Test Group	(mg/kg/day)	Male	Female
Control	0	10	10
Low	0.1	10	10
Middle 1	1.0	10	10
Middle 2	5.0	10	10
High	15.0	10	10

B. Preparation of Test Diets: A concentrated dietary premix was prepared by thoroughly mixing an appropriate amount of chlorpyrifos with basal diet. The premix was diluted with basal diet to achieve the desired final concentration of chlorpyrifos in the test diets. Control animals received basal diet only. Initial concentrations of chlorpyrifos in the diet were determined from pre-study feed consumption and body weight data. During the study, concentrations of chlorpyrifos were adjusted weekly, based

upon the most recent feed consumption and body weight data. The concentrated premix was prepared every 3 to 4 weeks and the test diets, weekly.

C. Observations: Starting the day before exposure (Day -1) through terminal sacrifice, cage-side observations were performed twice daily; detailed clinical exams were performed once weekly and as part of the behavioral testing. Ophthalmic examinations were preformed on all rats pretreatment and at terminal sacrifice. Animals were weighed on Day -1, at the start of the study, and weekly, thereafter.

D. Behavioral Tests: Behavioral tests consisted of the Functional Observational Battery (FOB) and evaluation of motor activity. Because of the complexity of these tests, animals were randomly divided into four subgroups of 25 rats each; each subgroup was sequentially stagger-started, at the same time each day, over a four day period. FOB and motor activity were evaluated during the pre-study and during Weeks 4, 8, and 13.

1. Functional Observational Battery: The following parameters were evaluated by a trained observer:

Hand-held observations
- General appearance
- Palpebral closure
- Pupil size
- Lacrimation
- Salivation
- Skin/haircoat abnormalities
- Perineal staining
- Abnormal movements
  - Convulsions
  - Tremors
  - Muscle tone
- Abnormal respiration
- Reactivity to handling

Open-field observations
- Level of activity
- Startle response
- Touch response
- Tail pinch response
- Gait abnormalities
- Abnormal behavior
- Quantity of urine/fecal pellets

Measurements
- Body weight
- Hindlimb/forelimb grip strengths
- Landing hind foot splay

2. Motor Activity: Motor activity was measured using automated photobeam activity recording devices; devices were calibrated before use. Animals were monitored individually during a 48-minute session, which consisted of six 8-minute intervals. The length of the session was selected so that untreated animals approached asymptotic activity levels after 30 to 40 minutes.

3. Positive controls: Positive control data were included in the study. The following positive controls were used: Amphetamine (0.1, 0.32 or 1 mg/kg, ip) and chlorpromazine (0.5, 2.24 or 5 mg/kg, ip) for motor

activity; amphetamine (8 mg/kg, ip), chlorpromazine (4 mg/kg, ip), or atropine (2 mg/kg, ip) plus physostigmine (0.75 mg/kg, sc) for FOB; and trimethyltin (7 mg/kg, po) or acrylamide (35 mg/kg, po) 5 times/week for 3 weeks for neuropathology. The positive control materials accurately validated the FOB, motor activity and neuropathology findings.

E. Sacrifice and Pathology: At terminal sacrifice, 5 animals/group/sex were necropsied for routine pathological examinations; the tissues listed below were immersion fixed in neutral phosphate-buffered 10% formalin. The remaining 5 animals/group/sex animals were necropsied for neuropathological examinations; the tissues listed below were perfusion fixed with 4% paraformaldehyde/1.5% glutaraldehyde. The tissues of control and high-dose animals were examined microscopically.

ROUTINE PATHOLOGY (Immersion Fixed)

Digestive system
- Tongue
- Salivary glands
- Esophagus
- Stomach
- Duodenum
- Jejunum
- Ileum
- Cecum
- Colon
- Rectum
- Liver
- Pancreas

Respiratory
- Trachea
- Lungs
- Nasal tissues
- Larynx

Cardiovas./Hematol
- Aorta
- Heart
- Bone marrow
- Lymph nodes
- Spleen
- Thymus

Urogenital
- Kidneys
- Urinary bladder
- Testes
- Epididymides
- Prostate
- Seminal vesicle
- Ovaries
- Uterus
- Cervix
- Oviducts
- Vagina

Neurologic
- Brain (cerebrum, brainstem, cerebellum)
- Peripheral nerve
- Spinal cord (3 sections)
- Pituitary
- Eyes

Glandular
- Adrenals
- Mammary gland
- Parathyroids
- Thyroids
- Harderian/lacrimal glands
- Coagulating gland
- Auditory sebaceous glands

Other
- Bone
- Skeletal muscle
- Skin
- Gross lesions

NERVOUS SYSTEM TISSUES (Perfusion Fixed)

- Brain (9 Sections)
  - Olfactory bulb
  - Cerebral cortex
    - Frontal lobe
    - Parietal lobe
    - Temporal lobe
    - Occipital lobe
  - Thalamus/Hypothalamus
  - Midbrain
  - Pons
  - Cerebellum
  - Medulla oblongata
  - Nucleus gracilis
  - Nucleus cuneatus
- Eyes (retina and optic nerve)
- Dorsal root ganglia
  - Cervical & lumbar swelling
- Dorsal & ventral roots
  - Cervical & lumbar swelling
- Spinal cord
  - Cervical & lumbar swelling
- Peripheral nerves
  - Sciatic
  - Tibial
  - Sural
- Skeletal muscle
  - Gastrocnemius
  - Anterior tibial
- Olfactory epithelium
- Trigeminal ganglia and nerve

F. Statistical Evaluations: The means and standard deviations of parametric data (body weight, hindlimb and forelimb grip strength, landing hindfoot splay, and motor activity) were determined and variances (F-max test) evaluated for homogeneity. Grip strength was normalized for body weight; motor activity was expressed as the square root of the counts. Repeated measures analyses (ANOVA or MANOVA) were used to evaluate dependent variables over time (treatment × time, treatment × time × sex, and/or treatment × time × epoch). To reduce the number of false positives, the type I error rate (α) was set at 0.02.

III. Regulatory Compliances

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

IV. RESULTS

A. Analytical Chemistry: The test diets were analyzed for stability, homogeneity and concentration. Chlorpyrifos was stable in the basal diet for at least 56 days. Chlorpyrifos was homogeneously distributed in the low test diet (coefficient of variation = 6.67%). The mean concentrations of chlorpyrifos in the test diets were within 95 to 103% of the target.

B. Clinical Signs and Mortality

1. Clinical signs: There were no treatment-related differences in clinical signs noted during the study. Urine incontinence was observed in two 5 mg/kg/day females, one from Days 80 to 92 and the other from Days 92 to 93, and one high-dose female from Days 86 to 93.

2. Mortality (survival): All animals survived until the scheduled sacrifice.

C. Ophthalmology: Pre-study and terminal sacrifice ophthalmic examinations did not reveal any abnormalities.

D. Body Weight: No treatment-related differences in mean body weights were noted during the study.

E. FOB Results: During the FOB evaluations, urine incontinence was observed in several 5 and 15 mg/kg/day females (Table 2). Only one high-dose animal showed urine incontinence at Weeks 4, 8, and 13; another, only at Weeks 4 and 8. None of the other animals showed urine incontinence in more than one FOB session. It should be noted that one control animal, not tabulated in the Text Table on page 30 of the study, also showed urine incontinence at Week 8. Although the study suggested that these observations were

treatment-related, there was no clear dose- or time-relationship. Furthermore, the incidences were low and none statistically significantly different from the control.

Table 2: FOB: Incidence of Urine Incontinence in Females[a]

Week	0	0.1	1.0	5.0	15.0
4	0/10	0/10	0/10	0/10	2/10
8	1/10	0/10	0/10	2/10	3/10
13	0/10	0/10	0/10	1/10	1/10

[a] Data summarized from Table II-3 and Appendix III-2 of study.

F. Motor Activity: A statistically significant depression in motor activity was observed in high-dose animals at Week 4 (Table 3). The effect, however, was transient; no significant differences were noted at Weeks 8 or 13.

Table 3: Motor Activity (Square root of total counts) at Week 4[a]

Sex	0	0.1	1.0	5.0	15.0
Males	13.42	14.12	14.07	14.44	11.27*
Females	15.20	14.63	15.09	13.54	12.24*

[a] Data summarized from Table III-2 of the study
* $p \leq 0.005$

G. Pathological Examinations

1. Gross pathology: The incidence of gross pathological observations did not show any treatment-related effects.

2. Histopathology: Microscopic examination did not reveal any treatment-related effects.

V. DISCUSSION and CONCLUSIONS: In this subchronic neurotoxicity study, male and female Fischer 344 rats were treated for 13 weeks with diets containing sufficient chlorpyrifos to yield doses of 0, 0.1, 1.0, 5.0 or 15 mg/kg/day. During the study, body weights, clinical signs, FOB, motor activity and neuropathology were examined. FOB, performed at pre-study and Weeks 4, 8, 13, consisted of hand-held and open field observations and measurement of grip performance and landing foot splay.

The study indicated that treatment-related effects included decreased motor activity and an increased incidence of urine

incontinence in females. Although a statistically significant depression in motor activity was evident in high-dose animals at Week 4, the effect was not present at Weeks 8 and 13. The transitory nature of the effect suggests that the differences were not treatment-related. In addition, a low, and statistically non-significant increase in the incidence of urine incontinence was observed in several 5 and 15 mg/kg/day females during the clinical examinations and FOB evaluations. The effect was sporadic in nature. Only one high-dose female showed urine incontinence at Weeks 4, 8, and 13 and another, only at Weeks 4 and 8. None of the other animals showed urine incontinence in more than one FOB session. There was no clear dose- or time-relationships which would suggest that the incontinence was treatment-related. Body weights of treated animals were comparable to controls. Both the routine and neuropathological examinations did not reveal any differences which might be attributed to treatment.

It should be noted that the doses of chlopyrifos used in this study produced significant decreases in cholinesterase activities. In a previously submitted and reviewed subchronic feeding study (MRID No.: 409528-01, HED Doc No: 07102), male and female Fischer 344 rats were treated with chlorpyrifos at doses of 0.1, 1.0, 5.0, or 15 mg/kg/day. Even though this study demonstrated that chlorpyrifos, at 5 and 15 mg/kg/day, produced significant decreases in plasma, RBC and brain cholinesterase activities (Table 4), no signs of neurotoxicity were evident from the present study.

Table 4: Cholinesterase Activity (% of control)[a]

Dose mg/kg/day	Plasma (U/ml)		RBC (U/ml)		Brain (U/g)	
	Male	Female	Male	Female	Male	Female
0	0.64	3.31	1.33	1.81	11.37	11.91
0.1	0.57 (-10%)	3.01 (-9%)	1.59 (+20%)	1.84 (+2%)	11.29 (-1%)	11.84 (-1%)
1.0	0.27* (-58%)	0.62* (-81%)	1.13 (-15%)	1.16* (-36%)	11.08 (-3%)	11.77 (-1%)
5.0	0.14* (-79%)	0.20* (-94%)	0.79* (-41%)	0.93* (-49%)	6.79* (-40%)	7.08* (-41%)
15	0.11* (-83%)	0.13* (-96%)	0.73* (-45%)	0.88* (-51%)	4.31* (-62%)	4.18* (-65%)

[a] Data summarized from a previously submitted subchronic toxicity study (MRID No.: 409528-01, HED Doc No: 07102) Tables 18 and 19.
* $p \leq 0.05$, compared to control

Based on the results of this study, the NOEL for neurotoxicity was established at 15 mg/kg/day (high dose tested); the LOEL was not established.

This study is classified as Core - Guideline and satisfies guideline requirements (§82-7) for a subchronic neurotoxicity screening battery in the rat.