3-29-94

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

010878

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: **CHLORPYRIFOS** - Upgrading Acute Neurotoxicity Study

EPA ID NOs: MRID No.: 429431-01
Tox. Chem: 219AA
P.C. Code: 059101
DP Barcode: D195791
Submission No.: S449760

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 24Mar94
Toxicology Branch II, Section IV
Health Effects Division (7509C)

TO: Linda Propst
Product Manager (73)
Registration Division (7505C)

THRU: Susan L. Makris, M.S. Susan L Makris 3/25/94
Toxicology Branch II, Head, Section IV
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 3/29/94
Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: DowElanco

Chemical: Chlorpyrifos

Action Requested: Review additional data submitted by Registrant to support upgrading of an acute neurotoxicity study.

Comments: The Registrant submitted an acute neurotoxicity study (§81-1) with chlorpyrifos in rats and was classified as Core - Supplementary because positive control data were not provided. The positive control data (MRID No.: 429431-01) have been submitted, reviewed and found to be sufficient to support upgrading of the acute neurotoxicity study (MRID No.: 426691-01). This study should be reclassified as **Core - Guideline** and satisfies guideline requirements (§81-8) for an acute neurotoxicity study in rats.

REVIEWER

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

010281

MAY 26 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Chlorpyrifos: Review of generic data submission to support reregistration

EPA IDENTIFICATION NUMBERS: Caswell No.: 219AA
P.C. Code: 059101
D.P. Barcode: D188148, D188791
Submission: S435336, S436324

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 25 May 93
Toxicology Branch II, Section IV
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TO: Joanne Edwards
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THRU: Jess Rowland Jess Rowland 5/25/93
Toxicology Branch II, Head Section IV
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and

Marcia van Gemert, Ph.D. Marcia van Gemert 5/25/93
Chief, Toxicology Branch II
Health Effects Division (H7509C)

Registrant: DowElanco

Chemical: Chlorpyrifos (Dursban)

Action Requested: Review range-finding and definitive Neurotoxicity Screening Battery (§81-8) toxicology studies in rat to support reregistration.

1. The following studies were reviewed: Chlorpyrifos: Acute neurotoxicity study in Fischer 344 rats (MRID No.: 426691-01) and Chlorpyrifos: Acute oral toxicity (range-finding) study in Fischer 344 rats (MRID No.: 424954-04)

RESULTS: Male and female Fischer 344 rats were treated once, by oral gavage, with test compound at doses of 0, 10, 50, or 100

mg/kg and evaluated for neurotoxicity on Days 1 (at the peak time of toxicity, approximately 6 hours after dosing), 8 and 15. Systemic toxicity consisted of decreased body weights of animals in the 50 and 100 mg/kg groups. Neurotoxic effects consisted of decreased motor activity on Day 1 through Day 8 (females only). Significant FOB changes were limited to high dose females, of which six out of ten could not perform the landing hind leg splay on Day 1 of the study. Grip performance on Day 1 revealed a possible treatment-related decrease with increasing dose. Neuropathological examinations did not reveal any treatment-related effects.

2. Conclusions: The systemic and neurotoxic NOEL and LOEL are as follows:

	NOEL	LOEL
Male and Female	10 mg/kg (LDT)	50 mg/kg (MDT)

LOEL is based on decreases in both body weight and motor activity and increased incidence of adverse clinical signs consistent with organophosphorus intoxication.

CLASSIFICATION: core - supplementary; study did not include positive controls.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 25 May 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland Jess Rowland 5/25/93.
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chlorpyrifos: Neurotoxicity Screening Battery - Rat (81-8) (NOTE: This study was incorrectly submitted to satisfy guideline requirements in §82-7).

SUBMISSION: S435336, S436324 **DP BARCODES:** D188148, D188791

P.C. CODE: 059101 **CASWELL NO.:** 219AA

MRID NO.: 426691-01 (main study)
424954-04 (R-F)

TEST MATERIAL: Chlorpyrifos

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

STUDY NUMBERS: K-044793-093B (Main Study), K-044793-093A (R-F)

SPONSOR: DowElanco, 9002 Purdue Road, Indianapolis, IN

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

TITLE OF REPORT: Chlorpyrifos: Acute neurotoxicity study in Fischer 344 rats

AUTHORS: J,W. Wilmer, N.M. Berdasco, J.W. Crissman, and J.P. Maurissen

REPORT ISSUED: 11 September 1992

CONCLUSIONS: Male and female Fischer 344 rats were treated once, by oral gavage, with test compound at doses of 0, 10, 50, or 100 mg/kg and evaluated for neurotoxicity on Days 1 (at the peak time of toxicity, approximately 6 hours after dosing), 8 and 15. Systemic toxicity consisted of decreased body weights of animals in the 50 and 100 mg/kg groups. Neurotoxic effects consisted of decreased motor activity on Day 1 through Day 8 (females only). Significant FOB changes were limited to high dose females, of which six out of ten could not perform the landing hind leg splay on Day 1 of the study. Grip performance on Day 1 revealed a possible treatment-related decrease with increasing dose. Neuropathological examinations did not reveal any treatment-related effects.

CLASSIFICATION: core - Supplementary; positive control data not included in the study as required by Subdivision F Guidelines.

This study does not satisfy guideline requirements (81-8) for a neurotoxicity screening battery in the rat and is not acceptable for regulatory purposes.

I. MATERIALS AND METHODS

A. Test Compound: Chlorpyrifos, technical Description: whitish-tan granular crystals Batch #: MM-890115-616 Purity: 98.2% Contaminants: Not given

B. Test Animals: Species: Rat Strain: Fischer 344, Age: 9 weeks Weight (g): 195 - 228 (males), 132 - 150 (females) Source: Charles River Laboratories, Kingston, NY

C. Study Design:

1. Dose selection: A preliminary range-finding study (MRID No.: 424954-04) was carried out to establish the benchmark dose for the definitive study and the time to peak effect. Animals (2/sex/dose), fasted overnight, were orally gavaged with test compound (5% in corn oil) to yield final doses of 0, 50, 100, 150, or 200 mg/kg. Toxic effects peaked at approximately six hours after dosing and consisted of decreased activity, incoordination, lacrimation, perineal soiling, salivation and tremors. Toxic effects were most evident in animals in 100, 150 and 200 mg/kg dose groups. Based on the data obtained from the range-finding study, a high dose of 100 mg/kg was selected for the main study.

2. Study design: Four groups of 10 animals/sex/group were randomly assigned to control and treatment groups. Following an overnight fast, animals were orally gavaged with test compound at doses of 0, 10, 50, or 100 mg/kg; the weight of test compound was adjusted for purity of active ingredient. Because of the complexity of the study, animals were randomly subdivided into four groups of 20 rats each; each group was sequentially stagger-started over a four day period.

3. Observations: Starting the day before exposure through terminal sacrifice, all animals were observed twice daily for signs of toxicity. Animals were weighed one day before treatment (Day -1), the day of treatment (Day 1), Day 8 and Day 15. Motor activity and Functional Observational Battery (FOB) were measured on all animals on Days -1, 1, 8 and 15. Motor activity was evaluated approximately five hours after dosing and consisted of six 8-minute epochs, totalling 48 minutes (asymptote at 30 to 40 minutes). Approximately six hours after dosing, FOB was performed and consisted of the following parameters:

Hand-held observations
- General
- 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
- Abnormal gait
- Abnormal behavior
- Urine quantity and number of fecal pellets voided during FOB

Measurements/counts
- Hindlimb and forelimb grip strengths
- Landing hind leg splay

4. Positive controls: Either concurrent or historical positive control data were not included in the study, as required by the guidelines.

D. Statistical Evaluations: The means and standard deviations of parametric data (body weight, hindlimb and forelimb grip strength, landing hid leg 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 roots of the counts. Repeated measures analyses (ANOVA or MANOVA) were used to evaluate different interactions (treatment × time, treatment × time × sex, and/or treatment × time × epoch).

E. Regulatory Compliances

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

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

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

II. RESULTS

A. Mortality: No deaths occurred in any of the groups.

B. Body Weight Changes: Treatment-related effects on the mean body weights of male and female rats were observed only on Day 2. Animals in the 50 and 100 mg/kg groups showed significantly decreased mean body weights (Table 1).

C. Motor Activity Results: When measured at the peak time of effect, motor activity of the 50 and 100 mg/kg animals

was significantly lower than that of the controls. The decreased motor activity was still present in the 50 and 100 mg/kg females on Day 8.

Table 1: Mean Body Weights on Day 2 of Study (Data summarized from Appendix II-3 and Table II-4 of study)

	Dose (mg/kg)			
Sex	0	10	50	100
Male	201.1	197.0	190.8*	186.6*
Female	137.0	134.7	127.2*	119.1*

* p value ≤ 0.0001 compared to control

D. Clinical Observations and FOB Results: Treatment-related clinical and handheld FOB observations during the study were consistent with organophosphate intoxication (tremors, incoordination, lacrimation, salivation, perineal soiling, and gait abnormalities). The incidence of adverse effects was most apparent on Day 1, with the high-dose females being more severely affected than males (Table 2).

Statistical analysis of grip performance (Appendix 1) indicated a significant difference in the hindlimb grip strength between study groups. Analysis by days, however, did not reveal any treatment-related differences on any of the observation days. Forelimb grip strength did not show any statistically significant differences due to treatment.

Table 2: Summary of Clinical Observations and FOB Results (Data summarized from Tables II-2 and II-3 of the study)

			Dose (mg/kg)			
Observation	Sex	Day	0	10	50	100
Perineal soiling	Male	2	1/10	2/10	5/10	5/10
		3	0/10	0/10	2/10	2/10
	Female	1	2/10	4/10	6/10	8/10
		2	0/10	0/10	6/10	7/10
		3	0/10	0/10	0/10	1/10
Decreased activity	Male	2	0/10	1/10	1/10	2/10
	Female	2	0/10	1/10	4/10	3/10
Decreased muscle tone	Female	2	0/10	0/10	0/10	1/10
		3	0/10	0/10	0/10	1/10
Lacrimation	Male	1	0/10	0/10	0/10	2/10
	Female	1	0/10	0/10	1/10	8/10
Salivation	Female	1	0/10	0/10	0/10	8/10
Gait Abnormalities	Female	1	0/10	0/10	2/10	8/10

Combination of the hind- and forelimb grip performance for males and females showed a significant treatment-by-day effect. Although further analysis did not reveal any statistically significant differences on any of the study days, the results on Day 1 revealed a possible treatment-related decrease in grip performance with increasing dose. The landing foot splay for males was not affected by treatment on any of the observation days. Results for high-dose females, however, showed that on the day of treatment (Day 1), six of 10 could not perform the test (Appendix 2).

E. Sacrifice and Pathology: At the end of the two week study period, 5 animals/group/sex were selected for neuropathological examination of the central and peripheral nervous tissues. Detailed gross pathological examinations were performed. All of the tissues listed below were collected and preserved at necropsy. Nervous system tissue was also collected and examined microscopically.

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
- Coagulating gland

Neurologic
- Brain
- Periph. nerve
- Spinal cord
- Pituitary
- Eyes

Glandular
- Adrenals
- Mammary gland
- Parathyroids
- Thyroids

Other
- Bone
- Skeletal muscle
- Skin
- Gross lesions
- Harderian/lacrimal glands
- Skull
- Auditory sebaceous glands

Nervous System Tissues Collected

- Olfactory epithelium
- Olfactory bulb
- Cerebrum, anterior
- Cerebrum, middle
- Pituitary gland
- Trigeminal ganglia
- Midbrain & posterior cerebrum
- Cerebellum & pons
- Cerebellum & medulla
- Nucleus gracilis
- Eyes

Dorsal root ganglia
- Cervical & lumbar swelling

Dorsal & ventral roots
- Cervical & lumbar swelling

Spinal cord
- Cervical & lumbar swelling

Peripheral nerves
- Proximal sciatic, tibial, peroneal, sural, caudal, optic

Skeletal muscle
- Anterior tibial & gastrocnemius

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

2. Neuropathology: Microscopic evaluation of central and peripheral nervous system tissues did not reveal and treatment-related effects.

III. DISCUSSION and CONCLUSIONS: Neurotoxicity was evaluated measuring motor activity and FOB evaluation. At the conclusion of the study, a complete neuropathological examination was performed. Treatment-related toxicity consisted of significant weight loss in males and females in the 50 and 100 mg/kg dose groups on Day 2 of the study. Clinical signs observed during the study were consistent with organophosphate intoxication (tremors, incoordination, lacrimation, salivation, perineal soiling, and gait abnormalities). The incidence of these effects was most apparent on Day 1, with the high-dose females being more severely affected than males. The number of adverse clinical signs decreased with time and were normal by Day 4. Significant decreases in motor activity were observed males and females in 50 and 100 mg/kg groups when measured at the peak time of effect. Decreased motor activity was still present in the 50 and 100 mg/kg females on Day 8. Significant FOB changes were limited to high dose females, of which six out of ten could not perform the landing hind leg splay on Day 1 of the study. The hind- and forelimb grip strengths did not show any significant treatment-related effects, however, the results on Day 1 revealed a possible treatment-related decrease in grip performance with increasing dose. Neuropathological examinations did not reveal any treatment-related effects.

The systemic and neurotoxic NOEL and LOEL are as follows:

	NOEL	LOEL
Male and Female	10 mg/kg (LDT)	50 mg/kg (MDT)

LOEL is based on decreases in both body weight and motor activity and increased incidence of adverse clinical signs consistent with organophosphorus intoxication.

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CHLORPYRIFOS TXR 010378

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Pages 10 through 15 are not included.

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