CASWELL FILE

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

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JAN 24 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: METHYL PARATHION - ACUTE DELAYED NEUROTOXICITY STUDY - RESPONSE TO REGISTRATION STANDARD

TO: JOANNE EDWARDS
PRODUCT MANAGER (74)
REGISTRATION DIVISION (H7508C)

FROM: LINDA L. TAYLOR, PH.D.
TOXICOLOGY BRANCH II, SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL
TOXICOLOGY BRANCH II, HEAD SECTION II
HEALTH EFFECTS DIVISION (H7509C)
1/15/91

AND

MARCIA VAN GEMERT, PH.D.
CHIEF, HEALTH EFFECTS DIVISION (H7509C)

REGISTRANT:	A/S CHEMINOVA
CHEMICAL:	O,O-DIMETHYL-O-P-NITROPHENYL PHOSPHOROTHIOATE
SYNONYMS:	METHYL PARATHION
PROJECT:	1-0295
CASWELL NO.:	372
RECORD NO.:	NOT PROVIDED; CASE: 818831; SUBMISSION: S386866
ACTION REQUESTED:	PLEASE REVIEW - COULD BE TIERED STUDY.

COMMENT: AN ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WAS SUBMITTED AS REQUIRED BY THE REGISTRATION STANDARD ON METHYL PARATHION. THIS STUDY HAS BEEN REVIEWED, AND THE DER IS ATTACHED.

UNDER THE CONDITIONS OF THE STUDY, METHYL PARATHION DID NOT ELICIT CLINICAL SIGNS (ATAXIA) OR HISTOPATHOLOGICAL LESIONS IN THE BRAIN, SPINAL CORD, OR PERPHERAL NERVE TISSUE INDICATIVE OF ACUTE DELAYED NEUROTOXICITY, AT DOSE LEVELS AT OR ABOVE THE LD_{50}. CLINICAL SIGNS TYPICAL OF CHOLINESTERASE INHIBITION WERE DISPLAYED IMMEDIATELY AFTER DOSING, BUT RECOVERY OCCURRED WITHIN 7 DAYS. DEATH OCCURRED IN 50% OF THE HENS. THIS STUDY DOES NOT SATISFY THE GUIDELINE REQUIREMENTS (81-7) FOR ACUTE DELAYED NEUROTOXICITY, BUT IT MAY BE UPGRADED FOLLOWING SUBMISSION OF DATA/INFORMATION CONFIRMING THE CONCENTRATIONS OF THE DOSING SOLUTIONS.

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REVIEWED BY: LINDA L. TAYLOR, PH.D. 1/15/91
TOX. BRANCH II, SECTION II, (H7509C)
SECONDARY REVIEWER: K. CLARK SWENTZEL 1/15/91
TOX. BRANCH II, HEAD SECTION II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: ACUTE DELAYED NEUROTOXICITY - HENS TOX. CHEM. NO.: 372

M.ID NO.: 416058-01

TEST MATERIAL: METHYL PARATHION

STUDY NUMBER: PROJECT #: 232-111

SPONSOR: A/S CHEMINOVA

TESTING FACILITY: [illegible] LABORATORIES, INC.

TITLE OF REPORT: METHYL PARATHION: AN ACUTE DELAYED NEUROTOXICITY STUDY IN THE LAYING HEN (GALLUS GALLUS DOMESTICUS)

AUTHORS: JE BEAVERS, J FOSTER, BY COCKRELL, AND MJ JABER

REPORT ISSUED: MAY 1, 1990

QUALITY ASSURANCE: A QUALITY ASSURANCE STATEMENT WAS PROVIDED.

CONCLUSIONS: UNDER THE CONDITIONS OF THE STUDY, METHYL PARATHION, AT DOSE LEVELS AT OR GREATER THAN THE LD_{50}, DID NOT ELICIT CLINICAL SIGNS (ATAXIA) OR HISTOPATHOLOGICAL LESIONS IN THE BRAIN, SPINAL CORD, OR PERIPHERAL NERVE TISSUE INDICATIVE OF ACUTE DELAYED NEUROTOXICITY. THIS STUDY DOES NOT SATISFY THE GUIDELINE REQUIREMENTS (81-7) FOR ACUTE DELAYED NEUROTOXICITY, BUT IT MAY BE UPGRADED FOLLOWING SUBMISSION OF DATA/INFORMATION CONFIRMING CONCENTRATIONS OF TEST MATERIAL USED.

CLASSIFICATION: CORE: SUPPLEMENTARY, PENDING SUBMISSION OF DATA TO CONFIRM DOSE LEVELS OF METHYL PARATHION USED.

A. MATERIALS:

1. Test compound: methyl parathion technical, without [redacted]
Description: amber liquid; Batch No. 95-1A-57; Purity: 95.8%.

2. Test animal: Species: chicken; Strain: not given; from same hatch; Age: 61-62 weeks; Weight: 1265-2019 grams (males); Source: Truslow Farms, Inc., Rt. 4, Box 129, Chestertown, MD. NOTE: The report does not identify the test animal further; the protocol and Pathology Report refer to the test animals as white leghorn hens (single comb).

B. STUDY DESIGN:

Methodology

Hens were assigned randomly to each of the treatment and control groups and housed in individual pens. The vehicle control was corn oil; the positive control was tri-o-tolyl phosphate (TOCP).

	Dose (mg/kg)	Number of hens
Control	0	10
Methyl parathion	250/215	10
Methyl parathion	250/215	6*
TOCP	600	10

The doses of methyl parathion used were based on a preliminary toxicity study (LD_{50}); the dose of TOCP was based on its known toxicity. Due to mortalities following the initial dose of methyl parathion, which was 16% greater than the LD_{50}, the second dose was reduced to 215 mg/kg. Single doses were administered orally via intubation directly into crop or proventriculus (following 15-hour fast; constant dosage volume of 4 mL/kg body weight). All methyl parathion-treated birds were administered concurrently an intramuscular injection of atropine sulfate (5 mg/kg) and as needed (2 mg/kg).

* second group dosed following deaths in original group

Since no clinical signs of delayed neurotoxicity were observed during the 21-day observation period, treated and control birds were redosed at 215 mg/kg or 0 mg/kg, respectively and observed for an additional 21 days. Atropine was administered as before. Because clinical signs of delayed neurotoxicity were observed in the TOCP-treated birds, they were sacrificed after 21 days.

All hens had access to feed (ration formulated to test facility specifications) and water ad libitum.

Observations

All birds were observed at least twice daily for mortality, signs of toxicity, or abnormal behavior. All were monitored closely on the days of dosing for signs of cholinesterase inhibition. Twice each week the hens were evaluated (individually) for locomotor impairment during a period of forced motor

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activity, which included the following:

1) dropped from a height of about 1 meter and evaluated for landing ability;
2) encouraged to walk a distance of about 8 meters and evaluated;
3) forced to hop onto a flat surface about 10 centimeters off the floor; followed by another hop to a surface about 10 centimeters above first surface;
4) encouraged to walk an additional distance of about 8 meters.

Each activity was scored, based upon an ataxia point system (Table 4, copy attached). If a hen failed to perform a given activity, it was given the maximum score.

Body weights were recorded initially and on days 3, 7, 14, 21, 22, 25, 29, 36, and 43, and feed consumption was estimated (per pen) for days 0-3, 4-7, 8-14, 15-21, 22-25, 26-29, 30-36, 37-43.

Histopathology

The positive control birds were sacrificed on day 21, and all surviving methyl parathion-treated and vehicle control birds were sacrificed on day 43. Hens were anesthetized and perfused through the heart. Tissues collected at necropsy were brain (cerebrum, medulla/pons, cerebellum), spinal cord (cervical, thoracic, lumbosacral), and right and left sciatic nerves (proximal and distal). These were examined histologically.

Statistics

Data were not analyzed statistically.

C. RESULTS

Preliminary LD_{50} determination

Dose levels of 60, 90, 135, 203, and 304 mg/kg were administered to 5 hens per dose level. Signs typical of cholinesterase inhibition were observed at all dose levels. The LD_{50} was calculated by probit analyses to be 215 mg/kg with confidence limits of 154 mg/kg to 392 mg/kg and a slope of 5.5.

Survival

There were no deaths in the vehicle control group. Four deaths occurred in the first methyl parathion group (2 on day 1 and one on both day 2 and 3 following dosing. An additional death occurred following the second dose (day 23). In the second methyl parathion group, two deaths occurred following the initial dose (days 2 and 3) and one death occurred after the second dose (day 24). No deaths occurred in the TOCP group.

Clinical Observations

No overt clinical signs were displayed by the vehicle control birds.

The methyl parathion birds that survived treatment displayed clinical signs normally associated with cholinesterase inhibition starting about 1 hour post dose (lethargy, depression, reduced reaction to external stimuli (sound and movement), wing droop, loss of coordination, lower limb weakness, prostrate posture, loss of righting reflex, salivation, shallow and rapid respiration, and a cyanotic comb). After the initial dose, most birds were asymptomatic within 6 days. After the second dose, most birds were normal within 6 days. One hen displayed stiffness in the left leg after the second dose until study termination. All other birds were normal from day 29 until day 43, study termination.

One TOCP bird displayed slight lower limb weakness the day of dosing, which remained intermittent and was accompanied by occasional loss of coordination through day 17. Two additional birds displayed these signs on day 12. By terminal sacrifice on day 21, signs ranging from slight loss of coordination to a more marked loss of coordination accompanied by lower limb weakness was observed in 6 of the birds during at least one observation interval. One additional bird displayed reduced reaction to external stimuli, wing droop, loss of coordination, lower limb weakness, minor muscle fasiculations, and lower limb rigidity.

Body Weight and Feed Consumption

Compared to vehicle controls, a slight loss of body weight was observed in the methyl parathion birds for a 3-day period following each dosing. A marked reduction in feed consumption was observed during the first 3 days following each dosing, with a slight reduction continuing for an additional 4 days. In the TOCP-treated birds, a very slight loss of body weight was observed for the 3-day period following dosing, but no apparent effect was observed on feed consumption.

Motor Activity

METHYL PARATHION

First Dose: With the exception of one bird, all birds displayed marked signs of acute toxicity and were not removed from their pens for evaluation of forced locomotor activity. All were given the maximum score of 5 for each activity. One bird had recovered sufficiently on day 3 for an assessment. This hen was reluctant to walk initially, but completed the course without exhibiting signs of ataxia and was given a score of zero for all tasks. This bird subsequently died following the second dose. On day 7, one bird

WAS RELUNTANT TO WALK AND REFUSED TO HOP. A SECOND BIRD SHOWED SIGNS OF TOXICITY AND WAS NOT REMOVED FROM ITS PEN. ON DAY 17, THE BIRD RELUCTANT TO WALK ON DAY 7 REFUSED TO HOP; ON DAY 21, ANOTHER BIRD REFUSED TO HOP. ALL OTHER BIRDS APPEARED NORMAL AT ALL INTERVALS TESTED, AND NO CLINICAL SIGNS OF ATAXIA TYPICAL OF DELAYED NEUROTOXIC EFFECTS WERE OBSERVED IN ANY OF THE METHYL PARATHION-TREATED BIRDS.

SECOND DOSE: ALL BIRDS DISPLAYED MARKED SIGNS OF ACUTE TOXICITY AND WERE NOT REMOVED FROM THEIR CAGES FOR EVALUATION ON DAY 24, THREE DAYS AFTER DOSING. ONLY ONE BIRD DISPLAYED SIGNS OF TOXICITY THEREAFTER; ON DAY 28 THIS BIRD SHOWED SEVERE LAMENESS OF THE LEFT LEG (SCORE OF 15 OUT OF 20). ON DAY 31, THE SCORE WAS 17 AND BY DAY 36 IT WAS 4. THE BIRD IMPROVED GRADUALLY WITH TIME AND WAS ABLE TO PERFORM ALL TASKS BY DAY 38, SHOWING ONLY SLIGHT LAMENESS OF THE LEFT LEG. ALL OTHER BIRDS APPEARED NORMAL AT ALL TESTED INTERVALS, AND NO CLINICAL SIGNS OF ATAXIA TYPICAL OF DELAYED NEUROTOXIC EFFECTS WERE OBSERVED IN ANY METHYL PARATHION-TREATED BIRD.

TRI-O-TOLYL PHOSPHATE

ON THE FIRST DAY OF LOCOMOTOR ACTIVITY ASSESSMENT (DAY 3), TWO BIRDS SHOWED SLIGHT LAMENESS OF THE RIGHT LEG AND ONE OF THESE ALSO SQUATTED FREQUENTLY. THIS LATTER BIRD CONTINUED TO SQUAT ON DAY 7, BUT LAMENESS WAS NOT OBSERVED. ANOTHER BIRD WAS RELUCTANT TO WALK ON DAY 7, WHICH CONTINUED DURING DAY 10 ASSESSMENT. THROUGH DAY 10, NO BIRDS DISPLAYED SIGNS OF ATAXIA AND ALL BIRDS RECEIVED SCORES OF ZERO FOR ALL ACTIVITIES. ON DAY 14, ONE BIRD REFUSED TO WALK (SCORE 16) AND SLIGHT ATAXIA WAS OBSERVED IN 3 OTHER BIRDS (SCORE 5 EACH). ON DAY 17, 3 BIRDS EXHIBITED SLIGHT ATAXIA (TWO HAD PREVIOUSLY DEMONSTRATED ATAXIA ON DAY 14), ONE EXHIBITED MODERATE ATAXIA, AND ONE (PREVIOUS SIGNS OF ATAXIA ON DAY 14) EXHIBITED PROFOUND ATAXIA, ACCOMPANIED BY LEG TREMORS (SCORE 18). ALL OTHER BIRDS DID NOT EXHIBIT ATAXIA (SCORE 0). DURING THE FINAL ASSESSMENT (DAY 21), 5 BIRDS DID NOT EXHIBIT ATAXIA. SLIGHT ATAXIA WAS OBSERVED IN 1 BIRD, MODERATE ATAXIA WAS OBSERVED IN 3 BIRDS, AND ONE EXHIBITED PROFOUND ATAXIA (SAME BIRD AS ON DAY 17).

HISTOPATHOLOGY

NO NEURAL DEGENERATIVE CHANGES WERE OBSERVED IN ANY OF THE VEHICLE CONTROL AND THE METHYL PARATHION-TREATED HENS. SOME UNRELATED PERIVASCULAR INFILTRATES WERE OBSERVED, WHICH WERE COMMON TO ALL GROUPS.

THE PREDOMINANT LESIONS IN THE TOCP-TREATED BIRDS APPEARED IN THE SCIATIC NERVES, PARTICULARLY THE DISTAL SEGMENTS, WHICH CONSISTED OF SWELLING OF THE AXIC (SIC) CYLINDER WITH NERVE FIBER DEGENERATION AND SCHWANN CELL PROLIFERATION. CHARACTERISTIC LESIONS WERE OBSERVED IN 4 OUT OF 5 TOCP-TREATED BIRDS THAT EXHIBITED CLINICAL SIGNS OF ATAXIA. LESIONS WERE ALSO DETECTED IN 3 ADDITIONAL BIRDS. THE BIRD DISPLAYING THE PROFOUND ATAXIA HAD THE MOST CHARACTERISTIC LESIONS OF TOCP PERIPHERAL NEUROPATHY, WITH DEGENERATIVE NEURAL LESIONS AS HIGH AS THE LUMBOSACRAL SPINAL CORD. NERVES FROM SEVERAL TOCP-TREATED BIRDS CONTAINED NO SIGNIFICANT NEURAL DEGENERATIVE LESIONS.

CONCLUSION

Hens exposed to two doses of methyl parathion, at or above the LD_{50} dose, displayed clinical signs typical of cholinesterase inhibition during the period of time immediately after dosing, but recovered completely within 7 days. Death occurred in 50% of the treated hens. No signs indicative of acute delayed neurotoxicity were observed in these hens. Microscopic examination of the brain, spinal cord, and bilateral peripheral nerve tissue failed to reveal the presence of neural degenerative changes. The positive control TOCP birds displayed clinical signs typical of delayed neurotoxicity, as well as typical histopathological lesions. It is concluded that methyl parathion showed no evidence of acute delayed neurotoxicity under the conditions of the study. This study does not satisfy the guideline requirements (81-7) for acute delayed neurotoxicity, since there is no documentation of the stability of the test material and no confirmation of the concentration of dosing solutions used. The study can be [illegible] the submission of this information.

TABLE 4

METHYL PARATHION - AN ACUTE DELAYED NEUROTOXICITY STUDY IN LAYING HENS
LOCOMOTOR ACTIVITY ASSESSMENT

POINTS	ATAXIA ASSESSMENT
0	No ataxia.
1	Slight incoordination; occasional stumbling or wing drooping, especially after exertion.
2	Staggering gait, tail and leg reflexes may be affected; birds land awkardly.
3	Continuous staggering gait, birds rest often, tail and leg reflexes usually noticeably affected.
4	Birds stand for short periods only, normally moves by shuffling on hocks; tail and leg reflexes usually noticeably affected.
5	Birds unable to stand, weak limb movements; tail and leg reflexes virtually nonexistent.

(Continued)

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Tox Chem No. 372 Methyl Parathion File Last Updated _____ Current Date 1/14/91

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute Delayed Neurotox. Species: hen Wildlife Intern. Ltd./ Exper. Path. Lab., Inc. Project #: 232-111; 5/1/90	Methyl Parathion 95.8%	416068-01	Under the conditions of the study, methyl parathion, at dose levels at or greater than LD_{50}, did not elicit clinical signs (ataxia) or histopathological lesions indicative of acute delayed neurotoxicity; clinical signs of cholinesterase inhibition were observed, and deaths occurred in 50% of the treated hens. This study does not satisfy the guideline requirements (81-7) for acute delayed neurotoxicity but can be upgraded following submission of data to confirm the concentration of test material used.		Supplementary