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

[redacted] 8/24/1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: METHYL PARATHION - 6(A)2: Acute Neurotoxicity Study - Rats

EPA ID NOs: MRID NO.: 4432544-01
Pesticide Chemical Code: 053501
Toxicology Chemical Code: 372
DP Barcode: D205593
Submission No.: S470065

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 24Aug 94
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and

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Health Effects Division (7509C)

Registrant: Cheminova Agro A/S
P.O. Box 9, DK-7620
Lemvig, Denmark

Chemical: Methyl parathion
O,O-Dimethyl O-*p*-nitrophenylphosphorothioate

Action Requested: Review acute neurotoxicity study (§81-8) in the rat. **Study was submitted as a 6(A)(2) action.**

1. **Results:** The Registrant submitted a study entitled "Acute Neurotoxicity Study of Methyl Parathion in Rats". In study, male and female Sprague-Dawley rats (10 animals/ sex/group) were orally gavaged once with methyl parathion at doses of 0, 0.025, 7.5, 10 (males only), or 15 (females only) mg/kg. Neuro-behavioral evaluations, consisting FOB and motor activity, were

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conducted at pre-study, at the peak time of effect (1.5 hrs post-dosing) on Day 0 and on Days 7 and 14. At 15 ± 3 days animals were euthanized and neuropathological examination performed on control and high-dose animals (6/dose/sex). Plasma and erythrocyte (RBC) cholinesterase (ChEase) activities were determined at Day -2; plasma, RBC and brain (six different regions) activities were measured at the peak time of effect and at Day 14.

No significant differences were noted in the mean body weights of the treated animals; body weight gain in high-dose males was significantly lower than controls.

Neurobehavioral evaluation revealed treatment-related FOB and motor activity findings at the mid- and high-dose levels. The effects were transient and observed only at the peak time of effect. Neurobehavioral findings are consistent with those observed following cholinesterase inhibition (i.e. lacrimation, salivation, miosis, tremors/convulsions, muscle fasciculations, muscle weakness, and ataxia).

ChEase activities showed, treatment-related decreases in mid- and high-dose animals. Plasma, RBC, and brain ChEase activities were decreased at the peak time of effect. At Day 14, some recovery was observed, however, plasma (females only), RBC and brain ChEase activities was still significantly decreased.

Neuropathology findings included focal demyelination of the dorsal and ventral root fibers of the cervical and lumbar spinal cord and focal demyelination of the sural and tibial nerves. No treatment-related gross pathological findings were observed.

2. Conclusions: Based on the results of this study, the systemic LOEL 10 mg/kg (males) and 15 mg/kg (females); the systemic NOEL was 7.5 mg/kg. In males and females, the LOEL for neurotoxicity was 7.5 mg/kg; the NOEL for neurotoxicity was 0.025 mg/kg. No further regulatory action is needed.

This study is classified as Core - Guideline and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 23 Aug 94
Section IV, Tox. Branch II (7509C)

Secondary Reviewer: Clark Swentzel Clark Swentzel 8/23/94
Section II, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Study - Rat (§81-8)

EPA ID NOs: MRID NO.: 432544-01
Pesticide Chemical Code: 053501
Toxicology Chemical Code: 372
DP Barcode: D205593
Submission No.: S470065

TEST MATERIAL: Methyl parathion

SYNONYMS: O,O-Dimethyl O-*p*-nitrophenylphosphorothioate

STUDY NOs: HWA 2688-102

SPONSOR: Cheminova Agro A/S, P.O. Box 9, DK-7620
Lemvig, Denmark

TESTING LAB: Hazelton Washington, Inc.
Vienna, Virginia 22182-1699

REPORT TITLE: Acute Neurotoxicity Study of Methyl Parathion in Rats

AUTHORS: D.J. Minnema

REPORT ISSUED: 31 May 1994

EXECUTIVE SUMMARY: In this acute neurotoxicity study (MRID No: 432544-01), male and female Sprague-Dawley rats (10 animals/sex/group) were orally gavaged once with methyl parathion at doses of 0, 0.025, 7.5, 10 (males only), or 15 (females only) mg/kg. Neurobehavioral evaluations, consisting FOB and motor activity, were conducted at pre-study, at the peak time of effect (1.5 hrs post-dosing) on Day 0 and on Days 7 and 14. At 15 ± 3 days animals were euthanized and neuropathological examination performed on control and high-dose animals (6/dose/sex). Plasma and erythrocyte (RBC) cholinesterase (ChEase) activities were determined at Day -2; plasma, RBC and brain (six different regions) activities were measured at the peak time of effect and at Day 14.

No significant differences were noted in the mean body weights of the treated animals; body weight gain in high-dose males was significantly lower than controls.

Neurobehavioral evaluation revealed treatment-related FOB and motor activity findings at the mid- and high-dose levels. The effects were transient and observed only at the peak time of

effect. Neurobehavioral findings are consistent with those observed following cholinesterase inhibition (i.e. lacrimation, salivation, miosis, tremors/convulsions, muscle fasciculations, muscle weakness, and ataxia).

ChEase activities showed, treatment-related decreases in mid- and high-dose animals. At the peak time of effect, plasma, RBC, and brain ChEase activities were decreased greater than 67, 56 and 76%, respectively. At Day 14, some recovery was observed, however, plasma (females only), RBC and brain ChEase activities was still significantly decreased by 45%, 15 to 28%, and 7 to 39%, respectively.

No treatment-related gross pathological findings were observed. Neuropathological findings consisted of focal demyelination in the dorsal root fibers of the cervical spine in 3/6 high-doses males and lumbar spine in 3/6 low-, 4/6 mid- and 5/6 high-dose males. Focal demyelination was also observed in the ventral root fibers of the cervical spine in 2/6 high-dose males and of the lumbar spine in control (males, 2/6; females, 1/6), low- (males, 3/6), mid- (males, 4/6), and high- (males, 4/6; females, 3/6) dose groups. Focal demyelination of the lumbar spinal cord and spinal nerve were observed in high-dose males; the incidence of each of these observations was only 1/6. Focal demyelination was observed in the tibial nerves of 1/6 mid- and 3/6 high-dose males and in the sural nerves of 2/6 high-dose males.

In summary, systemic toxicity was observed in high-dose males (decreased body weight gain) and females (increased incidence of clinical signs). Neurotoxic effects (abnormal FOB findings, decreased motor activity, inhibition ChEase activities, and neuro-pathological findings) were observed in mid- and high-dose males and females.

Based on the results of this study, the systemic LOEL 10 mg/kg (males) and 15 mg/kg (females); the systemic NOEL was 7.5 mg/kg. In males and females, the LOEL for neurotoxicity was 7.5 mg/kg; the NOEL for neurotoxicity was 0.025 mg/kg.

This study is classified as Core - Guideline and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.

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I. MATERIALS

A. TEST COMPOUND: Methyl parathion, technical Description: brown solid Lot No: 95-1A-84 Purity: 93.1% Contaminants: Not given

B. VEHICLE: Pure corn oil Description: yellow liquid Lot No: 6202 Purity: 100% (assumed)

C. TEST ANIMALS: Species: Rat Strain: Sprague-Dawley, Crl:CD BR Age: 7 to 8 weeks Weight at initiation (g): 244 to 331 (males), 157 to 201 (females) Source: Charles River Breeding Laboratories, Inc., Raleigh, NC Housing: Individually in mesh-bottom cages Feed: Agway certified rodent feed (RMH 3200) *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature, 64.5 to 78.0°F; Humidity, 24.1 to 74.6%; Light cycle (reversed) 12 hr/12 hr, light/dark; Air changes, ≥ 10/hr.

II. METHODS

A. STUDY DESIGN: Animals were assigned to control and treatment groups using a computerized weight randomization program. Animals were orally gavaged once on Day 0 with methyl parathion at the doses indicated in Table 1. The original study protocol established high doses of 15 mg/kg for males and 30 mg/kg for females. After administration of 15 mg/kg, 4/5 males died on the day of dosing. Because of the excessive lethality, the high-dose was lowered to 10 mg/kg and 15 mg/kg for males and females, respectively.

Main study animals underwent neurobehavioral and neuropathological evaluations. Neurobehavioral assessment included evaluation in a Functional Observational Battery (FOB) and determination of motor activity. In the cholinesterase (ChEase) study, plasma, erythrocyte (RBC) and brain ChEase activities were measured before and after treatment.

B. DOSING PREPARATIONS: Sufficient amount of methyl parathion was suspended in corn oil vehicle to yield the intended doses when administered in a dose volume of 2 ml/kg. Dosing

Table 1: Animal Assignment to Study Groups

Test Group	Dose[a] (mg/kg)	Main Study		ChEase Study	
		Male	Female	Male	Female
Control	0	10	10	10	10
Low	0.025	10	10	5	5
Mid	7.5	10	10	5	5
High (male)	10.0	10	0	10	0
High (female)	15.0	0	10	0	10

[a] Doses not corrected for percent purity of active ingredient.

preparations were prepared prior to each of the dosing days. Dosing solutions were analyzed for homogeneity, stability, and achieved concentration. Samples of each dosing solution were stored frozen until analysis.

C. OBSERVATIONS

1. Clinical Signs: Animals were observed twice daily for signs of toxicity and mortality and once daily for clinical signs. Detailed clinical examinations were performed at each weighing interval.

2. Body Weights: Animals were weighed on Days 0 (before dosing), 7 and 14.

D. NEUROBEHAVIORAL ASSESSMENT: Neurobehavioral assessment, consisting of the Functional Observational Battery (FOB) and evaluation of motor activity, were performed at pre-study, on Day 0 at time of peak effect (1.5 hrs) and on Days 7 and 14. Because of the complexity of the tests, five animals of each dose group were stagger-started over a two- day period.

1. Functional Observational Battery: The following parameters were evaluated:

Home Cage/Hand-Held Observations
- Appearance of fur
- Color of tears
- Convulsions/tremors
- Ease of handling/body tone
- Ease of removal from cage
- Excessive vocalizations
- Exophthalmus
- Lacrimation
- Palpebral closure
- Piloerection
- Respiration
- Salivation
- Writhing
- Hind/forelimb grip strengths
- Hindlimb landing foot splay
- Body temperature
- Tail flick latency
- Other signs

Open-field Observations
- Arousal
- Circling
- Convulsions/tremors
- Gait
- Posture
- Abnormal/Stereotypic behavior
- Mobility
- Urination/defecation
- Other signs

Response Observations
- Light approach response
- Catalepsy
- Olfactory response
- Pupillary reflex
- Air Righting reflex
- Touch response
- Auditory startle response
- Other signs

2. Motor Activity: Motor activity was measured using automated photobeam activity recording devices. Animals were monitored individually over a 40 min session, consisting of eight 5-minute intervals.

3. Positive Controls: A positive control study (Study No.: HWA 0001-692, entitled "Neurotoxicity Study of Acrylamide (Positive Control) in Rats", dated 12 April 1994) submitted by the performing laboratory adequately

validated the FOB, motor activity and neuropathology findings.

E. CLINICAL PATHOLOGY - CHOLINESTERASE ACTIVITIES: Plasma and RBC ChEase activities were determined at Days -2 and 14 in control and high-dose animals and at the peak time of effect in all dose groups. Brain (six regions) ChEase activity was measured in all dose groups at the peak time of effect and at Day 14 in control and high-dose groups. Plasma and RBC were separated by centrifugation and stored separately at -70°C until assayed. Brains were dissected to isolate the olfactory bulbs, cerebellum, cortex, striatum, hippocampus and midbrain plus brainstem; tissues were weighed, frozen in liquid nitrogen and stored at -70°C until assayed. Samples of each of the brain tissues were homogenized (0.1 g tissue/ml phosphate buffer) and centrifuged. Aliquots of the plasma, lysed RBC and brain homogenates were assayed for ChEase activities using the BMD/Hitachi 704 Chemistry Analyzer.

F. SACRIFICE AND PATHOLOGY: Animals found dead or euthanized *in extremis* were examined grossly. At termination of the study (15 ± 3 days post-dosing), main study animals were weighed and euthanized with and an IP injection of pentobarbital. The first six main study animals were perfusion fixed *in situ* for neuropathological evaluation, the remaining animals were examined grossly. The tissues listed below were examined from control and high-dose animals. Tissues from intermediate dose groups were collected and examined if necessary.

Gross Pathology
- Carcass
- Cervical tissues and organs
- Cranial cavity
- External body surface
- External surface of the brain
- Nasal cavity and parinasal sinuses
- Thoracic, abdominal and pelvic cavities and viscera

Neuropathology
- Muscle: Anterior tibialis and gastrocnemius
- Brain with brainstem
- Cervical and lumbar dorsal roots
- Cervical and lumbar ventral root fibers
- Cervical and lumbar dorsal root ganglia
- Gasserian ganglion
- Trigeminal nerves
- Spinal cord: Cervical (C_3 - C_8) and lumbar (T_{13} - L_4) swellings
- Eyes/Optic nerves
- Peripheral nerves: Sciatic, tibial, sural, and peroneal
- Pituitary
- Other: Tail and forelimbs (Preserved but not examined)

G. STATISTICAL EVALUATIONS: Parametric data were first evaluated using Levene's test to determine if the variances were homogeneous. Heterogeneous data were sequentially transformed ($\log_{10}x$, x^2, $\sqrt{x}$, $1/x$, arcsine, or rank) and reevaluated using Levene's test. The homogeneous data were evaluated using analysis of variance (ANOVA) and, if significant differences were observed, pair-wise comparisons

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were carried out using Dunnett's multiple t-test. Continuous behavioral data were analyzed by factorial analysis of variance with repeated measures. Motor activity data were square-root transformed before statistical analysis. Dose effects and dose x time effects were detected using univariant analysis.

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.

C. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. There are no established flagging criteria or reporting code for this type of study.

IV. RESULTS

A. ANALYTICAL CHEMISTRY: Dosing preparations were analyzed for stability, homogeneity and concentration. Samples taken from the top, middle and bottom of the low- and high- (30 mg/kg) dosing preparations indicated that the methyl parathion was homogeneously distributed with relative standard deviations of 2.64% and 2.32%, respectively. Analysis of the dosing preparations showed that the percent of target concentrations were 115 - 118% (low-dose), 92.6% (mid-dose), 88.7 - 91.0% (high-dose, male), and 91.0 - 93.4% (30 mg/kg, female). Following four days of refrigerated storage, concentrations were within 1% of the original values.

B. CLINICAL SIGNS AND MORTALITY

1. Clinical Signs: Clinical signs, observed at the peak time of effect, included ataxia, muscle fasciculations, tremors, and hypoactivity in mid- and high-dose animals; high-dose females additionally exhibited labored breathing and salivation (Table 2). During the cage side observations on Day 0, mid- and/or high-dose females showed red crust around the nose, chromodacryorrhea and urine stains on the fur.

2. Mortality (Survival): On Day 0, three males and three females in the high-dose group were found dead. All other animals survived until the scheduled sacrifice.

C. BODY WEIGHT AND BODY WEIGHT GAIN: No statistically significant differences in mean body weight were observed during the study. For Days 0 to 7, the mean body weight gain for high-dose males (22.4 g) was significantly ($p \leq 0.05$) lower than that of controls (43.6 g).

Table 2: Clinical Signs at Post-Dosing and Day 0 Cage-Side Observations[a]

OBSERVATION	Sex	Dose Level (mg/kg)				
		0	0.025	7.5	10	15
POST-DOSING						
Ataxia	Male	0/10	0/10	8/10	6/10	---
	Female	0/10	0/10	4/10	---	3/10
Muscle Fasciculations	Male	0/10	0/10	5/10	6/10	---
	Female	0/10	0/10	7/10	---	8/10
Tremors	Male	0/10	0/10	10/10	6/10	---
	Female	0/10	0/10	9/10	---	8/10
Hypoactivity	Male	0/10	0/10	9/10	6/10	---
	Female	0/10	0/10	8/10	---	7/10
Labored Breathing	Female	0/10	0/10	0/10	---	2/10
Salivation	Male	0/10	0/10	8/10	5/10	---
	Female	0/10	0/10	8/10	---	8/10
CAGE SIDE (DAY 0)						
Red perineal crust	Female	0/10	0/10	0/10	---	3/10
Chromodacryorrhea	Female	0/10	0/10	0/10	---	3/10
Urine stains on fur	Female	0/10	0/10	0/10	---	3/10

[a] Data taken from Table 3 of the study.

D. NEUROBEHAVIORAL RESULTS

1. FOB Findings: Significant FOB findings were observed in mid- and high-dose animals only at the peak time of effect (Table 3). Treatment-related effects consisted of increased incidences in home cage/hand held observations (limp body tone, lacrimation, salivation, impaired breathing and convulsions/ tremors), open field observations (abnormal posture, impaired gait, tremors, muscle fasciculations, hypo-alertness and number of rears), and sensory observations (abnormal righting reflex, constricted/ unresponsive pupils). Significant, treatment-related performance observations consisted of increased tail flick latency and decreased forelimb grip strength in mid-and high-dose females and decreased rectal temperature and hindlimb grip strength in mid- and high-dose males and females.

2. Motor Activity: Treatment-related decreases in mean motor activity were observed only at the peak time of effect (Table 4); results at Weeks 1 and 2 were comparable between control and treated animals. Motor activity during each of the first four intervals (1-5, 6-10, 11-15 and 16-20 min) was significantly lower in mid- and high-dose males and females; activity in low-dose animals was comparable to controls.

Table 3: FOB Performed on Day 0 at the Peak Time of Effect[a]

OBSERVATION	Sex	Dose Level (mg/kg)				
		0	0.025	7.5	10.0	15.0
HOME CAGE/HAND HELD OBSERVATIONS						
Limp body tone	Male	0/10	0/10	2/10	4/7	---
	Female	0/10	0/10	8/10	---	7/7
Lacrimation	Female	0/10	0/10	6/10	---	4/7
Salivation	Male	0/10	0/10	5/10	---	4/7
	Female	0/10	0/10	5/10	---	6/7
Clear tears	Female	0/10	0/10	6/10	---	4/7
Impaired respiration	Male	0/10	0/10	2/10	5/7	---
	Female	0/10	0/10	1/10	---	6/7
Convulsions/Tremors	Male	0/10	0/10	4/10	6/7	---
	Female	0/10	0/10	8/10	---	7/7
OPEN FIELD OBSERVATIONS						
Abnormal posture	Male	0/10	0/10	3/10	5/7	---
	Female	0/10	0/10	2/10	---	6/7
Impaired gait	Male	0/10	0/10	5/10	7/7	---
	Female	0/10	0/10	8/10	---	7/7
Tremors	Male	0/10	0/10	3/10	6/7	---
	Female	0/10	0/10	8/10	---	7/7
Muscle fasciculations	Male	0/10	0/10	4/10	5/7	---
	Female	0/10	0/10	4/10	---	7/7
Arousal (hypo-alertness)	Male	0/10	1/10	6/10	3/7	---
	Female	0/10	0/10	0/10	---	5/7
Number of rears	Male	3.5	3.3	0.4*	0.4*	---
	Female	5.3	5.0	1.1*	---	0.0*
Sensory Observations						
Pupil response absent (one or both eyes)	Male	0/10	0/10	6/10	4/7	---
	Female	0/10	0/10	3/10	---	5/7
Abnormal righting reflex (Uncoordinated, lands on side or back)	Male	2/10	2/10	7/10	7/7	---
	Female	0/10	1/10	9/10	---	7/7
Pupils constricted (one or both eyes)	Male	0/10	0/10	6/10	4/7	---
	Female	0/10	0/10	3/10	---	5/7
PERFORMANCE OBSERVATIONS						
Tail flick latency (sec)	Female	11.9	11.2	18.9*	---	24.0*
Rectal temperature (°C)	Male	38.9	39.1	36.8*	36.3*	---
	Female	39.2	39.3	36.5*	---	35.5*
Forelimb grip strength (grams)	Female	558	563	385*	---	279*
Hindlimb grip strength (grams)	Male	638	675	538	455*	---
	Female	504	611*	368*	---	257*

[a] Data summarized from Tables 6B, 6D, 6E, 6F, 6G, 6I, 7B, 7C, 7D, 7H, 7I, 7N, 8E, 8F, 8G, 9A, 9B, 9C, and 9D of the study.

* $p \leq 0.05$ compared to control

Table 4: Mean Motor Activity Counts at Peak Time of Effect[a]

Sex	Interval (min)	Dose Level (mg/kg)				
		0	0.025	7.5	10	15
Male	1-5	435	385	72.1*	50.7*	---
	6-10	270	260	24.0*	21.8*	---
	11-15	145	214	33.4*	35.3*	---
	16-20	102	103	23.2*	15.1*	---
	21-25	66.2	85.7	48.4	15.8	---
	26-30	24.2	40.3	16.8	21.0	---
	31-35	22.7	38.5	10.3	16.4	---
	36-40	29.0	5.9	25.9	15.0	---
Female	1-5	330	402	30.9*	---	25.3*
	6-10	288	332	40.1*	---	20.0*
	11-15	176	220	12.4*	---	16.9*
	16-20	126	142	43.8*	---	19.4*
	21-25	74.8	91.2	19.3	---	20.9
	26-30	52.0	49.5	23.8	---	22.6
	31-35	72.1	38.2	32.1	---	22.7
	36-40	46.8	53.4	16.8	---	31.7

[a] Data summarized from Table 11 of the study.
* $p \leq 0.05$

E. CLINICAL PATHOLOGY - CHOLINESTERASE ACTIVITIES: ChEase activities showed consistent, treatment-related decreases in mid- and high-dose animals (Table 5). At the peak time of effect, plasma and RBC ChEase activities were decreased by greater than 76% and 56%, respectively for high-dose animals and 67% and 56%, respectively for mid-dose animals. Regional brain ChEase activities were all uniformly inhibited at the mid- (> 76%) and high- (> 88%) doses. On Day 14, some recovery in ChEase activity was observed. Plasma activity in high-dose males was comparable to controls, while activity in females was still decreased by 45%. In the high-dose group, RBC ChEase activities were decreased by 15% in males and 28% in females. Decreases in brain ChEase activity ranged from 7 to 32% (males) and 10 to 39% (females) in the high-dose group.

F. SACRIFICE AND PATHOLOGY

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

2. Histopathology: Pertinent neuropathological findings are summarized in Table 6. Neuropathological findings consisted of focal demyelination in the dorsal root fibers of the cervical spine in 3/6 high-doses males and lumbar spine in 3/6 low-, 4/6 mid- and 5/6 high-dose males. Focal demyelination was also observed in the ventral root fibers of the cervical spine in 2/6 high-dose males and of the lumbar spine in control (males, 2/6; females, 1/6), low- (males, 3/6), mid- (males, 4/6), and high- (males, 4/6; females, 3/6) dose

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groups. Focal demyelination of the lumbar spinal cord and spinal nerve were observed in high-dose males; the incidence of each of these observations was only 1/6. Focal demyelination was observed in the tibial nerves of 1/6 mid- and 3/6 high-dose males and in the sural nerves of 2/6 high-dose males.

Table 5: Plasma, RBC and Brain Cholinesterase Activities[a]

ChEase[b]	Sex	Day[c]	Dose Level (-mg/kg)				
			0	0.025	7.5	10	15
Plasma	Male	0	686	514 (-25)[d]	229* (-67)	161* (-77)	---
		14	485	---	---	513* (-6)	---
	Female	0	1902	1737 (-9)	557* (-71)	---	459* (-76)
		14	2248	---	---	---	1239* (-45)
RBC	Male	0	1688	1714 (+2)	739* (-56)	735* (-56)	---
		14	1655	---	---	1412* (-15)	---
	Female	0	1856	1856 (-0)	790* (-57)	---	781* (-58)
		14	1911	---	---	---	1369* (-28)
Cerebral Cortex	Male	0	54.2	52.3 (-3)	6.68* (-88)	3.84* (-93)	---
		14	57.2	---	---	46.5* (-19)	---
	Female	0	60.9	59.2 (-3)	11.2* (-82)	---	5.92* (-90)
		14	53.0	---	---	---	32.1* (-39)
Cerebellum	Male	0	41.4	43.9 (+6)	7.49* (-82)	3.84* (-91)	---
		14	43.6	---	---	40.5* (-7)	---
	Female	0	43.9	47.1 (+7)	9.40* (-79)	---	5.24* (-88)
		14	41.0	---	---	---	36.8 (-10)
Hippocampus	Male	0	71.2	72.1 (+1)	9.44* (-87)	4.92* (-93)	---
		14	75.4	---	---	56.8* (-25)	---
	Female	0	72.1	72.4 (-0)	15.2* (-79)	---	6.36* (-91)
		14	76.6	---	---	---	51.0* (-33)
Striatum	Male	0	301.4	294.4 (-2)	29.2* (-90)	13.7* (-95)	---
		14	293.1	---	---	200.0 (-32)	---
	Female	0	315.8	336.4 (+7)	47.8* (-85)	---	22.2* (-93)
		14	284.1	---	---	---	183.9* (-35)
Olfactory Bulb	Male	0	443.6	45.0 (+1)	5.92* (-87)	4.00* (-91)	---
		14	472.8	---	---	33.5* (-29)	---
	Female	0	429.6	45.1 (+5)	9.88* (-77)	---	4.48* (-90)
		14	470.4	---	---	---	33.5* (-29)
Brainstem	Male	0	107.3	106.0 (-1)	14.2* (-87)	6.96* (-94)	---
		14	117.6	---	---	92.6* (-21)	---
	Female	0	114.2	112.4 (-2)	27.0* (-76)	---	11.0* (-90)
		14	102.1	---	---	---	85.9* (-16)

[a] Data summarized from Table 5 of the study.
[b] Units of activity are in U/l for plasma and RBC and U/g tissue for brain tissues.
[c] Day 0 activities determined at the peak time of effect (1.5 hrs, post-dosing).
[d] Values in parentheses, calculated by reviewer, represent the percent increase (+) or decrease (-) from control.
* $p \leq 0.05$

Table 6: Neuropathology[a]

OBSERVATION	Sex	Dose Level (mg/kg) 0	0.025	7.5	10	15
DORSAL ROOT FIBER						
- Cervical, focal demyelination	Male	0/6	0/6	0/6	3/6	---
- Lumbar, focal demyelination	Male	0/6	3/6	4/6	5/6	---
VENTRAL ROOT FIBER						
- Cervical, focal demyelination	Male	0/6	0/6	0/6	2/6	---
- Lumbar, focal demyelination	Male	2/6	3/6	4/6	4/6	---
	Female	1/6	0/6	0/6	---	3/6
LUMBAR SPINAL CORD						
- Focal demyelination	Male	0/6	0/6	0/6	1/6	---
- Focal demyelination, spinal nerve	Male	0/6	0/6	0/6	1/6	---
TIBIAL NERVE						
- Focal demyelination	Male	0/6	0/6	1/6	3/6	---
SURAL NERVE						
- Focal demyelination	Male	0/6	0/6	0/6	2/6	

[a] Data summarized from Table 13 of the study.

V. DISCUSSION and CONCLUSIONS: In this acute neurotoxicity study, male and female Sprague-Dawley rats (10 animals/sex/group) were orally gavaged once with methyl parathion at doses of 0, 0.025, 7.5, 10 (males only) or 15 (females only) mg/kg. Neurobehavioral evaluations, consisting FOB and motor activity, were conducted at pre-study, at the peak time of effect (1.5 hrs post-dosing) on Day 0 and on Days 7 and 14. At 15 ± 3 days animals were euthanized and neuropathological examination performed on control and high-dose animals (6/dose/sex). In a sub-study, ChEase activities were determined at Day -2 in plasma and RBC samples and at the peak time of effect and at Day 14 in plasma, RBC and brain. Brain ChEase activities were determined in six different regions.

No significant differences were noted in the mean body weights of the treated animals; body weight gain in high-dose males was significantly lower than controls.

Neurobehavioral evaluation revealed treatment-related FOB and motor activity findings at the mid- and high-dose levels. The effects were transient and observed only at the peak time of effect (1.5 hrs post-dosing). The FOB findings, when taken together, are consistent with those observed following cholinesterase inhibition (i.e. lacrimation, salivation, miosis, tremors/convulsions, muscle fasciculations, muscle weakness, and ataxia).

Motor activity was significantly decreased in mid- and high-dose males, when measured at the peak time of effect during the first 4 sessions (1-5, 6-10, 11-15 and 16-20 min) of the evaluation. At Days 7 and 14, motor activity of treated animals was

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comparable to those of the controls.

ChEase activities showed, treatment-related decreases in mid- and high-dose animals. At the peak time of effect, plasma, RBC, and brain ChEase activities were decreased greater than 67, 56 and 76%, respectively. At Day 14, some recovery was observed, however, plasma (females only), RBC and brain ChEase activities was still significantly decreased by 45%, 15 to 28%, and 7 to 39%, respectively.

The incidence of gross pathological observations did not show any treatment-related effects. Neuropathological findings consisted of focal demyelination in the dorsal root fibers of the cervical spine in 3/6 high-doses males and lumbar spine in 3/6 low-, 4/6 mid- and 5/6 high-dose males. Focal demyelination was also observed in the ventral root fibers of the cervical spine in 2/6 high-dose males and of the lumbar spine in control (males, 2/6; females, 1/6), low- (males, 3/6), mid- (males, 4/6), and high- (males, 4/6; females, 3/6) dose groups. Focal demyelination of the lumbar spinal cord and spinal nerve were observed in high-dose males; the incidence of each of these observations was only 1/6. Focal demyelination was observed in the tibial nerves of 1/6 mid- and 3/6 high-dose males and in the sural nerves of 2/6 high-dose males.

In summary, systemic toxicity was observed in high-dose males (decreased body weight gain) and females (increased incidence of clinical signs). Neurotoxic effects (abnormal FOB findings, decreased motor activity, inhibition ChEase activities, and neuro-pathological findings) were observed in mid- and high-dose males and females.

Based on the results of this study, the systemic LOEL 10 mg/kg (males) and 15 mg/kg (females); the systemic NOEL was 7.5 mg/kg. In males and females, the LOEL for neurotoxicity was 7.5 mg/kg; the NOEL for neurotoxicity was 0.025 mg/kg.

This study is classified as Core - Guideline and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.