2-17-95

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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Parathion, 90-day Neurotoxicity Study in Rats

TO: Joshua First PM 61
Reregistration Branch
Special Review and Reregistration Division (7508C)

1/31/95

FROM: Robert P. Zendzian Ph.D.
Senior Pharmacologist
Toxicology Branch I
Health Effects Division (7509C)

THROUGH: Karl Baetcke Ph.D.
Chief
Toxicology Branch I
Health Effects Division (7509C)

2/14/95

Compound; Parathion

Tox Chem #637

Registration #; 057501

Registrant; Chemnova

MRID 434915-01

DP Barcode; D210490

Action Requested

Review the following study;

Citation

Subchronic neurotoxicity study of dietary parathion in rats. D.J. Minnema. Hazleton Washington Inc, HWA 2688-101, December 19, 1994. MRID 434915-01

Study Type Subchronic neurotoxicity, Guideline 82-7

Core Classification Acceptable

Conclusions

Rats dosed at 0, 1, 50 and 100 ppm males and 0, 1, 25 and 50 ppm females for 13 weeks. Control and high dose recovery

animals carried on basel diet for an additional 4 weeks. Body weight gain decreased in both sexes at high dose. Erythrocyte cholinesterase activity decreased significantly males and hypocampus females. LOEL 1 ppm (LDT). Blood and brain all areas cholinesterase activity decreased significantly at 50 ppm males and 25 ppm females. Only plasma and cerebellum activity recovered at the high dose in both sexes at 17 weeks. Neurotoxicity, pupillary response LOEL 50 ppm males 25 ppm females, NOEL 1 ppm both sexes. Forelimb grip strength, LOEL 50 ppm females, NOEL 25 ppm. hindlimb grip strength, LOEL 100 ppm males 50 ppm females, NOELs 50 ppm males, 25 ppm females.

Discussion

Dose related depression of cholinesterase activity was clearly the most sensitive indication of parathion toxicity in this study showing significant depression at the respective mid and high doses for both sexes with minimal recovery. Dose related and statistically significant depression of cholinesterase activity in plasma, erythrocytes and all brain sections in both sexes at their respective intermediate and high doses (males 50 and 100 ppm and females 25 and 50 ppm). In the 1 ppm males erythrocyte cholinesterase activity was statistically significantly depressed at 8 and 14 weeks, 86 and 85 percent of controls respectively. In the 1 ppm females hippocampus activity was statistically significantly depressed 14 weeks (78 percent of control). In the high dose recovery animals at 17 weeks, plasma and cerebellum cholinesterase activity had recovered. Erythrocyte and the remaining brain area activity had recovered to some extent but the remaining depression was statistically significant.

Body weight and weight gains were depressed in the respective high doses in males and females.

Very few of the parameters in the functional observational battery gave any indication of a treatment related effect. The only clearly observed effects were fore and hindlimb grip strength at the respective high doses in both sexes.

At 8 weeks one high dose female (50ppm) and 2 high dose males (100 ppm) showed tremors. At 13 weeks one mid dose (25 ppm) and one high dose female (50 ppm) showed tremors. At 8 and 13 weeks there were 'subjective' observations of slow and/or partial pupillary constriction in intermediate and high dose animals in both sexes. The number of animals showing the effect appeared to be dose related.

Forelimb grip strength showed a statistically significant decrease ($p \leq 0.5$) in the high dose females (50 ppm) at 4, 8 and 13 weeks. A dose-response relationship not apparent in the lower doses.

Hindlimb grip strength showed a statistically significant decrease ($p \leq 0.5$) in the high dose females (50 ppm) at 4, 8 and 13 weeks. A dose-response relationship not apparent in the lower doses. Statistically significant decrease ($p \leq 0.5$) in the high dose males (100 ppm) at 4 and 13 weeks. A dose-response relationship not apparent in the lower doses.

Data Evaluation Report

Compound Parathion

Study Type Subchronic neurotoxicity, Guideline 82-7

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Reviewed by Robert P. Zendzian PhD
Senior Pharmacologist

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Rats dosed at 0, 1, 50 and '00 ppm males and 0, 1, 25 and 50 ppm females for 13 weeks. Control and high dose recovery animals carried on basel diet for an additional 4 weeks. Body weight gain decreased in both sexes at high dose. Erythrocyte cholinesterase activity decreased significantly males and hypocampus females, LOEL 1 ppm (LDT). Blood and brain all areas cholinesterase activity decreased significantly at 50 ppm males and 25 ppm females. Only plasma and cerebellum activity recovered at the high dose in both sexes at 17 weeks. Neurotoxicity, pupillary response LOEL 50 ppm males 25 ppm females, NOEL 1 ppm both sexes. Forelimb grip strength, LOEL 50 ppm females, NOEL 25 ppm. hindlimb grip strength, LOEL 100 ppm males 50 ppm females, NOELs 50 ppm males, 25 ppm females.

Materials

Ethyl parathion (parathion)
Technical
Batch 70813-01
brown liquid
purity 96.2%
from Cheminova Agro A/S May 3, 1993

Vehicle
Agway® Prolab® Certified rodent feed (RMH 3200 Meal)

Sprague-Dawley Crl:CD®BR rats
4-week old males and females
(7 weeks when placed on study)
From Charles River Laboratories
Raleigh NC,
Nov 30, 1993

Experimental design

Group	Dose level ppm	Number of Animals: Neurotox Male	Neurotox Female	Cholinesterase Male	Cholinesterase Female
1 control	0	10[a]	10[a]	-	-
		-	-	5[b]	5[b]
		-	-	5[c]	5[c]
		5[d]	5[d]		
2 low	1	10[a]	10[a]	-	-
		-	-	5[b]	5[b]
3 mid-low	25	-	10[a]	-	-
		-	-	-	5[b]
4 mid-high	50	10[a]	10[a]	-	-
		-	-	5[b]	5[b]
		-	-	-	5[c]
		-	5[d]	-	-
5 high	100	10[a]	-	-	-
		-	-	5[b]	-
		-	-	5[c]	-
		5[d]	-	-	-

a. neurotox animals
b. cholinesterase animals
c. recovery cholinesterase animals
d. recovery behavior animals

Dose preparation

Dietary mixes were prepared weekly. No adjustment made for purity of technical material. Group 5 mix was used as stock for group five mix. Groups 3-5 mixes were prepared individually. Fresh diets were presented weekly and were available 7 days/week for at least 13 weeks until the day before necropsy. The recovery animals were returned to basel diet after at least 13 weeks on treated diet and maintained until sacrifice during week 17.

Homogeneity and stability analysis were performed on the 1, 25, and 100 ppm dose concentrations. Duplicate samples of each concentraton (including control) from weeks 1,4, 8 and 14 were analyzed for concentration of test material. Samples of each dietary formulation were stored frozen for possible additional analysis.

Observations

Rats were observed twice daily for morbidity and mortality. A through clinical analysis was performed at each weighing.

Rats were weighed before treatment and weekly thereafter. Food consumption was measured weekly.

"An indirect opthalmoscopic examination was performed on each rat prior to treatment and on the neurotox control, mid-high-dose female and high-dose male rats prior to termination (week 14) using 1% Mydriacyl® as the mydriatic agent."

Cholinesterase determinations

Blood samples were taken from the cholinesterase animals before dosing and at weeks 4, 8 and 13 for plasma and RBC cholinesterase determination. Blood samples were taken from the recovery cholinesterase animals at week 17 for plasma and RBC cholinesterase determination. Brain samples were collected from all cholinesterase animals at termination (13 and 17 weeks respectively). Blood samples were collected from the orbital sinus, during the last three hours of the animals light cycle under CO_2/O_2 anesthesia.

Shortly after collection of the terminal blood samples, cholinesterase animals were anesthized with CO_2/O_2 and decapitated. The brains, including the olfactory lobes were removed and six regions (olfactory bulbs, cerebelum. cortex, striatum, hippocampus and midbrain plus brainstem) were isolated (based on a modification of the method of Glowinski & Iverson 1) for individual cholinesterase determination.

Chlinesterase analysis were performed on the BMD/Hitachi® 704 Chemistry Analyser.

Neurobehavioral Assessment

The Functional Observational Battery (FOB) was conducted on 10 animals prior to dosing and at weeks 4, 8 and 13. In adition the pupil response test was performed on the recovery aninals during weeks 13 and 16. Testing was performed blind during the dark cycle at approximately the same time of day for each interval. With the exception of the performance measures all neurobehavioral assessments were performed under red-light and white-noise conditions.

Observations

"As each animal was removed from its cage, the following parameters were evaluated;

appearance of fur	lacrimation
color of tears/deposits around eyes	other signs
convlusions/tremors	palpebral closure

1. Glowinski & Iverson, Regional studies of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in verious regions of the brain, J. Nuerochem, 13: 655-669 1966

ease of handling/body tone	piloerection
ease of removal from cage	respiration
excessive vocalizations	salivation
exopthalmus	writhing"

"After home cage observations, each animal was placed into a Plexiglas arena (66L X 48W X 30.5 H cm) on a flat surface covered with a clean absorbent paper for a 1-minute period to evaluate the following parasmeters;

arousal	other signs
circling	posture
convulsions	stereotypy
gait	tremors"

"The following responses were evaluated in the testing arena at the end of the 1-minute period;

light aproach response	pupil response
catalepsy	righting reflex
olfactory response	touch reflex
other signs"	

"The following parameters were evaluated at the same intervals as the FOB testing:

forelimb grip strength	rectal body temperature
hindlimb grip strength	tail flick latency
landing foot splay"	

Criterial for neurolotoxicological findings are presented in Appendix I of this DER (pages 26 and 27 of the report).

"Automated Auditory Startle Response -The auditory startle response was measured by placing each animal in a sound-attenuated automated audifory startle chamber which measures the muscular fource (i.e. the force generated by motor movement or "startle") that occurs in response to the presentation of an auditory stimulus (i.e. tone). The parameters measured and reported for both the prestimilus (control habituaion period, no tone) and stimilus (tone) trials included the maximum input voltage [mv] (MXI, the time to reach maximum response), the maximum input time [msec], (MXIT, the time to reach maximum amplitude of the muscular response following the presentation of the auditorystimulis) and the average input voltage [mv], (AVI, the average amplitude of the muscular response over a 100-msec recording window). The auditory startle equiptment was calibrated prior to each interval by using a standard stimuli in each chamber. Animals were assigned

to placement in the devices by rotating through the groups to ensure that the treatment groups were ballanced across the separate chambers."

"Locomotor Activity - Locomotor activity was monitored prior to initiation of treatment and at weeks 4, 8 and 13. Each animal was placed in an automated photocell activity monitoring device (San Diego Instruments, Model PAS) for a period of 40 minutes. The movement of each animal was recorded as activity counts (photo beam breaks) in 1-minute intervals. The motor activity equiptment was calilbrated and the diagnostics program run prior to each interval to ensure that all photobeams and receptors functioned properly. The lccomotor activity counts were converted into eights blocks of 5 minutes each for statistical analysis and tabular presentation. Animals were placed in the devices by rotating through the groups to ensure that the treatment groups were ballanced across the separate chambers."

Termination

"On the day of scheduled necropsy (week 14), following an overnight fast, all neurotox animals were weighed and given an intraperitoneal injection of sodium pentobarbital. Preceding gross necropsy, a whole body perfusion was performed on six (first six successful perfusions) rats/sex/group. Heparinized saline was perfused through the left ventricle, followed immediately by buffered glutaraldehyde-paraformaldehyde solution. All remaining neurotox rats in each group were esanguinated and a gross necropsy was conducted on each animal --------. Designated recovery behavioral animals (controls, high dose males and mid-high dose females) were sacrificed via CO_2/O_2 inhalation and exsanguination during week 17 without a gross necropsy. Necropises included examination of the following;

all orifices
carcass
cervical tissues and organs
cranial cavity
external surface of the body
external surface of the brain (at necropsy)
nasial cavity and paranasel sinuses
thoracic, abdominal and pelvic cavities and their viscera"

"The following tissues (when present) from each perfused animal were collected and preserved in 10% neutral-buffered formalin;

Anterion tibialis muscles
brain with brainstem (medulla/pons cerebellar cortex and cerebral cortex)
lumbar dorssal and ventral root fibers
lumbar spinal cord
lumbar dorsal root ganglia

cervical dorsal root and ventral root fibers
cervical spinal cord
cervical dorsal root ganglia
eyes with portion of optic nerve
gasserian ganglion
gastrocnemius muscles
macroscopic lesions
mid-thoracic spinal cord
pituitary
sciatic nerve
sural nerve
tibial nerve"

Macroscopic lesions in nonperfused animals were saved.

Histopathology

"The proximal sciatic, sural and tibial nerves from the perfused animals in Group 1, Group 4 females and Group 5 males were embedded in both plastic (glycol methacrylate) and parafin and examined microscopically. For cross sections, the tissues embedde in plastic were sectioned at approximately 1 u, mounted and stained with cresyl fast violet. For longitudinal sections, the tissues embedded in parafin were sectioned at aproximately 5 u, mounted and stained with luxol fast blue and counter stained with periodic acid-Schiff."

"All other preserved tissues from the perfused animals in Group 1, Group 4 females and Group 5 males were imbedded in parafin, sectioned at 5 u, mounted and stained with hemotoxylin and eosin and examined microscopically."

Statistical Analysis

"Body weight, body weight change, food consumption and cholinesterase data of the control group were compared statistically with the data from the same sex of the treated groups." An outline of the statistical procedure is presented in Figure 1 from the report (Appendix II).

Results

Analysis of the diet showed that homogeneity, stability and concentration were within 10 % of target doses.

No morbidity or mortality was observed in the study. No evidence of treatement related toxicity was observed clinically.

Opthalmoscopic examination reported that the eyes of control and high dose treated animals showed no differences that could be attributed to treatment. However individual observations were not reported.

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Body weights and weight gain was depressed at the high dose in the males (100 ppm) and the females (50 ppm). Food intake appeared depressed in the males at the high dose but not in the femles.

Cholinesterase activity is summarized in Text Table 1 from the report (Appendix II, page 46). Statistically significant decreases in activity (related to controls) were seen at 50 and 100 ppm in all parameters (blood and brain) tested in the males. Similar results were observed in the females at 25 and 50 ppm except in the cerebellum where a 71%, of control, value was not statistically significant.

Cholinesterase activity at 1 ppm in both sexes showed a three values significantly depressed and two other values depressed is an extent that is suspicious but not statisticaly significant. For these latter parameters individual animal values were examined.

In males at 1 ppm erythrocyte cholinesterase was significantly depressed at 8 and 14 weeks. Terminal cerebellum cholinesterase activity was 61% of control at week 14 but the difference was not statistically significant. Individual animal values were as follows;

0 ppm		1 ppm		50 ppm		100 ppm	
#	U/L	#	U/L	#	U/L	#	U/L
B43792	1660	B43842	1600	B43887	960	B43927	520
B43793	9480	B43843	2480	B43888	620	B43928	680
B43794	1880	B43844	2100	B43889	880	B43929	740
B43795	2380	B43845	2220	B43890	900	B43930	860
B43796	2920	B43846	2720	B43891	1020	B43931	640
mean	3664		2224		876		688
percent	100		61		24*		19*
mean$_1$	2210		2224		876		688
percent	100		101		40		31

animal number U/L cholinesterase activity units/liter
* statistically significant $p \leq 0.05$
1. Mean calculated omitting control animal # B43793

Animal # B43793 is clearly a high value outlier, over 3 times higher than the next highest value (2920). Omitting this outlier removes the difference between control and 1 ppm while the depressed activity is clearly present at 50 and 100 ppm.

In females at 1 ppm hippocampus cholinesterase was significantly depressed at 14 weeks (78%). Terminal olfactroy bulb cholinesterase activity was also 78% of control but the difference was not statistically significant. Individual animal values for olfactory bulb were as follows;

0 ppm		1 ppm		25 ppm		50 ppm	
#	U/L	#	U/L	#	U/L	#	U/L
B43817	6460	B43857	4660	B43872	900	B43902	400
B43818	3740	B43858	3300	B43873	1760	B43903	400
B43818	6540	B43859	3920	B43874	2480	B43904	620
B43820	5180	B43860	4540	B43875	NT	B43905	700
B43821	4980	B43861	4540	B43876	2160	B43906	720
mean	5380		4192		1825		568
percent	100		78		34*		11*

animal number U/L cholinesterase activity units/liter
* statistically significant $p \leq 0.05$

In ths case there is no obvious outlier, with high to low ratios of 1.7, 1.4, 2.8 and 1.8 for the 0, 1, 25 and 50 ppm doses respectively. One may consider the depression real but not statistically significant.

Neurobehavioral Assessment

Observations at removal from cage

Observation	Results (all doses)
appearance of fur	normal
color of tears/deposits around eyes	none
convlusions/tremors	none
ease of handling/body tone	normal
ease of removal from cage	normal
excessive vocalizations	none
exopthalmus	none
lacrimation	none
other signs	none
palpebral closure	normal
piloerection	none
respiration	normal
salivation	none
writhing	none

After home cage observations, each animal was placed into a Plexiglas arena (66L X 48W X 30.5 H cm) on a flat surface covered with a clean absorbent paper for a 1-minute period to evaluate the following parasmeters;

Observation	Results
arousal	normal
circling	none
convulsions	none
diarrhea	none
gait	normal
latency to first step	no dose-related differences
number fecal boli	no differences

number rears	no differences
number urine pools	no differences
other signs	none
polyurea	none
posture	normal
stereotypy	none
tremor	At 8 weeks one high dose female (50ppm) and 2 high dose males (100 ppm) showed tremors. At 13 weeks one mid dose (25 ppm) and one high dose female (50 ppm) showed tremors.

"The following responses were evaluated in the testing arena at the end of the 1-minute period:

Observation	Results
light approach response	no differences
catalepsy time	no differences
olfactory response	present
other signs	number showing signs/number dosed

	males	females
8 weeks		
25 ppm	not dosed	2/10
50 ppm	2/10	7/10
100 ppm	5/10	not dosed
13 weeks		
25 ppm	not dosed	2/10
50 ppm	3/10	8/10
100 ppm	8/10	not dosed

observations consisted mainly in a "subjective" observation of slow and/or partial pupillary constriction usually in both eyes.

pupil response	present
righting reflex	At 13 weeks 4 females at the high dose (50 ppm) showed mildly abnormal righting response
touch reflex	no differences

"The following parameters were evaluated at the same intervals as the FOB testing:

Observation	Results
forelimb grip strength	Statistically significant decrease ($p < 0.5$) in the high dose females (50 ppm) at 4, 8 and 13 weeks. A dose-response relationship not apparent in the lower doses.

hindlimb grip strength	Statistically significant decrease ($p \leq 0.5$) in the high dose females (50 ppm) at 4, 8 and 13 weeks. A dose-response relationship not apparent in the lower doses. Statistically significant decrease ($p \leq 0.5$) in the high dose males (100 ppm) at 4 and 13 weeks. A dose-response relationship not apparent in the lower doses.
landing foot splay	no differences
rectal body temperature	no differences
tail flick latency	no differences

Auditory Startle Response

No evidence of a treatment related effect was observed in the auditory startle response. However, in many cases the Standard Deviation was equal to or larger then the mean value indicating a large degree of individual animal variation. Individual animal data confirm this. The first page of Appendex 7 from the report (Appendix IV, page 372) presents the individual male and female control data. Individual animal variation is readily apparent and appears to be without any pattern or consistancy within the single animal derived numbers. The same observation can be made for the treated groups. It must be concluded that normal variation in the tested parameters is so large as to make it imposible to detect effects less than comatose or dead animals.

Locomotor Activity Counts

No evidence of a treatment related effect was observed in the locomotor activity counts. A single block, high dose males, 11-15 minutes, week 4 was significantly decreased from the concurrent control value. This value is considered an anomaly and of no toxicological significnce. Individual animal data show a large variation in activity in this period. Some animals are essentially inactive but subsiquently 'come back to life'. Even in the control groups, individual animal activity is extremely variable with only a general pattern of the animals being more active at the beginning of the test and decreasing in activity with time. It must be concluded that the test is not sensitive to small changes in activity.

Gross necropsy

No abnormalities were observed at gross necropsy.

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Histopthology

No evidence of treatment related abnormalities were observed in the nervious tissue examined.

Discussion

Dose related depression of cholinesterase activity was clearly the most sensitive indication of parathion toxicity in this study showing significant depression at the respective mid and high doses for both sexes with minimal recovery. Dose related and statistically significant depression of cholinesterase activity in plasma, erythrocytes and all brain sections in both sexes at their respective intermediate and high doses (males 50 and 100 ppm and females 25 and 50 ppm). In the 1 ppm males erythrocyte cholinesterase activity was statistically significantly depressed at 8 and 14 weeks, 86 and 85 percent of controls respectively. In the 1 ppm females hippocampus activity was statistically significantly depressed 14 weeks (78 percent of control). In the high dose recovery animals at 17 weeks, plasma and cerebellum cholinesterase activity had recovered. Erythrocyte and the remaining brain area activity had recovered to some extent but the remaining depression was statistically significant.

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Hindlimb grip strength showed a statistically significant decrease ($p \leq 0.5$) in the high dose females (50 ppm) at 4, 8 and 13 weeks. A dose-response relationship not apparent in the lower doses. Statistically significant decrease ($p \leq 0.5$) in the high dose males (100 ppm) at 4 and 13 weeks. A dose-response relationship not apparent in the lower doses.

Parathion

RIN 3393-97

Page _____ is not included in this copy.

Pages 15 through 19 are not included in this copy.

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