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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity - rat (82-7)

SHAUGHNESSY NO./TOX. CHEM. NO.: 128501/893C

ACCESSION NO./MRID NO.: 431512-02 (sulfosate); 430133-01 thru -  
05 for positive controls

DP BARCODE/SUBMISSION NO.: D200555, D200557, D200558, D200561,  
D201511, D201514, D194075, D194071

TEST MATERIAL: Glyphosate Trimesium

SYNONYMS: Sulfosate

STUDY NUMBER(S): PRO887

REPORT NUMBER: CTL/P/3831

SPONSOR: Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: Zeneca Central Toxicology Laboratory,  
Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Glyphosate Trimesium: Subchronic  
Neurotoxicity Study in Rats

AUTHOR(S): S. A. Horner

REPORT ISSUED: 2/15/93

CONCLUSION: Technical glyphosate trimesium (sulfosate, 59.4%) was tested in a 90 day neurotoxicity feeding study in Alpk:APfSD rats. The rats received either 0, 200, 600 or 2000 ppm (0, 15.6, 47.6 or 153.2 mg/kg/day for males; 0, 18.2, 54.4 or 171.0 mg/kg/day for females) in the diet. Twelve males and 12 females were tested per dose group. Clinical signs of toxicity, body weights, food consumption, functional battery, motor activity and neuropathology parameters were measured and recorded regularly. Positive control data were provided.

At 2000 ppm, decreases in body weights (16% for males and 9% for females), food consumption and utilization were observed. In addition, mean forelimb grip strength values for high dose females were statistically significantly decreased over the control values during weeks 5-14 (75 - 82% of controls). Since there were no effects in mean hindlimb grip strength for high

dose females, in either of the mean grip strength values at any time period for males, in any of the other functional battery parameters, in motor activity values or in neuropathology microscopic examinations for either sex, it is unlikely that these decreases in mean forelimb grip strength values for high dose females constitute a neurotoxicological effect. Adequate positive control studies were submitted under separate cover for this particular laboratory.

The NOEL is 600 ppm (47.6 mg/kg/day) and the LEL is 2000 ppm (153.2 mg/kg/day) based on decreases in mean body weight, food consumption, food utilization and mean forelimb grip strength values. There was no microscopic evidence of neurotoxicity. The evidence for neurotoxicity is not clear.

This study is classified as Core Guideline and satisfies the regulatory requirements for a subchronic mammalian neurotoxicity study (82-7).

A. MATERIALS AND METHODS:

1. Test Compound: N-(phosphono-methyl) glycine, sulfonium salt

Description: Amber colored liquid

Batch #(s), Other #(s): F47 D7534/36; CTL Y06380/036

Purity: 59.4%

Source: ICI Agrochemicals

Vehicle: None

Positive Control(s): chlordiazepoxide hydrochloride, morphine sulfate, amphetamine sulphate, chlorpromazine hydrochloride, trimethyltin chloride and acrylamide

2. Test Animals

Species and Strain (sexes): Male and female  
Alpk:APfSD rats

Age: 28 days old upon receipt.

Source(s): ICI Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

- a. Dietary Preparation: The diets were prepared in 15 - 25 kg batches from premixes prepared by triturating the appropriate amount of the test substance with 1 kg of CT1 diet. The premixes were then added to additional CT1 diet and mixed thoroughly.

Frequency of preparation: Not stated.

Storage conditions: The first and second batches were stored frozen until required. The diet was removed from the freezer and allowed to thaw prior to use. After the results from the stability analyses were available, all subsequent batches were stored at room temperature.

Stability Analyses: Stability studies of the chemical in the diet were conducted on the 200 and 2000 ppm dose levels that had been stored frozen and at room temperature after 82 days of storage .

Homogeneity Analyses: Homogeneity analyses were conducted on samples from the 200 and 2000 ppm dose levels from the fifth batch of the experimental diets.

Concentration Analyses: Samples from all dietary levels were taken at intervals and analyzed for concentration of the test material.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of results from studies conducted in the same laboratory on the same strain of rat.

c. Animal Assignment and Dose Levels:

Test Group	Dose Admin- istered ppm	Main Study	
		90 days	
		male	female
Control	0	12	12
1	200	12	12
2	600	12	12
3	2000	12	12

\*Six animals/sex in each group were designated for terminal neuropathology.

d. Clinical Signs of Toxicity and Mortality: All rats were examined prior to the start of the study and cageside checks were conducted daily during the study for clinical signs of toxicity, behavior changes and mortality. At weekly intervals, each rat was removed from its cage and physically examined for changes in general health status.

e. Body Weight Determinations: Bodyweights were recorded immediately before feeding the experimental diet, weekly thereafter on the same day and at termination.

- f. Food and/or Water Consumption: Food consumption was recorded continuously throughout the study and calculated weekly.
- g. Functional Observational Battery: The report stated that "detailed clinical observations ...and quantitative assessments of landing foot splay, sensory perception (tail flick test) and muscle weakness (fore and hindlimb grip strength) were made in weeks -1, 5, 9 and 14. The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour etc; reactivity to stimuli; changes in level of arousal; sensorimotor responses; [and] alterations in respiration. The observations were made by one observer who was 'blind' with respect to the animal's treatment, and recorded on a computer system by personnel not directly involved in the clinical observations. The observations were carried out in a room separate from that in which the animals were housed and animals were presented to the observer with no indication of the treatment group. The observations were coded and the degree of condition noted (slight, moderate or extreme) where appropriate. This included the recording of no abnormalities detected."
- h. Motor Activity: An automated activity recording apparatus was used to measure locomotor activity. The animals were tested in week -1, 5, 9 and 14 of the exposure period. The report stated that "each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices, and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimize disturbances."
- i. Neuropathology:  
At termination, six animals/sex/group were anesthetized with halothane, exsanguinated and subjected to a full post mortem examination. The tissues listed below were removed and fixed in 10% neutral buffered formol saline. The brains were

weighed and the length and width were recorded with calipers.

Six other animals/sex/group were deeply anesthetized with intraperitoneal barbiturate and killed by perfusion fixation with modified Karnovsky's fixative. The tissues listed below were removed and brain weight, length and width were recorded. The tissues from these latter groups were further microscopically examined. The oral cavity and nasal passages were stored and not processed. The neuropathological examination was performed on the control and the 2000 ppm groups only. All sections were examined by light microscopy. The brain and gastrocnemius muscle were embedded in paraffin wax, and 5 micrometer thick sections were cut and stained with H & E stain. Transverse sections of the vertebral column containing samples from the lumbar and cervical regions, with dorsal root ganglia and spinal roots attached, were decalcified, embedded in paraffin wax and 5 micrometer thick sections were also cut and stained with H & E. The remaining tissues were embedded in ARALDITE and semi-thin sections (1-2 micrometers) were cut and stained with toluidine blue. Samples of the spinal cord and peripheral nerves were also embedded in Araldite and semi-thin sections cut and stained with toluidine blue. An initial examination of the brain was conducted on 1 male and 1 female from the 2000 ppm dose group. The brain was examined in the transverse plane at 12 levels. On the basis of this examination, the remaining 5 animals/sex from this group and 6 rats/sex from the control group were examined in the transverse plane at the following 6 levels: 2, 5, 6, 7, 8 and 9. The spinal cord from the cervical region (C3-C6) and from the lumbar region (L1-L4) was also examined in the transverse plane. Spinal roots and the dorsal root ganglia were examined from the C3-C6 and L1-L4 levels and the gasserian ganglia were examined from the trigeminal nerve. Transverse and longitudinal sections of the sciatic nerve and transverse sections of the sural and tibial nerves were also examined. In addition, samples of the gastrocnemius muscle were examined in the transverse plane.

The following tissues were removed and examined microscopically:

- x Brain
- x Gasserian ganglia
- x Vertebral column including spinal cord
- x Dorsal root ganglea including spinal roots
- x Gastrocnemius muscle
- x Sciatic nerve
- x Sural nerve
- x Tibial nerve
- x Oral cavity and nasal passages

- k. Statistical Analyses: Day 1 bodyweights, brain weight, length and width and replicate structure of the study design were analyzed by analysis of covariance. Motor activity measurements, weekly food consumption, food utilization during the period weeks 1-4, 5-8, 9-13 and 1-13, tail flick response, landing foot splay and fore and hindlimb grip strength, were all analyzed by analysis of variance. Least squares means for each group were calculated. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

## B. RESULTS:

1. Dietary Preparation: The mean analyzed concentrations for the 200 ppm dose group ranged from 200 to 235 ppm (100 - 118% of the nominal concentration). The mean analyzed concentrations for the 600 ppm dose group ranged from 598 - 621 ppm (99.7 - 103.5% of the nominal concentration). The mean analyzed concentrations for the 2000 ppm dose group ranged from 1834 - 2063 ppm (91.7 - 103.2% of the nominal concentration).

The homogeneity study indicated the following: at 200 ppm, the mean analyzed concentrations from the top, middle and bottom of the mixing chamber were 233, 213 and 218 ppm, respectively and at 2000 ppm, the mean analyzed concentrations from the top, middle and bottom of the mixing chamber were 2189, 2238 and 1989 ppm, respectively.

The chemical stability study indicated that the test chemical was stable in the diet at both freezer and room temperatures. At room temperature, the 200 ppm dose level remained stable after 82 days (96% of the

initial concentration) and the 2000 ppm dose level remained stable after 82 days (96.4% of initial concentration). In the freezer, the 200 ppm dose level remained stable after 82 days (104.0% of the initial concentration) and the 2000 ppm remained stable after 82 days (94.5% of the initial concentration).

2. Clinical Observations and Mortality: There were no unscheduled deaths during the course of the study. In the high dose females, slight signs of urinary incontinence were occasionally observed in 2 animals from week 9, and slight upward curvature of the spine was seen for 1 other female in week 14. Since these were only seen occasionally, they are not considered to be related to treatment. The following table summarizes selected clinical signs.

#### Clinical Signs of Toxicity

Observation	Dose Levels (ppm)			
	0	200	600	2000
Males				
Reduced Splay Reflex				
# Observations	2	2		2
# Animals	1	1		1
Weeks <sup>a</sup>	14-14	14-14		14-14
Females				
Reduced Splay Reflex				
# Observations	2	4		3
# Animals	1	2		2
Weeks	14-14	14-14		5-10
Signs of Urinary Incontinence				
# Observations				6
# Animals				2
Weeks				9-14
Upward Curvature of Spine				
# Observations				1
# Animals				1
Weeks				14-14

<sup>a</sup>Expressed as from (No.) - to (No.)

3. Body Weight Determinations: When adjusted for initial weight, body weights in the 2000 ppm dose group were significantly less than the control groups. At week 14, mean bodyweights for males and females were approximately 16% and 9% less than that of the control

groups, respectively. No treatment-related effects were observed at the two lower dose levels. The effects observed in males at 200 ppm are considered to be due to the lower initial bodyweights for this group. The following table summarizes the findings.

Intergroup Comparison of Bodyweights (g)

Week	Dose Levels (ppm)			
	0	200	600	2000
Males				
Week 1	213.8	208.7	213.3	212.1
Week 4	331.3	320.5	328.8	296.8** (89.6%)
Week 8	428.2	407.3	426.7	365.8** (85.4%)
Week 14	512.6	484.3	505.6	428.4** (83.6%)
Females				
Week 1	164.4	164.6	165.4	161.4
Week 4	209.4	214.1	212.9	195.1* (93.2%)
Week 8	243.3	251.5	250.7	227.3* (93.4%)
Week 14	270.0	271.9	276.0	245.5** (90.9%)

4. Food and/or Water Consumption: At the highest dose level, food consumption was less than controls throughout the study in both sexes, although in females it was not as consistent as in males. Food consumption was less than controls in the low dose males, but this was not considered to be related to treatment. Food utilization was statistically significantly less than controls for the high dose group males from weeks 1-4 and from weeks 1-13. There were no effects in females. The following table summarizes selected food utilization values for both sexes.

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Intergroup Comparison of Food Utilization (g Growth/100 Food)

Dietary Concentration				
Weeks	0	200	600	2000
Males				
1-4	17.34	17.34	17.49	14.69**
5-8	9.70	9.65	9.56	8.55
9-13	6.30	6.01	6.08	5.57
1-13	10.76	10.65	10.67	9.28**
Females				
1-4	9.01	9.34	10.46	7.62
5-8	5.17	5.32	4.10*	5.57
9-13	2.80	2.65	2.88	2.63
1-13	5.50	5.62	5.70	5.11

\*\*Statistically significant (p < 0.01)

5. Functional Observational Battery

Time to Tail Flick: No treatment-related effects were observed. The following table summarizes selected values.

Intergroup Comparison of Time to Tail Flick

Dietary Concentration (ppm)				
Week	0	200	600	2000
Males				
-1	7.10	6.23	3.48**	6.82
5	5.06	5.33	5.52	5.40
9	4.75	5.60	5.48	6.89
14	4.70	5.12	5.56	5.86
Females				
-1	6.62	8.83	6.81	6.85
5	4.15	6.03	5.90	5.47
9	5.38	5.46	4.41	6.65
14	5.04	4.80	5.42	5.10

\*\*Statistically significant (p < 0.01)

Landing Foot Splay: No treatment-related effects were observed. The following table summarizes the results.

Intergroup Comparison of Landing Foot Splay

Week	Dietary Concentration (ppm)			
	0	200	600	2000
Males				
-1	49.4	53.8	54.3	48.8
5	74.1	67.5	73.9	72.5
9	67.5	63.3	58.7	61.8
14	66.9	62.0	72.7	55.8
Females				
-1	51.7	48.3	46.6	48.0
5	60.6	54.7	57.5	54.0
9	63.4	61.8	58.9	58.1
14	59.8	54.8	67.2	61.6

Grip Strength Measurements: No treatment-related effects were observed for mean hindlimb grip strength. Mean forelimb grip strength values for high dose females were statistically significantly decreased over the control values during weeks 5-14. Mean forelimb grip strength values for 200 and 600 ppm females were statistically significantly lower than controls in week 9 (both) and in week 14 (200 ppm). In these cases, although all doses were significantly decreased, no dose-response was observed. If two controls which had unusually high values were excluded during week 14, then the statistically significant value for the 200 ppm females would not be significantly lower. The following tables summarize the data.

Intergroup Comparison of Forelimb Grip Strength

Week	Dietary Concentration (ppm)			
	0	200	600	2000
Males				
-1	477	494	457	488
5	940	993	1015	950
9	1053	1033	1136	1075
14	1175	1133	1010	956

Intergroup Comparison of Forelimb Grip Strength  
Dietary Concentration (ppm)

Week	0	200	600	2000
Females				
-1	470	480	436	445
5	888	897	830	692**
9	1168	903**	1022*	954**
14	1130	932*	1035	851**

\*Statistically significant from controls ( $p < 0.05$ )

\*\*Statistically significant from controls ( $p < 0.01$ )

Intergroup Comparison of Hindlimb Grip Strength  
Dietary Concentration (ppm)

Week	0	200	600	2000
Males				
-1	337	381*	406**	384*
5	860	838	835	799
9	967	919	951	917
14	988	1008	943	978
Females				
-1	389	352	364	358
5	670	686	685	618
9	698	786	704	664
14	868	977	887	885

\*Statistically significant from controls ( $p < 0.05$ )

\*\*Statistically significant from controls ( $p < 0.01$ )

6. Motor Activity: There was no evidence of a treatment-related effect in motor activity. The following tables summarize selected values.

Intergroup Comparison of Motor Activity (Movements/Animal)

Males		Dietary Concentration (ppm)			
Minutes	0	200	600	2000	
<b>Week 5</b>					
1-5	79.6	75.9	75.7	74.8	
6-10	71.0	69.1	69.1	65.8	
41-45	23.1	27.5	32.8	29.3	
46-50	18.7	20.6	30.3	27.2	
1-50	492.8	480.1	484.8	510.2	
<b>Week 9</b>					
1-5	65.9	66.1	58.1	63.0	
6-10	49.6	59.3	41.6	48.7	
41-45	11.8	21.2	14.6	13.4	
46-50	10.8	21.6	13.3	18.9	
1-50	293.9	366.9	263.3	298.5	
<b>Week 14</b>					
1-5	61.8	62.5	61.8	61.2	
6-10	50.3	57.3	46.7	57.4	
41-45	7.3	23.8	19.4	19.1	
46-50	2.2	20.7	15.9	19.2	
1-50	247.7	355.0	293.4	318.8	

Intergroup Comparison of Motor Activity (Movements/Animal)

Females		Dietary Concentration (ppm)			
Minutes	0	200	600	2000	
<b>Week 5</b>					
1-5	71.5	69.6	72.5	70.3	
6-10	73.3	70.6	69.8	66.4	
41-45	56.5	58.3	49.6	64.8	
46-50	61.7	57.8	59.0	63.7	
1-50	627.2	637.3	650.3	650.3	
<b>Week 9</b>					
1-5	70.8	72.3	70.0	72.3	
6-10	59.9	61.2	68.3	65.4	
41-45	46.5	46.6	60.5	56.8	
46-50	44.0	46.4	56.1	55.1	
1-50	522.7	515.4	630.3	582.6	
<b>Week 14</b>					
1-5	61.6	70.0	72.7*	70.1	
6-10	64.2	73.9	68.4	63.5	
41-45	39.4	55.5	51.0	51.5	
46-50	41.0	50.6	41.1	48.6	
1-50	490.3	590.5	604.3	566.6	

\*Statistically significant (p < 0.05)

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8. Brain Measurements: There was no evidence of a treatment-related effect. The following table summarizes the results.

Intergroup Comparison of Brain Parameters

Brain Parameter	Dietary Concentration (ppm)			
	0	200	600	2000
Males				
Brain Weight (g)	2.15	2.12	2.14	2.08
Brain Length (mm)	28.7	28.6	28.5	28.8
Brain Width (mm)	15.4	15.5	15.6	15.4
Females				
Brain Weight (g)	1.95	1.97	1.93	1.91
Brain Length (mm)	27.8	28.0	27.8	27.5
Brain Width (mm)	15.2	15.2	15.2	15.2

9. Neuropathology: No treatment-related effects were observed. One single degenerate peripheral nerve fiber was observed in a section of sciatic nerve from one high dose male. In light of historical control data (see table below), this lesion is not considered to be related to treatment. The following table summarizes pertinent findings. No other microscopic findings were found in the examined tissues.

Intergroup Comparison of Microscopic Findings

	Dose Level (ppm)			
	Males		Females	
	0	2000	0	2000
Animals on study	12	12	12	12
Animals completed	6	6	6	6
Sciatic nerve				
Examined	6	6	6	6
No Abnormalities detected	6	5	6	6
Nerve fibre degeneration (total)	0	1	0	0
minimal	0	1	0	0

In response to a question concerning these same type of lesions observed with another pesticide submitted by Zeneca and tested in the same laboratory, historical control data were submitted on these lesions. The following table summarizes these data.

# Historical Control Incidence of Nerve Fiber Degeneration in Sciatic Nerve of Alderley Park Rats<sup>a</sup>

Subchronic Oral Studies: N = 6 Rats/Sex/Group

Month/Year	Males	Females
April 1992	0	0
May 1992	0	0
July 1992	0	1
April 1993	4	0
February 1993	0	1

<sup>a</sup>The data refer to the AlpK:APfSD (Wistar-derived) strain of rat. Nerve fiber degeneration is defined as foci of either Wallerian type degeneration/axonal swellings and/or areas of demyelination. The grading of nerve fiber degeneration seen was minimal for all animals. A grading criteria of minimal represents one to several small foci of Wallerian type degeneration originating from 1-2 nerve fibers.

9. Quality Assurance Measures: Signed Quality Assurance and GLP statements were provided.

C. DISCUSSION: This study was conducted according to the testing guidelines. It is graded Core Guideline. There appeared to be toxicity in both sexes at the highest dose level. This was evident by the decreases in body weight, food consumption and food utilization, particularly in the males. In addition, mean forelimb grip strength values for high dose females were statistically significantly decreased over the control values during weeks 5-14. Since there were no effects in mean hindlimb grip strength for high dose females, in either of the mean grip strength values at any time period for males, in any of the other functional battery parameters, in motor activity values or in neuropathology microscopic examinations for either sex, it is unlikely that these decreases in mean forelimb grip strength values for high dose females constitute a neurotoxicological effect. Adequate positive control studies were submitted under separate cover for this particular laboratory. These are summarized with the acute mammalian neurotoxicity study on glyphosate trimesium.