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DATA EVALUATION RECORD

STUDY TYPE: Acute Delayed Neurotoxicity - hen (81-7)

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

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

TEST MATERIAL: Technical ICIA-0224

SYNONYMS: Sulfosate, Touchdown, SC-0224

LABORATORY PROJECT ID #: Division Rep: SA41/88; CTL Ref.: YO6380/001/001

REPORT NUMBER: T-12324

SPONSOR: Zeneca Ag Products, Wilmington, DE

TESTING FACILITY: ICI Americas, Inc., Toxicology Laboratory, Richmond, CA

TITLE OF REPORT: Acute Delayed Neurotoxicity of ICIA-0224

AUTHOR(S): L.C. Mutter

REPORT ISSUED: 4/18/89

CONCLUSION: Technical ICIA-0224 (sulfosate, 56.9% pure) was tested in an acute delayed neurotoxicity study in adult white leghorn hens (Hyline strain). The test material was administered by gavage at 0, 500 or 5000 mg/kg in 5 ml/kg water. The high dose level was applied without dilution. TOCP (500 mg/kg) was administered as a positive control. Six hens/group were tested in the control groups and 8 hens/group were tested in the treated groups. Each group was divided in half and the dosing was staggered a day apart. Each animal was dosed twice during the study, on day 1 (or 2) and on day 22 (or 24). Each animal was evaluated up to day 41 (or 42).

At 500 mg/kg, diarrhea was observed for 2-3 days, starting a few days after each dosing. No other treatment-related effects were observed. At 5000 mg/kg, diarrhea, changes in comb appearance, early decrease in food consumption and decrease in egg production were observed. No indications of neurotoxicity were observed. The positive control indicated the appropriate clinical signs of

toxicity, increased ataxia and microscopic observations for an organophosphate. **The NOEL for systemic toxicity is 500 mg/kg. The LEL for systemic toxicity is 5000 mg/kg based on diarrhea, changes in comb appearance, early decrease in food consumption and decrease in egg production. There were no indications of neurotoxicity at any dose level.**

The study is core minimum because it was conducted prior to the publication of the new neurotoxicity guidelines which were published in 1991. The regulatory requirement for an acute delayed neurotoxicity study in hens has been satisfied.

A. MATERIALS AND METHODS:

1. Test Compound(s): Technical material

   Chemical Name: N-(phosphono-methyl) glycine, sulfonium salt

   Description: yellow liquid
   Lot #: Lot # 4921-50-2; 8289-35-1
   Purity: 56.9%
   Source: Zeneca Ag Products
   Vehicle: water
   Positive Control: Tri-ortho cresyl phosphate (TOCP)

2. Test Animals

   Species and Strain (sexes): Adult white leghorn hens (Hyline strain) in full egg production.

   Age: Not stated
   Weight(s): Not stated
   Source(s): Feather Hill Farms, Petaluma, CA

3. Procedure:

   a. Diet and Analysis of Dosing Solutions: Purina Layena® Poultry Feed was provided ad libitum. The test material was applied neat at the high dose. Therefore, it was assumed that this dose level did not need to be analyzed. The low dose solution and the positive control solution were each prepared on the day that they were used. The concentration and stability of these dosing solutions were verified by chemical analysis.

   b. Basis For Selection of Dose Levels: The hens were dosed up to the limit dose (5000 mg/kg).

c. Animal Assignment and Dose Levels: Four groups of hens were used. They were as follows: vehicle control (water, 5 ml/kg, 6 animals); positive control (TOCP, 500 mg/kg, 6 animals); low dose (500 mg/kg, 8 animals) and high dose (5000 mg/kg, 8 animals). The test material was applied by gavage.

d. Protocol: Each group was divided in half and assigned to a section (I or II) in order to stagger the dosing. Each animal was dosed twice during the study, on day 1 (or 2) and on day 22 (or 24). Each animal was evaluated up to day 41 (or 42).

e. Clinical Observations and Mortality: Two baseline observations were made for each hen at weekly intervals before treatment. After treatment, the hens were evaluated daily for clinical signs of toxicity or any unusual behavior.

f. Body Weight Determinations: Two baseline observations were made for each hen at weekly intervals before treatment. For group I, body weights were measured weekly after dosing. For group II, body weights were also measured weekly during dosing, however, an additional body weight measurement was taken the day before dosing.

g. Food and/or Water Consumption: Food consumption was measured for both groups on days -13, -12, -6 and -5 before treatment; on days 3, 4, 7-10, 15-17, 23-25, 29-31 and 36-38 for group I; and on days 4-5, 7-10, 16-18, 25-27, 29-31 and 36-38 for group II.

h. Egg Production: This observation was measured daily.

i. Walking Behavior: Twice weekly, the hens were forced to walk on an enclosed, horizontal surface and their motor activity was evaluated. Two baseline observations were made for each hen at weekly intervals before treatment.

j. Histopathology: The hens were terminated on day 42. They were anesthetized with sodium pentobarbitol and then perfused with cold formalin. Following in situ fixation, the tissues were removed from the carcass and placed in cold formalin. The brain and sciatic nerve sections

were trimmed and fixed. The spinal column was removed intact, fixed for 3 days and decalcified for 1 week. After decalcification, the cord was trimmed and processed. The following sections were saved for microscopic evaluation: transverse sections of the cerebral hemispheres, cerebellum and brain stem; longitudinal and transverse sections of the medulla, 3 levels of spinal cord and both right and left sciatic nerves. The spinal cord sections were taken at vertebrae C5-7 (cervical), T1-6 (thoracic) and in the middle of the synsacrum including the glycogen body (lumbosacral). The sciatic nerve sections included the peroneal and tibial branches. Duplicate sections were taken from the central nervous system and the peripheral nerve. The first sections were stained with hematoxylin and eosin. The second sections were either stained with Luxol Fast Blue (central nervous system) or Bodian's silver stain (peripheral nerve).

The microscopic lesions were evaluated by light microscopy and scored according to the following 4-point grading system: 1 = rare, minimal; 2 = few, mild; 3 = several, moderate; 4 = numerous, extensive. Only grades 2 and above were summarized in the summary tables.

k. Statistical Analyses: The mean body weight values were analyzed using the Dunnett's Test ($p < 0.05$) after an analysis of variance. Group scores for walking behavior were analyzed using the Mann-Whitney U-Test.

B. RESULTS:

1. Dose Solution Analysis: Analyses of the low-dose solutions indicated that the concentrations ranged from 97 to 106% of the expected concentrations, which is an acceptable range.

2. Clinical Observations and Mortality: None of the treated hens died during the study. The hen LD_{50} is greater than 5000 mg/kg, the limit dose. Diarrhea was observed in both treated groups following dosing, which was not observed in the vehicle control group. In the low dose group, the diarrhea was generally observed from days 2-6 and 26-27, a few days after dosing. Two hens had flacid comb, one on days 40-42 and one on day24. In the high dose group, the diarrhea lasted a little longer and was observed in more hens. In addition to the diarrhea, some changes were observed in

the appearance of the hens (comb) following treatment, particularly in the high dose group. The positive control exhibited clinical signs of neurotoxicity, which were not observed in the other treated groups or in the vehicle controls. Some of these neurotoxic signs appeared on approximately day 14 and continued until termination. The vehicle control also exhibited some clinical signs which were attributed to the stress of handling (i.e. forced walking). The following table taken directly from the report summarizes the observed clinical signs.

Summary of Occurrence of Clinical Signs				
	Incidence of Signs[a]			
Observed Sign	Water 5 mg/kg	TOCP 500 mg/kg	ICIA-0224 500 mg/kg	ICIA-0224 5000 mg/kg/
Appearance				
Feather loss	6/6	5/6	8/8	8/8
Flaccid comb	0/6	3/6	2/8	3/8
Blue tipped comb	0/6	5/6	0/8	3/8
Dry and/or atrophied comb	0/6	3/6	0/8	2/8
Curled toes	0/6	1/6	0/8	0/8
Behavior				
Hypoactivity	4/6	6/6	3/8	8/8
Posture & Coordination				
Reduced stability	0/6	6/6	0/8	0/8
Sitting on hocks	0/6	6/6	0/8	0/8
Leaning back	0/6	6/6	0/8	0/8
Spreads wings for balance	0/6	1/6	0/8	0/8
Physiological				
Diarrhea	0/6	6/6	5/8	8/8

[a]No. hens with sign/# hens in group (incidence of sign at any time during study period)

3. Body Weight Determinations: No treatment-related decreases in body weight or body weight gain were observed in the treated groups. Significantly decreased body weight gains were observed in the positive control group. The following table taken directly from the report summarizes the results.

Hen Body Weights				
Average Body Weight (grams)				
Study Day	Water[a] 5 ml/kg	TOCP[a] 500 mg/kg	ICIA-0224[b] 500 mg/kg	ICIA-0224[b] 5000 mg/kg
-15	1624	1640	1663	1623
-7	1623	1619	1649	1644
1	1577	1578	1613	1571
8	1611	1557	1667	1582
15	1651	1628	1698	1646
22	1612	1532	1664	1599
29	1672	1405	1728	1582
35	-	1375	-	-
36	1693	1358	1815	1702
42	1732	-	1803	1736
% Weight change[c]	+4	-17*	+9	+5

[a]n = 6
[b]n = 8
[c]Change in body weight from day -15 to day 36.
*Statistically significant ($p < 0.05$)
- Data not available

4. Food and/or Water Consumption: The report stated that the high dose hens ate less for several days after treatment (60% decrease which quickly returned to control levels). No treatment-related differences were observed with the low dose group when compared to controls. The positive control group ate significantly less after the second TOCP treatment. For these hens, the feeders had to be placed on the floor because of severe ataxia. As a result, food comsumption could not be measured because of spillage. The following table

taken directly from the report summarizes food consumption.

Daily Food Consumption				
	Treatment Group			
Study Day	Water[a] 5 ml/kg	TOCP[a] 500 mg/kg	ICIA-0224[b] 500 mg/kg	ICIA-0224[b] 5000 mg/kg
-14[c]/-13[d]	116 ± 21[e]	91 ± 32	107 ± 13	116 ± 10
-7[c]/-6[d]	130 ± 15	112 ± 17	127 ± 9	125 ± 10
3	124 ± 12	74 ± 14	124 ± 15	42 ± 25
7	111 ± 18	107 ± 21	118 ± 12	111 ± 14
8	110 ± 13	114 ± 13	113 ± 11	124 ± 12
9	118 ± 13	113 ± 17	121 ± 14	109 ± 14
15	121 ± 16	98 ± 16	125 ± 9	104 ± 14
16	120 ± 12	108 ± 7	118 ± 13	105 ± 10
23	125 ± 14	23 ± 22	116 ± 29	43 ± 16
24	124 ± 11	39 ± 25	134 ± 11	90 ± 33
29	143 ± 15	67 ± 65	135 ± 13	144 ± 27
30	133 ± 18	84 ± 38	138 ± 26	135 ± 32
35	-	15 ± 24	-	-
36	122 ± 15	N/A[f]	137 ± 20	127 ± 30
37	131 ± 16	N/A[f]	152 ± 18	127 ± 28

[a]6 hens/group
[b]8 hens/group
[c]Section II hens
[d]Section I hens
[e]Grams of feed/hen/day, x ± S.D.
[f]Unscheduled termination - only one animal left in each section
- Data not available

5. Egg Production: No treatment-related effects were observed in the low dose group. Hens from the high dose group layed fewer eggs after treatment (50% reduction in the weekly rate). This decrease was maximal 2 weeks after treatment and then recovered. The positive control group first showed a transient decrease in egg production and then no eggs were produced after the second treatment. The following table taken directly from the report summarizes the results.

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Weekly Egg Production				
Average Eggs/Week/Hen				
Study Week	Water[a] 5 ml/kg	TOCP[a] 500 mg/kg	ICIA-0224[b] 500 mg/kg	ICIA-0224[b] 5000 mg/kg
-2	4.5	4.7	4.3	4.9
-1	5.8	4.8	5.9	5.8
1	4.7	3.2	5.0	2.8
2	4.8	2.8	5.6	2.0
3	6.0	4.5	6.5	4.9
4	5.8	1.0	5.4	3.6
5	5.5	0.2	5.6	2.6
6	4.3	-[c]	5.0	3.8

[a]6 hens/group
[b]8 hens/group
[c]Early termination

6. Walking Behavior: No treatment-related differences in walking behavior were observed between the treated and control groups. However, the positive control group had "motor deficits that were progressive". These deficits were first observed on day 10 and continued to become worse. After the second dose, the hens became severely ataxic and never recovered. The major signs of motor dysfunction were loss of equilibrium, coordination and strength. The following table taken directly from the report summarizes the results.

Walking Behavior Summary				
	Average Score			
Study Day	Water 5 ml/kg	TOCP 500 mg/kg	ICIA-0224 500 mg/kg	ICIA-0224 5000 mg/kg
-12	0	0	0	0
-4[a]/-5[b]	0	0	0	0
3	0	0	0	0
7	0	0.2	0	0
10	0	2.2	0.1	0.1
14	0	5.8	0.1	0
17	0	7.0	0	0
18[a]/21[b]	0	7.2	0	0
24[a]/25[b]	0	7.2	0	0
28	0	7.5	0	0
31	0	8.3	0	0
35	0	10.8	0	0
37	0	9.5[c]	0	0
38	0	8.0[d]	0	0

[a]Section I hens
[b]Section II hens
[c]n=2
[d]n=1

7. Histopathology: No treatment-related neurological lesions were observed in the treated groups. For the treated groups, the report stated "generally, most tissue sections showed minimal or no pathology except for non-specific reactive changes, i.e. lymphocytic perivascular cuffing (brain) or foci (sciatic nerve) and neuronal swelling (spinal cord). The high dose group had two hens with focal gliosis in brain tissue, one of which also had axonal degeneration in the lumbo-sacral level of spinal cord. In distinct contrast to the TOCP induced axonal degeneration, these sacral cord lesions were found in random locations, not the ventro-medial funiculi." Therefore, the changes were not considered to be biologically significant. The positive control group had specific histopathological changes typical of organophosphate induced delayed neurotoxicity, as evidenced by lesions in every tissue examined. The following table summarizes microscopic results taken directly from the report.

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Summary of Neuropathological Lesion Incidence				
	Number of Hens with Lesion/Group[a]			
Tissue Lesion	Water 5 ml/kg	TOCP 500 mg/kg	ICIA-0224 500 mg/kg	ICIA-0224 5000 mg/kg
Brain				
Axonal degeneration (cerebellar peduncles)	0/6	6/6	0/8	0/8
Lymphocytic perivascular cuffing	1/6	2/6	1/8	3/8
Focal gliosis	0/6	6/6	0/8	2/8
Cervical Spinal Cord				
Axonal degeneration (dorsal funiculi)	0/6	6/6	0/8	0/8
Focal gliosis	0/6	3/6	0/8	0/8
Thoracic Spinal Cord				
Axonal degeneration (ventral & lateral funiculi)	0/6	6/6	0/8	0/8
Focal gliosis	0/6	2/6	0/8	0/8
Neuronal swelling/chromatolysis	0/6	0/6	0/8	1/8
Vacuolation of white matter	0/6	1/6	0/8	0/8
Lumbo-Sacral Spinal Cord				
Axonal degeneration (ventro-medial funiculi)	0/6	1/6	0/8	0/8
Axonal degeneration (random location)	0/6	0/6	0/8	1/8
Neuronal swelling/chromatolysis	1/6	0/6	2/8	2/8
Vacuolation of white matter	0/6	1/6	0/8	1/8
Peripheral Nerve - Sciatic[b]				
Swelling of axis cylinder	0/0[b]	2/2	0/0	0/0
Nerve fiber degeneration (digestion chamber)	0/0	6/6	0/0	0/0
Lymphocytic foci (perineural or interstitial)	3/2	3/4	5/5	6/6
Schwann cell hyperplasia	0/0	6/6	0/0	0/0

[a]Only grade ≥ 2 lesions summarized.
[b]R = right nerve; L = left nerve

8. Quality Assurance Measures: Signed Quality Assurance and Good Laboratory Practice statements were provided.

C. DISCUSSION:

This study was conducted in 1988, prior to the publication of the March 1991 neurotoxicity guidelines. Therefore, the procedures used for the study are according to the old guidelines. As a result, measurements of acetylcholinesterase (AchE) and neurotoxic esterase (NTE) were not conducted. Therefore, on this basis, the study is graded Core Minimum and is considered to have fulfilled the regulatory requirement for an acute delayed neurotoxicity study in hens (81-8).

In the high dose group, two hens with focal gliosis in the brain tissue, one of which also had axonal degeneration in the lumbo-sacral level of spinal cord. In light of the fact that the 1 hen which had axonal degeneration had it in a random place and that there were no clinical signs of

neurotoxicity in this dose group, it is unlikely that this is a biologically significant effect.