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

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: 3F04238; 9F03796; 0F03660; 1H5606; 0F3890; 3F4238; 4F04343; 010182-00324; 010182-00325. Touchdown® Herbicide. Petition for Tolerances In or On Stonefruit and Nut Crop Group (Except Almonds); Resubmission of Petition for Tolerances In or On Corn and Soybeans, Grapes and Citrus; and Review of Submitted Neurotoxicity Studies

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Submission Nos. S460634, S446081, S446079, S460461, S462572, S462590, S460635, S460639

TO: Edward Allen, PM Team # 25
Registration Division (7505C)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 8/23/94
Section I, Toxicology Branch I
Health Effects Division (7509C)

THRU: Roger L. Gardner, Section Head
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Health Effects Division (7509C)

Background and Request:

Zeneca Ag Products has either submitted new petitions or resubmitted old petitions for a variety of tolerances for glyphosate-trimesium. The requested tolerances are as follows: for residues of (N-phosphonomethyl) glycine (carboxymethylamino methyl phosphonate) resulting from application of the trimethylsulfonium salt in or on stone fruit, the nut crop group (except almonds), corn (grain, forage and fodder), soybeans (seed, forage and hay), grapes and citrus fruits. The submitted label for the stonefruit and nut crop group is for Touchdown® Herbicide, containing 57.6% active ingredient. It contains 6 pounds active ingredient per gallon. It is to be applied as a

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broadcast and/or a spot spray. For soybeans, the pesticide may also be applied using a wiper or "wick" applicator. For nonbearing crops and bearing tree fruit, no more than 5-1/3 pints of Touchdown® are to be used per acre/year. For soybeans, citrus and grapes no more than 4 pounds a.i./acre/year will be applied. The amounts to be used on corn were not available. Based on the labels and on previous Toxicology Branch (TB-I) reviews, it appears that the formulation, Touchdown 4LCE (39.8% a.i.) will be used on corn, soybeans and citrus fruits and that Touchdown® concentrate and Touchdown® 6 (both end-use products that are supported by the available toxicology data base) will be used on grapes and citrus fruits. It is noted that in a phone call to the Registrant on August 23, 1994, the Registrant stated that if the labels refer to the 4LC formulation, it is actually the 4LCE formulation. The 4LC formulation is not a currently registered product.

The requested tolerances are as follows:

Stonefruit: 0.05 ppm
Nut Crop Group (Except Almonds): 0.05 ppm
From TB-I response dated 5/13/91:
Corn, grain: 0.1 ppm
Corn, forage: 0.1 ppm
Corn, fodder: 0.2 ppm
From TB-I response memos, all dated 08/03/92:
Soybean seed: 2.0 ppm
Soybean forage: 1.0 ppm
Soybean hay: 3.0 ppm
Whole grapes: 0.2 ppm
Dried pomace: 0.4 ppm
Citrus fruits: 0.5 ppm

In addition to the petitions for tolerances, Zeneca Ag Products has submitted the acute delayed neurotoxicity study in hens (81-7), the acute mammalian neurotoxicity study in rats (81-8) and the subchronic mammalian neurotoxicity study in rats (82-7) in response to the TB-I request for these studies for the requested tolerances. TB-I had also requested an acute inhalation study on Touchdown 4LCE, the 39.9% a.i. formulation. The Registrant states that this study had already been submitted and reviewed by the Agency (MRID 414260-01) and accepted.

TB-I has been asked to review the neurotoxicity studies and to determine whether or not the toxicology data base supports these requested tolerances.

Toxicology Branch Response:

TB-I has reviewed the submitted neurotoxicity studies conducted with glyphosate-trimesium and has determined that they are acceptable for regulatory purposes. They satisfy the regulatory requirements for neurotoxicity studies 81-7, 81-8 and 82-7.

The toxicology data base for this chemical is complete. TB-I has no objection to granting the requested petitions for tolerances on stone fruit, the nut crop group (except almonds), corn and soybeans, grapes and citrus.

An acute inhalation study on a 480 g/l SL formulation of sulfosate (MRID 414260-01) was reviewed by TB-I in a memorandum from N. Thoa to R. Taylor, dated 6/19/90. It was classified as Acceptable and is in Toxicity Category II. In a phone call to the Registrant on August 23, 1994, the Registrant verified that the 480 g/l SL formulation is the same as the SC-0224 4LC-E formulation. Therefore, the regulatory requirement for this study has been satisfied.

In previous responses by TB-I, it is noted that in the Toxicity Profile, a 21-day dermal study needs to be conducted on the 4LC formulation. A 21-day dermal study has already been conducted on the technical material and on the 4LCE formulation. Since the 4LC formulation is not going to be registered for use, this issue has been resolved.

Glyphosate-trimesium was reviewed by the Health Effects Division (HED) RfD/Peer Review Committee on March 10, 1994. The RfD is calculated to be 0.1 mg/kg/day based on a no-observable effect level (NOEL) of 10 mg/kg/day from the 1 year dog study. The uncertainty factor was 100. The LEL for the dog study was 50 mg/kg/day based on single episodes of emesis and transient salivation. These results were supported by the results of the 28-day and 90-day studies in the dog. The Committee classified glyphosate-trimesium in Category E for carcinogenicity.

The RfD/Peer Review Committee changed the systemic NOEL and LEL for the chronic feeding/carcinogenicity study in the rat, the paternal NOEL and LEL for the 2-generation reproduction study in the rat and the NOEL and LEL for developmental toxicity in the rabbit. These changes are as follows:

Study	Old NOEL/LEL	New NOEL/LEL
2-Year Chronic Feeding: rat	100 ppm / 500 ppm	1000 ppm (NOEL: HDT)
2-Generation Reproduction: rat	Paternal: 800 ppm / 2000 ppm	Paternal: 150 ppm / 800 ppm
Developmental Toxicity: rabbit	Developmental Toxicity: 100 mg/kg/day (NOEL - HDT)	Developmental Toxicity: 40 mg/kg/day / 100 mg/kg/day

Glyphosate-trimesium was also reviewed by the Less-than Lifetime Committee for short term risk assessments. A risk assessment for acute dietary exposure is not required because no appropriate toxicological endpoints were found. The short term (1 to 7 Days) and the intermediate term occupational or residential (1 Week to Several Months) risk assessments are not required because when compared with the oral studies, the 21-day dermal studies indicate that there is not much dermal absorption.

The following paragraphs summarize the neurotoxicity studies reviewed for the tolerance requests.

Acute Delayed Neurotoxicity Study in Hens (81-7)

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.

Acute Mammalian Neurotoxicity Study in Rats (81-8)

Glyphosate Trimesium was tested in an acute neurotoxicity study in male and female Alderley Park Alpk:APfSD rats. Ten rats/sex were tested at each dose level, one time by gavage at 1 ml/100 g body weight. The following doses were tested: 0, 30, 100 or 300 mg/kg. Positive control data were provided.

At 300 mg/kg, the following effects were observed: death (2 on day 1); clinical signs of toxicity (ptosis (day 1), decreased activity (days 1-2), reduced splay reflex (days 1-4), upward curvature of the spine (days 1-5), chromodacryorrhea (days 1-3), shaking (days 1-3), sides pinched in (days 1-4), signs of urinary incontinence (day 1), irregular breathing (day 1), hunched posture (days 1-7), abnormal or staggering gait (days 2-7) and staining around the nose, in some cases up to days 4-7); reduction in bodyweight in males on days 8 (9.5% less) and 15 (5.4% less); up to a 75.9% reduction in food consumption in males; increase in time to tail flick (273 - 281% of controls, 1-2 hours after dosing on day 1); reduction in landing foot splay (77 - 83% of controls, 1-2 hours post dosing on day 1); reductions in forelimb grip strength (82 - 85% of controls, 1-2 hours post-dosing on day 1); reduction in hindlimb grip strength (82% of controls, day 1) and reduction in motor activity (5.9 - 48.4% of controls, first hour after dosing on day 1). The results of the latter screening battery for neurotoxicity were not apparent on days 8 or 15 post-dosing, indicating that the effects were reversible. There was no microscopic evidence of neurotoxicity. No effects were observed at dose levels of 100 mg/kg or below.

The NOEL is 100 mg/kg and the LEL is 300 mg/kg based on death, clinical signs of toxicity, reduction in bodyweight and food consumption and effects on time to tail flick, landing foot splay, forelimb grip strength, hindlimb grip strength and motor activity during the first day after dosing. These were reversible by day 8. There was no microscopic evidence of neurotoxicity. There were no indications of neurotoxicity below at lethal dose.

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

Subchronic Mammalian Neurotoxicity Study in Rats (82-7)

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).

The following summarizes the toxicity testing requirements for the requested uses and tolerances. A Toxicity Profile is attached for the technical material and for 1 formulation. In addition, the Data Evaluation Records (DER's) for the newly reviewed neurotoxicity studies along with the studies that have been revised by the RfD Committee are also attached.

Data Requirements (CFR 158.135):

Technical: Glyphosate Trimesium
Use Pattern: Herbicide; broadcast and/or spot spray; wiper or "wick" applicator (soybeans only).
Action Type: Tolerance requests

Last Updated: 08/22/94

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Yes
81-3	Acute Inhalation Toxicity	Yes	Yes
81-4	Primary Eye Irritation	Yes	Yes
81-5	Primary Dermal Irritation	Yes	Yes
81-6	Dermal Sensitization	Yes	Yes
81-7	Acute Delayed Neurotoxicity	Yes	Yes
81-8	Acute Mammalian Neurotoxicity	Yes	Yes
82-1(a)	Subchronic Oral (rodent)	Yes	Yes
82-1(b)	Subchronic Oral (non-rodent)	Yes	Yes
82-2	21-Day Dermal	Yes	Yes
82-7	Subchronic Mammalian Neurotoxicity	Yes	Yes
83-1(a)	Chronic Toxicity (rodent)	Yes	Yes
83-1(b)	Chronic Toxicity (nonrodent)	Yes	Yes
83-2	Oncogenicity (mouse)	Yes	Yes
83-5	Oncogenicity (rat)	Yes	Yes
83-3(a)	Teratology (first species)	Yes	Yes
83-3(b)	Teratology (second species)	Yes	Yes
83-4	Multigeneration Reproduction	Yes	Yes
84-2(a)	Mutagenicity - Gene Mutation	Yes	Yes
84-2(b)	Mutagenicity - Structural Chromosomal Aberrations	Yes	Yes
84-2(c)	Mutagenicity - Other Genotoxic Effects	Yes	Yes
85-1	Metabolism	Yes	Yes

Formulation: Touchdown 4LCE (39.8% a.i.)

Last Updated: 8/22/94

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Yes
81-3	Acute Inhalation Toxicity	Yes	Yes
81-4	Primary Eye Irritation	Yes	Yes
81-5	Primary Dermal Irritation	Yes	Yes
81-6	Dermal Sensitization	Yes	Yes
82-2	21-Day Dermal	Yes	Yes

SULFOSATE (Caswell No. 693C; PD No. 128501)

SULFOSATE TECHNICAL (52% a.i.)
Updated 08/22/94

81-1	Acute Oral Toxicity in Rats. MRID 249802 STAUFFER CHEMICALS # T11185 November, 1982. Acceptable	LD_{50} = 748 mg/kg (males) LD_{50} = 755 mg/kg (females) Doses used: 500, 550, 600, 700, 800, and 900 mg/kg by gavage Signs: mild to severe depression, prostration, tremors, and slow/shallow respiration. Product tested: SC-0224 62% a.i. TOXICITY CATEGORY: 3
81-2	Acute Dermal Toxicity in Rabbits MRID 249802, 260508 Stauffer CHEMICALS # T-11185 November, 1982. Acceptable	LD_{50} > 2000 mg/kg (Both sexes; intact or abraded skin). Doses used: 800 -2200 mg/kg. Signs: Rabbits with braded skin showed mild to sever: depression at all doses levels and mild to moderate erythema. Rabbits with skin intact showed mild depression and mild erythema. Product tested: SC-0224 62% a.i. TOXICITY CATEGORY: 3
81-3	Acute inhalation toxicity in rats MRID 249802 Stauffer Chem No. T-11084 November, 1982 Acceptable	LC_{50} > 6.9 mg/L (both sexes, 4-hr, whole body exposure) Actual chamber concentration: 6.9 mg/L MMAD = 3.5 um at 64 min. 2.8 um at 184 min. SIGNS: wet fur, salivation, chromorhinorrhea Product tested: Sulfosate (62% a.i.) TOXICITY CATEGORY 3

81-3	Acute inhalation toxicity in rats MRID 412359-01 ICI No: CTL/P/2254 08/25/88 Unacceptable	LC_{50}> 5.18 mg/L (4-hr, nose only exposure) Actual chamber concentration: 2.65-6.3 mg/L MMAD: 4.56 ± 2.06 um [20% ≤ 2.5 um (inhalable) & 3.9% ≤ 1 um (respirable)] No mortality observed. SIGNS: (CNS & Autonomic) salivation, splayed gait, head & paw flicking, tail erection, shaking, subdued behavior, slow/deep breathing, decrease response to sound. Effects subsided on day 2. A limit test was not reached since only 3.9% of the aerolised sulfosate particles were of respirable size (EPA requires 25%). Product tested: Sulfosate 57.6% a.i. and [redacted]. This study may be upgraded to acceptable when evidences are provided to show that optimum technology was used in generating the sulfosate containing aerosol. TOXICITY CATEGORY:
81-4	Primary Eye Irritation in Rabbits MRID 249802 STAUFFER CHEMICALS # T-11185 November, 1982. Acceptable	No effect on cornea. Effects on unwashed eyes: mild iritis (1/6 rabbits), and mild conjunctivitis (6/6 rabbits) at 24 hr (Draize score). All effects reversible by day 7. Effects on eyes washed after 20-30 sec. exposure: mild conjunctivitis (3/3 rabbits) lasting 3 days. Dose used: 0.1 ml SC-0224 62% a.i. TOXICITY CATEGORY: 3 (based on mild irritation of conjunctiva).

81-5	Primary Dermal Irritation in Rabbits MRID 249802 STAUFFER CHEMICALS # T-11185 November, 1982. Acceptable	24-hr exposure. Effects at 24 hr: intact and abraded skin showed mild erythema. Mild edema observed in 3/6 rabbits with skin abraded and 1/6 rabbits with skin intact. All dermal effects reversed within 72 hrs. Primary Irritation Score: 0.67. Dose used: 0.5 ml SC-0224 62% a.i. TOXICITY CATEGORY: 4
81-6	Dermal Sensitization in Guinea Pigs MRID 258398 Richmond Tox. Labs. # T-11269 October 12, 1984. Acceptable	SC-0224 Technical (56.3% a.i) is a mild skin sensitizer (Open Epicutaneous Test)

81-7	Acute Delayed Neurotoxicity Study in Hens MRID 431512-01 ICI Toxicology Labs CA/# T-12324; 4/18/89 Core Minimum	NOEL: for systemic toxicity: 500 mg/kg LEL: for systemic toxicity: 5000 mg/kg Effects: Administered by gavage at 0, 500 or 5000 mg/kg in 5 ml/kg water. TOCP (500 mg/kg) was positive control. 6 hens/group in control groups, 8 hens/group in treated groups. Each animal dosed twice during study; day 1 & day 22. Each animal evaluated up to day 41 (or 42). 500 mg/kg: diarrhea for 2-3 days, starting a few days after each dosing. 5000 mg/kg: diarrhea, changes in comb appearance, early ↓ food consumption & ↓ in egg production 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. Study is core minimum because it was conducted prior to the publication of the new neurotoxicity guidelines which were published in 1991.
81-8	Acute Mammalian Neurotoxicity Study in Rats MRID 431323-01 Zeneca Central Tox. Labs #'s AR5425; CTL/P/3813 2/15/93 Core Guideline	NOEL: 100 mg/kg LEL: 300 mg/kg Effects: Alpk:APfSD rats. Doses: 0, 30, 100 or 300 mg/kg. Positive control data provided. 300 mg/kg: death, ptosis, ↓ activity, ↓ splay reflex, upward curvature of spine, chromodacryorrhea, shaking, sides pinched in, signs of urinary incontinence, irregular breathing, hunched posture, abnormal or staggering gait, staining around nose, ↓ bodyweight (♂),↓ food consumption (♂), ↑ time to tail flick, ↓ landing foot splay,↓ forelimb grip strength, ↓ hindlimb grip strength, ↓ motor activity. Effects reversible. No microscopic evidence of neurotoxicity. No indications of neurotoxicity below a lethal dose (300 mg/kg).

82-1(A)	Subchronic feeding rat MRID 412099-02 Stauffer Chem No. T-10888 4-3-87 Acceptable	NOELs: 800 ppm (MDT, 36 mg/kg/day) in males and 2000 ppm (HDT. 108 mg/kg/day) in females. LOEL: 2000 ppm (88 mg/kg/day) in males, based on a significant overall decrease in body weight gain (22% below controls). The HDT only caused sporadic and minimal decreases in body weight in females (secondary to a feed palability - related reduction in feed intake) and no significant overall decrease in B.W. gain. No significant changes were observed in clinical chemistry, hematology, urinalysis, organ weights, or macroscopic/microscopic histopathology. Doses tested: 0, 150, 350, 800, and 2000 ppm. MTD was reached for males only. Product tested: Sulfosate (19.2% a.i., [redacted])
82-1(b)	Subchronic feeding dog MRID 412099-02/03 Stauffer Chem No. T-11002 4-3-87 Acceptable	NOEL: 10 mg/kg/day (MDT) LOEL: 50 mg/kg/day (HDT) based on increase incidences and earlier onset of emesis and salivation. No changes in B.W., food consumption, clinical chemistry, hematology, urinalysis, organ weights, or macroscopic/microscopic histopathology were observed. Doses tested: 0, 2, 10, and 50 mg/kg/day by gavage. Dog's Strain: Beagle Product tested: Sulfosate (19.2% a.i., [redacted]).
82-2	21-day dermal - rabbit MRID 408937-02 Hazleton No. HLA6142-107 3-1-88 Guideline	Systemic NOEL: 1000 mg/kg/day (HDT) Mild erythema at application sites in all treated groups. Doses tested: 0, 10, 100, and 1000 mg/kg/day 6 hrs/day; 5 days/week; 3 weeks. Product tested: Sulfosate (57.0% a.i.).

82-7	Subchronic mammalian neurotoxicity rat MRID 431512-02 Zeneca Central Tox. Labs Study #'s PRO887; CTL/P/3831 2/15/93 Core Guideline	NOEL: 600 ppm (47.6 mg/kg/day) LOEL: 2000 ppm (153.2 mg/kg/day) Effects: Alpk:APfSD rats: 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 diet for 90 days. 12/sex/dose group. Positive control data were provided. 2000 ppm: ↓ body weights, food consumption & utilization, ↓ mean forelimb grip strength values (♀). No microscopic evidence of neurotoxicity. Evidence for neurotoxicity is not clear.
83-1a 83-2b	Feeding/Oncogenic (2-year) in Mice MRID 402140-06 412099-07 Stauffer Chem No. T-11813 4/3/87 Guideline	Systemic NOEL: 1000 ppm (MDT) Systemic LOEL: 8000 ppm based on decreases in B.W. and feed consumption (both sexes), increases incidences of white matter degeneration in lumbar spinal cord (males only), and increase incidences of duodenal epithelial hyperplasia (females only). Not oncogenic at dose levels up to and including 8000 ppm. Highest dose level may have been excessive. Doses used: 0, 100, 1000, and 8000 ppm Mice strain: Charles River Test material: Sulfosate 56.17% a.i.
83-1a 83-2a	Feeding/Oncogenic (2-year) in Rats MRID 402140-07 412099-05 Stauffer Chem No: T-11082 4/4/87 Guideline	Systemic NOEL: 1000 ppm (HDT) There were decreases in bodyweight (both sexes) and increased incidences of chronic laryngeal and nasopharyngeal inflammation (males). Bodyweight decrease was considered to be secondary to reduction in food consumption. However, study was acceptable because top dose may be approaching at least 1/2 of an adequate dose for carcinogenicity testing (based on results from subchronic, reproduction studies). Not oncogenic at any level tested. Doses used: 0, 100, 500, and 1000 ppm Rats strain: Charles River CrL:CD (SD)BR. Test material: Sulfosate 56.17% a.i.

83-1(b)	Chronic Feeding (1-year) in Dogs MRID 402140-05 Stauffer Chem. No: ECH T-11075 4/3/87 Minimum	Systemic NOEL: 10 mg/kg/day (MD) Systemic LOEL: 50 mg/kg/day (HD) based on decreases in LDH. Doses used: 0, 2, 10, and 50 mg/kg/day, by gavage. Selection of above dose range was based on (i) a 28-Day oral gavage study in which 150 mg/kg/day was lethal within 3 days and 75 mg/kg/day produced emesis, and (ii) a 90-Day study in which 50 mg/kg/day produced increase in emesis and salivation. Dog's Strain: Beagle Test material: Sulfosate 56.2% a.i.
83-3(a)	Teratogenicity in Rats MRID 249802 Stauffer Environ. Health Cen. No: T-11050 November 1982 Minimum	Developmental NOEL: 100 mg/kg/day Developmental LOEL: 333 mg/kg/day based on significant decreases in fetal bodyweight Maternal NOEL: 100 mg/kg/day. Maternal LOEL: 333 mg/kg/day based on undetermined deaths of 2 dams at HDT; decreases in bodyweight, bodyweight gain and feed intake; increased salivation, chromorhinorrhea and lethargy (HDT). Doses used: 0, 30, 100, and 333 mg/kg/day by gavage to S-D rats. Test material: Sulfosate 19.2% a.i.
83-3(b)	Teratogenicity in Rabbits MRID 260966 Stauffer Chem. No: T-11052 6/21/83 Guideline	Developmental NOEL: 40 mg/kg/day Developmental LOEL: 100 mg/kg/day (reduction in number of live fetuses/doe, 4 abortions & having only 7 litters does not give sufficiently high # of animals to absolutely conclude that no developmental toxicity is occurring. Maternal NOEL: 40 mg/kg/day (MDT) Maternal LOEL: 100 mg/kg/day (HDT) (6 deaths/17 pregnant does, 4 abortions in 11 survivors, decreased body weight, body weight gain, food consumption). Doses used: 0, 10, 40, and 100 mg/kg/day by gavage to Dla;(NZW)SPF rabbits. Test material: Sulfosate 56.2% a.i.

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83-4	Reproduction (2-gen) in Rats Accession Nos: 258399 & 258399 Stauffer Chem. No: T-110-51 4/19/84 Guideline	Reproductive/Developmental NOEL: 150 ppm (LDT) Reproductive/Developmental LOEL: 800 ppm (MDT) based on decreased litter size in the F_{0a} and F_{1b} litters at 2000 ppm and on decreased mean pup weights during lactation in the second litters at 800 ppm and in all litters at 2000 ppm. Systemic NOEL: 150 ppm (LDT) Systemic LOEL: 800 ppm (MDT) based on reduced feed intake, body weights & body weight gains, reduced absolute and sometimes relative thymus, heart, liver & kidney weights. Doses used: 0, 150, 800, and 2000 ppm in Crl CD(SD)Br strain. Test material: sulfosate 19.2% a.i.
84-2(a)	Mutagenicity Reverse mut. (Ames Test) in Salmon. Typhi. MRID 249802 Stauffer Chem. No:T-10487 1/19/82 Acceptable	Not mutagenic at concentrations of 0.12, 0.37, 1.11, 3.33, and 10 mg/plate without S9, and of 0.56, 1.11, 1.67, 3.33, 5.0, 10, and 15 mg/plate with S9. Tester Bacteria: TA1535, TA1537, TA1538, TA98, and TA100 from Dr. Ames. Pos. controls: Na azide, 9-aminoacridine (9-AA), 2-nitrofluorene (2-NF), and 2-aminoanthracene (2-AA). Test material:sulfosate 90% a.i (estimated purity).
84-2(a)	Mutagenicity Reverse mut. (Ames Test) in Salmon. Typhi. MRID 260966 Stauffer Chem. No: T-12660 9/25/85 Acceptable	Not mutagenic at concentrations of 2.5, 5, 10, 20, and 40 ul/plate, with or without S9. Tester Bacteria: TA1535, TA1537, TA 98, and TA100. Pos. controls: Na azide, 9-AA, 2-NF. Cytotoxic Dose: HDT Test material: Sulfosate 55.6% a.i.

84-2(a)	Gene Mutation (SLRL) in Drosophila melanoga MRID 249802 Litton Bionetics No: 22169 6/13/82 Acceptable	Not mutagenic at doses of 25 and 50 mg/ml in "Sex linked recessive lethal test". Pos. control: EMS
84-2(a)	Gene Mutation (Forward Mut.) Mouse Lymphoma MRID 249802 Stauffer Chem T-10848 2/8/1982 Acceptable	Not mutagenic without S9. Significant reproducible increase in mutation frequency in presence of S9. Test medium pH not mentioned but was probably in the acid range. Indicator cells: L5178Y ($TK^{+}/^{-}$) mouse lymphoma cell line from Dr. Clive, RTP, No.Carolina). Concentrations used: 0.38, 0.75, 1.50, 3, 6, 8, 8.5, 9, and 10 mg/ml in presence of S9, and 0.38, 0.75, 1.5, 3, 6, 7, 8, 9, and 10 mg/ml w/o S9. Cytotoxic concentrations: >7 mg/ml
84-2(a)	Gene Mutation (forward mut.) Mouse Lymphoma MRID 260966 Stauffer Chem. No. T-12661 12/19/1985 Acceptable	Introduction of sulfosate in the test incubation medium reduced its pH to an acid range (5.67 -7.07). Under this experimental condition, sulfosate was positively mutagenic both in the presence of S9, at concentrations of 3-5 ul test material/ml, or without S9, at concentrations of 3.5 to 5ul/ml). When the pH of test incubation medium was readjusted to a physiological level of 7.4 (Addendum of 3/20,1987), concentrations from 5 to 10 ul/ml lost their mutagenic effect Indicator cells: L5178Y($TK^{+}/^{-}$) mouse lymphoma cell line (Dr. Clive, RTP, No.Carolina). Test material:Sulfosate 55.6% a.i. Cytotoxic concentrations: Unadjusted acidic medium: >5ul/ml pH adjusted medium: >7.75 ul/ml Pos. controls: N-Nitrosodimethyl-amine (DMN) with S9 and Ethyl-methanesulfonate (EMS) wo S9.

84-2(b)	Mutagenicity Cytogenetic Rat bone marrow MRID 249802 Stauffer Chem. No: T-10884 september 1982 Acceptable	Test animals: 6-wk old CD-Crl:CoBScd(SD)BR male rats. Not mutagenic (did not induce any structural chromosome aberrations in rats' bone marrow cells. Doses used: 21, 63, and 188 mg/kg (LD_{50}= 565 mg/kg). Test material: sulfosate 58.5% a.i. Pos. control: cyclophosphamide
84-2(b)	Mutagenicity (Micronucleus assay) Mouse bone marrow MRID 402140-04 412099-08 Stauffer Chem. No: EHC-T-12689 4/23/87 Acceptable	Test animals: Charles River D-1 str. Not mutagenic (did not induce any increase in the number of PCE containing micronuclei). Doses used: 700, 900, and 1100 mg/kg in males and 400, 600, and 800 mg/kg in females, based on results of a range finding study in which doses >1400 mg/kg killed 3/3 males within 48 hrs and doses >1000 mg/kg killed 2/3 females.
84-2(b)	Mutagenicity (Cytogenetic) in CHO cells MRID 249802 Stauffer Chem. No: T-10875 7/6/1982 Acceptable	Positive mutagenicity (induces structural chromosomal aberration in CHO cells both in the absence of S9, at the concentration of 4 mg/ml, and in its presence, at concentrations of 10 and 12 mg/ml. Sister chromatid exchange (SCE) was not determined. Concentrations used: 2, 4, and 6 mg/ml w/o S9 and 2, 4, 6, 8, 10, and 12 mg/ml with S9. Test material: Sulfosate 58.5% a.i.
84-2(b)	Mutagenicity (Cytogenetic) in CHO cells MRID 249802 Stauffer Chem. No: T-11019 7/22/82 Acceptable	Positive mutagenicity (Induces structural chromosomal aberration in CHO cells both in the absence of S9, at concentrations of 6-8 ul/ml, and in its presence, at 1-8 ul/ml. No increase in SCE was observed. Concentrations used: 2, 4, 6, 8, 10, and 12 ul/ml. Test material: Sulfosate 72% a.i.

84-2(b)	Mutagenicity (cytogenetic) in CHO cells MRID 260966 Stauffer Chem. No: EHC T-12663 12/18/1985 Acceptable	pH of treatment medium was readjusted to 7.4-7.6 prior testing. Not mutagenic (did not induce any structural chromosome abberrations in CHO cells or any increase in SCE) at concentrations of 4-10 ul/ml, with or w/o S9. Cytotoxic concentrations: None Pos. controls: Mitomycin C and Cyclophosphamide. Test material: sulfosate 55.6% a.i.
84-2(b)	Mutagenicity (cytogenetic) Mouse Lymphoma MRID 260966 Stauffer Chem. No: EHC T-12662 12/19/82 Acceptable	Indicator cells: L 5178Y (TK+/-) mouse lymphoma cell line from Dr. Clive, RTP, No.Carolina). Sulfosate concentrations of 5 ul/ml (w/o S9) and >3 ul/ml (w S9) induced chromosomal aberrations in the mouse lymphoma cells and increased the number of SCEs when the pH of the test medium was not readjusted (5.62-7.07). When the pH was readjusted to 7.4 concentrations from 4-10 ul/ml were not mutagenic. Cytotoxic concentrations: >5 ul/ml at acidic pH, and < 10 ul/ml at physiological pH. Pos. controls: Ethyl methane-sulfonate & N-nitrosodimethylamine. Test material: 55.6% a.i.
84-4	Mutagenicity BALB/3T cells (morphological transformation) MRID 249802 Stauffer Chem. No: T-10849 1/4/82 Acceptable	Indicator cells: 1-1 subclone of clone A-31 of BALB/3T3 mouse cells from Dr. Kanunaga (NCI). Not mutagenic (did not induce an increase in the number of transformed foci) Concentrations used: 0.313, 0.625, 1.25, 2.5, and 5 mg/ml . Cytotoxic concentrations: >3 mg/ml Test material: sulfosate 90% estimated purity.

85-1	Metabolism in Rats MRID 258398 Stauffer Chem. PMS-148 2/4/85 Acceptable	Test material: (Methyl ^{14}C) trimethylsulfonium Carboxymethylaminomethylphosphonate) 96.5% purity, 20 mci/mmol. Identification of the (Methyl ^{14}C) trimethylsulfonium ion (^{14}C-TMS) in urine and fecal extracts done by TLC, GC/MS, autoradiography, and K iodoplatinate spray. After oral administration of 35 mg/kg (LDT) or 350 mg/kg (HDT) test material to S-D rats of both sexes, the ^{14}C-TMS ion is rapidly and almost completely absorbed from the GI tract and rapidly excreted unmetabolized mostly via the kidney. Urine recovery of ^{14}C (expressed as % of administered dose were: 80.8-95% at 24 hr and 91.4-98.5 at 120 hr. Most (95.3-97%) of the total radioactivity was unmetabolized ^{14}C-TMS ion. Fecal recovery of ^{14}C (expressed as % of administered dose were: 0.72-4.03% at 24 hr and 0.95-7.19% at 120 hr. All the radioactivity was unmetabolized ^{14}C-TMS ion. $^{14}CO_2$ in expired air was negligible. Tissues residues were negligible: 0-0.148 (LD) and 0-10.6 ppm (HD) sulfosate equivalents. The lack of metabolism may be explained by the hydrophilic nature of TMS ion. Acute toxic effects at the HDT: lethargy, ataxia, slow/labored breathing, salivation, occasional tremors. Signs lessened after 24 hrs.

85-1 Metabolism in Rats
MRID 412359-03
ICI Americas Inc.
No: T-12906
12/20/88

Acceptable

Test material: Trimethylsulfonium Carboxymethylaminomethylphosphonate ^{14}C-radiolabeled on the anionic moity (Carboxymethylaminomethyl-phosphonate), 93.2% radiopurity, 9.8 mCi/mmol.
Identification of anion by TLC, autoradiograhy, and GC/MS.
Males and females S-D rats iv-treated with 25 mg/kg (LDT) test material excreted 90% of the administered dose in urine.
After oral administration of the LDT or the HDT (250 mg/kg), the test material was rapidly excreted in urine and feces (70-82% of the total radioactivity administered was excreted within 24 hrs, and 85-94% within 120 hrs).
Absorption was incomplete: only 47-57% of total radioactivity was recovered in urine. Fecal excretion was 36-42% of the administered dose.
Most of the recovered radioactivity was unmetabolized carboxymethyl-aminomethylphosphonate (80-90% of urine and 77-96% of feces total radioactivity).One fecal metabolite was aminomethylphoshonic acid (8.5% of total fecal radioactivity in female rats dosed repeatedly (14 single daily LD of unlabeled test material followed by a single LD of labeled test material._
$^{14}CO_2$ in expired air was negligible.
Combined tissue residues were only $\geq$0.32% of administered dose.
Carcasses contained 2.25% of the administered dose, most of it located in bones.
Acute toxic signs observed with the HD: lethargy, moderate/severe depression, tremors, dehydration, and reduced feed consumption. Signs lasted 72 hours.

FORMULATION TOUCHDOWN 4LCE (39.8% a.i.)
Updated 8/22/94

81-1	Acute Oral Toxicity in Rats. MRID 408938-02 STAUFFER CHEMICALS # T12589 2/12, 1987. Acceptable	LD_{50} = 1760 mg/kg (males) LD_{50} = 1298 mg/kg (females) SIGNS: depression, hypersensitivity to touch and sound. NECROPSY: dark livers, spleens, and/or lungs, and test-like material in GI tract. TOXICITY CATEGORY: 3
81-2	Acute Dermal Toxicity in Rabbits MRID 408938-02 Stauffer CHEMICALS # T-12589 November, 1987. Acceptable	LD_{50}> 2000 mg/kg (Both sexes) SIGNS: mild depression and diarrhea. TOXICITY CATEGORY: 3
81-3	Acute inhalation toxicity in rats MRID 408938-03 Stauffer Chem No. T-12983 6/22/1987 Unacceptable	A respirable aerosol could not be generated: the test material was highly viscous and formed excessive foam. Registrant was advised to pursue additional testing. Ways to reduce foaming were suggested (Dilute test material, reduce surfactants) as well as ways to improve particulation (form dense fog and run through a cyclone separator to remove large particles). TOXICITY CATEGORY: Not classified

81-3	Acute inhalation toxicity in rats MRID 414260-01 ICI Central Tox. Labs CTL/P/2848 2/7/90 Acceptable	ICIA0224, 480 g/l SL formulation (unclear if this is 4LCE (39.8% a.i.formulation) tested nose only for 4 hours at atmospheric total particles concentration of 0.83 ± 0.15 mg/l (MMAD ± SD = 1.62 μm, of which 13.8% were ≤ 1 μm. No deaths. Salivation, lacrymation, reduction in activity, tail erection which were short lasting and piloerection, upward curvature of spine, mucoid nasal discharge which were resolved after several days. LC_{50} > 0.83 ± 0.15 mg/l. Toxicity Category II. Distribution of respirable size particles below required 25% level. However, best efforts were made to realize the highest attainable stable concentration of the test materical which had the smallest achieved particle size. TOXICITY CATEGORY: II
81-4	Primary Eye Irritation in Rabbits MRID 408938-02 STAUFFER CHEMICALS # T-12589 2/12/1987. Acceptable	Unwashed eyes: Moderate iritis, and mild to moderate conjunctival irritation. Effects cleared by day 7. Eyes washed: (Exposure of 20-30 sec.) Mild to moderate conjunctival irritation. Dose: 0.1 ml (pH of test material= 5.85). TOXICITY CATEGORY: 3
81-5	Primary Dermal Irritation in Rabbits MRID 408398-02 STAUFFER CHEMICALS # T-12589 2/12/1987 Acceptable	Non-irritating (4-hr exposure) TOXICITY CATEGORY: 4

81-6	Dermal Sensitization in Guinea Pigs MRID 408398-04 Stauffer Chem. No.T-12588 8/4/1987 Acceptable	Not a skin sensitizer (Modified Buehler test).
82-2	21-Day Dermal in Rats MRID 412099-04 Ciba-Geigy Corp. No: CTL/P/2496, LR0535 7/7/89 Acceptable	Doses: 25, 250, 1000 mg/kg/day (6hr/day/21 days) in 0.0021, 0.0027, and 0.0826 ml/100 g B.W. NOEL= 250 mg/kg (MDT) EFFECTS: dermal irritation in HDT males (dermal histology was normal). Slight increase in testes weight at 25 and 1000 mg/kg/day with normal histology. Occasional sciatic nerve fiber degeneration (1 male and 2 fem. out of a total of 10) at 1000 mg/kg/day.

Data Gaps: None

Actions Being Taken to Obtain Additional Information or Clarification: N/A

Reference Dose (RfD):

The recommended RfD (to the RfD Workgroup) is 0.1 mg/kg/day. This value was calculated by using the chronic dog feeding study NOEL of 10 mg/kg/day and a safety factor of 100. This RfD has been verified or approved by the Health Effects Division RfD Committee.

Pending Regulatory Actions: None

Toxicologic Issues Pertinent to This Request:

This chemical has been classified as a Group E Carcinogen: no evidence of carcinogenicity in rat and mouse studies.

Technical sulfosate is usually supplied as an aqueous solution containing about 52% active ingredient. The very viscous nature of sulfosate precludes the practical manufacture of a technical grade with a standard a.i. content (sulfosate forms an intractable glass-like product if its [redacted] content is ≤ 30%). The various "technical grade sulfosates" in the "Toxicological Profile" above are either an aqueous sulfosate concentrate containing 62% a.i. or aqueous dilutions of this concentrate to a.i. concentrations of 19.2, 52 or 56.17%.

The previous data gaps for neurotoxicity have been fulfilled.

In some of the in vitro mutagenicity tests conducted in 1982, sulfosate induced a false positive mutagenic effect. These studies were submitted with accession number 249802 (study numbers T-10848 (forward mutation/mouse lymphoma cells), T-10875 (structural chromosomal aberrations/CHO cells) and T-11019 (structural chromosomal aberrations/CHO cells)). A common feature of these tests was that the pHs of the test incubation media were acidic (pH 5.67-7.07) due to the addition of sulfosate. These positive results were no longer observed [accession number 260966 (study numbers T-12661 (forward mutation/mouse lymphoma cells), T-12662 (structural chromosomal aberrations/CHO cells) and T-12663 (structural chromosomal aberrations/mouse lymphoma cells)] when the pH was readjusted to a more physiological level (7.4) before the mutagenicity tests were conducted.

Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 8/19/91
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger Gardner, Head
Review Section I, Toxicology Branch
Health Effects Division (7509C)

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.: L200555, 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 neurcpathology 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 YO6380/036
   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 Administered 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[a]	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

[a]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. For The following table summarizes selected food utilization values for both sexes.

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

Weeks	Dietary Concentration 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

Week	Dietary Concentration (ppm) 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

Week	Dietary Concentration (ppm) 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

Week	Dietary Concentration (ppm) 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
Week 5				
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
Week 9				
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
Week 14				
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
Week 5				
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
Week 9				
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
Week 14				
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 siatic 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[a]

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

[a]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.

011387

Primary Review by: Roger Gardner and Pam Hurley Pamela M. Hurley 6/9/94
Section Head, Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Roger Gardner
Toxicology Branch I/HED

DATA EVALUATION RECORD
(Addendum of June 3, 1993)

Study Type: Developmental Toxicity
Guideline §83-3
Species: Rat

EPA Identification No.s: EPA Accession No. 249802
EPA Pesticide Chemical Code: 128501
Toxicology Chemical Code: 893C

Test Material: SC-0224 (19.2% purity); Lot #EHC-0355-25

Synonyms: Trimethylsulfonium carboxymethylaminomethylphosphonate; sulfosate; Touchdown

Sponsor: Stauffer Chemical Co.

Study Number(s): T-11050

Testing Facility: Stauffer Chemical Co., Environmental Health Center, Farmington, CN

Title of Report: A Teratology Study in CD® Rats with SC-0224.

Author(s): J. R. Downs and J. L. Minor

Report Issued: November 5, 1982

Conclusions: Sulfosate was administered by gavage to groups of pregnant Sprague-Dawley rats on gestation days 6 through 20 at dose levels of 0, 30, 100, or 333 mg/kg/day. The test material was dissolved in water and administered in a volume of 5 ml/kg.

The maternal NOEL is 100 mg/kg/day, and the maternal LOEL is 333 mg/kg/day (undetermined death of 2 dams, decreased body weight, feed consumption and body weight gain along with increased incidences of salivation, chromorhinorrhea, and lethargy after dosing).

The developmental toxicity NOEL is 100 mg/kg/day, and the developmental LOEL is 333 mg/kg/day (decreased fetal body weight).

Core Classification: Minimum. (NOTE: This study was originally classified as core supplementary because of a question regarding determination of the doses administered in terms of the active ingredient or the test substance which contained 19.2% active ingredient. A laboratory audit report verified that the doses

were based on the amount of active ingredient administered, and the study was upgraded from supplementary to minimum. Technical grade sulfosate is usually supplied as an aqueous solution. Because its viscous nature precludes the practical manufacture of a technical grade with a standard a.i. content (sulfosate froms an intractable glass-like product if its [redacted] content is ≤ 30%). From 1982 to the present time, the studies submitted to support registration had different a.i. contents ranging from 19.2 to 72%).

This study satisfies the guideline requirements (§83-3) for a developmental toxicity study in rats.

Discussion: The report and original DER noted significant reductions in body weight and feed consumption at the highest dose level (333 mg/kg/day) along with increased incidences of dams with salivation, lethargy after dosing, and chromorhinorrhea. Selected clinical signs, body weight and body weight gain and food consumption data are summarized in the following tables:

Summary of Clinical Signs in Dams

Dose (mg/kg/day)	0	30	100	300
No. examined	24	24	22	20
Chromorhinorrhea	2	2	2	9*
Salivation	0	0	0	7*
Lethargic after dosing	0	0	0	8*

* Statistically significantly different from control value, $p \leq 0.05$, Mann-Whitney U test.

Summary of Body Weight and Body Weight Gain (g)[a]

	Dose Level (mg/kg/day)			
	0	30	100	333
Pregnant Survivors	24	24	22	20
Body Weight (g)				
Day				
0	234	238	234	234
6	266	268	266	268
9	271	272	274	252*
12	289	292	289	266*
16	314	320	306	277*
21	387	386	370	322*
Body Weight Gain (g)				
Days				
6-9	5	3	8	-15*
6-12	23	24	23	-2*
12-16	25	28	17	11*
6-16	48	52	40	9*
16-21	73	66	64	45*
Uterine Weight	91	89	82	74
Net Body Weight Change	29	29	22	-19*

[a]Extracted from Table 5 of study.
*Statistically significant ($p < 0.05$)

Food Intake (g/day)[a]

	0 mg/kg	30 mg/kg	100 mg/kg	333 mg/kg
Day				
0-6	23	22	23	23
6-9	24	22	21	9*
9-12	24	25	22	12*
12-16	26	26	24	15*
16-21	27	28	25	18*

[a]Extracted from Table 5 of study.
* $p < 0.05$

Based on these results plus the undetermined death of 2 dams at the high dose, the maternal NOEL is 100 mg/kg/day, and the maternal LOEL is 333 mg/kg/day (decreased body weight, feed consumption and body weight gain along with increased incidences of salivation, chromorhinorrhea, and lethargy after dosing).

The only developmental toxicity noted in the report was decreased fetal weights at the 333 mg/kg/day dose level, and the original DER reported increases (not statistically significantly increased in comparison to the control group value) in the number of early resorptions per dam at the 100 and 333 mg/kg/day dose levels. *In utero* results are also summarized in the attached copy of Tables 5, 6, 7 and 8 from the report.

The increases in resorptions at the mid and high dose levels are not toxicologically significant because:

(1) There were no significant differences in group mean litter sizes,

(2) There were no statistically significant differences noted between the control and each of the treated groups (statistics were conducted on % resorptions, not on total resorptions/dam). The standard deviations for all groups, including controls were larger than the means.

(3) In examining the individual litter data for the early resorptions, it is noted that in both the mid- and high dose groups, there were 1 or 2 outliers. In the high dose group, 1 dam had 11/14 early resorptions/implantations and 2 had 3 (3/12 and 3/15). The rest had 2, 1 and 0. In the mid-dose group, 1 dam had 7/14 early resorptions and 1 had 3/17. The rest had 2, 1 and 0. In the low dose group, 2 had 3 (3/11 and 3/13) and the rest had either 2, 1 or 0 early resorptions and in the control group, they all had either 2, 1 or 0 resorptions.

Cesarean Section Observations[a]

	Control	LDT	MDT	HDT
Dose (mg/kg/day):	0	30	100	333
# Animals Assigned	25	25	25	26
# Animals Mated/Inseminated	24	24	22	23
Pregnancy Rate (%)	96	96	88	88
Maternal Wastage				
# Died	0	0	0	3
# Died/Pregnant	0	0	0	3[b]
# Non Pregnant	1	1	3	3
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total Corpora Lutea	365	384	329	306
Corpora Lutea/Dam	15.2	16.0	15.0	15.3
Total Implantations	337	345	299	304
Implantations/Dam	14.0	14.4	13.6	14.5
Total Live Fetuses	318	323	275	277
Total Dead Fetuses	0	1	1	1
Live Fetuses (%)	94	96	91	88
Dead Fetuses (%)	0	0.3	0.3	0.4
Total Resorptions	19	21	23	26
Early (%)	4.3	5.6	7.7	9.0
Mid (%)	0.9	0	0	2.0
Late (%)	0.5	0.3	0.9	0
Resorptions/Dam	0.79	0.88	1.05	1.3
Live Litters				
Live Fetuses/Dam	13.3	13.5	12.5	12.8
Dead Fetuses/Dam	0	0.04	0.05	0.05
Mean Fetal Weight (gm)	5.0	4.9	4.9	4.2*
Sex Ratio (% Female)	51	53	50	48

[a]Data extracted from tables 2 and 5
* $p < 0.05$

Based on these results, the developmental toxicity NOEL is 100 mg/kg/day, and the developmental LOEL is 333 mg/kg/day (decreased fetal body weight).

TOF Review 011337

Page____ is not included in this copy.

Pages 44 through 48 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

A Teratology Study in CD Rats with SC-0224. Study T-11050. Conducted by Stauffer Chemical Co. Environmental Health Center, Farmington, Conn. 06032. Study conducted by J. R. Downs, J. L. Minor, G. M. Zwicker, et al. November 1982. Accession No. 249802. Caswell #893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224. Lot #EHC-0355-25. SC-0224. Purity (potency) 19.2% in [redacted].

Sprague-Dawley rats were obtained from Charles River Breeding Laboratories in Kingston, N.Y. Thirty males and 145 females were used for mating. One or 2 females were placed with one male. The next morning the females were examined for the presence of a copulatory plug or sperm in a vaginal smear. The day sperm or a plug were found was considered day 0 of gestation for that female. About 25 mated females were assigned to each of 4 dose groups and were intubated with 0, 30, 100, or 333 mg/kg SC-0224 active chemical in water on day 6 through 20 of gestation. Body weights and feed consumption were recorded on days 0, 6, 9, 12, 16 and 21. The females were killed on day 21 of gestation and necropsied. The reproductive tract was weighed and examined, and resorption sites were noted. Corpora lutea were counted on the ovaries. Fetuses were weighed, sexed, and examined for external malformations. One-half of the fetuses were eviscerated, fixed in alcohol, and then cleared and stained with 1% KOH and alizarin red S for skeletal anomaly examination. The heads of the rest of the fetuses were removed and fixed in Bouin's fixative; the trunks of these fetuses underwent internal organ examination under a dissecting microscope prior to being eviscerated and processed for skeletal examination.

Statistical Analysis:

The Fisher exact probability test was used to evaluate significance of enumeration data, such as litters with anomalies.

For quantitative data such as body weights, feed intakes and percent incidence data, the significance was based on multiple comparisons with a nonparametric rank test. ($p < 0.05$, two-tailed).

A test for a linear trend in proportions was used to determine if there was a dose-related change in the proportion of affected litters in groups treated with SC-0224.

Mortality:

Three females, all in the 333 mg/kg/day dose group, died during the study. One female found dead following the 14th dose had a perforated esophagus, indicating death was due to a dosing accident. The other two females were found dead after the second dosing day. Both of these had lungs which were partly dark red, and a darkened (brown or red) cortico-medullary junction in their kidneys. Definite cause of death could not be determined.

Fertility:

Pregnancy percentages were 96, 96, 88, and 88 percent for controls, 30 mg, 100 mg, and 333 mg/kg/day dose groups, respectively. Any differences in fertility between groups were not statistically significant.

Maternal Effects:

Maternal clinical signs which were seen in significantly greater numbers in high dose females (333 mg/kg/day) than in controls or lower doses were chromorhinorrhea, salivation, and lethargy. ($p < 0.05$, two-tailed). However, on necropsy at termination of the study there were no gross findings which could be related to treatment.

In the dams, body weights and feed intake were significantly reduced in the high dose group. The high dose group also showed significantly lower mean uterine weights and liver weights. The relative liver weight for dams receiving 100 mg/kg/day was significantly increased, although the actual mean weight was not affected. A dose-response effect on liver weights was not demonstrated.

Fetal Effects:

There was an increased number of resorptions per dam at the 100 and 333 mg/kg dose levels. (5.7%, 5.6%, 8.6%, and 11.4% for controls, 30 mg, 100 mg, and 333 mg/kg/day dose levels, respectively.) These were mainly early resorptions, and may be related to decreased feed intake in the treated rats. The increases in resorptions are not statistically significant.

There was a statistically significant reduction in mean fetal weight in the 333 mg/kg/day dose group. This, too, could be related to the decreased feed consumption of the dams.

Anomalies:

A number of soft-tissue anomalies were seen in all groups, including controls. These could not be related to treatment or dose. The most numerous ones are shown below: (L = litters; F = fetuses).

	Dose (mg/kg/day)							
	0		33		100		333	
	L	F	L	F	L	F	L	F
Brain: dilated 4th ventricle	17	65	16	61	19	56	14	49
Left kidney: dilated pelvis	6	7	3	3	7	9	4	4
Right Kidney: dilated pelvis	4	5	4	4	2	2	3	3
Dilated ureters, left	17	55	14	43	14	41	9	24
Dilated ureters, right	14	46	13	41	13	37	7	20
Convoluted ureters, left	19	76	19	60	14	54	9	27
Convoluted ureters, right	18	61	18	55	14	51	9	26

The most severe soft tissue anomalies were a cleft palate in one fetus in the low dose (33 mg) group and reduced oronasal tissues in one fetus in the high dose (333 mg) group. None of these can be considered a result of treatment.

Regarding skeletal anomalies, those seen in greatest incidence were incompletely ossified sternebrae and lobed vertebral centra; the incidences were similar in controls and all treatment groups.

Five litters in each of the 100 mg and the 333 mg dose groups showed wavy ribs, considered slight but statistically signficant ($p < 0.05$, two-tailed). The laboratory quotes Khera in referring to these as fetal aberrations due to delays in normal growth or slight deviations in normal morphogenesis.

No structural teratogenicity was seen in any of the treatment groups.

Conclusions:

1. Maternal toxicity in terms of reduced body weights and feed intake was seen in the high dose group (333 mg/kg/day).

2. Toxic signs were salivation, lethargy after dosing, and increased chromorhinorrhea.

3. Fetotoxicity was seen as reduced fetal body weights at the high dose level. This was apparently a direct result of maternal toxicity.

4. Teratogenicity was not seen in this study.

5. The material tested was stated to be 19.2% pure. Applicant should verify whether doses administered were weights of the 19.2% material or were based on active ingredient.

Data are supplementary.

Roland A. Gessert

Roland A. Gessert, DVM
Veterinary Medical Officer
Review Section #1
Toxicology Branch/HED (TS-769)

011337

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

004585

July 30, 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT : Laboratory Data Audit. Stauffer Chemical Co. Environmental Health Center; Farmington, CT. February 19-22, 1985.

FROM : Roland A. Gessert, D.V.M.; Veterinary Medical Officer/Toxicologist

TO : John A. McCann, Director; National Laboratory Audit Program

This report provides information previously provided to the Inspector at the inspection site. While at the laboratory we ascertained that Stauffer's acute studies were conducted at their Richmond, California laboratory. Multiple dosing and inhalation toxicity studies are conducted at the Farmington, CT facility.

While we were at the laboratory, Stauffer was acquired by Chesebrough Ponds Co.

All the studies scheduled for audit were audited. These included:

132-1 → Ro-Neet 2-year oral toxicity study in rats

R-29148 2-year chronic toxicity/oncogenicity study in rats

R-40244 (Racer 2-E) multi-generation reproduction study in rats

MV-678 Teratogenicity study in New Zealand rabbits

83-3 c SC-0224 Teratogenicity study in CD rats

In the initial 2-year rat study with Ro-Neet, a NOEL was not demonstrated, peripheral neuromyopathy being demonstrated at all doses. (Hazleton Study 132-134. Accession # 240914). Therefore, Stauffer conducted a repeat 2-year rat study # T-10114. In the repeat study a neuropathy NOEL was demonstrated at 10 ppm and a myopathy NOEL at 60 ppm. The repeat study was Core graded Supplementary because histopathology was not done. However, these two studies combined can be graded Core Minimum or Core Guidelines. (In the one-liners, the Hazleton study is graded Core Guidelines.)

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In the multigeneration reproduction study of Racer 2-E (R-40244) in rats. we inquired why there was no sperm analyses for P_2 males. It is not required. Only data for 2 generations are now required, and were provided. In this study we compared testes/epidymides weights and lesions with histopathology at the high dose. Everything check O.K.

The teratogenicity study of SC-0224 in CD rats which I had reviewed, I had declared Core Supplementary data because it was not clearly stated whether doses administered were based on the 19.2% material or on the active ingredient. The data audit verified that doses were based on the active ingredient. The data for this study now are Core Minimum.

Dr. M. Adrian Gross and I worked together in auditing the Stauffer studies. Therefore, our reports should be combined for a complete audit report.

Roland A. Gessert

Roland A. Gessert, D.V.M.

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011337

005584

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

MAR 10 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Applications for Pesticide Registrations for the Nonselective Foliar Systemic Herbicides, SC-0224 4-LC and SC-0224 Concentrate, for Weed Control in Non Food Crop Areas. Trimethylsulfonium carboxymethylamino-methylphosphonate. R-50224. CASWELL #893C. Accession Nos. 250544, 250545, 250547, 250548, 260508.

TO: Robert Taylor, PM 25
Registration Division (TS-767)

FROM: Brian Dementi, Ph.D. Brian Dementi 3/7/86
Review Section #1
Toxicology Branch/HED (TS-769)

THRU: Robert B. Jaeger, Section Head RBJ 3/7/86
Review Section #1
Toxicology Branch/HED (TS-769) 3/10/86

Applicant: Stauffer Chemical Company
1200 S. 47th Street
Richmond, California 94804

Stauffer Chemical Company requests pesticide registration for SC-0224 and SC-0224 4-LC, non-selective foliar systemic herbicides, for weed control in non-crop areas.

Recommendations:

Pesticide registrations for weed control in non food crop areas for the herbicides SC-0224 and SC-0224 4-LC are supported by available toxicity data. See the February 8, 1984 review by Dr. R. A. Gessert and Dr. John Chen on experimental use permits for SC-0224 LC, CASWELL #893C.

Deficiencies identified in the above review have been satisfied. Specifically, these include: 1) Information regarding eight mutagenicity studies (identified in Section 2, A-F of the Review Recommendations). Supplementary information submitted by Stauffer in response to the indicated mutagenicity deficiencies has been reviewed and appraised acceptable by Dr. John Chen (3/4/86 review of Dr. Chen); 2) Individual

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animal data requested in support of the acute dermal toxicity study. Review of this information supports the classification of core minimum as indicated; and 3) Information requested as to dosage used in the the teratology study. Stauffer has affirmed that the dosages contained in the original report were based on, or expressed in terms of, the actual amount of active ingredient. It is recommended that the core rating for this study be upgraded from supplementary to guideline.

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011934

Primary Review by: Roger Gardner and Pam Hurley Pamela M. Hurley 8/1/94
Section Head, Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Roger Gardner
Toxicology Branch I/HED

DATA EVALUATION RECORD
(Addendum of June 3, 1993)

Study Type: Developmental Toxicity
Guideline §83-3
Species: Rabbit

EPA Identification No.s: EPA Accession No. 260966
EPA Pesticide Chemical Code: 128501
Toxicology Chemical Code: 893C

Test Material: SC-0224 (56.2% purity); Lot #EHC-0355-25. Note: Technical grade sulfosate is usually supplied as an aqueous solution. Because its viscous nature precludes the practical manufacture of a technical grade with a standard a.i. content (sulfosate froms an intractable glass-like product if its [redacted] content is ≤ 30%). From 1982 to the present time, the studies submitted to support registration had different a.i. contents ranging from 19.2 to 72%).

Synonyms: Trimethylsulfonium carboxymethylaminomethylphosphonate; sulfosate; Touchdown

Sponsor: Stauffer Chemical Co.

Study Number(s): T-11052

Testing Facility: Stauffer Chemical Co., Environmental Health Center, Farmington, CN

Title of Report: A Teratology Study in New Zealand White Rabbits with SC-0224.

Author(s): J. L. Minor and J. R. Downs

Report Issued: June 21, 1983

Conclusions: Sulfosate was administered by gavage to groups of mated New Zealand White rabbits (21 does in the highest dose group) on gestation days 7 through 19 at dose levels of 0, 10, 40, or 100 mg/kg/day. The test material was dissolved in water and administered in a volume of 2 ml/kg.

The maternal NOEL is 40 mg/kg/day according to the report, and the maternal LOEL is 100 mg/kg/day (6 deaths in 17 pregnant does, 4 abortions in the 11 survivors along with decreased body weight, feed consumption and body weight gain).

The developmental NOEL is 40 mg/kg/day and the LOEL is 100 mg/kg/day. This is based on the following: at 100 mg/kg/day, there was a reduction in the number of live fetuses/doe for the 7 surviving rabbits when compared with the controls (5.4 versus 7.4), 4 rabbits aborted their litters (apparently all of the fetuses were dead in those litters and if those are included in the calculations there would be a statistically significant decrease in live fetuses/doe and postimplantation loss), and having only 7 litters does not give a sufficiently high number of animals to absolutely conclude that no developmental toxicity is occurring, particularly in light of the massive losses to death and abortions.

Core Classification: Minimum.

This study satisfies the guideline requirements (§83-3) for a developmental toxicity study in rabbits.

Discussion: The original DER cited the increased incidence of clinical signs in the low dose group as the basis for setting the NOEL < 10 mg/kg/day (lowest dose tested). However, incidences reported (see table below, taken from Table 2 of the original report) indicate that those signs do not occur in a dose-related manner or in numbers that exceed control group values. It should also be noted that 4 of the 8 reported deaths appeared to be associated with dosing accidents (see attached Table 3 from the original report).

Attached Table 5 from the original report and the tables below summarize the effects of the test compound on body weight, weight change, and food consumption, and *in utero* results are summarized in the table below and in Table 7 in the Appendix.

All of these data suggest a maternal NOEL of 40 mg/kg/day and an LOEL of 100 mg/kg/day.

Data summarized in attached Tables 8, 9 and 10 from the original report indicate that possible developmental toxicity was observed at the highest dose tested (100 mg/kg/day).

Clinical Signs for Does Gravid on Day 30

	Dose Levels (mg/kg/day)			
	0	10[a]	40	100
Females Examined	14	14	14	7
Without signs	13	7*	10	4
General				
Anorexia	0	0	1	2
Diarrhea	0	1	2	2
Lethargy	0	0	1	1
Head tilt	0	1	0	0
Nasal discharge after dosing	0	1	0	0
Wet stains, chin	0	1	0	0
Scab, mouth	0	1	0	0
Red urine	0	1	0	0
Red stains on cage pad	0	1	1	0
Respiratory				
Rales while dosing	1	0	0	0

*$p \leq 0.05$
[a]Each one of the clinical signs was observed in a different animal. No one animal in the 10 mg/kg/day dose group had more than 1 clinical sign.

Body Weight Gains (Kg)[a]

Group:	Prior to Dosing Period	Days 7 - 21	Days 21-30	Days 7 - 30	Corrected Body Weight Gain[1]
Control	0.3	0.2	0.0	0.2	-0.2
LDT	0.3	0.1	0.1	0.1	-0.3
MDT	0.3	0.1	0.1	0.2	-0.3
HDT	0.4	-0.3*	0.3*	0.0*	-0.4

[1]corrected body weight gain for entire gestation period = body weight gain for days 7 - 30 minus gravid uterus weight.
[a]Data extracted from table 5.
* $p < 0.05$

Food Consumption Data (g/day)[a]

Group:	Days 0 - 7	Days 7 - 21	Days 21 - 30	Days 7 - 30
Control	190	165	104	3200
LDT	191	136	119	2600
MDT	174	136	134	3000
HDT	199	54*	180	2000*

[a]Data extracted from table 5.
* $p < 0.05$

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Cesarean Section Observations[a]

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	Control	LDT	MDT	HDT
Dose (mg/kg/day)	0	10	40	100
# Animals Assigned	15	15	15	21
# Animals Mated/Inseminated	15	15	15	15
Pregnancy Rate (%)	14(93)	14(93)	14(93)	17(81)
Maternal Wastage				
# Died	0	0	0	8*[b]
# Died/pregnant	0	0	0	6
# Non pregnant.	1	1	1	4
# Aborted	0	0	0	4*
# Dams with live fetuses	14	13	14	7
Total Corpora Lutea	119	132	113	73[c]
Corpora Lutea/dam	8.5	9.4	8.1	8.0
Total Implantation	117	103	104	133[d]
Implantations/Dam	8.4	7.4	7.4	7.1[d]
Total Live Fetuses	103	86	100	38
Live Fetuses/Dam	7.4	6.6	7.2	5.4[d]
Total Resorptions	14	17	4	12
Early	8	3	3	6
Mid	3	1	0	1
Late	3	13	1	5
Resorptions/Dam	1.0	1.3	0.3	1.7
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	43	47	46	48
Preimplantation Loss(%)	2	22*	6	9
Postimplantation Loss(%)	12	6	4	22[d]
Standard Deviation	13	9	6	20
Sex Ratio (% Female)	56	60	56	61

[a]Data extracted from table 7.
* $p < 0.05$
[b]4 of 8 deaths were from dosing accidents.
[c]Some of these data were not available.
[d]Implantation sites were found in 17 dams. Later, only 7 dams had litters to term. If the values include the 4 females which aborted, then the following would values would be used: implants/dam - 7.6 ± 1.6, live fetuses/dam - 3.4* ± 3.0 and postimplantation loss (%) - 49* ± 42.

TH Review 011337

Page ____ is not included in this copy.

Pages 61 through 69 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

005450

Study: A Teratology Study in New Zeland White Rabbit 011337 0224.

Laboratory: Environmental Health Center
Stauffer Chemical Company
Farmington, Connecticut.

Study Number and Date: T-11052, June 21, 1983. - 011337

Accession Number: 260966 (Appendix 1)

Material Tested: SC-0224, 56.2% Pure

Animals: New Zeland White Rabbit [Dla: (NZW)SPF]

Procedure (As paraphrased or quoted from the study protocol):

Following a 45-day quarantine period, 71 virgin females ranging in age 185-213 days were cohabited with 6 males to obtain a total of 66 successfully mated females which were then assigned to the study.

Fifteen mated females were assigned to each of 4 dosage groups, with 6 additional animals being assigned to the highest dosage group. The date of mating was designated as day 0 of gestation. These females subsequently received 13 consecutive daily doses of 0, 10, 40 or 100 mg/kg of SC-0224 in tap water on days 7 through 19 of gestation. Aqueous solutions used for administration of the test compound were prepared to contain 0, 5.0, 20.0 and 50.0 mg/ml. Analytical determinations were made to assure these levels.

Animals were observed daily for clinical evidence of change. Body weights were determined on days 0, 7, 14, 21 and 30. Feed intakes were recorded for gestational intervals 0-7, 7-14, 14-21 and 21-30. The females were sacrificed on day 30 of gestation and necropsied. "The liver, kidneys, spleen, ovaries, and heart were weighed and preserved in 10% neutral buffered formalin or 2.5% buffered glutaraldehyde. Paired organs were weighed separately. Placentas were weighed collectively for live fetuses, individually for dead fetuses, or attached for resorptions. Uteri without conceptuses and ovaries were weighed.

Ovaries were examined to determine the number of corpora lutea. The uterus was opened and examined for the number and distribution of fetuses and resorptions. Resorption sites were noted as early, if there was no fetal tissue present; as mid, if fetal tissue was present, but without recognizable features; or as late, if the conceptuses showed either external degenerative changes or an arrested state of development. Placenta and associated fluids were inspected for any unusual appearances. Obvious malformations in late resorptions were described, when present, but not examined further or included

in statistical analyses of structural deviations.

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Fully developed fetuses were classified as dead if reflexes were absent when the neck was pressed at the time it was removed from the uterus. Dead fetuses were subsequently examined for anomalies, but not included with live fetuses for statistical analyses of structural malformations.

All fetuses were weighed and examined for external malformations. Pups within a litter which weighed less than three-fourths of the litter mean were designated as calculated runts. Live pups were sacrificed by intrathoracic injection of sodium pentobarbital. Identity of the pups was maintained throughout the examination process.

Each fetus was examined for both soft-tissue and skeletal anomalies. The head of each fetus was removed and fixed in Bouin's fixative for examination of the organ structure by a modification of the Wilson (1965) serial cross-section technique. The trunk of each fetus was examined internally by a modification of the Staples (1974) fresh dissection technique. The sex of each fetus was determined at this time. Following the internal examination, the fetus was eviscerated and processed by a modification of the Kimmel (1981) procedure for skeletal examinations" p.3.

Principle findings are presented as follows:

1. Among the control and three dose groups of females, deaths occured during the observation period only in the high dose group where mortality was 38%, a statistically significant finding. In addition, fertility was numerically reduced in the high dose group (81%) as compared with controls (93%), but the reduction was not statistically significant.

2. Four females (36%) of the high dose group aborted, an increase which was statistically significant.

3. Of 14 surviving pregnant females of the low dose group on gravid day 30, seven were observed to exhibit clinical signs of toxicity including diarrhea, head tilt, nasal discharge, wet stains (chin), scab (mouth), red urine and red stains on cage pad. These clinical findings constituted a significant increase for the low dose group with respect to the control group. Toxic signs were essentially absent in the control group. Some of the same toxic signs were observed in the middle and high dose groups, but were not reported as statistically significant

in the study itself. However, in the high dose group 10 of 17 pregnant females were apparently eliminated from gravid day 30 observations due to unscheduled sacrifice (Table 2, p. 14). There was much evidence of toxicity among the ten gravid females which underwent unscheduled sacrifice. Notable findings included red foci of the lungs and reddened trachea/laryrx (Table 3, p.16).

Necropsy findings for terminally sacrificed animals, as revealed in Table 4 (p. 17), suggest increased numbers of lesions in the low dose group, but probably is not statistically significant. Necropsy evaluations on ten rabbits (unscehduled sacrifice, Table 3, p. 15) of the high dose group certainly indicate toxic effects of SC-0224.

Maternal Body Weights and Feed Intakes

Mean body weight determinations on pregnant survivors (14 rabbits each from control, low dose and middle dose groups and 7 rabbits from the high dose group) did not reveal any adverse effects related to dosing.

Feed intake was significantly reduced in the high dose group during days 7-14 and 14-21, but numerically (not signigicant) increased on days 21-30. Total feed intake for days 7-30 was significantly reduced for this group. Feed intake was essentially unaltered in the other groups excepting a significant reduction during days 7-14 in the 40 mg/kg dose. Similarly feed intake on a per kg body weight basis was significantly reduced in the high dose group, with consistency (Table 5, p. 19).

Maternal Organ Weights

Absolute organ weights measured of pregnant survivors were unaltered by dosing. There was a numerical reduction in mean reproductive tract weight of the high dose group (379 $\pm$ 108 gms vs. control 434 + 80), but was not a statistically significant change. Similarly, relative organ weight data did not reveal any compound related adverse effect. (Table 6 p. 20).

Intrauterine Data

Intrauterine findings as disclosed on the basis of a variety of expressions, eg. corpora lutea/dam, implants/dam, live fetuses, dead fetuses, implants/corpora lutea, % viable implants, % resorptions, fetuses/dam, live fetuses/implants, mean placental weight/live fetus, etc. did not disclose any

meaningful compound related effects. (Perhaps it should be noted that live fetuses/dam and live fetuses/implants (%) were numerically reduced in the high dose group with respect to the control group (5.4 ± 1.5 vs. 7.4 ± 2.0 and 78 ± 20 vs. 88 ± 13, respectively) (Table 7, p. 21).

External Anomalies

External examinations revealed numerically more anomalies among the yet smaller number of fetuses in the high dose group. Identified anomalies included domed cranium w/wo prominent vasculature, meningocele, prominent eye, enlarged fontanels, dark distended abdomen, arthrogryposis of forelimb. This increased number of anomalies was not reported as statistically significant, but may indeed reflect adverse effects of SC-0224 at the highest dose. There were no meaningful findings at the lower doses. (Table 8, p. 22).

Soft Tissue Anomalies

Table 9 (p. 23) reveals the number of male and female fetuses in each group. Percentages of males in each group are: group 1 (45.6%), group 2 (43%), group 3 (45%) and group 4 (39.5%). We do not consider that the numerically smaller percentage of males in the high dose group to be significant.

The number of specific anomalous findings in the various groups does not suggest an adverse effect of SC-0224. Examples of anomalies identified include: head (hydrocephalus, cleft palate, dilated 4th ventricle, etc.); thorax (fluid-filled pericardium, reddened lungs); abdomen (necrotic focus on liver, small pear shaped gallbladder, pale spleen, etc.). The total number of rats in the highest dose group with anomalies, expressed as % of fetuses examined, is suggestive of an effect of SC-0224 at the highest dose. The percentage of fetuses having anomalies ranked 3 and 4 are 10.5% and 7.9%, respectively. The comparable percentages for the control group are both 2%. The numerically higher percentage of more severe anomalies in group 4 is not indicated as significant. There appear to be no specific anomalies accounting for this difference as tabulated.

Skeletal Anomalies

No remarkable findings at any dose with respect to skeletal anomalies were reported. Table 10, p. 25.

Summary

A. Statistically Significant Findings

1. Increased maternal mortality, LOEL = 100 mg/kg/day
NOEL = 40 mg/kg/day

2. Increased spontaneous abortion, LOEL = 100 mg/kg/day
   NOEL = 40 mg/kg/day

3. Clinical evidence of maternal toxicity, LOEL = 10 mg/kg/day
   NOEL = undetermined

4. Reduced feed intake during days 7-30, LOEL = 40 mg/kg/day
   NOEL = 10 mg/kg/day

Developmental Toxicity Index*

$$\text{A/D Ratio} = \frac{\text{LOEL, maternal toxicity}}{\text{LOEL, fetotoxicity}} = \frac{10 \text{ mg/kg/day}}{> 100 \text{ mg/kg/day}} = < 0.1$$

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Core Rating: Guideline

* Chitlik, et al. (1985) HED/SEP Teratology Studies. EPA-540/9-84-018.

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

005450

SEP 10 1986

- 011386 OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 476-EEEL/476-EEEA

TO: Robert J. Taylor, PM #25
Fungicides-Herbicides Branch
Registration Division (TS-767C)

THRU: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

DAR for RBJ 9-9-86

FROM: Brian Dementi, Ph.D.
Review Section #1
Toxicology Branch/HED (TS-769)

Brian Dementi 9/8/86

E.R. 9/9/86

Hafa WH 9/10/86

The following studies submitted by Stauffer Chemical Company in support of registration of SC-0224 concentrate and SC-0224 4-LC nonselective foliar systemic herbicides (Ref: Letter of Ralph L. Riggs to Mr. Robert J. Taylor, January 13, 1986) have been reviewed and are herewith submitted to your office.

1. MUTAGELICITY EVALUATION of SC-0224 in SALMONELLA TYPHIMURIUM. Report No. T-12660, Septmeber 25, 1985, Accession No. 260966.

Evaluation: The test compound, SC-0224 (55.6% purity), was considered non-mutagenic in the Ames Test at the concentration tested. The study is acceptable.

2. MOUSE LYMPHOMA MUTATION ASSAY with SC-0224. Report No. T-12661, December 19, 1985. Accession No. 260966.

Evaluation: The test compound, SC-0224, was considered mutagenic in this assay with and without metabolic activation under the normal test conditions and concentrations tested. The study is acceptable.

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3. CYTOGENETIC ASSAYS (CHROMOSOMAL ABERRATION and SISTER CHROMATID EXCHANGE) with SC-0224 in the MOUSE LYMPHOMA (L5178Y) CULTURED CELL SYSTEM. Report No. T-12662, December 19, 1985, Accession No. 260966.

Evaluation: The test compound, SC-0224, was considered clastogenic in mouse lymphoma cells with and without metabolic activation at the dose levels tested. SC-0224 was also positive for sister chromatid exchanges in lymphoma cells with and without metabolic activation. Both studies are acceptable.

4. CYTOGENETIC ASSAYS (CHROMOSOMAL ABERRATION and SISTER CHROMATID EXCHANGE) with SC-0224 in the CHINESE HAMSTER OVARY CELL SYSTEM. Report No. T-12663, December 18, 1985, Accession No. 260966.

Evaluation: The two studies in question are considered inadequate due essentially to lack of conformity to procedures for in vitro cytogenetic and sister chromatid exchange assays as recommended by EPA (EPA HEALTH EFFECTS TEST GUIDELINES 560/6-83-001). See reviews for further clarification.

5. A TERATOLOGY STUDY in NEW ZELAND WHITE RABBITS with SC-0224. Report No. T-11052, June 21, 1983, Accession No. 260966 (Appendix 1).

Evaluation: Findings are summarized as follows:

a) Increased maternal mortality
LOEL = 100 mg/kg/day, NOEL = 40 mg/kg/day

b) Increased spontaneous abortion
LOEL = 100 mg/kg/day, NOEL = 40 mg/kg/day

c) Maternal Toxicity
LOEL = 10 mg/kg/day, NOEL = undetermined

d) Reduced feed intake during days 7-30.
LOEL = 40 mg/kg/day, NOEL = 10 mg/kg/day

Core Rating: Guideline

TS-769:DEMENTI:s11:X73710:9/5/86

Primary Review by: Roger Gardner and Pam Hurley Pamela M. Hurley 8/9/94
Section Head, Review Section 1, Toxicology Branch 1/HED
Secondary Review by Roger Gardner
Toxicology Branch I/HED

DATA EVALUATION RECORD
(Addendum of June 11, 1993)

011337

Study Type: Multigeneration Reproduction
Guideline §83-4
Species: Rat

EPA Identification No.s: EPA Accession No. 258398, 258399
EPA Pesticide Chemical Code: 128501
Toxicology Chemical Code: 893C

Test Material: SC-0224 (19.2% purity); Lot # EHC-0355-25

Synonyms: Trimethylsulfonium carboxymethylaminomethylphosphonate; sulfosate; Touchdown

Sponsor: Stauffer Chemical Co.

Study Number(s): T-11051

Testing Facility: Stauffer Chemical Co., Environmental Health Center, Farmington, CN

Title of Report: SC-0224: Two-Generation Reproduction Study in Rats

Author(s): J. L. Minor, J. R. Downs, et al.

Report Issued: April 19, 1984

Conclusions: Sulfosate was administered in the diet to groups of 20 male and 30 female Sprague-Dawley rats through two matings in each of two successive generations. Dose levels were 0, 150, 800, or 2000 ppm. The NOEL for systemic effects is 150 ppm and the LEL is 800 ppm based on consistent decreases in absolute and sometimes relative organ weights in both generations (thymus, heart, kidney and liver) at 800 and 2000 ppm and decreases in body weights and body weight gains during the premating period at 2000 ppm. The NOEL for reproductive/developmental effects is 150 ppm and the LOEL is 800 ppm based on decreased litter size in the F_{0a} and F_{1b} litters at 2000 ppm and on decreased mean pup weights during lactation in the second litters at 800 ppm and in all litters at 2000 ppm.

Core Classification: Guideline

Discussion:

Systemic Toxicity: The decreases in body weight and body weight gain appear to be related to a palatability problem since food efficiency did not appear to be affected. However, although the food efficiency was not statistically significantly affected at any time point, it was quite a bit less than the controls at several time points. The NOEL was set at 150 ppm because the decreases in organ weights were consistent across both generations. The point discussed in the first Data Evaluation Record concerning platelet counts is probably not biologically significant because it was not consistent across generations.

Reproductive/Developmental Toxicity: The statistically significant decreases in pup weights at the 800 ppm level were borderline biologically significant because at no time were either the body weights or body weight gains less than 90% of the control values and because the effect was not apparent in all litters.

Investigators' Conclusions: The investigators summarized their conclusions as follows:

> The principal effects attributed to SC-0224 were reductions in body weights and feed intakes at 800 and 2000 ppm. Reductions in body weight became progressively more apparent throughout the study, initially appearing as a significant reduction for P0 males after five weeks and at both 800 and 2000 ppm for P0 females during reproductive phases. Body weights for P1 males at 800 and 2000 ppm and females at 2000 ppm were significantly reduced throughout their study period. Reductions in feed intakes generally accompanied the reductions in body weights. Pup weights were significantly reduced after lactational day 7 at 2000 ppm for all litters and for both second litters at 800 ppm. Both the slight reductions in litter size at 2000 ppm and the reductions in pup weights at 800 and 2000 ppm appeared to be secondary to the health of the dams. There was no evidence of altered intrauterine developmnent, increased stillborns, or pup anomalies.
>
> Based on reduced feed intakes and body weights in both parents and pups observed at 800 and 2000 ppm, the no-effect level was 150 ppm SC-0224 in the diet, corresponding to an approximately (sic) daily intake of 7.5 mg/kg/day.

Previous reviews (Document Nos. 005173 and 005690) also described decreases in body weight, feed consumption and organ weights in adult males and females of the P0 and P1 generation. Selected data are summarized in Tables 1 and 2 below to supplement the previous conclusions regarding these endpoints. Table 3 summarizes body weight, feed consumption and efficiency data for P0 and P1 females during gestation, Table 4 summarizes body weight, feed consumption and efficiency data for P0 and P1 females during lactation, Table 5 summarizes the litter size data and Table 6 summarizes the pup weight data mentioned in the investigators' conclusions above. Also provided are copies of

the fertility and reproductive behavior and pup survival and developmental indices tables taken directly from the report.

Table 1: Selected body weight, feed consumption, and feed efficiency means from report tables 5-10 and 23-28.

Observation	Dose level (ppm) 0	150	800	2000
F_0 Generation Males - premating				
Body weight (g) on day				
0	143	141	144	141
118	543	551	537	488**
Body weight gain (g)				
Day 0-118	400	410	393	347
Feed consumption (g/day)				
Day 8	16	16	15	13**
29	22	18**	17**	18**
62	23	23	19**	16**
91	22	23	23	14**
118	20	18**	19	16**
Feed efficiency+				
Day 8	62	55	56	60
29	36	37	32	36
118	14	6	9	4
F_0 Generation Females - premating				
Body weight (g) on day				
0	122	122	122	120
118	280	280	273	263*
Body weight gain (g)				
Day 0-118	158	162	151	143
Feed consumption (g/day)				
Day 8	12	12	11	11
29	14	11**	10**	13
62	17	16	13**	12**
118	16	14	15	14
Feed efficiency+				
Day 8	37	36	36	40
29	19	19	16	20
62	15	17	4	4

* Significantly different from control, $p \leq 0.05$, two tailed.
** Significantly different from control, $p \leq 0.01$, two tailed.
+ According to the report, feed efficiency was calculated as 100 x interval body weight change ÷ interval days ÷ daily feed intake.

Table 1: Selected body weight, feed consumption, and feed efficiency means from report tables 7-10 and 23-28. 011337

Observation	Dose level (ppm) 0	150	800	2000
F_1 Generation Males - premating				
Body weight (g) on day				
0	181	171	164**	128**
62	508	503	451**	380**
Body weight gain (g)				
Day 0-62	327	332	287	252
Feed consumption (g/day)				
Day 5	17	17	14**	10**
27	19	19	17**	15**
62	19	19	18	16**
Feed efficiency+				
Day 6	53	55	56	52
27	29	30	29	28
62	15	16	15	18
F_1 Generation Females - premating				
Body weight (g) on day				
0	138	137	132	121**
62	277	271	249**	234**
Body weight gain (g)				
Day 0-62	139	134	117	113
Feed consumption (g/day)				
Day 7	12	12	11*	10**
28	13	13	12*	11*
62	14	13	12*	11*
Feed efficiency+				
Day 7	31	30	30	35
28	16	14	17	15
62	10	8	10	8

* Significantly different from control, $p \leq 0.05$, two tailed.
** Significantly different from control, $p \leq 0.01$, two tailed.
+ According to the report, feed efficiency was calculated as 100 x interval body weight change ÷ interval days ÷ daily feed intake.

BD

Table 2: Selected absolute organ weights (g) from Tables 15, 16, 33 and 34 of the original report.

Organ	Dose level (ppm) 0	150	800	2000
	F_0 Generation Males			
Whole body	634.303	634.956	605.508	533.490**
Heart	1.637	1.694	1.609	1.500
Kidney				
Left	1.501	1.488	1.486	1.436
Right	1.535	1.560	1.479	1.444
Liver	14.727	15.475	14.007	12.583
Spleen	0.855	0.831	0.840	0.824
Thymus	0.500	0.524	0.446	0.339**[a]
	F_1 Generation Males			
Whole body	648.511	634.630	584.991**	476.453**
Heart	1.727	1.738	1.542**	1.402**[a]
Kidney				
Left	1.608	1.597	1.467	1.296**[a]
Right	1.651	1.620	1.471**	1.308**
Liver	14.086	14.159	12.235**	9.822**
Spleen	0.828	0.863	0.745	0.672**
Thymus	0.466	0.461	0.420*	0.332**
	F_0 Generation Females			
Whole body	309.304	309.410	299.583	282.189**
Heart	1.209	1.164	1.081**[a]	1.026**
Kidney				
Left	0.944	0.937	0.937	0.869**
Right	0.952	0.947	0.922	0.900
Liver	7.873	7.802	7.454	7.384
	F_1 Generation Females			
Whole body	331.341	323.829	295.506**	269.477**
Heart	1.234	1.164	1.118**	1.067**
Kidney				
Left	1.031	1.008	1.008	0.851**
Right	1.089	1.019	0.973**	0.882**
Liver	7.912	7.518	7.341*[a]	6.904**[a]

* Significantly different from control, $p \leq 0.05$, two tailed.
** Significantly different from control, $p \leq 0.01$, two tailed.
[a]These organ weights were also statistically significantly decreased when expressed as relative organ weights.

Table 3: Selected female body weight, feed consumption, and feed efficiency means during gestation from report tables 41, 43, 45, 47, 49 and 51.

Observation		Dose level (ppm) 0	150	800	2000
		F_{0a} Generation			
Body weight (g) on gestation day	0	245	256	250	236
	20	312	348*	311	322
Body weight gain (g) gestation day 0-20		67	92	61	86
Feed consumption (g/day) gestation day	6	16	16	14**	13**
	13	17	17	14**	14**
	20	8	11*	9	13**
Feed efficiency+ gestation day	6	29	29	29	27
	13	27	25	25	45
	20	-56	42	18	46
		F_{0b} Generation			
Body weight (g) on gestation day	0	303	288	272	265*
	20	404	408	380	361**
Body weight gain (g) gestation day 0-20		101	120	108	96
Feed consumption (g/day) gestation day	6	16	17	16	14**
	13	17	17	16	14**
	20	16	16	14*	15
Feed efficiency+ gestation day	6	29	26	27	26
	13	25	24	23	21
	20	56	58	57	50

* Significantly different from control, $p \leq 0.05$, two tailed.
** Significantly different from control, $p \leq 0.01$, two tailed.
+ According to the report, feed efficiency was calculated as 100 x interval body weight change ÷ interval days ÷ daily feed intake.

Table 3: Selected female body weight, feed consumption, and feed efficiency means during gestation from report tables 41 - 52.

Observation	Dose level (ppm) 0	150	800	2000
F_{1a} Generation				
Body weight (g) on gestation day 0	268	261	247*	230**
20	386	373	349**	320**
Body weight gain (g) gestation day 0-20	118	112	102	90
Feed consumption (g/day) gestation day 6	14	14	13	13
13	17	16	16	15
20	16	16	14*	14**
Feed efficiency+ gestation day 6	33	32	33	32
13	22	23	21	20
20	56	54	53	45
F_{1b} Generation				
Body weight (g) on gestation day 0	316	304	280**	269**
20	432	418	385**	362**
Body weight gain (g) gestation day 0-20	116	114	105	93
Feed consumption (g/day) gestation day 6	18	17	16	16
13	17	18	16	16
20	16	16	15	16
Feed efficiency+ gestation day 6	24	24	21	25
13	20	18	19	18
20	60	56	56	45

* Significantly different from control, $p \leq 0.05$, two tailed.
** Significantly different from control, $p \leq 0.01$, two tailed.
+ According to the report, feed efficiency was calculated as 100 x interval body weight change ÷ interval days ÷ daily feed intake.

Table 4: Selected female body weight, feed consumption, and feed efficiency means during lactation from report tables 57 - 68.

Observation	Dose level (ppm) 0	150	800	2000
	F_{0a} Generation			
Body weight (g) on lactation day 0	264	288**	261	252
21	283	286	275	260**
Body weight gain (g) lactation day 0-21	19	-2	14	16
Feed consumption (g/day) lactation day 7	34	35	29*	24**
14	41	45	36**	27**
21	53	56	49	36**
Feed efficiency+ lactation day 7	8	3	-8	6
14	1	3	3	0
21	-3	-5	-1	2
	F_{0b} Generation			
Body weight (g) on lactation day 0	323	326	305	293**
21	313	316	303	289**
Body weight gain (g) lactation day 0-21	-10	-10	-2	-4
Feed consumption (g/day) lactation day 7	30	31	27	25*
14	39	39	34*	29*
21	47	49	45	35*
Feed efficiency+ lactation day 7	6	3	7	?
14	2	1	3	1
21	-6	-3	-1	-1

* Significantly different from control, $p \leq 0.05$, two tailed.
** Significantly different from control, $p \leq 0.01$, two tailed.
+ According to the report, feed efficiency was calculated as 100 x interval body weight change ÷ interval days ÷ daily feed intake.

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Table 4: Selected female body weight, feed consumption, and feed efficiency means during lactation from report tables 42, 44, 46, 48, 50 and 52.

Observation	Dose level (ppm) 0	150	800	2000
F_{1a} Generation				
Body weight (g) on lactation day 0	314	314	280**	263**
21	301	286	275**	247**
Body weight gain (g) lactation day 0-21	-13	-28	-5	-16
Feed consumption (g/day) lactation day 7	31	33	28	21**
14	41	39	36*	28**
21	57	55	50*	39**
Feed efficiency+ lactation day 7	1	0	1	4
14	1	-3	1	2
21	-2	-5	0	-6
F_{1b} Generation				
Body weight (g) on lactation day 0	342	339	306**	294**
21	325	316	298**	289**
Body weight gain (g) lactation day 0-21	-17	-23	-8	-5
Feed consumption (g/day) lactation day 7	2?	30	27	24**
14	38	38	34**	29**
21	53	50	47*	40**
Feed efficiency+ lactation day 7	-5	3	3	-4
14	0	0	2	-1
21	3	3	-2	0

* Significantly different from control, $p \leq 0.05$, two tailed.
** Significantly different from control, $p \leq 0.01$, two tailed.
+ According to the report, feed efficiency was calculated as 100 x interval body weight change ÷ interval days ÷ daily feed intake.

Table 5: Summary of Mean Litter Sizes

Observation and study time	Dose Levels: Control	Low	Mid	High
	F_1 Generation			
Litter A				
Day 0	11.9	12.6	11.7	10.6*
Day 4 pre-cull	12.0	12.2	11.4	10.4*
Day 4 post-cull	12.0	12.2	11.4	10.4*
Day 7	11.9	12.2	11.3	10.8
Day 14	11.9	12.2	11.3	10.8
Day 21	11.9	12.2	11.3	10.6*
Litter B				
Day 0	11.9	12.9	11.8	11.3
Day 4 pre-cull[a]	11.8	12.6	11.8	11.2
Day 7	7.8	7.7	7.4	7.6
Day 14	7.8	7.7	7.4	7.6
Day 21	7.7	7.7	7.4	7.6
	F_2 Generation			
Litter A				
Day 0	11.2	12.6	10.6	10.3
Day 4 pre-cull	10.8	12.3	10.7	10.7
Day 4 post-cull	10.8	12.3	10.7	10.7
Day 7	11.2	12.6	10.7	10.7
Day 14	11.2	12.6	10.7	10.7
Day 21	11.2	12.6	10.7	10.7
Litter B				
Day 0	13.9	12.2*	12.4	10.3**
Day 4 pre-cull[a]	13.5	12.3	12.2	10.2**
Day 7	7.9	7.8	7.6	7.6
Day 14	7.9	7.8	7.6	7.6
Day 21	7.9	7.8	7.6	7.5

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Both B litters were culled to 8 pups at day 4, however, the mean litter sizes were not given (assumed 8).

Table 6: Mean Pup Weights During Lactation

Observation and Study Time	Contr.	Dose Levels Low	Mid	High
F_1 Generation				
Litter A				
Mean pup weight (day 0)	5.6	5.8	5.6	5.7
Mean pup weight (day 4 pre-cull)	9.5	9.8	10.0	8.1**
Mean pup weight (day 7)	14.2	14.5	14.2	11.8**
Mean pup weight (day 14)	26.8	27.6	25.7	20.3**
Mean pup weight (day 21)	42.7	44.7	41.6	30.9**
Weight gain: days 1 - 21	37.1	38.9	36.0	25.2[a]
Litter B				
Mean pup weight (day 0)	6.3	5.8*	5.8*	5.9*
Mean pup weight (day 4 pre-cull)	10.7	9.7*	9.9	9.3**
Mean pup weight (day 7)	17.7	16.7	16.3*	14.8
Mean pup weight (day 14)	35.3	34.5	32.0**	27.6**
Mean pup weight (day 21)	56.3	55.5	51.3**	41.6**
Weight gain: days 1 - 21	50.0	49.7	45.5	35.7
F_2 Generation				
Litter A				
Mean pup weight (day 0)	5.9	6.0	5.7	5.8
Mean pup weight (day 4 pre-cull)	10.0	9.5	9.9	9.3
Mean pup weight (day 7)	15.4	14.2	14.5	12.9**
Mean pup weight (day 14)	29.1	26.7	26.9	21.7**
Mean pup weight (day 21)	48.2	43.1*	43.9	33.0**
Weight gain: days 1 - 21	42.3	37.1	38.2	27.2
Litter B				
Mean pup weight (day 0)	5.7	6.1*	5.7	6.1
Mean pup weight (day 4 pre-cull)	10.0	10.5	9.7	10.3
Mean pup weight (day 7)	16.8	17.5	15.9	15.9
Mean pup weight (day 14)	34.3	35.4	32.0*	29.0**
Mean pup weight (day 21)	56.7	58.4	52.2**	44.4**
Weight gain: days 1 - 21	51.0	52.3	46.5	38.3

* Statistically significantly different from control, $p<0.05$.
** Statistically significantly different from control, $p<0.01$.
[a]Pup weight gain was calculated by EPA toxicologist (statistical significance was not calculated).

Tox Review 011337

Page ____ is not included in this copy.

Pages 88 through 97 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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__✓__ FIFRA registration data.

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

005690

JAN 27 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of SC-0224 Two-Generation Reproduction Study in Rats, Addendum I: Response to EPA Comments Submitted by Stauffer Chemical Company.
EPA Reg. No.: 476-2225/476-2226
TOX-Chemical No. 893C

TO: Robert Taylor, PM 25
Herbicide/Fungicide Branch
Registration Division (TS-767)

FROM: Brian Dementi, Ph.D. Brian Dementi, 1/21/87
Review Section #1
Toxicology Branch/HED (TS-769)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) 1/21/87

Applicant: Stauffer Chemical Company
1200 S. 47th Street
Richmond, CA

Stauffer Chemical Co. has provided a company response to questions raised in our 5/30/86 review (Dementi) regarding the 2-generation reproduction study. TOX Branch concluded in that review that no NOEL had been established, on the basis of finding reduced relative spleen weight in F2B weanling males at the lowest dose level of 150 ppm. A NOEL for increased platelet count in F2B adults (M,F) was estimated at the lowest level administered (150 ppm). Additionally, TOX Branch noted a statistically significant reduction in thymus weight (absolute and relative) for F2B adult males at 2000 ppm. In view of these effects, TOX Branch expressed concern that SC-0224 may be exerting an adverse effect on the reticuloendothelial system.

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In the present submission, Stauffer provides results of additional study and suggests that these data support their previous conclusion that a NOEL of 150 ppm was established.

The following responses are provided for each concern raised in our 5/30/86 review:

I. Reduction in Relative Spleen Weight

Stauffer claims that statistically significant decreases in relative spleen weight which were evident at 150, 800 and 2000 ppm in F2B weanling males is attributed to atypically elevated mean absolute spleen weight and low mean body weight for the control animals. Stauffer compared spleen weights of F1B and F2B weanling males and females to help illustrate this point.

Absolute Spleen Weight, Grams

Group	F1B		F2B	
	M	F	M	F
Control	.329	.315	.474	.354

Spleen weights of F2B males are numerically greater than F1B males, and except for F2B males the other control values are comparable to absolute spleen weights of the Low Dose group.

Furthermore, Stauffer argues that body weights of control F2B weanlings (M,F) were relatively low, perhaps due to increased litter size of this group:

Body Weight, grams

Group	M	F
Control F1B Weanlings	78.9	79.3
" F2B "	70.2	66.6

In addition to these data purporting to explain the reduced relative spleen weights in the various dose groups, Stauffer provided additional data on body weights and spleen weights of control animals. In 50 weanling male rats having a mean body weight of 81.8 grams (a figure comparable to F1B weanling mean weight of 78.9 grams), the mean spleen weight was 0.339 grams. This figure does support Stauffer's position that a mean spleen weight of 0.474 grams in rats of comparable size is unusually high. Consequently, a relative spleen weight of 0.418 as derived

from the supplemental spleen and body weight data appears to be a more reliable control value than that reported for weanling F2B male controls (0.67%) in the original study.

EFB branch is satisfied that Stauffer has adequately addressed this concern and that SC-0224, at doses evaluated in the present study, did not exert an adverse effect on spleen weight at 800 ppm.

I. Thymus

In our previous review we noted that for F2B male adults, absolute and relative thymus weights were reduced, being statistically significant at the high dose.

The Registrant has tabulated thymus weight changes for the different generations at the various dose levels. Statistically significant absolute thymus weight decreases are noted for P1 (M,F) at 800 and 2000 ppm and for P2 (M), F1b weanlings (F) and F2B (F) adults at 2000 ppm. When expressed on a relative weight basis, the only significant decreases were for groups P2 (M), F1b (F) and F2B adult (F) at the 2000 ppm dose level.

EFB Branch agrees with the Registrant that a NOEL = 150 ppm has been demonstrated for thymus weight changes in this study.

J. Platelet Count

In the original 2-generation study, EFB Branch identified significant increases in platelet count at 800 and 2000 ppm in both sexes of F2B adults. (Appendices 103, 114)

ppm Level	Males	N	Females	N
0	855 ± 169	5	863 ± 87	5
150	888 ± 65	5	899 ± 178	5
800	1017 ± 69	5	1042 ± 49	5
2000	1092 ± 68	4	1107 ± 162	5

In Addendum I, Stauffer provided historical platelet count control data for male and female rats in the age groups 1-3 months and 4-14 months, and notes that all of the platelet values for animals in the 800 and 2000 ppm dose groups fall within the reference range for the laboratory. Furthermore, Stauffer emphasizes that one of the male reference ranges used has a skewed distribution. The petitioner also points out

that the study controls appear to be atypical and that this particular group of controls is derived from the same group of animals in which the atypical spleen weights were observed. For these reasons Stauffer would have the Agency accept that an apparent dose related increase in platelet count in both sexes has no biological significance.

Tox Branch adheres to the conclusion reached in our original review that the increases in platelet count observed in both sexes at 800 and 2000 ppm are significant. Therefore NOEL=150 ppm for this biological effect. Since platelet count was altered at the higher doses and there is uncertainty as to what dose represents the LOEL, any additional studies which include assessments of this and/or related parameters should be carefully evaluated.

TOX Branch conclusions: NOEL = 150 ppm, LOEL = 800 ppm [reduced feed intake and body weight in both parents and pups; reduced absolute thymus weight, P1 (M,F); platelet count increase, F2B adults (M,F)]

Core rating: upgrade from supplementary to guideline

011337

005173

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

MAY 30 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Reviews of Studies performed on Herbicides SC-0224 and SC-0224 4LC submitted by Stauffer Chemical Company.
EPA ID Number: 476 EEEL/476 EEEA

TO: Robert Taylor, PM 25
Registration Division (TS-767)

FROM: Brian Dementi, Ph.D.
Review Section #1
Toxicology Branch/HED (TS-769)

Brian Dementi, 5/23/86

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

5-29-86

APPLICANT: Stauffer Chemical Company
1200 S. 47th Street
Richmond, California 94804

Stauffer Chemical Company has requested review of the accompanying studies, submitted in anticipation of a petition for the use of Herbicides SC-0224 and SC-0224 4LC. Of eight studies submitted, seven are reviewed herein. The remaining study, a teratology study (T-11050, November 5, 1992) on SC-0224 was previously submitted by Stauffer and reviewed by Toxicology Branch. See the February 8, 1984 review by Roland A. Gessert, Caswell 893C.

Summary of Results:

1) SC-0224 Two-Generation Reproduction Study in Rats (T-11051)
Overall Reproductive NOEL = < 150 ppm (F2B male, weanlings, relative spleen weight reduction)
Overall Clinical NOEL = 150 ppm (platelet count increase, combined male and female adult F2B generation)
Core: supplementary

2) Acute Inhalation Study, Rat with SC-0224 (T-11728)
LC_{50} > 0.81 mg/L
Core = guideline

3) Acute Inhalation Study with SC-0224 4LC (T-11870)
LC_{50} = 1.30 mg/L (male); LC_{50} = 1.56 mg/L (female)
Core: guideline

* Second Gen. weanling relative spleen wt reduction should be considered as a reproduction effect only fertility, gestation, live birth and lactation indices should be used to set the reproduction end point
1 ppb only 40

011337

Study: SC-0224 Two-Generation Reproduction Study in Rats

Laboratory: Environmental Health Center
Stauffer Chemical Company
Farmington, Connecticut

Study No. and Date: T-11051, April 19, 1984

Accession No.: 258398 (Appendix 8), 258399

Material Tested: Aqueous SC-0224 technical containing 19.2% active ingredient by weight.

Animals: Rat [Crl CD (SD)Br]

The purpose of this study was to determine if SC-0224 technical has effects on rat reproduction when admininstered in the diet.

Materials and Methods: (as paraphrased from Study Procedure)

Husbandry: Standard GLP

During mating, one male was housed with one or more females. Subsequent to mating, females were individually housed. On gestational day 20, pregnant females were transferred to larger cages for delivery and weaning. Weanling pups were subsequently housed two per cage. At approximately 40 days of age pups were housed singly for future breeding.

A. Experimental Design (Excerpted from Study No. T-11051, pp. 2-9).

Following quarantine, "80 males and 120 females were assigned to four dose groups of 20 males and 30 females each. Beginning at 43 days of age the animals were fed purified rodent meal containing 0, 150, 800, 2000 ppm of active SC-0224. At the end of 62 days of treatment, 105 days of age, the 20 males and 30 females of each group were randomly mated to yield the litters of the F1a generation. F1a pups were weaned at 21 days and discarded. At 160 days of age, study day 118, P0 animals were randomly mated a second time to yield the F1b litters. On postpartum day 4, F1b litters were culled to eight pups (where applicable) leaving, as nearly as possible, four males and four females per litter. The culled pups were necropsied under the supervision of the Study Director. Following weaning, 20 male and 30 female weanlings of each group were randomly selected and continued on their respective treatments as the P1 animals. Additionally, five male and five female

weanlings from each dose group were randomly selected and necropsied by the Pathology Section. The remaining weanlings were necropsied under the supervision of the Study Director. Five weanlings of each sex were saved as possible replacements for weanlings lost either before necropsy or prior to the designated P1 day 0 reference date of March 15, 1983. P0 male and female animals were sacrificed and necropsied three to four weeks following the weaning of the F1b pups.

"The P1 animals were 38 to 43 days of age on the day 0 reference date. The P1 animals were maintained on their respective diets for 62 days and then mated as described above to yield the F2a litters. After 119 days on treatment, they were again mated to yield the F2b litters. The litters were handled as described above for the F1 generation. Additionally, five weanlings of each sex were selected from each group to continue as adult F2b animals. P1 male and female animals were sacrificed and necropsied three to four weeks following the weaning of the F2b pups.

"The continuing F2b weanlings had a designated day 0 reference date of September 12, 1983. Ages of animals on this reference date ranged from 37 to 41 days. The F2b adult animals continued on treatment until sacrificed and necropsied 56 to 57 days after the reference date; at 93 to 98 days of age."

B. In-Life Observations

"All parent animals were observed daily for overt signs of toxicity or ill health. Thorough examinations for clinical signs occurred whenever body weights were determined.

1. During the Growth Phases

The body weight and food consumption of each parental animal were determined weekly.

2. During the Mating Phases

Before cohabitation, a thorough external examination was performed on the animals to detect any abnormal signs. Male precopulatory behavior was noted as present or absent when the female was added to the male's cage. Each morning of the first ten mornings of cohabitation, a vaginal smear was taken from each female to determine the stage of estrus, or to note a positive mating sign. The day sperm or a

copulatory plug were detected was considered day 0 of gestation. The cohabitation period was 24 hours/day for up to three weeks. Weekly body weights were continued on males and unmated females.

3. During the Gestational Phases

Body weights were taken on gravid days 0, 6, 13, and 20. Food consumption was determined for the gravid day intervals 0-6, 6-13, and 13-20.

4. During the Perinatal Phases

On gravid day 20, females were transferred to a large cage. Beginning on day 21, the females were monitored for normal behavior in the sequence of events during parturition. Following delivery, the dam and litters were examined as soon as possible. The day of delivery was considered postpartum, or lactational, day 0. Deliveries beginning before 3:00 p.m. were assigned that calendar date; those after, the next calendar date.

5. During the Lactational Phases

The dam and litter were examined after the dam had cleaned and assembled the litter; usually on day 0, but occasionally on day 1. On lactational days 0 or 1, 4, 7, 14, and 21 the dam body weight, total litter size, numbers of live and dead pups, and pup anomalies were recorded. For F1a and F2a litters, the total live litter weight was taken on days 0 or 1, 4, 7, and 14, and individual pup sexes and weights were recorded on day 21.

F1b and F2b litters were given more extensive examinations. On day 0 or 1, pups were sexed individually, but weighed collectively. Individual pup sexes and weights were recorded on days 4, 7, 14, and 21. The litters were culled on day 4 to eight pups leaving, when possible, four males and four females in each litter. In addition, on the day indicated, completion of the following maturational landmarks were determined for the pups: day 4, unfolding of the external pinna of the ear; day 7, incisor eruption; day 14, opening of the eye. At each weighing before day 14, the presence or absence of milk in the stomach of the pups was noted.

Food consumption was determined for the dams during the lactational day intervals of 0-4 or 1-4, 4-7, 7-14, and 14-21."

C. Terminal Procedures and Observations

"1. Scheduled Terminations

a. Necropsy Examination with Organ Weights and/or Tissue Collection: Five male and five female F1b and F2b weanlings selected randomly at each dose group and all P0, P1, and adult F2b parental animals were anesthesized with intraperitoneally administered sodium pentobarbital and exsanguinated. Necropsy laboratory personnel collected weights for the following organs: liver, heart, brain, pituitary, lungs, thymus, spleen, right and left kidney, adrenals, and gonads. The following tissues were collected from the P0, P1, and adult F2b animals and samples placed in the indicated fixative. Tissues were not routinely collected from the weanlings.

Organ System	Tissue	Fixative*
Integument	Skin and mammary gland	NBF
Musculoskeletal	Skeletal muscle (thigh)	NBF
	Bone	
	tibia/femur and joint	NBF
	sternum	NBF
Respiratory	Lungs	NBF
Cardiovascular	Heart	NBF
Hemic/lymphatic	Thymus	NBF
	Spleen	NBF
	Bone marrow sternum	NBF
	Mesenteric lymph node	NBF
	Mediastinal lymph node	NBF
Digestive	Mandibular salivary gland	NBF
	Esophagus	NBF
	Stomach	NBF
	Duodenum	NBF
	Jejunum	NBF
	Ileum	NBF
	Cecum	NBF
	Colon	NBF

Organ System	Tissue	Fixative*
Digestive (cont'd)	Rectum	NBF
	Pancreas	NBF
	Liver	NBF
Urogenital	Kidneys	NBF
	Urinary bladder	NBF
	Testes	BG
	Epididymides	BG
	Prostate	NBF
	Seminal vesicles	NBF
	Coagulating glands	NBF
	Ovaries	BG
	Vagina	NBF
	Cervix	NBF
	Uterus	NBF
Endocrine	Pituitary	BG
	Thyroids	NBF
	Parathyroids	NBF
	Adrenals	BG
Nervous	Brain	NBF
	Sciatic nerve	NBF
Special Senses	Eyes	BG
	Harderian glands	BG
	Gross lesions (as specified by the pathologist	

b. Necropsies without Organ Weights or Tissue Collection: After carbon dioxide asphyxiation, both the F1b and F2b pups culled on day 4 and those not selected for either organ weight determination or the next parental generation on day 21 were necropsied under the supervision of the Study Director. The heads of culled pups were fixed in Bouin's fixative and then examined by free-hand razor blade sectioning according to Wilson (1965). The thoracic and abdominal viscera were examined according to Staples (1974).

*BG - 2.5% buffered gluteraldehyde, NBF - 10% neutral buffered formalin.

c. Clinical Laboratory Tests (Adult F2b's Only):

Adult F2b's were fasted overnight prior to their terminal sacrifice. At sacrifice, blood samples were collected from the abdominal aorta of each animal and the following hematologic and blood chemistry tests were performed:

Hematology

1) Hematocrit
2) Hemoglobin
3) Erythrocyte count
4) Leukocyte count (total and differential)
5) Platelet count
6) Prothrombin time
7) Partial thromboplastin time

Blood Chemistry

1) Aspartate aminotransferase (SGOT)
   Alanine aminotransaminase (SGPT)
3) Gamma glutamyl transferase
4) Alkaline phosphatase
5) Total protein
6) Total bilirubin
7) Albumin
8) Blood urea nitrogen
9) Glucose
10) Sodium
11) Calcium
12) Potassium
13) Inorganic phosphate
14) Chloride
15) Creatinine
16) Total cholesterol
17) Triglycerides
18) Creatine phosphokinase
19) Lactate dehydrogenase
20) Plasma cholinesterase
21) Red blood cell cholinesterase
22) Albumin/globulin ratio
23) Uric acid
24) Serum protein electrophoresis

d. Histopathology: A single set of slides were prepared for the tissues in the collection list for five animals/sex/dose group for the PG, P1, and Adult F2b animals. The slides were sent to the Japanese collaborators for histological evaluation. Their findings are not included in this report."

"2. Unscheduled Terminations

a. Parental Animals: Moribund animals which were sacrificed and animals which were found dead were necropsied under the supervision of a Veterinary Pathologist.

b. Preweaning Pups: Preweaning F1b and F2b pups which died and stillborn pups were necropsied under the supervision of the Study Director. The heads of these pups were fixed in Bouin's fixative and examined according to the method of Wilson (1965). The thoracic and abdominal viscera were examined by the technique of Staples (1974)."

Data Interpretation

"1. Parameters

In addition to the raw data collected, other parameters were calculated and analyzed statistically, these parameters are defined in the tables.

"2. Statistical Analysis

Quantitative or continuous data such as body weights, feed intakes, pup weights, and organ weights were tested for significance using a one-way analysis of variance and Dunnett's procedure (Dunnett, 1964).

Enumeration data for each group, including clinical observations, necropsy findings, reproductive counts, and weanling findings were evaluated using the Fisher exact probability test (Siegel, 1965) with Bonferroni's correction for multiple comparisions to a control value (Ingelfinger, 1983).

Additionally, litter parameters including the number of male, female, and viable pups and litter incidences such as the live-born index, survival indices, and developmental landmarks were analyzed with a nonparametric rank test (Mann-Whitney U, Steel, 1960).

Fisher exact tests were one-tailed. All other tests were two-tailed. Statistical significance was based on a level of $p < 0.05$, but values also significant at $p < 0.01$ were so indicated." (pp 2-8)

Results:

Concentrations of SC-0224 as actually measured in the diet agree well with the intended concentration, i.e., 0, 150, 800, and 2000 (ppm); Table 1, p. 30 to 31.

I. CLINICAL OBSERVATIONS ON PARENTAL ANIMALS

P0 and P1 generation males receiving SC-0224 did not display at any dose adverse clinical signs meaningfully different from those observed in control animals (Tables 3 and 4 pp. 33 to 35).

A. General Effects in Parental Animals

All P0 males survived until scheduled sacrifice. On the other hand six P0 females did not survive to scheduled termina ion. The distribution for premature death was 150 ppm (4 rats), 800 ppm (1 rat) and 2000 ppm (1 rat). A dose-related trend for the deaths was not evident. Among the four premature deaths of the 150 ppm group, multifocal nephrosis plus other complications were identified as contributing causes of death. Red discolorations (foci, fluids) from various tissues were noted. Renal and pulmonary congestion and edema were implicated as contributing causes of death in the rat of the 800 ppm group. Cause of death in the 2000 ppm rat was undetermined (Table 21, pp. 84 to 87, necropsy).

One P1 male (2000 ppm group) died prematurely. Necropsy information indicated mottled red lungs and red fluid in thoracic cavity. Five P1 females died prior to scheduled sacrifice (0 ppm [1], 800 ppm [3], and 2000 ppm [1]). For these animals, red discolorations were noted in various areas, but necropsy did not disclose any findings that could be linked to dosing (Table 22, pp. 88 to 89).

B. Body Weights, Feed Intake

Body weight gain for P0 male animals in the 2000 ppm group was shown to be significantly reduced, which became apparent by about the 35th day of study and continued to near the end of study. A numerical reduction in body weights was observed in the 800 ppm group after about 132 days, but was not statistically significant. Males of the P1 generation in the 800 ppm and 2000 ppm group displayed significantly reduced body weight over the entire 175-day observation period. Consistent with the above findings were generalized reduced food intake in the high dose P0 and P1 males (Tables 7 and 8, pp. 43 to 49).

Among females, weight gain for the P0 generation was unaffected in the 150 and 800 ppm groups, but was repressed

in the 2000 ppm group from day 62 essentially to the end of the study during which time four of ten weight determinations were significantly reduced. For the P1 females, weight gain was inhibited in both the 800 ppm and 2000 ppm groups throughout the study period, whereas the reductions for most determinations were statistically significant (Tables 23 and 24, pp. 90 to 94). There were no consistent dose-related effects on food intake for P0 females. Among P1 females, food intake was significantly reduced for the first 62 days of observation, however, this effect was not apparent on days 118 to 191 (Tables 25 and 26, pp. 95 to 98). Thus in summary, for reduced weight gain:

P0 male LOEL = 800 ppm
female LOEL = 2000 ppm
P1 male LOEL = 800 ppm
female LOEL = 800 ppm

C. Feed Efficiencies

Feed efficiencies for P0 and P1 male and female rats (premating) were unaffected by dosing (Tables 9 and 10, pp. 50 to 56 and Tables 27 and 28, pp. 99 and 100). There were some puzzling numbers in Table 9. For example, at 84 days (p. 51) figures for the 0, 150, 800 and 2000 ppm groups were, respectively, -258, -15, -66, and 10.

D. SC-0224 Intake

Generally speaking, with respect to actual SC-0224 intake, it was observed that for P0 and P1 males, intake declined from the initial time point (day 8) to the time of mating (day 62) and then remained fairly constant. Overall mean intakes were 0, 6.2, 35, and 85 mg/kg/day for P0 males and 0, 6.1, 35, and 92 mg/kg/day for P1 males. At the time of mating, the values of P0 females were 0, 9, 43, and 101 mg/kg/day and 0, 7, 39, and 95 mg/kg/day for P1 females.

E. Necropsy - P0, P1

Necropsy of P0 and P1 males did not disclose any unusual findings or increased incidence of adverse effects at any dose level (Tables 13 and 14, pp. 64 to 67). Similarly, necropsy findings for P0 and P1 females did not disclose any remarkable effects of SC-0224 at any dose level (Tables 31 and 32, pp. 103 to 107).

Absolute organ weights for P0 males were generally unaffected by SC-0224. A notable exception was that of the thymus, which was significantly reduced in the high dose-group (0.339 gm vs. 0.500 gm [control]). Among P1 males a number of organ weights were significantly reduced in the

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high-dose group. These included adrenal (right), brain, heart, kidney (both), and liver. Liver and heart weights were also significantly reduced in the 800 ppm group. Liver weight for the 2000 ppm group was significantly reduced to 9.82 gm vs. 14.89 gm for control.

Among P0 females, absolute organ weights were not markedly affected by SC-0224. Heart weight was significantly reduced in the 800 and 2000 ppm dose groups, and left kidney weight was significantly less in the high dose group. Among P1 females, several absolute organ weights were reduced in the high dose group, these included heart, kidneys, liver, pituitary, spleen, and thymus. All were statistically significant changes except that of the spleen. In the 800 ppm dose group, the following organ weights were significantly reduced relative to the controls: heart, right kidney, liver and thymus (Tables 33 and 34, pp. 108 to 113).

According to the study author, where organ weight changes for P0 and P1 males and females are concerned, "These changes can be attributed to the reductions in body weight, or were not toxicologically significant" (pp. 12 and 15). It may be true that organ weight losses are simply consonant with reduced weight gain, since no remarkable effects were observed on a relative organ weight basis.

F. Reproductive Effects

In terms of such parameters as mating index and fertility index for males and females and gestation index, behavior during delivery, length of gestation and length of delivery, there were no adverse dose-related effects observed with respect to either P0 or P1 generations (Tables 37 to 40, pp. 120 to 123).

Dam weights for P0 and P1 generations (1st and 2nd matings) during 20 days of gestation were unremarkable with respect to dosing. P0 generation dams in the 2nd mating exhibited reduced body weights in the 2000 ppm dose group, but this was in evidence from day 0 of gestation time, and did not appear to worsen in the course of time. Likewise, dams of the P1 generation (1st and 2nd mating) exhibited reduced body weights in the 800 ppm and 2000 ppm dose groups for the duration of the 20-day gestation period, but did not appear to worsen at either dose during the course of gestation. Reduced dam body weights appear to have been the consequence of dosing prior to mating (Tables 41 to 44, pp. 124 to 127).

G. Food Intake During Gestation

Food intake for P0, P1 dams was significantly reduced for the 800 ppm and 2000 ppm dose groups at various gestational

times. These changes may be the consequence of dosing and as the study authors indicate elsewhere may be related to palatability of the food, SC-0224 admixture (Tables 45 to 48, pp. 128 to 131). Data provided on food efficiency and SC-0224 intake during gestation did not disclose any points of appreciable concern.

H. Dam Weights During Lactation

Dam weights for postpartum days 4 to 21 were significantly reduced in the high dose group of P0 generation, 1st mating. The dam weight was not significantly reduced for this group at time 0. Dam weight of generations P0 (2nd mating) and P1 (1st and 2nd matings) for the 800 ppm and 2000 ppm dose groups were significantly less than control weights during the 0 to 21 days of lactation (Tables 57 to 60, pp. 140 to 143).

I. Dam Food Intake During Lactation

Generally, food intake, as measured during lactation days 4 to 21, was significantly reduced for both matings of the P0 and P1 generations in the high-dose group. There was some evidence of food intake reduction in the 800 ppm dose groups, particularly in the P1 generation (2nd mating), where at three of the four postpartum time points food intake was significantly reduced (Tables 61 to 64, pp. 144 to 147).

Food efficiency and SC-0224 intake data reported during lactation did not provide any remarkable findings.

II. LITTER AND PUP PARAMETERS

A. Litter Size

Mean litter size was significantly reduced in the 2000 ppm dose group of the P0 first mating. The mean litter size at birth was 10.5 ± 3.2 for the 2000 ppm dose group as compared to 12.5 ± 2.3 for the control group. By virtue of this at birth reduced litter size for the 2000 ppm group, litter size on live days 0 to 21 were in general significantly reduced. However, there was no evidence of increased pup mortality in this or any other dose group during the 21-day postpartum period. For P0 (2nd mating) and P1 (1st mating), mean litter sizes were not altered at any dose level, at birth or during 21-days postpartum. For P1 (2nd mating), there were two live day time points (0 and 4 days) where litter size was reduced in the high-dose group. This was not true at later time points. These significant reductions may simply reflect

continuance of the numerical* reduction in litter size seen in the high-dose group (10.4 ± 3.0 vs. 14.1 ± 2.8 control) at birth, and does not serve to indicate increased pup mortality in the course of time during the 21-day postpartum period (Tables 73 to 76, pp. 156 to 159). Sex ratios were not adversely affected.

B. Mean Pup Weights

P0 (1st mating) pups did not differ at any dose in mean weight at birth from that of the control group. However, by postpartum day 4 through day 21 mean pup weight was significantly reduced in the 2000 ppm dose groups.

Mean pup weights for male and female animals were lower in the 2000 ppm groups. No effects were observed at other dose levels for the P0 (1st mating) offspring. In the P0 (2nd mating), mean pup weight was significantly reduced in the 800 ppm and 2000 ppm dose groups. This was a generally consistent finding from birth through 21 days postpartum. In the two high-dose groups, mean pup weights expressed as percent of control mean pup weights are as follows:

	Mean Pup Weight, % of Control	
Postpartum Day	800 ppm	2000 ppm
Birth (0)	92	94
4	92	87
7	92	84
14	91	78
21	91	74

The above table shows that mean pup weight was affected in a dose-related manner and that in the course of time, the weight declined from 94 percent on day 0 to 74 percent of control values by day 21. These data clearly show an adverse effect of SC-0224 in terms of reduced pup weights at doses of 800 and especially 2000 ppm.

In the P1 (1st mating), offspring weight was not affected by any dose at the time of birth, however in the high-dose group, on postpartum days 7 to 21 there was a significant reduction in mean pup weight. Pups in other dose groups were not so affected.

*Note: While not reported to be significant in the Stauffer table (76, p. 159), our independent calculations show this reduced litter size in the high-dose group to be highly significant.

Surprisingly, in the P1 (2nd mating), mean pup weight at birth was unaffected by dosing. Furthermore, mean pup weights were not altered on postpartum day 4 or 7. On postpartum days 14 and 21, mean pup weight was significantly reduced in the 800 ppm and 2000 ppm dose groups. This inhibition was of greater magnitude in the 2000 ppm group than in the 800 ppm group (Tables 77 to 80, pp. 160 to 163).

C. Pup Survival and Development

Among offspring of the first and second matings of the P0 and P1 generations, there were no adverse effects of dosing evident with respect to the following parameters: liveborn index, viability index, lactation index, survival indices. Furthermore. offspring arising from the second matings of the P0 and P1 generations did not exhibit any adverse dose-related effects with respect to developmental landmarks including milk in stomach (days 0, 4, 7), detached pinna, incisor eruption, eye opening (Tables 81 to 84, pp. 164 to 169).

D. Macroscopic Findings in Pups

Macroscopic data of F1B and F2B pups exists for 1) pups found dead before weaning, 2) pups culled on day 4, 3) pups at weaning (Study Director), and 4) pups at weaning (pathology): Appendices 80 to 83 (pp. 895A to 926A) for F1B pups and Appendices 84 to 87 (pp. 927A to 950A) for F2B pups.

Generally speaking with respect to both F1B and F2B pups there were no definitive findings indicating a teratogenic or birth defects problem. Frequently observed phenomena in the control and dosed animals were convoluted and dilated ureters and dilated renal pelvis. However, there is no evidence that SC-0224 enhanced the frequency of those abnormalities.

Additional abnormalities worthy of notation are tabulated below.

	Dose, ppm (page)					
	0	150		800	2000	
F1B	Kinked tail (914)*	4 small pups	(906)		Short tail	(920)
	2-cerebellum small,	Small eyeball	(917)		Short tail	(920)
	depressed area (916)	Kinked tail	(917)		Absent tail	(921)
		Situs inversus	(906)			
F2B	Vestigial tail, anus	Pointed snout	(929)	Kinked tail (943)	Small pup	(930)
	imperforate, short	Small eyeballs	(929)	Runt (944)	Hind brain, cystic	
	thorax (929)	Hindbrain, cystic			dilation	(938)
	Testis absent (940)	dilation	(935)		Situs inversus	(945)
	Testis absent (940)				Tail absent	(946)

( )* - Litter ID Number.

These findings are not viewed as indicating an adverse reproductive or teratogenic effect of SC-0224. The absence of a tail is a serious defect and appears once in the high-dose group of each the F1B and F2B generations, however, these two findings in this study probably do not violate spontaneous occurrences of this defect.

E. Absolute Organ Weights, (F1B) Weanlings

Males: Whole body weight and liver and kidney organ weights of the 2000 ppm dose group were significantly reduced with respect to control values. Body and organ weights were not significantly reduced in the 800 ppm group. The 150 ppm dosing did not appear to affect organ weights (Table 87, pp. 172 to 173). Reduced organ weights at the higher doses appear to be consonant with general reduced body weights.

Females: Whole body weight and certain organ weights (kidneys, liver, lungs, and thymus) of the 2000 ppm dose group were significantly reduced with respect to control values. This compound did not appear to affect body or organ weights in females at doses lower than 2000 ppm (Table 91, pp. 180 to 181). The effects observed on organ weights evidently are consonant with body weight losses.

F. Absolute Organ Weights (F2B) Weanlings

Males: Whole body weight as well as the following absolute organ weights were significantly reduced in the high dose (2000 ppm) group: adrenal (L), kidneys, lungs and spleen. In the 800 ppm group the only remarkable finding was a significant reduction in spleen weight. Dosing at 150 ppm was unremarkable (Table 88, pp. 174 to 175).

Females: Whole body weight was numerically but not significantly reduced in the high-dose group. The only organ weight significantly reduced in the high-dose group was that of the spleen. The effects of lower doses on body weight and absolute organ weights were unremarkable (Table 92, pp. 182 and 183).

G. Relative Organ Weights, F1B and F2B Weanlings

The only remarkable findings were: 1) significantly increased brain weight at the high dose in F1B and F2B males and F1B females, and 2) a remarkable repression in relative spleen weight in all dose groups among F2B males and in the high-dose F2B female group, tabulated as follows:

Relative Spleen Weight (%), F2B Generation Weanlings

	Control	150 ppm	800 ppm	2000 ppm
Male	0.676	0.499*	0.504*	0.434*
Female	0.534	0.473	0.470	0.423*

*Significantly different from control, $P < 0.05$, two-tailed. (Table 90, pp. 178-179 and Table 94, pp. 186-187).

III. GENERAL FINDINGS IN F2B ADULT MALES

A. Clinical Observations

There were no remarkable observations in any dose group (Table 95, p. 188).

B. Body Weights

There was, for the high-dose group, a general repression of body weight increase of F2B generation animals, as evidenced by significant weight reductions relative to controls over a 55-day observation period. Weight gain in other dose groups was not so impaired (Table 96, p. 189).

C. Feed Intake

Feed intake was diminished in the high-dose group only, a finding consistent with reduced body weight gain for this groups (Table 97, p. 190), as evidenced by feed efficiency data (Table 98, p. 191).

D. Findings at Necropsy

Five animals of each dose group and control group were evaluated at scheduled necropsy. There were no remarkable observations (Table 100, p. 193).

E. Organ Weights of F2B Adult Males

Mean body weight of high dose males was significantly lower than that of controls. With respect to absolute organ weights, the notable findings were significant reductions in weight of the spleen and thymus in the high-dose (2000 ppm) group (Table 101, pp. 194 to 196).

Relative organ weight data also revealed a significant reduction for the thymus (Table 102, pp. 197 to 199).

F. Clinical Test Results, F2B Males

Among hematological parameters, platelet count data suggest a dose response, where the counts for the high-dose and middle-dose groups were significantly greater than that of the control: Control(855), 150 ppm(888), 800 ppm(1017) and 2000 ppm(1092). See note, page 19. Also, hemoglobin was significantly elevated in the high-dose group (Table 103, p. 200).

IV. GENERAL FINDINGS ON F2B ADULT FEMALES

A. Clinical Observations

There were no remarkable dose-related observations (Table 106, p. 204).

B. Body Weights

There was no evidence of an effect of SC-0224 at any dose or body weights or weight gain (Table 107, p. 205).

C. Feed Intake

Mean feed intake was not significantly altered by any dose level of SC-0224 (Table 108, p. 206). Mean feed efficiency data did not yield any remarkable findings (Table 109, p. 207).

D. Findings at Necropsy

Among the five animals/dose group necropsied, there were no remarkable findings that would indicate an adverse effect of SC-0224 at the dose administered (Table III, p. 209).

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E. Organ Weights

Absolute organ weight data do not indicate any effects on females which could be viewed as related to the administration of SC-0224 (Table 112, pp. 210 to 212). The same statement is applicable for Relative Organ Weight Data (Table 113, pp. 213 to 215).

F. Clinical Findings

Among hematological parameters, platelet count data suggest a dose response effect (as was noted for males): control control(863), 150 ppm(899), 800 ppm(1042) and 2000 ppm(1107). See note p. 19. Platelet counts ($10^3/mm^3$) by our calculations were significantly elevated in the high- and middle-dose groups. (Table 114, p.216)

Among blood chemistry values, BUN was significantly reduced for the mid- and high-dose groups, as were total protein and albumin.

Mean protein electrophoretic data indicated significantly reduced total protein in the mid- and high-dose groups (Table 116, p. 219).

Summary of Findings

Body weight gain for both male and female, P0 and P1 generation, rats was impaired by doses of SC-0224 as low as 800 ppm in the diet. Organ weight reductions among P0 and P1 rats were also observed at 800 ppm. Most notably affected in this manner were thymus, liver, heart, and kidneys. During gestation, food intake for P0 and P1 animals was less at 800 ppm. Similarly, during lactation, weight gain and food intake for P0 and P1 animals were less in the 800 ppm dosed animals.

Weight gain of P0 and P1 pups during the 21-day postpartum period was reduced in groups feed 800 ppm.

At scheduled sacrifice of F1B weanlings, body weight and organ weights were reduced in the 2000 ppm dose group. Similarly, F2B body weights were reduced in the high-dose group, but male spleen weight was significantly reduced in the 800 ppm dose group, otherwise, organ weights for both sexes were affected by 2000 ppm.

A notable finding for F2B weanling animals, under relative organ weights, was that in male rats of decreased spleen weight, LOEL < 150 ppm. For F2B females, spleen LOEL = 2000 ppm, with a trend toward lower spleen weights at the lower dose levels.

Evaluations on F2B adult rats revealed the following:

serious defect, this is not viewed as exceeding the incidence likely to occur spontaneously.

Necropsy of F2B adult rats did not disclose any remarkable defects.

In terms of such parameters as mating index and fertility index for males and females, and gestation index, behavior during nursing, length of gestation and length of delivery, there were no adverse dose-related effects observed with respect to P0 and P1 matings.

Overall Reproductive NOEL = < 150 ppm (F2B, male, weanlings relative spleen weight reduction)

Overall Clinical NOEL = 150 ppm (platelet count increase, male and female adult F2B generation)

Core Rating = Supplementary

Repairability = Nonrepairable

Reference:

Raab, S.O. The spleen and reticuloendothelial system. (1974) In: Pathologic physiology, mechanisms of disease. W.A. Sodeman, Jr., and W.A. Sodeman, eds. W.B. Saunders, Co., PA.

Note (as referenced on pp. 16 and 17): combined F2B adult male and female platelet count data revealed statistically significant reductions ($P < .05$) in the middle and high dose groups.

for male rats, body weight, feed intake and organ weights were decreased in the high-dose group. In adult F2B females body weight and feed intake were not affected even in the high-dose group. A notable finding was that for both male and female adult F2B rats, platelet count was statistically significantly increased in the 800 ppm and 2000 ppm dose group.

The findings of reduced spleen weight and increased platelet count in response to SC-0224 merit comment, as these two parameters are coordinated.

It is recognized that the spleen sequesters platelets. The spleen normally contains about one third of the individual's total platelet mass. Epinephrine administration, for example, will stimulate release (not synthesis) of sequestered platelets from the spleen resulting in an increased circulating platelet concentration. Furthermore, in splenectomy and in congenital splenic agenesis (barrenness, impotence), thrombocytosis may be striking initially. Following splenactomy, platelet count rises fairly rapidly (24 to 48 hours), reaching a peak in 5 to 7 days. (Raab, 1974).

This line of evidence showing that reduced spleen competence and increased platelet count are coordinated serves to indicate that the effects noted on spleen weight and platelet count following SC-0224 dosing are likely related. This evidence reinforces the validity of each of the study findings. These effects on the spleen and platelet count, taken in conjunction with reduced weight of the thymus as noted, collectively suggest a general adverse effect of SC-0224 on the reticuloendothelial system for which the LOEL has not been determined.

Among adult male F2B rats, hemoglobin was increased and certain enzymes decreased in the 2000 ppm dose group. In female F2B rats, BUN, protein and albumin were significantly reduced for the 800 ppm group.

There was no evidence of a compound-related increase in pup mortality, postdelivery, for P0 and P1, 1st and 2nd generations.

There were statistically significant decreases in mean litter size at <u>birth</u> for the P0 (1st mating) and P1 (2nd mating) at the high dose in both cases.

Necropsy of P0 and P1 (male and female) rats did not disclose any compound-related effects. With respect to F1B and F2B pups there were no definitive findings indicating a teratogenic response. In each of the F1B and F2B high-dose groups there was one pup with the tail absent. While a

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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 8/19/97
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner, Section Head
Section I, Tox. Branch (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic feeding/oncogenicity - rat (83-5)
Supplemental DER - Changes generated from RfD Meeting

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

ACCESSION NO./MRID NO.: 412099-07, 412099-05, 402140-07

TEST MATERIAL: Sulfosate

SYNONYMS: SC-0224

STUDY NUMBER(S): T-11082

SPONSOR: Stauffer Chemical Co

TESTING FACILITY: Stauffer Laboratory, Farmington, CT

TITLE OF REPORT: Two-Year Chronic Toxicity and Oncogenicity Dietary Study with SC-0224 in Rats

AUTHOR(S): Pavkov, K.L.; Wyand, S.

REPORT ISSUED: April 3, 1987

CONCLUSION: Technical SC-0224 (Sulfosate, 56.2% pure) was tested in a 2-year chronic feeding/oncogenicity study in male and female Charles River Sprague-Dawley (Crl:CD[SD]BR) rats. Sixty animals/sex were tested in control group 1 (basal diet, no vehicle), 80/sex were tested in control group 2 (basal diet plus propylene glycol at 1% w/w vehicle) and in the low and mid-dose groups, and 90/sex were tested in the high dose group. The following dose levels were tested: 0, 100, 500 or 1000 ppm a.i.. There were interim sacrifices of variable numbers of rats at 6, 12, and 18 months. The number of rats sceduled for the full 24-month study duration was 50/sex/group.

It appears that the rats may have tolerated higher dose levels. At 1000 ppm, there were decreases in bodyweight (both sexes) and increased incidences of chronic laryngeal and nasopharyngeal inflammation (males). The bodyweight decrease was considered to be secondary to reduction in food consumption. However, the study is considered to be acceptable because the top dose may be approaching at least one-half of an adequate dose for carcinogenicity testing (based on results from the subchronic and reproduction studies). In addition, the mouse carcinogenicity study was tested above the limit dose (8000 ppm). Therefore, it is believed that this chemical was adequately tested for

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carcinogenicity. This study satisfies the regulatory requirement for a chronic feeding/oncogenicity study in the rat.

Classification: Minimum

Testing Guideline Satisfied: 83-5

DER #1

Sulfosate: 2-Year Feeding/Oncogenicity Study in Rats
Stauffer Chemical Company. 1987. MRID No. 40214007, 41209905.
HED Doc. No. 006542, 008368.

011337

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

002348

Chronic Rat

- 011337

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Sulfosate - EPA File Symbols 10182-ETT and 10182-ETA (PP#9F3796) - Sulfosate in/on Corn - Touchdown 4LC and Touchdown Concentrate - Additional Toxicology Information and Partial Evaluation of Data

Caswell No.: 893C
Project No.: 0-0523
Record Nos.: 162448, 162449, 250410

FROM: William Dykstra, Reviewer
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Roger Gardner, Acting Section Head
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Requested Action

Review submitted toxicology data in support of tolerance request for use of sulfosate in/on corn.

Conclusions and Recommendations

1. The supplemental information to the 2-year combined chronic toxicity/oncogenicity studies in rats and mice are adequate to upgrade the core-supplementary status of those studies to core-guideline.

2. The 1-year dog study can be upgraded to core-minimum data and supports the registration.

3. The following submitted studies have been sent to Dynamac for review:

Study	Review Hours
1. 21-Day Dermal Rat	24
2. Acute Inhalation	4
3. Metabolism (rat)	24
4. 3-Month Dog	120
5. 3-Month Rat	120
Total	292

4. The company response to the review by Dr. Chen of the mouse micronucleus mutagenicity study has been transmitted to Dr. Chen for further comment.

5. Following resolution of items 2, 3, and 4, Toxicology Branch (TB) will evaluate the tolerance request for sulfosate in/on corn.

Review

I. TWO-YEAR COMBINED CHRONIC TOXICITY/ONCOGENICITY STUDIES IN RATS AND MICE

A. Supplemental Information

MRID Nos. 412099-07 and 412099-05; histopathology of individual animals with codes for individual animals.

1. T-11813; Addendum to Final Report of 2-Year Chronic Toxicity and Oncogenicity Dietary Study with SC-0224 in Mice; prepared by ICI Americas.

2. T-11082; Addendum to Final Report of 2-Year Chronic Toxicity and Oncogenicity Dietary Study with SC-0224 in Rats; prepared by ICI Americas.

3. Dictionary Codes for Histopathology Reports (hand carried on March 22, 1990 by Barbara Kaminski, ICI Americas) (attached).

B. Discussion

The January 5, 1988 review by W. Dykstra of the two 2-year chronic studies concluded the following:

"The 2-year rat feeding is considered a supplementary study. Evaluation of individual rat pathology sheets (Appendix N) did not provide a clear indication that tissue masses identified in the antemortem examination (Appendix I) and noted in the postmortem gross necropsy (Appendix L) were further evaluated microscopically. These deficiencies are required to be resolved." [End of quotation.]

"The 22-month mouse feeding study is considered a supplementary study. The tissue masses listed in Table I (clinical observations) and Table I (necropsy observations) were not clearly identified in the histopathology observations (Table N) as being histologically examined. This deficiency has to be resolved." [End of quotation.]

C. TR Conclusion

In the recent submission (MRID No. 412099-01), ICI stated that "in volumes 7 through 9, information will be submitted which we believe will greatly facilitate the tracking of tissue masses." [End of quotation.]

According to this submission:

"The following are being submitted for each study:

"1. Trail for individual clinical mass observations.

"2. Clarifications/annotations to trail.

"3. Necropsy detail report by animal <u>with codes</u>.

"4. Histopathology detail report by animal <u>with codes</u>.

"Necropsy and histopathology detail reports by animal were included in the original reports without codes. In the coded section to the extreme right of the enclose printouts, lesion numbers are listed which will clarify our tracking system. The Trail for Individual Clinical Mass Observation is an ancillary table prepared for EPA convenience." [End of quotation.]

The only data received by TR at this time is item 4: Histopathological detail report by animal with codes for each study.

Additionally, the dictionary code, which was hand delivered, provides codes only for the individual histopathological findings for each animal in the addenda. A check between the original histopathological report and the newly submitted histopathological addendum, by using the dictionary code, shows that the original histopathological findings and the histopathological findings in the addenda are the same. Therefore, the coded information in the histopathological addenda can be verified.

However, items 1, 2, and 3 listed above of ICI's present submission are required to be submitted to complete the evaluation of tracking the tissue masses. In response to this situation, telephone communication on August 1, 1990 with Dr. Ann Manley, Toxicologist with ICI, provided the correct MRID Numbers for completing the evaluation of the 2-year rat and mouse studies. The MRID Numbers are 412099-05 (Rats) and 412099-07 (Mice). These MRID Numbers contained the individual animal data for tissue masses and gross necropsy findings for all rats on the study.

Analysis of randomly selected individual male and female rats and mice for tracking of tissue masses to gross necropsy findings to histopathological findings showed that the tracking of tissue masses could be correctly accomplished. This issue is considered resolved and the 2-year rat and 2-year mouse studies can be upgraded to core-guideline.

II. TWELVE-MONTH DOG STUDY

A. HED Review

Classification of Data: Supplementary

Deficiencies: The MTD was not employed for this study. The volume of urine for all animals at the treatment intervals was missing in this study report. Historical control data are needed to evaluate the incidence of abnormal protrusion of pituitary and the incidences of hamartoma and dermal histiocytoma of pinna described in this study.

Reviewed by: William Dykstra
Section II, Toxicology Branch (TS-769C)
Secondary reviewer: Edwin Budd
Section II, Toxicology Branch (TS-769C)

006542

DATA EVALUATION REPORT

011337

Study Type: 83-5, Chronic Toxicity/Oncogenicity

TOX Chem No.: 893C
MRID No.: None

Accession Number: 402140-07 (Vol. 1-7)

Test Material: Sulfosate

Synonyms: SC-0224

Study Number: T-11082

Sponsor: Stauffer Chemical Company

Testing Facility: Stauffer Laboratory
Farmington, CT

Title of Report: Two-Year Chronic Toxicity and Oncogenicity Dietary Study with SC-0224 in Rats

Authors: Pavkov, K.L.; Wyand, S.

Report Issued: April 3, 1987

Conclusions: [Tentative]

The oncogenic potential was negative at the highest dose tested (HDT) of 1000 ppm. The NOEL for systemic toxicity is 100 ppm. At the LEL of 500 ppm, lactate dehydrogenase levels in male and female rats at 6 and 12 months were decreased in a dose-related manner.

At 1000 ppm there were decreased body weights for males (8 to 10%) for the first 49 weeks of the study and decreased body weights for females (8 to 9%) for the first 75 weeks of the study. The body weight decreases for 1000 ppm (HDT) male and female rats are considered sufficient evidence that an MTD was reached in this study.

Histologically, at 1000 ppm, there was an increased incidence of chronic inflammation of the larynx and nasopharynx in male rats.

Classification: Core-Supplementary, because the tissue masses listed in Appendix I (clinical observations) and Appendix L (necropsy observations) were not clearly

identified in the histopathology sheets (Appendix N) as being histologically examined. This deficiency has to be resolved by the registrant.

Special Review Criteria (40 CFR 154.7): N/A

REVIEW

- 011337

I. Two-Year Chronic Toxicity and Oncogenicity Study With SC-0224 in Rats (Stauffer Labs Report No. T-11082; April 3, 1987).

Test Material: Technical SC-0224 (Trimethylsulfonium carboxymethyl-aminomethylphosphonate, active ingredient); Lot No. WRC 8108-24-1, EHC Code No. EHC 0469-15. Clear aqueous solution, 56.2% active ingredient (ai) on a w/w basis.

Experimental Design:

Randomized groups of male and female Charles River (Kensington, New York) Sprague-Dawley-derived rats (Crl:CD[SD]BR) were used in the study. The rats were identified by an ear tag and were housed individually.

The test material was administered continuously in the diet (Purina Certified Rodent Chow Meal 5002) for 24 months. The experimental design is shown below.

		Number of Animals	
Dose Group	ppm ai	Male	Female
0	Control[a]	60	60
1	0[b]	80	80
2	100	80	80
3	500	80	80
4	1000	90	90

[a]Basal diet, no vehicle.
[b]Basal diet plus vehicle (propylene glycol at 1% w/w).

There were interim sacrifices of variable numbers of rats at 6, 12, and 18 months. The number of rats scheduled for the full 24-month study duration was 50/sex/group.

Rats were observed twice daily for toxic signs. A general physical examination was performed on all animals once per week including palpatation for nodules or tissue masses. Moribund rats were sacrificed to avoid tissue autolysis.

Individual body weights were recorded weekly for the first 13 weeks of the study and every other week thereafter. Body weights at the time of necropsy were recorded for animals sacrificed at 6, 12, or 18 months and at study termination. Individual food consumption was measured

weekly during the first 13 weeks of the study and on alternate weeks thereafter by determining the sum of allocated feed for a 7-day interval minus the residual from the 7-day period. Feed efficiency was calculated at each interval.

Blood samples were drawn for hematologic analyses (listed below) from 20 fasted animals of each sex during the quarantine-acclimation period and from 20 in each dose group and vehicle control (0 ppm) at 3, 6, 12, 18, and 24 months (the control basal diet group was evaluated only at 12 and 24 months). As much as possible, the same rats were sampled at each time interval.

Hematology parameters evaluated included:

Hematocrit (Hct)
Hemoglobin (Hgb)
Erythrocyte count (RBC)
Total leukocyte count (WBC)
Differential leukocyte count (also prior to termination of any animal)
- Immature neutrophils (Bands)
- Mature neutrophils (Segs)
- Lymphocytes (Lymph)
- Monocytes (Mono)
- Basophils (Baso)
- Eosinophils (Eos)

Platelet count (PLT)
Prothrombin time (PT) (10/sex/dose at termination)
Partial thromboplastin time (PTT) (10 sex/dose at termination)

Samples for blood chemistry were obtained from 10 fasted rats/sex/dose group (same animals used for hematologic analyses) at 6, 12, 18, and 24 months. The blood chemistry parameters evaluated are listed below. When sample volume was insufficient, those parameters of the highest priority were measured in the following order:

Asparate aminotransferase (SGOT)
Alanine aminotransaminase (SGPT)
Gamma glutamyl transferase (GGT)
Alkaline phosphatase (Alk. Phos.)
Total protein (T. Prot.)
Total bilirubin (T. Bili.)
Albumin (Alb)
Globulin
Blood urea nitrogen (BUN)
Glucose (Glu)
Sodium (Na)

Calcium (Ca)
Potassium (K)
Inorganic phosphorus (Phos)
Chloride (Cl)
Creatinine (Creat)
Cholesterol (Choles)
Triglycerides (Triglyc)
Creatinine phosphokinase (CPK)
Lactate dehydrogenase (LDH)
Plasma cholinesterase (P ChE)
Red blood cell cholinesterase (RBC ChE)
A/G ratio
Uric acid
Protein electrophoresis

The right or left half of the brain from five rats/sex/dose level was homogenized at 6, 12, 18, and 24 months to measure the cholinesterase activity per gram of protein (determined by the Lowry method).

Urinalyses were performed for 20 fasted rats/sex/dose level (same animals mentioned above in hematology). The parameters evaluated included:

Appearance
Microscopic examination of sediment
Specific gravity (SpGr)
pH
Protein (Prot)
Glucose (Glu)
Ketones (Ket)
Occult blood (Occ Bl)
Urobilinogen (U-blin)
Bilirubin (Bili)

All rats were necropsied by trained prosectors under the direction of a veterinary pathologist. The animals were anesthetized by injecting saline-diluted sodium pentobarbital IP and exsanguinated by severing the abdominal aorta and vena cava. They were examined fo. external abnormalities, including palpable masses. Viscera and body cavities were also examined.

The sacrifice schedule is shown below.

		Sacrifice Interval (Months)			
Group	ppm	6	12	18	24
0	Control	--	20(1)	--	Survivors

(1) 10/sex/group

Group	ppm	Sacrifice Interval (Months) 6	12	18	24
1	0	20(1)	20	20(1)	Survivors
2	100	20	20	20	Survivors
3	500	20	20	20	Survivors
4	1000	20	40(2)	20	Survivors

(1) 10/sex/group.
(2) 20/sex/group.

The following tissues were fixed in 10% neutral buffered formalin or 2.5% buffered glutaraldehyde (BG):

Skin
Mammary gland
Muscle-thigh
Tibiofemoral joint
Sternum
*Lungs
*Heart
Aorta - ascending and thoracic
*Spleen
Thymus
Bone marrow - Sternal
Lymph nodes - mesenteric and mediastinal
Salivary glands - partotid and mandibular
Buccal/alveolar mucosa
Tongue
Esophagus
Stomach
Duodenum
Jejunum
Ileum

Cecum
Colon
Rectum
Pancreas
*Liver
*Kidneys

Nasal passage
Paranasal sinus
Nasopharynx
Larynx
Trachea
Urinary bladder
*Testes (BG)
Epididymides (BG)
Prostate
Seminal vesicles
Coagulating glands
*Ovaries (BG)
Vagina
Cervix
*Uterus
*Pituitary (BG)
Thyroids
Parathyroids
*Adrenals (BG)
*Brain
Spinal cord - cervical, thoracic and lumbar
Sciatic nerve
Eyes (BG)
Harderian glands (BG)
Zymbal's glands

Middle ear(s)
Gross lesions (as specified by the pathologist)

Those organs marked (*) above were weighed for rats sacrificed at the interim and final terminations. The

paired organs were weighed together for the 6-month interim sacrifice but were weighed separately thereafter. All tissues on the above list were routinely processed for light microscopic examination for all animals.

Statistical Analysis

Continuous data were analyzed using a one-way analysis of variance (ANOVA; Winer, 1962) and Dunnett's Test (Dunnett, 1964) to compare test groups with controls. Test group data were compared to the 0-ppm (Control) dose groups at 3, 6, and 18 months and were compared to the basal diet (Control) groups at 12 and 24 months. The criterion for statistical significance was $p < 0.05$. Values of $p < 0.01$ were also indicated. The statistical significance was not determined at the $p < 0.001$ level because all Dunnetts' tables only include 0.05 and 0.01 values.

Results:

In the initial 2 to 3 weeks, mean body weights of male and female rats were statistically significantly decreased in all test groups in comparison to the respective control groups. These decreases ranged from 4.4 to 8.3 percent for males and 2.7 to 3.7 percent for females.

Mean body weights of the 1000 ppm dose groups for both males and females remained significantly lower through 49 weeks (males) and through 75 weeks (females). These decreases ranged from 8.3 to 10.1 percent for males during this period (49 weeks) and from 8.2 to 9.4 percent for females during this period (75 weeks).

At 24 months, absolute weight gain was comparable for both males and females of all groups in comparison to week 0 of the study. Males showed mean increases of 457, 507, 403, 406, and 473 g for groups control, 0, 100, 500, and 1000 ppm, respectively. Females showed mean increases of 234, 231, 314, 266, and 244 g for groups control, 0, 100, 500, and 1000 ppm, respectively.

The body weight decreases for high-dose male and female rats during the study are considered sufficient evidence that an MTD was reached in the study.

The average concentrations of SC-0224 active ingredient (measured by separate anion and cation analyses) were within 15 percent (anion analysis) and 18 percent (cation analysis) of the nominal values measured at regular intervals during the study.

Mean Body Weights in Rats

Weeks	Dose Level (ppm)				
	Control	0[a]	100	500	1000
			Males		
0	236	218**	213**	221**	228** (97)
4	403	396	392	390	384** (95)
8	486	496	478	473	460** (95)
12	538	552	530	520	504** (94)
25	633	657	622	612	589** (93)
51	716	753	732	697	683 (95)
71	773	811	759	735	732 (95)
91	696	700	704	684	663
105	695	725	616	627	701

[a]Vehicle control
* p < 0.01, **p < 0.01

Mean Body Weights in Rats

Weeks	Dose Level (ppm)				
	Control	0[a]	100	500	1000
			Females		
0	165	154**	159**	159**	161
4	232	225	229	224	220** (95)
8	265	254	263	260	250** (94)
12	286	277	286	279	273 (95)
25	337	324	336	321	313* (93)
51	435	416	434	426	399* (92)
71	481	472	492	460	430* (89)
91	493	463	479	459	448 (91)
105	399	385	473	425	407 (102)

[a]Vehicle control
* p < 0.05, **p < 0.01

The calculated intake of active ingredient on a mg/kg/day basis is shown below:

Nominal ppm ai	Males	Females
100	4.2	5.4
500	21.2	27.0
1000	41.8	55.7

Food consumption of male and female rats at 1000 ppm was occasionally decreased during the study in comparison to control levels. The decreased food consumption was not considered responsible for the decreased body weights of male and female rats at 1000 ppm observed during the study.

Feed efficiency was comparable for males and females between controls and treated groups during the study.

There were no compound-related effects on survival for male and female rats during the study. At study termination (weeks 105 or 106), the number of survivors in each group of males was 17, 18, 12, 19, and 23 for control, 0, 100, 500, and 1000 ppm, respectively. For each group of females at study termination (weeks 105 or 106), the number of survivors was 16, 15, 25, 15 and 16 for control, 0, 100, 500, and 1000 ppm, respectively.

There were no compound-related clinical signs, including the onset, number, and location of palpable masses, for both male and female rats during the study. The most common observations were abrasions, anorexia, alopecia, broken teeth, chromodacryorrhea, chromorhinorrhea, dehydration, emaciation, exophthalmus, hair loss, hematuria, loose stool, malocclusion, pallor, rough/wet hair coat, swollen or torn ears, and scabs.

Ophthalmoscopic examinations at 6, 12, 18, and 24 months did not show any compound-related effects. The most common observation was conjunctivitis and was unrelated to treatment.

Evaluation of hematological data showed that at 3 months, the leukocyte counts (WBC) for the males of the 500 and 1000 ppm dose group and the females of the 1000 ppm dose group were reduced to 85.6, 85.6, and 84 percent of the respective 0 ppm dose group values. These changes are shown below.

Mean Food Consumption

Weeks	Dose Level (ppm)				
	Control	0	100	500	1000
			Males		
1	24	23	23	23	22** (92)
4	27	26	28	26	25** (93)
8	27	28	27	26**	25** (93)
12	27	27	26	26**	25** (93)
25	26	26	25	24**	24** (92)
51	26	25	26	25	25* (96)
71	28	30	28	27	27 (96)
91	28	29	30	28	27
105	26	31	29	27	26

* $p < 0.05$
** $p < 0.01$

Mean Food Consumption

Weeks	Dose Level (ppm)				
	Control	0	100	500	1000
			Females		
1	18	17	17	17*	15**
4	19	18	19	18	17**
8	19	18	18	18	17**
12	19	19	19	18**	18*
25	20	19	18**	18**	18**
51	22	20	21	20	20*
71	24	22	23	22	21*
91	24	24	23	23	23
105	18	-[a]	23	18	-[a]

* $p < 0.05$
** $p < 0.01$
[a]These values were not available in the original table taken from the report.

Mean Food Efficiency

Weeks	Dose Level (ppm)				
	Control	0	100	500	1000
			Males		
1	27	28	27	28	29
4	15	18**	18*	17*	17
8	9.2	9.4	9.3	8.3	8.6
12	4.4	6.3*	4.9	4.5	5.3
25	2.8	1.8	1.7*	2.0	2.1
51	1.4	1.7	1.1	1.4	1.8
71	0.9	2.4	1.4	2.1	2.1
91	2.4	2.1	3.4	3.0	1.5
103	1.7	2.2	-	1.3	0.5

* $p < 0.05$, ** $p < 0.01$

Mean Food Efficiency

Weeks	Dose Level (ppm)				
	Control	0	100	500	1000
			Females		
1	15	18**	17	16	15
4	9.6	8.8	9.4	9.9	9.0
8	7.2	6.0	6.7	6.1	6.3
12	4.4	3.0	3.5	3.4	4.4
25	2.4	1.9	1.7	1.1*	1.8
51	1.5	2.0	2.8*	3.1**	2.4
71	0.5	1.2	2.3*	1.0	2.3
91	1.8	3.7	2.4	3.0	3.7
103	2.0	1.0	2.3	0.5	1.3

* $p < 0.05$, ** $p < 0.01$

Leukocytes ($10^3/mm^3$)

	Males	3 Months	Females
Control	---	Mean + S.D.	---
0	13.2 ± 2.7		9.4 ± 2.8
100	11.9 ± 1.3		8.2 ± 2.4
500	11.3* ± 2.5		8.6 ± 2.0
1000	11.3* ± 2.4		7.9 ± 2.6

*$p < 0.05$

Evaluation of individual leukocyte data for male rats at 3 months showed a 0 ppm mean of 13.2 ± 2.7 $10^3/mm^3$ and a range of 8.3 to 21.3 $10^3/mm^3$.

In comparison, values at 500 ppm had a mean of 11.3 ± 2.5 $10^3/mm^3$ and a range of 8.5 to 16.8 $10^3/mm^3$, whereas values at 1000 ppm had a mean of 11.3 ± 2.4 $10^3/mm^3$ with a range of 7.6 to 15.0 $10^3/mm^3$.

The decrease in mean leukocyte values for males at 500 and 1000 ppm are not considered toxicologically significant since (1) mean values ± SD were within control mean ± SD values; (2) individual values ranged generally within the control range; and (3) the transient response at 3 months was not observed at 6, 12, 18, or 24 months as a dose-related finding.

Similarly, evaluation of individual leukocyte data for female rats at 3 months showed that the values for 0 ppm ranged from 5.4 to 12.5 (with animal number 1090 showing 17.5) $10^3/mm^3$. At 1000 ppm, the range was 4.0 to 14.3 $10^3/mm^3$. It can be seen that the individual values at 1000 ppm ranged generally within the control values with the exception of the single high value value of 17.5 $10^3/mm^3$ for animal #1090. Also, as with male rats, the mean values ± SD were within control mean ± SD and the finding did appear in a dose-related manner at 6, 12, 18, and 24 months.

At 12 months, decreases in mean hemoglobin and hematocrit values of females at 100 ppm were statistically significantly different in comparison to control values but were not considered toxicologically significant since they were not dose-related.

At 6 months, the activated partial thromboplastin times (PTT) for female rats in the 1000 ppm group were statistically significantly decreased ($p < 0.05$) in comparison to controls. At 1000 ppm, the mean value was 13 ± 1 seconds compared to 15 ± 2 seconds in the control.

Individual control values ranged from 13 to 18 seconds in comparison to the values at 1000 ppm which ranged from 11 to 14 seconds. These slight effects were not considered toxicologically significant.

At 12 months, the PTT times for female rats at 0, 100, and 500 ppm (but not 1000 ppm) were statistically significantly decreased ($p < 0.05$) in comparison to controls (79 to 89% of the control values). The mean values at 0, 100, and 500 ppm were 16 $\pm$ 1, 15 $\pm$ 1, and 17 $\pm$ 1, respectively, in comparison to the control value which was 19 $\pm$ 1. Individual values at 0, 100, and 500 ppm ranged between 15 and 16, 14 and 17, and 15 and 19, respectively, in comparison to the control range which was 17 to 20. These slight effects were not considered toxicologically significant.

All other hematological parameters for males and females were comparable to control values for all groups and for each time interval (3, 6, 12, 18, and 24 months).

The following serum enzyme parameters showed comparable values between control and treated groups of both sexes: AST/SGOT, ALT/SGPT, GGT, SAP, albumin, glucose, calcium, phosphorus, and sodium.

Lactate dehydrogenase levels (IU/L) showed statistically significant decreases at 6 and 12 months as shown on page 13.

The decreases in males and females at 6 and 12 months are in a dose-related manner and are statistically significant. These findings are considered clinically significant and may be indicative of progressive systemic toxicity related to treatment. The NOEL for this effect is 100 ppm.

Statistically significant decreases in creatine phosphokinase values (IU/L) at 1000 ppm in males and females at 6 months were not considered toxicologically significant. Creatine phosphokinase data are shown on page 14.

With respect to total bilirubin, the statistically significantly decreased values for females at 0, 100, 500, and 1000 ppm at 12 months and 500 ppm at 18 months, are within the range of control values. Control values ranged from 0.2 to 1.1 mg/dl at 12 months and 0.1 to 0.4 mg/dl at 18 months. Therefore, the decreased values observed do not indicate toxicological significance. Total bilirubin values are shown on page 15.

Mean values for BUN (mg/dl) show decreased values for females at 500 and 1000 ppm at 6 months and also at 1000 ppm at 18 months. As shown with other serum chemistries, most of the decreased values are within the range of 0 ppm values for females. Therefore, the decreased values in the treated groups are not considered toxicologically significant. BUN values are presented on page 16.

Mean values for creatinine show statistically significant decreases at 12 months in females at 0, 100, 500, and 1000 ppm. Control values for creatinine in females at 12 months range between 0.5 and 1.9 mg/dl and encompass the range of values for the females at 0 ppm (0.7 to 1.0 mg/dl), 100 ppm (0.6 to 1.0 mg/dl), 500 ppm (0.6 to 0.8 mg/dl) and 1000 ppm (0.6 to 0.90 mg/dl). Therefore, the decreased mean values are not considered toxicologically significant. Creatinine values for the study are shown on page 17.

The mean value for uric acid in females at 12 months at 1000 ppm was statistically significantly decreased in comparison to the control values. At 1000 ppm, the values ranged from 0.2 to 1.0 mg/dl, whereas the control values at 12 months for females ranged from 0.8 to 1.9 mg/dl.

Although the range of uric acid values at 1000 ppm is less than the range of control values, the transient decrease at only 12 months (which was not either dose-related at that time or was extended into 18 or 24 months) is not considered toxicologically significant. Uric acid acid data are presented on page 18.

Cholesterol mean values were significantly decreased in 1000 ppm males at 6 months and in 500 and 1000 ppm females at 18 months. Male 0 ppm values at 6 months ranged between 60 and 115 mg/dl in comparison to the range of 53 to 77 mg/dl values for males at 1000 ppm.

The decreases at the 1000 ppm level in males at 6 months are not considered clinically significant in comparison to 0 ppm values. For females at 18 months, 0 ppm values for cholesterol ranged from 71 to 121 mg/dl. In comparison to this, the range of female values at 500 ppm were 49 to 123 mg/dl and at 1000 ppm were 50 to 96 mg/dl. The statistically significant decreases at 18 months in females are not considered clinically significant. The data for cholesterol values for the study are shown on page 19.

Decreased triglycerides were observed to be significantly decreased at 12 months in 100 and 1000 ppm females and at 18 months in 1000 ppm females.

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Control values for females at 12 months ranged from 50 to 804 mg/dl, with a mean and S.D. of 410 ± 254 mg/dl. It should be noted that female control rat #942 had a 50 mg/dl value for triglyceride whereas the next lowest value in control females was 206 mg/dl for female rat #945. The decreased values of female rats at 12 months in the 100 and 1000 ppm groups ranged from 70 to 526 mg/dl and 30 to 332 mg/dl, respectively. Therefore, the range of control values for triglycerides, although higher than all groups including 0 ppm, essentially encompasses the range of decreased values observed in females at 100 and 1000 ppm. These decreased values at 100 and 1000 ppm are not considered toxicologically significant.

Similarly, at 18 months, the 0 ppm range for females is 30 to 405 mg/dl, with a mean and S.D. of 212 ± 147 mg/dl. The range of values in females at 1000 ppm is 33 to 225 mg/dl, with a mean S.D. of 94 ± 63 mg/dl. The decreased values observed in females at 1000 ppm are within the range of 0 ppm values observed and are not considered toxicologically significant. The study data for serum triglycerides are shown on page 20.

Mean and S.D. values for total protein and globulin were increased in a dose-related fashion at 12 months only in treated females. Additionally, the values were statistically significantly increased for both total protein and globulin at 500 and 1000 ppm ($p < 0.05$ and $p < 0.01$, respectively).

The data for total protein and globulin are presented on pages 22 and 23 as obtained from the report.

It can be seen from the above-mentioned tables that the mean total protein and globulin values for females at 12 months at control and 0 ppm levels are within the range of control and 0 ppm values at other (6, 18, and 24 months) sampling intervals. Additionally, the increases observed at 500 and 1000 ppm at 12 months exceed the mean values for the 500 and 1000 ppm levels at other sampling intervals (6, 18, and 24 months).

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Summary of Serum Lactate Dehydrogenase (IU/L) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	402 (221)		488 (206)		861 (442)[a]
	0		591 (246)	368 (174)	483 (226)	883 (286)[a]
	100		616 (237)	308 (234)	768 (392)	836 (307)[a]
	500		337 (154)*	206 (113)*	311 (185)	892 (409)
	1000		248 (180)**	138 (80)**	567 (341)	933 (326)
Females	Control	607 (397)		509 (286)		1086 (330)
	0		536 (126)	453 (288)	492 (268)	1032 (491)[b]
	100		481 (152)	503 (326)	419 (204)	881 (473)
	500		323 (173)**	387 (225)	698 (366)	973 (308)
	1000		160 (75)**	196 (114)	401 (305)	867 (258)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
a n = 9
b n = 7
c n = 8

Summary of Serum Creatine Phosphokinase (IU/L) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	359 (400)		152 (18)		155 (59)[a]
	0		213 (72)	109 (75)	200 (80)	179 (60)[a]
	100		260 (118)	73 (43)	306 (143)	177 (76)[a]
	500		149 (97)[a]	82 (37)	158 (155)	233 (102)
	1000		114 (43)*	66 (45)	219 (135)	226 (62)[a]
Females	Control	388 (208)		129 (69)		326 (102)
	0		168 (62)	111 (42)	264 (273)	294 (118)[b]
	100		192 (66)	113 (63)	205 (116)	266 (174)
	500		118 (50)	89 (64)	232 (121)	295 (81)
	1000		95 (43)*	96 (59)	235 (169)	307 (136)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
[a] n = 9
[b] n = 7
[c] n = 8

Summary of Serum Total Bilirubin (mg/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	0.2 (0.1)		0.3 (0.1)		0.2 (0.1)[a]
	0		0.3 (0.1)	0.3 (< 0.1)	0.2 (0.1)	0.1 (0.1)[a]
	100		0.2 (0.1)	0.3 (0.1)	0.2 (0.1)	0.1 (0.1)[a]
	500		0.3 (0.1)[a]	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
	1000		0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.0)
Females	Control	0.2 (0.1)		0.5 (0.3)		0.3 (0.2)
	0		0.2 (< 0.1)	0.3 (0.1)*	0.3 (0.1)	0.2 (0.1)[b]
	100		0.2 (0.0)	0.3 (0.1)*	0.2 (0.1)	0.3 (0.3)
	500		0.2 (< 0.1)	0.4 (0.2)*	0.2 (0.1)*	0.2 (0.2)
	1000		0.2 (0.1)	0.3 (0.1)**	0.2 (0.0)	0.2 (0.1)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
[a] n = 9
[b] n = 7
[c] n = 8

Summary of Serum Blood Urea Nitrogen (mg/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	14 (2)		16 (2)		24 (16)[a]
	0		15 (2)	15 (4)	21 (18)	22 (13)[a]
	100		14 (2)	14 (3)	16 (4)	20 (4)[a]
	500		15 (2)	15 (2)	20 (12)	22 (11)
	1000		15 (3)	14 (3)	16 (5)	24 (15)
Females	Control	17 (3)		14 (2)		13 (5)
	0		20 (2)	15 (2)	14 (2)	14 (5)[b]
	100		18 (2)	15 (4)	14 (3)	15 (4)
	500		17 (1)*	15 (3)	13 (2)	13 (4)
	1000		18 (4)*	14 (3)	11 (2)*	15 (5)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
[a] n = 9
[b] n = 7
[c] n = 8

Summary of Serum Creatinine (mg/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	0.7 (0.1)		0.8 (0.1)		1.1 (0.8)[a]
	0		0.8 (0.1)	0.7 (0.1)	1.1 (0.9)	0.9 (0.4)[a]
	100		0.8 (0.2)	0.7 (0.1)	0.9 (0.2)	0.8 (0.1)[a]
	500		0.8 (0.1)[a]	0.8 (0.1)	1.0 (0.6)	0.8 (0.3)
	1000		0.8 (0.1)	0.7 (0.1)	0.8 (0.1)	1.0 (0.5)
Females	Control	0.2 (0.1)		1.0 (0.4)		0.6 (0.1)
	0		0.8 (0.1)	0.7 (0.1)*	0.7 (0.1)	0.7 (0.2)[b]
	100		0.8 (< 0.1)[a]	0.8 (0.1)*	0.8 (0.1)	0.6 (0.1)
	500		0.7 (0.1)	0.7 (0.1)*	0.7 (0.1)	0.6 (0.1)
	1000		0.7 (0.1)	0.7 (0.1)**	0.7 (0.1)	0.6 (0.1)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
a n = 9
b n = 7
c n = 8

Summary of Serum Uric Acid (mg/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control		1.6 (0.7)		1.7 (0.4)[a]
	0	2.1 (0.4)	1.1 (0.3)	2.2 (1.6)	1.4 (0.2)[a]
	100	1.8 (0.3)[a]	1.1 (0.4)	1.3 (0.6)	1.8 (0.4)[a]
	500	1.4 (0.7)[b]	1.4 (0.5)	1.4 (0.6)	1.3 (0.6)
	1000	1.9 (0.8)	1.0 (0.4)	1.3 (1.2)	1.4 (0.7)
Females	Control		1.1 (0.3)		1.5 (0.4)
	0	1.3 (0.7)[b]	0.9 (0.3)	1.5 (0.7)	1.4 (0.5)[b]
	100	0.9 (0.6)[b]	0.8 (0.3)	1.2 (0.5)[b]	1.3 (0.5)
	500	1.1 (0.4)[a]	0.9 (0.5)	1.0 (0.4)	1.2 (0.4)
	1000	0.8 (0.2)	0.5 (0.3)*	1.0 (0.4)[b]	1.0 (0.3)[a]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
a n = 9
b n = 7
c n = 8

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Summary of Serum Cholesterol (mg/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	40 (11)		114 (55)		102 (40)[a]
	0		78 (18)	102 (36)	139 (80)	98 (40)[a]
	100		78 (17)	107 (36)	123 (143)	114 (43)[a]
	500		80 (15)[a]	99 (36)	125 (155)	136 (72)
	1000		61 (8)*	76 (18)	94 (135)	117 (44)
Females	Control	51 (13)		91 (26)		106 (49)
	0		77 (11)	84 (9)	100 (20)	95 (21)[b]
	100		69 (20)[a]	84 (35)	82 (16)	95 (54)
	500		71 (13)[a]	67 (19)	74 (23)*	79 (28)
	1000		81 (27)*	74 (25)	76 (15)*	68 (29)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
a n = 9
b n = 7
c n = 8

Summary of Serum Triglycerides (mg/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control			224 (81)		155 (95)[b]
	0		201 (144)	298 (123)	191 (78)	95 (68)[b]
	100		188 (87)	264 (194)	169 (93)[b]	97 (48)[b]
	500		173 (59)	238 (89)	145 (72)	151 (96)
	1000		128 (55)	217 (98)	128 (83)	111 (47)[b]
Females	Control			410 (254)		161 (154)
	0		42 (12)	243 (99)	212 (147)	135 (83)[c]
	100		70 (59)[b]	215 (139)*	135 (88)	219 (302)
	500		71 (40)	242 (116)	106 (69)	128 (175)
	1000		52 (47)	154 (110)**	94 (63)*	97 (77)[d]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
a Test not performed due to problem with reagent subsystem and insufficient sample for rerun.
b n = 9
c n = 7
d n = 8

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Although these findings tend to support the conclusion that the increased total protein and globulin values observed in females at 12 months at 500 and 1000 ppm are toxicologically significant, other pathological findings could not be correlated with these clinical chemistry findings. Specifically, there were no toxic signs or organ weight changes, including liver and kidney, for females at 12 months or at any other sampling interval. Additionally, there were no histopathological lesions in females which could be correlated with the clinical pathology data.

Since the increase in total protein and globulin did not occur at other sampling intervals during the study, the results at 12 months are not considered toxicologically significant. The increase in total protein and globulin at 12 months is not considered an effect.

Albumin levels were unaffected by treatment during the study and, as can be expected, the albumin/globulin ratio was significantly decreased at 12 months in females at 1000 ppm.

Transient increases in mean serum chloride values for the 0, 100, 500, and 1000 ppm female groups at 12 months in comparison to controls are considered to be due to the slight lowering in control values at this time (12 months). These findings in serum chloride are not considered toxicologically significant.

Although there were statistically significant increases and decreases of mean values for brain, RBC and plasma cholinesterase, no toxicologically significant dose-related trends were observed and most individual values of treated groups were within the range of control values.

There were no compound-related urinalyses findings at each of the measured intervals in male or female rats.

With respect to gross necropsy findings, there were no compound-related gross necropsy observations in male rats. In female rats, the incidences of focal, tan

Summary of Serum Total Protein (g/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	6.4 (0.3)		6.9 (0.2)		6.1 (0.5)[a]
	0		7.2 (0.4)	6.8 (0.3)	6.5 (0.4)	6.1 (0.4)[a]
	100		7.2 (0.4)	6.9 (0.3)	6.6 (0.4)	6.2 (0.4)[a]
	500		7.1 (0.3)	6.7 (0.3)	6.3 (0.3)	6.1 (0.4)
	1000		7.0 (0.3)	6.5 (0.5)	6.4 (0.3)	6.0 (0.5)
Females	Control	6.4 (0.2)		7.3 (0.4)		6.9 (0.2)
	0		7.6 (0.5)	7.4 (0.2)	7.2 (0.5)	6.6 (0.4)[b]
	100		7.7 (0.5)	7.5 (0.4)	6.9 (0.5)	6.8 (0.3)
	500		7.6 (0.6)	7.9 (0.5)*	7.0 (0.3)	7.0 (0.3)
	1000		7.5 (0.5)	8.0 (0.6)**	6.8 (0.4)	6.6 (0.6)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
a n = 9
b n = 7
c n = 8

Summary of Serum Globulin (g/dl) Mean Values for Rats Given SC-0224 in Diet

	Dosage Group (ppm)	Pretest n = 20	6 Months n = 10	12 Months n = 10	18 Months n = 10	24 Months n = 10
Males	Control	2.8 (0.2)		3.3 (0.3)		3.3 (0.2)[a]
	0		3.4 (0.3)	3.2 (0.4)	3.4 (0.3)	3.3 (0.4)[a]
	100		3.4 (0.4)	3.4 (0.4)	3.5 (0.5)	3.4 (0.4)[a]
	500		3.4 (0.3)	3.2 (0.2)	3.2 (0.2)	3.2 (0.4)
	1000		3.3 (0.4)	2.9 (0.2)	3.3 (0.2)	3.2 (0.4)
Females	Control	2.5 (0.2)		2.9 (0.5)		3.3 (0.4)
	0		2.9 (0.5)	3.0 (0.2)	3.3 (0.3)	3.2 (0.4)[b]
	100		3.1 (0.5)[a]	3.0 (0.4)	3.0 (0.4)	3.1 (0.5)
	500		3.0 (0.5)	3.4 (0.2)*	2.9 (0.2)*	2.9 (0.3)
	1000		3.0 (0.5)	3.5 (0.4)**	2.9 (0.2)*	3.0 (0.5)[c]

* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$
() Standard deviation
a n = 9
b n = 7
c n = 8

discoloration of the medial lobe of the liver showed the following incidences:

Dose (ppm)	Control	0	100	500	1000
No. Examined	60	80	80	80	90
Liver					
Tan focal discoloration	5	3	2	4	13
Percent response	8	4	3	5	14

The increased incidence at 1000 ppm in female rats is not considered compound-related because there is no other evidence in this study suggesting that the liver is a target organ. All other indicators of potential liver toxicity (with the possible exception of decreased lactate dehydrogenase) were essentially negative.

Organ weights showed occasionally slight decreases or increases in treated female animals in comparison to controls, but none of the differences at any interval (6, 12, 18, or 24 months) were statistically significant or compound related.

In male rats at 6 months, at 500 ppm, there was a significant increase in testes weight (left and right weighed together) in absolute weight (161% of control and relative to brain weight 116% of control), but not relative to body weight (114% of control). Since there was no significant effect at 1000 ppm, the finding at 500 ppm was not dose-related and is not considered toxicologically significant.

At 12 months in comparison to controls, absolute liver weight was decreased as well as absolute kidney weight (both left and right) at 1000 ppm in males. These decreased organ weights at 1000 ppm also were present as decreased relative to brain weight (88% of control for left kidney, 86% of control for right kidney, and 79% of control for liver) but not relative to body weight (100% of control for left kidney, 97% of control for right kidney, and 89% of control for liver). These decreased organ weights probably reflect the decreased body weight at 1000 ppm and are not likely to be a significant toxic effect at 12 months.

Also noted at 12 months in males were an increased (relative to body weight) weight of the right testes at 500 ppm. This increase was not dose-related and was not reflected as an increase relative to brain weight or in absolute testes weight at 12 months and is not considered compound related. There were no organ weight effects in males at 18 months. At 24 months, the absolute brain weight of the 0, 500, and 1000 ppm male groups were all increased (105% of control for each group). In comparison to relative body weight, the increases were not statistically significant and are not considered compound related.

Evaluation of individual pathology sheets for control and treated animals (Appendix N; Volume 7 of report) did not give a clear indication that tissue masses that were identified grossly both antemortem and postmortem were examined microscopically. The tissue masses in clinical observations (Appendix I) and gross necropsy observations (Appendix L) were not clearly presented in the histopathology report (Appendix N) as being histologically examined. This deficiency has to be resolved by the registrant.

With respect to non-neoplastic histological lesions in male rats, chronic inflammation of the larynx and chronic inflammation of the nasopharynx were compound related at 1000 ppm.

Larynx (Males)

Dose (ppm)	Control	0	100	500	1000
No. Examined	60	80	79	78	90
Chronic Inflammation	13	20	9	16	34
Percent response	22	25	11	21	38

The grades of the lesion were comparable among groups. The most frequent grade was minimal.

The NOEL for chronic inflammation of the larynx is 500 ppm.

Nasopharynx (Males)

Dose (ppm)	Control	0	100	500	1000
No. Examined	60	80	80	80	90
Chronic Inflammation	6	11	6	4	20
Percent response	10	14	8	5	22

The grade of the lesions was comparable among groups. The most frequent grade was minimal. The NOEL for chronic inflammation of the nasopharynx is 500 ppm.

In female rats at the 6-, 12-, and 18-month interim sacrifices, there was an increased incidence of cardiomyopathy in the 100 and 500 ppm dose groups. The incidence was 3 percent, 23 percent, 17 percent, and 10 percent for the 0 (vehicle control), 100, 500, and 1000 ppm groups, respectively.

At 24 months, the incidence of cardiomyopathy was comparable among groups. The incidences were 81 percent, 93 percent, 72 percent, 100 percent, and 100 percent for the control, 0, 100, 500, and 1000 ppm groups, respectively.

For all female rats on study, the incidence of cardiomyopathy was as shown below:

Heart (Females)

Dose (ppm)	Control	0	100	500	1000
No. Examined	60	80	80	80	90
Cardiomyopathy	34	33	44	39	36
Percent response	57	41	55	49	40

The grade of the lesion was comparable among groups. The most frequent grade was minimal. Due to the decreased incidence of cardiomyopathy at the high-dose (1000 ppm) in the interim sacrifice and the similar frequency at the 24-month sacrifice and in all female rats examined, the increased incidences in the interim sacrifices at 100 and 500 ppm are not considered compound-related.

The incidences and grades of the non-neoplastic lesions for other organs of male and female rats were comparable between groups.

There were no compound-related benign or malignant tumors in male and female rats. Additionally, there was no decrease in latency in any tumor for either sex of rats. The most frequently observed neoplasms were of the pituitary, mammary gland, and adrenals. The incidences of the most commonly found tumors are shown below:

Pituitary - Adenomas/Carcinomas

	Male					Female				
Dose (ppm)	Control	0	100	500	1000	Control	0	100	500	1000
No. examined	60	79	80	77	90	60	78	80	80	88
No. of tumor bearing animals	44	41	43	34	47	52	56	57	55	58
Percent	73%	52%	54%	44%	50%	87%	72%	71%	69%	66%

Female Mammary Gland - Adenomas/Carcinomas

Dose (ppm)	Control	0	100	500	1000
No. examined	59	80	80	80	90
No. of tumor bearing animals	28	29	33	33	22
Percent	48%	36%	41%	41%	24%

Adrenal Pheochromocytoma - Benign and Malignant

Dose (ppm)	Control	0	100	500	1000
No. examined (both sexes)	60	80	80	80	90
No. of males with tumor	18	18	13	14	13
No. of females with tumor	5	6	3	2	3

Discussion

Mean body weights of 1000 ppm male rats were decreased through the initial 49 weeks of the study by 8.3 to 10.1 percent. Mean body weights of 1000 ppm female rats were decreased through the initial 75 weeks by 8.2 to 9.4 percent. The body weight decreases for high-dose (1000 ppm) male and female rats during the study is considered as evidence of an MTD.

At study termination, the number of survivors in each group of male rats was 17, 18, 12, 19, and 23 for control, 0, 100, 500, and 1000 ppm, respectively. For females at study termination, the number of survivors in each group was 16, 15, 25, 15, and 16 for control, 0, 100, 500, and 1000 ppm, respectively.

Lactate dehydrogenase levels in male and female rats at 6 and 12 months were decreased in a dose-related manner. The NOEL for these effects was 100 ppm. Evaluation of individual pathology sheets for control and treated animals (Appendix N) did not give a clear indication that tissue masses that were identified grossly in the antemortem and postmortem examination were examined microscopically.

The tissue masses listed in Appendix I (clinical observations) and Appendix L (necropsy observations) were not clearly identified in the histopathology sheets (Appendix N) as being histologically examined. This deficiency has to be addressed by the registrant.

Histologically, at 1000 ppm, there was an increased incidence of chronic inflammation of the larynx and nasopharynx in males.

There were no compound-related benign or malignant tumors in male or female rats. Additionally, there was no decrease in latency in any tumor for either sex of rats. However, these are tentative conclusions since the study is Core-Supplementary.

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Section I, Tox. Branch (H7509C) Roger Gardner 5/27/94 011337

DATA EVALUATION RECORD

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

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

ACCESSION NO./MRID NO.: 431512-01

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 011337 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

erved Sign	Incidence of Signs[a]			
	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.

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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.

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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.

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.

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.

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Section I, Tox. Branch (7509C)
Health Effects Division

DATA EVALUATION RECORD

STUDY TYPE: Acute Mammalian Neurotoxicity - rat (81-8)

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

ACCESSION NO./MRID NO.: 431323-01 (sulfosate); 430133-01 thru -05 for positive controls; 430301-01 for historical control data

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

TEST MATERIAL: Glyphosate Trimesium

SYNONYMS: Sulfosate

STUDY NUMBER(S): AR5425

REPORT NUMBER: CTL/P/3813

SPONSOR: Zeneca Ag Products; Wilmington, DE

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

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

AUTHOR(S): S. A. Horner

REPORT ISSUED: 2/15/93

CONCLUSION: Glyphosate Trimesium was tested in an acute neurotoxicity study in male and female Alderley Park Alpk:APfSD rats. Ten rats/sex were tested at each dose level, one time by gavage at 1 ml/100 g body weight. The following doses were tested: 0, 30, 100 or 300 mg/kg. Positive control data were provided.

At 300 mg/kg, the following effects were observed: death (2 on day 1); clinical signs of toxicity (ptosis (day 1), decreased activity (days 1-2), reduced splay reflex (days 1-4), upward curvature of the spine (days 1-5), chromodacryorrhea (days 1-3), shaking (days 1-3), sides pinched in (days 1-4), signs of urinary incontinence (day 1), irregular breathing (day 1), hunched posture (days 1-7), abnormal or staggering gait (days 2-7) and staining around the nose, in some cases up to days 4-7);

reduction in bodyweight in males on days 8 (9.5% less) and 15 (5.4% less); up to a 75.9% reduction in food consumption in males; increase in time to tail flick (273 - 281% of controls, 1-2 hours after dosing on day 1); reduction in landing foot splay (77 - 83% of controls, 1-2 hours post dosing on day 1); reductions in forelimb grip strength (82 - 85% of controls, 1-2 hours post-dosing on day 1); reduction in hindlimb grip strength (82% of controls, day 1) and reduction in motor activity (5.9 - 48.4% of controls, first hour after dosing on day 1). The results of the latter screening battery for neurotoxicity were not apparent on days 8 or 15 post-dosing, indicating that the effects were reversible. There was no microscopic evidence of neurotoxicity. No effects were observed at dose levels of 100 mg/kg or below.

The NOEL is 100 mg/kg and the LEL is 300 mg/kg based on death, clinical signs of toxicity, reduction in bodyweight and food consumption and effects on time to tail flick, landing foot splay, forelimb grip strength, hindlimb grip strength and motor activity during the first day after dosing. These were reversible by day 8. There was no microscopic evidence of neurotoxicity. There were no indications of neurotoxicity below at lethal dose.

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

A. MATERIALS AND METHODS:

1. Test Compound(s)

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

Description: Amber yellow liquid
Batch #(s): F47 D7534/36 CTL Y06380/036
Purity: 59.4%
Source: ICI Agrochemicals
Vehicle (if applicable): Deionized water
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: 35 days old upon arrival; 42 - 49 days old at start of test.
Source(s): ICI Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

a. Preparation of Dose Levels: The test substance was weighed out for each dose level and an appropriate amount of the vehicle was added. Samples of each preparation were analyzed prior to the start of dosing in order to verify the concentrations desired. The chemical stability of glyphosate trimesium in deionized water was determined after a 7 day period. The homogeneity of the test chemical was not determined because the formulations were solutions.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of results of studies previously performed in the laboratory.

c. Animal Assignment and Dose Levels: Rats were dosed one time by gavage at 1 ml/100 g body weight.

Test Group	Dose Administered mg/kg	male	female
Control	0	10	10
1	30	10	10
2	100	10	10
3	300	10	10

*Five animals/sex from 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 daily during the study for clinical signs of toxicity and mortality.

e. Body Weight Determinations: Bodyweights were recorded on day -1, immediately prior to dosing, 1-2 hours after dosing and on days 8 and 15.

f. Food and/or Water Consumption: Food consumption was measured continuously throughout the study and calculated on a weekly basis.

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 on day -1, on day 1 (at 1 to 2 hours after

dosing), and on days 8 and 15. 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 on days -1, 1, 8 and 15. 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: Any rat requiring euthanasia during the study and up to 5 rats/sex/group terminated at the end of the study 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. Also, five other animals/sex/group were deeply anesthetized with sodium pentobarbitone 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 neuropathological examination was

performed on the control and highest dose 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 300 mg/kg group. The brain was examined in the transverse plane at 12 levels. On the basis of this examination, the remaining 4 animals/sex from this group and 5 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

j. Statistical Analyses: Day 1 bodyweights, functional observational battery data (day -1 measurements), brain measurements on method of kill and the replicate structure of the study design were analyzed by analysis of covariance. Motor activity measurements for each 5 minute period and overall (minutes 1-50), weekly food consumption and replicate structure of the study design 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. Dosage Preparation: The concentration analyses revealed that the mean achieved concentrations were within 3% of the nominal concentrations (97.7, 98.0 and 101.7% of the the nominal concentrations for the 3.00, 10.0 and 30.0 mg/ml concentrations, respectively). The 3.0 and 30.0 mg/ml formulations were stable for a period of 7 days, which covered the period of use during the study (99.0% and 94.8% of the initial concentrations for the 2.95 mg/ml and the 31.8 mg/ml solutions, respectively).

2. General Clinical Signs of Toxicity and Mortality: There were two unscheduled deaths in the study. These were considered to be due to treatment with the chemical. Two high dose animals died, one male and one female. The male died 7-8 hours after dosing on day 1 and the female was killed in extremis approximately 6 hours after dosing on day 1. Clinical signs of toxicity for both animals were observed approximately 1-2 hours after dosing. These included, ptosis, decreased activity, reduced splay reflex and upward curvature of the spine in both animals; shaking in the male and reduced splay index in the female. In addition, approximately 6 hours after dosing, chromodacryorrhea, shaking, sides pinched in, signs of urinary incontinence and irregular breathing were also observed in the female. For humane reasons the animal was sacrificed.

3. Body Weight Determinations: In males, a statistically significant reduction in bodyweight was observed on days 8 and 15 at the high dose level when compared to the control group. This decrease was approximately

9.5% less than the control value at day 8 and 5.4% less than the control value at day 15. High dose females also had reductions in body weight but they were not statistically significant at either time point. No effects were observed at the lower dose levels in either sex. Thus, in males, there was a borderline effect on bodyweights at the high dose and in females there was no effect on bodyweights at any dose level. The following table summarizes body weights.

Intergroup Comparison of Bodyweights

Dose Level of Glyphosate Trimesium (mg/kg)

Day	0	30	100	300
		Males		
1	149.4 ± 15.3	147.9 ± 14.6	146.7 ± 14.5	146.0 ± 16.3
8	222.0 ± 20.9	223.5 ± 17.9	221.3 ± 18.8	201.1** ± 21.0 (90.5%)
15	270.9 ± 22.3	274.6 ± 19.4	274.2 ± 21.1	256.4* ± 23.1 (94.6%)
		Females		
1	126.2 ± 7.5	125.8 ± 7.6	127.6 ± 9.6	125.7 ± 11.1
8	171.1 ± 7.6	170.1 ± 8.8	172.3 ± 11.1	166.7 ± 13.6 (97.4%)
15	193.1 ± 7.5	190.7 ± 12.8	194.2 ± 12.6	184.8 ± 12.3 (95.7%)

*Significantly different from control ($p < 0.05$)
**Significantly different from control ($p < 0.01$)
( ) = % of control value

4. Food and/or Water Consumption: During week 1, reduced food consumption was observed in both sexes of the high dose group when compared to controls. Food consumption after that time was comparable to the control group. No treatment-related effects were observed in the lower dose groups. The following table taken directly from the report summarizes food consumption.

Food Consumption (g/Rat/Day)

Week	Dose Levels (mg/kg) 0	30	100	300
		Males		
1	25.7	25.4	25.5	19.5* (75.9%)
2	27.3	27.7	27.7	27.1
		Females		
1	20.6	20.5	21.1	18.7* (90.8%)
2	20.5	20.0	20.7	19.7

*Statistically significant ($p < 0.05$)

5. Functional Observational Battery:

Clinical Observations: At the high dose, clinical signs were observed in both sexes. These were generally observed between 1-2 hours post dosing on day 1. Some signs continued to days 4-7. Recovery from the majority of the signs was observed within the first 24 to 48 hours post dosing. The signs included: ptosis, decreased activity, shaking, hunched posture, upward curvature of the spine, reduced splay reflex, sides pinched in and labored or irregular breathing. The report stated that abnormal, staggering gait was recorded from day 2 for 3 males and staining around the nose was also observed on day 2 for several animals. In addition, for several animals, abnormal gait, hunched posture, sides pinched in, upward curvature of the spine and reduced splay index were still apparent on days 4-7. No clinical signs were observed at any of the other dose levels. The following tables taken directly from the report summarize selected clinical signs of toxicity.

Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Males	0	30	100	300
Abnormal gait				
# Observations				11
# Animals				3
Days (from - to)				2-7
Activity decreased				
Observations				13
# Animals				8
Days (from - to)				1-2
Labored breathing				
Observations				2
# Animals				2
Days (from - to)				2-3
Chromodacryorrhea				
Observations				3
# Animals				2
Days (from - to)				2-3
Hunched				
Observations				7
# Animals				4
Days (from - to)				1-7
Ptosis				
Observations				9
# Animals				9
Days (from - to)				1-1
Shaking				
Observations				11
# Animals				8
Days (from - to)				1-3
Sides pinched in				
Observations				8
# Animals				3
Days (from - to)				1-4
Reduced splay index				
Observations		6		6
# Animals		1		5
Days (from - to)		8-15		1-2
Stains around nose				
Observations				5
# Animals				4
Days (from - to)				2-3

Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Males	0	30	100	300
Upward curvature of spine				
Observations				12
# Animals				7
Days (from - to)				1-5

Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Females	0	30	100	300
Activity decreased				
Observations				5
# Animals				5
Days (from - to)				1-1
Breathing irregular				
Observations				2
# Animals				2
Days (from - to)				1-1
Ptosis				
Observations				5
# Animals				5
Days (from - to)				1-1
Shaking				
Observations				7
# Animals				5
Days (from - to)				1-3
Sides pinched in				
Observations				3
# Animals				2
Days (from - to)				1-3
Reduced splay index				
Observations		4		7
# Animals		1		4
Days (from - to)		1-4		1-4
Stains around nose				
Observations				2
# Animals				1
Days (from - to)				2-3

Clinical Observation Incidence for Glyphosate Trimesium

Observation	Dose Level (mg/kg)			
Females	0	30	100	300
Upward curvature of spine				
Observations				10
# Animals				4
Days (from - to)				1-4

Tail Flick Response: In the high dose group, a statistically significant increase in time to tail flick was observed at 1-2 hours after dosing on day 1 for both sexes when compared with controls. By day 8, the time to tail flick response was slightly higher than controls in males, although not statistically significant. These animals had recovered by day 15. No treatment-related differences were observed for the lower dose groups. The following table taken directly from the report summarizes the most significant findings.

Intergroup Comparison of the Time to Tail Flick

Day	Dose Level (mg/kg) 0	30	100	300
		Males		
-1	7.4 ± 5.0	8.3 ± 5.3	7.8 ± 5.4	6.4 ± 3.7
1	5.6 ± 2.4	4.9 ± 1.7	6.5 ± 3.1	15.3 ± 5.9
A.M.[a]	5.7	4.6	6.4	15.7**
8	7.2 ± 3.4	7.6 ± 5.5	6.5 ± 2.8	10.9 ± 6.5
A.M.	7.2	7.5	6.4	11.1
15	5.3 ± 5.4	6.9 ± 5.7	4.0 ± 3.1	4.2 ± 1.5
A.M.	5.3	6.8	4.0	4.0
		Females		
-1	8.9 ± 6.3	7.3 ± 5.3	10.6 ± 6.6	7.0 ± 4.6
1	5.3 ± 2.7	6.6 ± 4.6	6.6 ± 5.6	14.9 ± 5.5
A.M.	5.3	6.6	6.7	14.8**
8	4.3 ± 1.9	6.9 ± 4.7	5.8 ± 3.7	5.2 ± 3.4
A.M.	4.3	7.1	5.6	5.4
15	4.9 ± 2.6	4.8 ± 4.0	4.3 ± 2.3	6.6 ± 5.2
A.M.	4.8	4.9	4.2	6.5

**Statistically significant ($p < 0.01$)
[a]A.M. = Adjusted mean.

Landing Foot Splay: A statistically significant reduction in landing foot splay was observed at 1-2 hours post dosing on day 1 in high dose males and in females in all dose groups. Considering that for all other tests there were no effects in the 2 lower dose groups in either males or females and that the low and mid-dose males were not affected in this particular test, the observed effects in females at the two lower dose levels are probably not biologically significant. By day 8, landing foot splay was comparable to the controls in all groups. The following table, taken directly from the report, summarizes landing foot splay measurements.

Intergroup Comparison of Landing Foot Splay (mm)

Day	Dose Level (mg/kg) 0	30	100	300
		Males		
-1	63.0 ± 14.6	64.5 ± 10.7	58.3 ± 19.0	64.0 ± 12.2
1	56.8 ± 11.5	58.2 ± 11.7	60.0 ± 11.8	47.2 ± 10.0
A.M.[a]	56.6	57.5	61.5	46.6*
8	61.5 ± 11.9	62.5 ± 16.2	68.8 ± 13.7	69.1 ± 14.8
A.M.	61.4	62.1	69.7	68.5
15	65.3 ± 13.1	64.8 ± 7.3	73.3 ± 14.6	73.3 ± 15.2
A.M.	65.2	64.1	74.9	72.4
		Females		
-1	58.0 ± 9.2	61.3 ± 13.9	61.0 ± 17.5	61.7 ± 11.1
1	58.5 ± 11.3	50.7 ± 11.0	43.0 ± 10.3	45.0 ± 7.1
A.M.	59.6	50.3*	42.8**	44.5**
8	61.7 ± 11.5	61.5 ± 13.3	54.2 ± 14.8	57.0 ± 11.9
A.M.	62.7	61.1	53.9	56.1
15	56.0 ± 5.7	61.3 ± 12.6	56.9 ± 13.7	61.1 ± 14.1
A.M.	56.7	61.0	56.7	60.6

[a]A.M. = adjusted mean
*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Grip Strength: Statistically significant reductions in forelimb grip strength was observed 1-2 hours post-dosing on day 1 in high dose males. Forelimb and hindlimb grip strength were significantly reduced in high dose females on day 1 as well. On day 15,

significant reductions in forelimb grip strength in 100 mg/kg males and in hindlimb grip strength in 30 or 300 mg/kg females were observed. These are not considered to be biologically significant because the changes are not consistent across dose levels, sex or time of observation. The following tables taken directly from the report summarize the results.

Intergroup Comparison of Forelimb Grip Strength (g)

Day	Dose Level (mg/kg) 0	30	100	300
		Males		
-1	610 ± 95	669 ± 83	599 ± 117	652 ± 75
1	801 ± 130	791 ± 160	720 ± 150	658 ± 80
A.M.[a]	818	763	745	643*
8	831 ± 207	876 ± 237	729 ± 153	787 ± 213
A.M.	835	868	735	767
15	1228 ± 210	1144 ± 217	1053 ± 174	1126 ± 112
A.M.	1242	1120	1074*	1120
		Females		
-1	641 ± 153	586 ± 98	637 ± 184	652 ± 144
1	771 ± 110	776 ± 99	691 ± 94	659 ± 129
A.M.	766	793	688	649**
8	814 ± 147	844 ± 171	798 ± 179	782 ± 206
A.M.	812	853	797	758
15	1158 ± 84	1123 ± 128	1106 ± 93	1084 ± 160
A.M.	1158	1120	1106	1086

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)
[a]A.M. = adjusted mean

Intergroup Comparison of Hindlimb Grip Strength (g)

Day	Dose Level (mg/kg) 0	30	100	300
		Males		
-1	431 ± 150	420 ± 110	397 ± 77	401 ± 73
1	483 ± 76	498 ± 138	438 ± 62	449 ± 89
A.M.[a]	477	495	44[illegible]	452
8	616 ± 104	643 ± 79	596 ± 90	631 ± 135
A.M.	618	643	594	628
15	670 ± 80	642 ± 205	662 ± 91	716 ± 150
A.M.	674	644	658	718
		Females		
-1	488 ± 87	428 ± 56	487 ± 109	442 ± 54
1	484 ± 71	461 ± 105	486 ± 66	395 ± 102
A.M.	479	466	482	398*
8	635 ± 112	644 ± 97	566 ± 116	629 ± 73
A.M.	632	648	563	632
15	743 ± 144	579 ± 113	723 ± 108	610 ± 86
A.M.	737	586**	717	619*

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)
[a]A.M. = Adjusted mean

6. Motor Activity: In the high dose group, motor activity was significantly reduced during the first hour after dosing on day 1 when compared to controls. For males, the difference was especially observed during the first 10 minutes and for females, the difference was especially observed during the first 5 minutes. During minutes 6 - 10, motor activity for females was reduced when compared to controls, but not significantly so. No treatment-related differences were observed in the other dose groups. The following table taken directly from the report summarizes selected values for motor activity.

Intergroup Comparison of Motor Activity (Movements/Animal)

Day Minutes	Dose Level (mg/kg) 0	30	100	300
		Males		
Day -1				
Min. 1-5	65.9 ± 12.1	72.1 ± 11.7	76.9 ± 8.2	68.7 ± 11.0
Min. 6-10	56.4 ± 27.9	51.9 ± 20.7	60.9 ± 15.9	50.6 ± 18.3
Day 1				
Min. 1-5	59.3 ± 13.6	66.3 ± 15.8	64.3 ± 6.7	3.5 ± 4.1**
Min. 6-10	22.6 ± 22.9	32.3 ± 25.3	21.9 ± 16.9	1.7 ± 3.0*
Min. 11-15	5.8 ± 8.5	12.4 ± 26.3	3.9 ± 11.3	0.9 ± 1.7
Min. 16-20	0.6 ± 1.1	4.1 ± 10.9	0.4 ± 1.0	1.7 ± 2.8
Min. 21-25	0.5 ± 1.0	0.3 ± 0.7	0.0 ± 0.0	5.0 ± 9.3*
Min. 26-30	0.2 ± 0.4	0.9 ± 2.2	0.9 ± 1.4	2.5 ± 4.4
Min. 46-50	0.0 ± 0.0	5.5 ± 8.3**	0.8 ± 1.3	0.5 ± 1.1
Min. 1-50	100.0 ± 54.5	127.8 ± 68.1	95.2 ± 28.8	17.7 ±15.9**
Day 8				
Min. 1-5	74.1 ± 8.9	73.9 ± 7.9	74.4 ± 7.9	69.7 ± 23.0
Min. 6-10	64.4 ± 10.2	54.9 ± 22.6	66.9 ± 13.0	47.1 ± 22.2*
Min. 46-50	5.0 ± 9.4	10.7 ± 21.3	10.7 ± 19.4	31.9 ±26.7**
Min. 1-50	298.5 ± 78.6	249.1 ± 76.9	280.2 ± 116.1	300.1 ±140.6
Day 15				
Min. 1-5	73.3 ± 14.4	75.5 ± 11.7	79.7 ± 7.2	73.0 ± 15.5
Min. 1-50	508.4 ± 158.9	508.8 ± 179.7	493.8 ± 191.6	506.9 ±142.0
		Females		
Day -1				
Min. 1-5	66.7 ± 12.8	67.1 ± 10.1	65.6 ± 11.8	65.7 ± 10.3
Min. 6-10	59.0 ± 12.7	63.7 ± 10.1	47.6 ± 20.0	56.9 ± 15.9
Day 1				
Min. 1-5	62.6 ± 11.3	71.1 ± 9.7	68.8 ± 12.8	30.3 ±31.8**
Min. 6-10	36.1 ± 30.0	51.9 ± 21.3	44.9 ± 25.0	25.0 ± 22.2
Min. 11-15	21.8 ± 28.5	29.8 ± 28.0	28.6 ± 31.1	16.9 ± 25.4
Min. 16-20	4.8 ± 10.1	23.2 ± 25.5*	11.7 ± 16.5	16.5 ± 22.7
Min. 21-25	2.1 ± 3.0	22.1 ± 28.4*	15.2 ± 18.7	14.0 ± 16.8
Min. 26-30	5.4 ± 15.0	17.1 ± 24.1	16.0 ± 21.9	9.8 ± 10.9
Min. 36-40	7.2 ± 15.7	23.7 ± 26.1*	11.4 ± 12.3	6.4 ± 11.0
Min. 41-45	2.2 ± 5.3	31.6 ± 34.4*	12.3 ± 25.7	15.3 ± 25.5
Min. 1-50	154.0 ± 84.7	315.2±161.0**	242.1 ± 150.2	150.9 ±132.8
Day 8				
Min. 1-5	65.3 ± 7.0	64.4 ± 20.5	68.0 ± 11.5	66.3 ± 16.3
Min. 11-15	46.7 ± 24.1	62.5 ± 13.7*	49.5 ± 16.2	57.9 ± 8.0
Min. 1-50	544.7 ± 131.7	621.9 ± 121.6	543.6 ± 120.5	595.6 ±125.7
Day 15				
Min. 1-5	70.3 ± 9.9	66.0 ± 17.0	71.8 ± 10.2	68.2 ± 10.5
Min. 1-50	565.8 ± 101.0	612.8 ± 164.7	585.1 ± 161.5	605.7 ±126.0

7. Brain Measurements: No treatment-related differences were observed in brain weight, length or width at any dose level in either sex. The following table taken directly from the report summarizes the values.

Intergroup Comparison of Brain Measurements

Observation	Dose Level (mg/kg) 0	30	100	300
	Males			
Brain Weight (g)	1.90	1.89	1.89	1.87
Brain Length (mm)	27.3	26.2	26.2	27.1
Brain Width (mm)	15.1	14.9	15.4	15.0
	Females			
Brain Weight (g)	1.77	1.76	1.78	1.78
Brain Length (mm)	26.2	25.9	27.0	26.3
Brain Width (mm)	14.9	14.8	14.8	15.1

8. Neuropathology: Minimal nerve fiber degeneration was observed in the sciatic nerve of 1 high dose male. The degeneration was minimal and consisted of a single, small focus of Wallerian type degeneration. The authors of the report stated that "such occasional nerve fiber degeneration is an incidental feature of the peripheral nervous system of a number of strains of rat, including the Alderley Park strain. In this study, the single small focus of Wallerian type degeneration seen, in one fiber, was considered to be a spontaneous finding and incidental to treatment...". In light of historical control data (see table below), this lesion is not considered to be related to treatment. The following table taken directly from the report summarizes the results.

Intergroup Comparison of Microscopic Findings

	Dose Level of Glyphosate Trimesium (mg/kg)			
	Males		Females	
	0	300	0	300
Animals on Study	10	10	10	10
Animals Completed	5	5	5	5
Sciatic Nerve Examined	5	5	5	5
No Abnormalities Detected	5	4	5	5
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[a]

Acute Oral Studies: N = 5 Rats/Sex/Group

Month/Year	Males	Females
May 1992	2	0
June 1992	0	0
July 1992	2	1
December 1992	1	1
February 1993	1	0

[a]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 Good Laboratory Practice and Quality Assurance Statements were provided.

C. DISCUSSION: This study was conducted according to the guidelines and is graded Core Guideline. It satisfies the regulatory requirements for an acute mammalian neurotoxicity study (81-8). Positive control data were provided under separate cover and are summarized in the Appendix. The NOEL is 100 mg/kg and the LEL is 300 mg/kg. Toxicity was observed at the highest dose level in both sexes (300 mg/kg).

There were positive results for the neurotoxicity screening battery on day 1. These effects had disappeared by day 8. Since the protocol does not call for observations in between days 1 and 8, it is not known how long these effects lasted. However, some of the clinical signs of toxicity that are similar to clinical signs of neurotoxicity persisted to day 7 (it is noted that in metabolism studies, this chemical is eliminated from the body within 1 to 5 days). In this case, the authors of the report stated that these effects were due to systemic toxicity and not neurotoxicity because the animals were tested at a dose level close to the LD_{50}. Since there were no microscopic indications of neurotoxicity, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity. It is noted here that the only change observed in the subchronic neurotoxicity study was a decrease in forelimb grip strength in high dose females at various time points. This was not seen in males and was not dose-related, although consistent at the high dose. No other differences were observed in the subchronic study. Therefore, if the results of the acute study are indicative of neurotoxicity, they are not validated by the results from the subchronic study. The positive control data submitted with these studies do not shed any light on how to interpret the data. Three of the positive control studies were terminated 1 hour after dosing and the other two were subchronic studies. In these, either the neurotoxic effects were different from the effects seen in this particular study or they appeared later in the study and not on the first day.

Appendix: Discussion of Positive Control Studies

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Section I, Tox. Branch (7509C) 5/26/94

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for Assessment of Sensory Perception in the Rat

ACCESSION NO./MRID NO.: 430133-02

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Morphine sulphate

STUDY NUMBER: XR2287

REPORT NUMBER: CTL/P/3689

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware

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

TITLE OF REPORT: Assessment of Sensory Perception in the Rat

AUTHOR(S): S. L. Allen

REPORT ISSUED: 6/26/92

CONCLUSION: Morphine sulphate (99%) was tested as a positive control in the tail flick test in male and female Alpk:APfSD rats. The rats received a single dose by gavage either 0, 50, 75 or 100 mg/kg of the test material in deionized water at a volume of 1 ml/100g bodyweight. The tail flick response test for pain perception was conducted one hour after dosing.

At 100 mg/kg, an increase in the tail flick response time (219 - 234% of control time) was observed in both sexes. No clear treatment-related effects were observed at the lower dose levels.

The NOEL for tail flick time response is 75 mg/kg and the LEL is 100 mg/kg.

This study is acceptable as a positive control study for the laboratory in which it was conducted, for chemicals that induce an analgesic or soporific effect. As a general comment, the animals were examined in the tail flick test at one hour after dosing and not at any time afterwards (it is assumed that this is

because the analgesic effect of morphine does not last for a long time). For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the LD_{50}. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for an acute neurotoxicity study). Therefore, when using this particular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Morphine sulphate

Description: White solid
Batch #(s), Other #(s): CTL Ref. No. YO5725/005
Purity: 99% w/w
Source: Sigma Chemical Company
Vehicle (if applicable): deionized water

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: Between 5 and 8 weeks
Weight(s): 130-184g (males); 107-164g (females)
Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfied, Cheshire, UK

3. Procedure:

a. Dosage Preparation: The test material was weighed out and added to an appropriate amount of deionized water.

Frequency of preparation: Only one time.

Storage conditions: The test material was stored at ambient temperature in the dark.

Stability Analyses: The Supplier had stated that the test material was stable for at least one year under the conditions of the storage used.

Homogeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.

c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

Test Group	Dose Administered mg/kg	Main Study male	female
Contr.	0	10	10
1	50	10	10
2	75	10	10
3	100	10	10

d. Measurement of Tail Flick Response: The tail flick time of each animal was measured the day before dosing. Any animal with a response time of greater than 9.5 seconds was replaced. Tail flick time of each animal was again measured 1 hour following dosing. The report stated that "the test involved the application of a thermal stimulus to the tail and measurement of the latency to withdraw the tail. A cut-off time of 20 seconds was used."

No other measurements were conducted.

e. Statistical Analyses: Time to tail flick was analyzed by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement of Tail Flick Response

The test chemical prolonged the tail flick response time in both sexes at the highest dose tested (100 mg/kg). There were no clear treatment-related effects at the lower dose levels. The following table, taken directly from the report summarizes the results.

Intergroup Comparison of Tail Flick Times (seconds)

Dose Level of Morphine Sulfate (mg/kg)			
0	50	75	100
	Males		
5.47 ± 2.42	7.31 ± 4.99	5.65 ± 2.28	12.83 ± 5.42**
	Females		
4.58 ± 2.10	6.53 ± 3.31	5.28 ± 1.21	10.06 ± 4.67**

**Statistically significant ($p < 0.01$)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that the tail flick response test is valid in this test system, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that sensory perception in the rat can be measured using the tail flick test. The report stated that "the characteristic analgesic effect of morphine sulphate was demonstrated in this study through an increase in response times at 100 mg/kg."

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DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for Assessment of Muscular Weakness in the Rat

ACCESSION NO./MRID NO.: 430133-01

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Chlordiazepoxide

STUDY NUMBER: XR2286

REPORT NUMBER: CTL/P/3688

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware

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

TITLE OF REPORT: Assessment of Muscular Weakness in the Rat

AUTHOR(S): S. L. Allen

REPORT ISSUED: 6/26/92

CONCLUSION: Chlordiazepoxide hydrochloride (98%) was tested as a positive control in hindlimb and forelimb grip tests in male and female Alpk:APfSD rats. The rats received a single dose by gavage either 0, 5, 10 or 20 mg/kg of the test material in corn oil at a volume of 1 ml/100g bodyweight. The grip strength tests for muscle weakness was conducted one hour after dosing in 3 replicate trials.

The test chemical reduced hindlimb grip strength in both sexes at all dose levels (74 - 83% of controls). In males, there was a clear dose response. Forelimb grip strength was less affected than hindlimb grip strength. Significant reductions in forelimb grip strength were only observed in males at 20 mg/kg only (71 - 75% of controls). A comparison of the replicate trials indicated that the data were reproducible.

No NOEL was established for reduction in hindlimb grip strength and the NOEL for reduction in forelimb grip strength was 10 mg/kg. The LEL for reduction in forelimb grip strength was 20 mg/kg.

This study is acceptable as a positive control study for the laboratory in which it was conducted. As a general comment, the animals were examined in the fore- and hindlimb grip strength tests at one hour after dosing and not at any time afterwards. For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the LD_{50}. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for an acute neurotoxicity study). Therefore, when using this particular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Chlordiazepoxide hydrochloride

Description: Solid
Batch #(s), Other #(s): CTL Ref. No. Y05672/003
Purity: > 98% w/w
Source: Sigma Chemical Company
Vehicle (if applicable): Corn oil

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: Between 5 and 8 weeks
Weight(s): 216-264g (males); 152-164g (females)
Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfied, Cheshire, UK

3. Procedure:

a. Dosage Preparation: The test material was weighed out and added to an appropriate amount of corn oil.

Frequency of preparation: Only one time.

Storage conditions: The test material was stored at ambient temperature in the dark.

Stability Analyses: The Supplier had stated that the test material was stable for at least one year under the conditions of the storage used.

Homcgeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.

c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

Test Group	Dose Administered mg/kg	Main Study male	female
Contr.	0	10	10
1	5	10	10
2	10	10	10
3	20	10	10

d. Measurement of Forelimb and Hindlimb Grip Strength: One hour following dosing, each rat was tested for muscle relaxation by measuring fore- and hindlimb grip strength. The report stated that the following was used as a procedure: "the apparatus consisted of two strain gauges, one with a triangular ring attached and the second with a T-bar attached, with a perspex channel between. A measurement of grip strength was made by placing tha animal into the channel with its forepaws inside the triangular grasping ring of the forelimb meter. The animal was grasped by the tail and steadily pulled from away from the ring. When the grip was broken the animal was continued to be pulled along the channel so that its hindlimbs grasped the T-bar. The trial was completed when the grip of the hindlimbs was broken." Replicate trials were conducted.

No other measurements were conducted.

e. Statistical Analyses: Forelimb and hindlimb grip strength were analyzed by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement of Forelimb and Hindlimb Grip Strength

The test chemical reduced hindlimb grip strength in both sexes at all dose levels. In males, there was a clear dose response. Forelimb grip strength was less affected than hindlimb grip strength. Significant reductions in forelimb grip strength were only observed in males at 20 mg/kg only. A comparison of the replicate trials indicated that the data were reproducible. The following tables, taken directly from the report summarize the results.

Intergroup Comparison of Grip Strength Data - Post Dosing Males

	Dose Level of Chlordiazepoxide (mg/kg)			
Grip Strength	0	5	10	20
Forelimb, trial 1	825	827	822	582**
Forelimb, trial 2	811	743	766	633**
Forelimb, trial 3	814	724	725	628**
Mean Forelimb	817	765	771	614**
Hindlimb, trial 1	652	632	577	501**
Hindlimb, trial 2	720	606*	557**	536**
Hindlimb, trial 3	699	597*	554**	531**
Mean Hindlimb	690	612*	562**	523**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Intergroup Comparison of Grip Strength Data - Post Dosing Females

Grip Strength	Dose Level of Chlordiazepoxide (mg/kg) 0	5	10	20
Forelimb, trial 1	720	765	817	705
Forelimb, trial 2	731	735	757	758
Forelimb, trial 3	702	710	711	779
Mean Forelimb	717	736	761	747
Hindlimb, trial 1	641	587	524**	517**
Hindlimb, trial 2	612	503*	476**	525
Hindlimb, trial 3	585	543	483**	490*
Mean Hindlimb	612	544*	494**	511**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that the hindlimb and forelimb grip strength tests are valid tests for assessment of muscular weakness, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that muscular weakness in the rat can be measured using the hindlimb and forelimb grip tests. The report stated that "the validity of grip strength measurement for the assessment of muscular weakness in the rat has been demonstrated using the known muscle relaxant chlordiazepoxide hydrochloride."

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DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for Assessment of Motor Activity in the Rat

ACCESSION NO./MRID NO.: 430133-03

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Amphetamine Sulphate or Chlorpromazine Hydrochloride

STUDY NUMBER: XR2285

REPORT NUMBER: CTL/P/3687

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware

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

TITLE OF REPORT: Measurement of Motor Activity in the Rat

AUTHOR(S): S. A. Horner

REPORT ISSUED: 8/7/92

CONCLUSION: Amphetamine sulphate (99%) or chlorpromazine hydrochloride (99%) were tested as positive controls in a motor activity test in male and female Alpk:APfSD rats. The rats received a single dose of either chemical by gavage at either 0, 0.1, 1.0 or 10.0 mg/kg of the test material in deionized water at a volume of 1 ml/100g bodyweight. The motor activity tests were conducted one hour after dosing.

Amphetamine sulphate induced a dose-dependent increase in motor activity in both sexes at both 10 and 1 mg/kg. At 10 mg/kg, activity for both sexes remained at 3-6 fold above controls throughout the study. At 1 mg/kg, activity scores were approximately twice the control values. Males showed in increase in activity during the first 35 minutes, whereas females showed an increase primarily during minutes 26-50. No effects were observed at 0.1 mg/kg. **The NOEL for amphetamine sulphate is 0.1 mg/kg and the LEL is 1 mg/kg based on increases in motor activity.**

At 10 mg/kg, treatment-related decreases in motor activity were observed in both males and females treated with chlorpromazine hydrochloride. Total activity was reduced to approximately 48 or 29% of control levels in males and females, respectively. No effects were observed at either 1.0 or 0.1 mg/kg. **The NOEL for chlorpromazine hydrochloride is 1.0 mg/kg and the LEL is 10.0 mg/kg based on decreases in motor activity.**

This study is acceptable as a positive control study for the laboratory in which it was conducted. As a general comment, the animals were examined in the motor activity tests at one hour after dosing and not at any time afterwards. For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the LD_{50}. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for ar acute neurotoxicity study). Therefore, when using this p rticular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Amphetamine sulphate or chlorpromazine hydrochloride

Description: Solids
Batch #(s), Other #(s): CTL Ref. No. YO1775/006/002 (amphetamine) and YO2531/002/009 (chlorpromazine)
Purity: 99% w/w (both)
Source: Sigma Chemical Company
Vehicle (if applicable): deionized water

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: Between 5 and 8 weeks
Weight(s): 174-275g (males); 127-215g (females)
Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfied, Cheshire, UK

3. Procedure:

a. Dosage Preparation: The test materials were weighed out and added to an apprcpriate amount of deionized water.

Frequency of preparation: Only one time each.

Storage conditions: The test materials were stored at ambient temperature in the dark.

Stability Analyses: The Supplier had stated that the test materials were stable for at least one year under the conditions of the storage used.

Homogeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.

c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

Test Group	Dose Administered mg/kg	Main Study male	female
	Amphetamine Sulphate		
Contr.	0	10	10
1	0.1	10	10
2	1.0	10	10
3	10.0	10	10
	Chlorpromazine Hydrochloride		
Contr.	0	10	10
1	0.1	10	10
2	1.0	10	10
3	10.0	10	10

d. Measurement of Motor Activity: One hour following dosing, each rat was allocated to an activity monitor and tested for motor activity. Each animal was assessed for ten 5 minute periods up to 50 minutes.

Clinical observations were recorded immediately prior to dosing for each animal and no abnormalities were recorded. No other measurements were conducted.

e. Statistical Analyses: Motor activity measurements for each 5 minute period and overall minutes (1-50) were considered at each measurement time by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement Motor Activity

For amphetamine sulphate, the report stated that during day -1, motor activity in all groups, including controls was highest during the first 5 minutes of the measurement period and attenuated thereafter. The decline in activity reached asymptomatic levels after 30 (males) to 40 (females) minutes. Amphetamine sulphate induced a dose-dependent increase in motor activity in both sexes. Effects were observed at both 10 and 1 mg/kg. In the high dose, activity for both sexes remained at a high level throughout the study (3-6 fold above controls). At 1 mg/kg, activity scores were approximately twice the control values. Males showed in increase in activity during the first 35 minutes, whereas females showed an increase primarily during minutes 26-50.

At 10 mg/kg, treatment-related decreases in motor activity were observed in both males and females treated with chlorpromazine hydrochloride. Total activity was reduced to approximately 48 or 29% of control levels in males and females, respectively.

The following tables, taken directly from the report summarize the results.

Intergroup Comparison of Motor Activity - Amphetamine Sulphate

Minutes	Dose Levels (mg/kg)			
	0	0.1	1.0	10.0
		Males		
Pre Dosing				
1-5	73.6	70.5	74.5	73.9
6-10	70.0	58.4	72.3	65.0
11-15	40.5	37.2	62.4*	52.6
36-40	2.1	1.1	1.8	0.1
1-50	214.6	199.6	252.5	243.0
Post Dosing				
1-5	54.5	62.3	69.2	59.8
6-10	40.2	27.4	55.1	67.5**
11-15	19.1	6.2	42.3**	65.1**
26-30	5.8	5.6	15.9	66.5**
46-50	3.4	1.0	2.1	68.9**
1-50	142.3	114.8	262.9**	652.7**
		Females		
Pre Dosing				
1-5	73.8	71.6	66.5	69.7
6-10	70.8	63.0	64.6	62.6
21-25	42.0	47.5	34.3	32.3
36-40	11.1	14.4	19.6	18.4
1-50	388.8	396.9	344.8	368.9
Post Dosing				
1-5	66.8	67.8	71.9	64.4
6-10	57.8	57.4	61.1	57.4
11-15	42.2	46.7	58.4	57.9
26-30	17.1	28.8	41.9	62.3**
41-45	11.0	24.4	44.1**	68.2**
1-50	278.1	393.6	490.0*	640.0**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Intergroup Comparison of Motor Activity - Chlorpromazine Hydrochloride

Minutes	Dose Levels (mg/kg)			
	0	0.1	1.0	10.0
		Males		
Pre Dosing				
1-5	78.7	71.4	67.7*	67.7*
6-10	70.7	57.5	55.4	58.5
11-15	60.0	41.0*	32.9**	36.4*
21-25	20.4	13.3	0.4*	3.5
1-50	283.2	215.6*	172.0**	201.6*
Post Dosing				
1-5	67.6	56.8	57.7	6.9**
6-10	49.0	34.2	33.9	5.2**
11-15	27.2	9.5*	19.0	6.5**
21-25	1.5	5.0	3.6	8.5
1-50	161.5	144.3	131.4	77.1*
		Females		
Pre Dosing				
1-5	73.3	71.8	73.8	70.4
6-10	69.4	67.9	70.2	69.4
21-25	20.3	34.1	42.4*	43.7*
1-50	342.0	365.1	371.5	374.3
Post Dosing				
1-5	67.0	73.1	63.6	9.5**
6-10	60.0	54.1	53.9	5.5**
11-15	37.1	48.5	46.2	7.3**
31-35	18.7	13.5	8.3	10.7
46-50	17.4	8.8	8.2	12.4
1-50	299.1	319.2	280.6	87.2**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that the motor activity test is a valid test for assessment of either stimulation or inhibition of the central nervous system, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity

of the study. The study shows that either stimulation or inhibition of the central nervous system in the rat by chemicals known to induce those reactions can be measured by using the motor activity test.

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DATA EVALUATION RECORD

STUDY TYPE: Positive Control Study: Subchronic Neurotoxicity in the Rat

ACCESSION NO./MRID NO.: 430133-04

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Trimethyltin Chloride

STUDY NUMBER(S): PRO874

REPORT NUMBER: CTL/P/3658

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, Delaware

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

TITLE OF REPORT: Trimethyltin Chloride: Neurotoxicity Study in Rats

AUTHOR(S): S. L. Allen

REPORT ISSUED: 7/30/92

CONCLUSION: Trimethyltin chloride (99%) was tested in a neurotoxicity feeding study in Alpk:APfSD rats for 29 days as a positive control. The following dose levels were administered in the diet: 0, 4 or 8 ppm (0, 0.2 or 0.4 mg/kg/day). Clinical signs of toxicity, body weights, food consumption, functional observational battery, motor activity and microscopic observations were measured and recorded.

At 0.4 mg/kg/day, severe toxicity was observed. As a result, all animals at this dose level were humanely killed prior to the end of the study. Clinical signs of toxicity included piloerection, urinary incontinence, hunched posture, aggression (males), shaking and clonic convulsions in both sexes.
Increases in motor activity were seen in females on day 15 (120-138% over controls). There was pronounced damage to the limbic system. The spinal cord showed minimal/slight vacuolation/degeneration of ventral horn motor neurons. In the peripheral nervous system there was minimal evidence of

peripheral neuropathy, characterized by Wallerian-type degeneration of peripheral nerve. There also was minimal evidence of degeneration in the sensory roots. The degeneration was confined to the junction of the root with the spinal cord.

The NOEL is 0.2 mg/kg/day and the LEL is 0.4 mg/kg/day based on clinical signs of toxicity and microscopic evidence of neurotoxicity.

This study is acceptible as a positive control study for this particular laboratory.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Trimethyltin chloride

Description: white solid
Batch #(s), Other #(s): CTL Y05954/001/002
Purity: 99%
Source: Aldrich Chemical Company
Vehicle: Ethanol

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 10 - 15 kg batches from premixes prepared by adding stock solutions of ethanol containing the appropriate amount of the test substance to 250g of milled diet. The premixes were then rotary evaporated, dried and added to 9.75 or 14.75 kg diet and mixed thoroughly.

Frequency of preparation: Not stated.

Storage conditions: Not stated.

Stability Analyses: Not conducted.

Homogeneity Analyses: Not conducted.

Concentration Analyses: Samples from all dietary levels were taken from each batch and retained for future analysis.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of results from published literature results and on a range-finding study conducted in the same laboratory.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 29 days male	female
Control	0	12	12
1	4	12	12
2	8	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 weekly, starting immediately before feeding the experimental diet and then on the same day of each week until termination.

f. Food and/or Water Consumption: Food consumption was recorded continuously 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 weekly. 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 on day -1, 15 and 29 of the exposure period. The report stated that "each observation period was divided into fifty scans of one 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 given full post mortem examinations. The brains were weighed and the length and width were recorded with calipers. The tissues listed below were left in situ and stored in 10% neutral buffered formol saline. These tissues were not microscopically examined.

Six other animals/sex/group were deeply anesthetized with barbituate i.p. 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 were microscopically examined. The neuropathological examination was performed on the control and the 8 ppm groups only. All sections were examined by light microscopy. The brain and gastrocnemius muscle were embedded in paraffin wax, cut and stained with H & E stain. The report stated that "the remaining tissues were post-fixed with osmium tetroxide, embedded in resin and semi-thin sections were cut and stained with toluidine blue. The brain was examined in the transverse plane at levels 2, 3, 5, 6 and 7 with sections

submitted including the olfactory bulb, olfactory tuberculum, pyriform cortex, hippocampal formation and amygdaloid nuclei. Spinal cord from the cervical region (C3-C6) and from the lumbar region (L1-L4) was examined in the transverse and longitudinal plane. Spinal roots and dorsal root ganglia were examined from the C3-C6 and L1-L4 levels and the gasserian ganglia from the trigeminal nerve. Transverse and longitudinal sections of the sciatic, sural and tibial nerves were also examined. Samples of the gastrocnemius muscle were examined in the transverse plane."

The following tissues were removed and examined microscopically:

|x| Brain (including forebrain, cerebrum, mid-brain, cerebellum, pons and medulla oblongata)
|x| Spinal cord form cervical region and lumbar region
|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

j. Statistical Analyses: Body weight gain was analyzed using a two-sided Student's t-test, separately for each sex. Brain weight, brain length and brain width were analyzed by analysis of covariance. Analysis of variance and covariance allowed for the replicate structure of the study design. Motor activity measurements, weekly food consumption, 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. Clinical Observations and Mortality: Severe toxicity was observed at the high dose. As a result, all the males were killed on days 22-24 and the females were killed on days 23-25 of the study. As scheduled, however, six/sex were killed by perfusion fixation and

six/sex were exsanguinated under terminal anesthesia. Clinical signs of toxicity were observed at the high dose. These included piloerection, urinary incontinence and hunched posture and did not occur until day 22. Aggression was also observed in males as well as shaking and clonic convulsions in both sexes. The following tables summarize selected clinical observations.

Selected Clinical Signs of Toxicity

Observation	Dose Level (ppm) 0	4	8
	Males		
Aggression	0[a]	0	1
Clonic Convulsions	0	0	1
Reduced Foot Withdrawal Reflex	0	1	0
Hunched	0	0	1
Salivation	0	0	1
Response to Sound	0	0	2
Shaking	0	0	24
Reduced Splay Reflex	0	2	0
Subdued	0	0	1
Signs of Urinary Incontinence	0	0	15
Piloerection	0	0	10

[a]Number of animals

Selected Clinical Signs of Toxicity

Observation	Dose Level (ppm) 0	4	8
	Females		
Clonic Convulsions	0	0	1
Hunched	0	0	1
Shaking	0	0	23
Reduced Splay Reflex	1	5	2
Signs of Urinary Incontinence	0	0	14

Selected Clinical Signs of Toxicity

	Dose Level (ppm)		
Observation	0	4	8
Piloerection	0	0	5

[a]Number of animals

2. Body Weight Determinations: No treatment-related decreases in body weights or body weight gains were observed. Bodyweight and bodyweight gain were significantly increased in the treated groups. Tables will not be provided in this DER because increases in bodyweight and bodyweight gain are not effects of interest in a positive control study for neurotoxicity.

3. Food and/or Water Consumption: Food consumption was increased in both sexes at the high dose in week 3.

4. Functional Observational Battery:

   Landing Foot Splay No consistent treatment-related effects were observed in landing foot splay measurements for either sex. In high dose females, at week 4, mean landing foot splay was less than controls; however, this was not observed in males or at any other time period.

   Time to Tail Flick No consistent dose-related differences in time to tail flick response were observed in the treated groups when compared to controls for either sex.

   Grip Strength Measurements No treatment-related effects in grip strength measurements were observed for either of the treated groups when compared to controls.

5. Motor Activity: On day 15, the motor activity of high dose females was increased during minutes 11-25 (periods 3-5). On day 29 increases in motor activity was observed in the 4 ppm males during minutes 1-20. The authors stated that this is mainly due to increased activity for a few individual animals. In looking at the individual animal data, it also appears that several of the values for the control group were particularly low for these time periods. The following table summarizes selected results.

Intergroup Comparison of Motor Activity

Minutes	Dietary Concentration (ppm) 0	4	8
	Males		
Day 15			
Minutes 1-5	63.6	64.2	70.1
Minutes 11-15	45.2	43.4	44.3
Minutes 16-20	29.8	21.3	37.6
Minutes 46-50	7.6	3.2	2.2
Minutes 1-50	261.3	205.8	261.5
Day 29			
Minutes 1-5	61.2	73.3	-[a]
Minutes 6-10	58.3	76.9*	-
Minutes 11-15	46.3	66.0*	-
Minutes 16-20	33.7	56.2*	-
Minutes 46-50	32.0	11.7	-
Minutes 1-50	375.0	414.5	-
	Females		
Day 15			
Minutes 1-5	75.3	72.0	75.5
Minutes 11-15	56.0	63.1	67.5*
Minutes 16-20	54.2	60.3	74.8*
Minutes 46-50	49.0	41.0	35.6
Minutes 1-50	504.1	563.8	586.8
Day 29			
Minutes 1-5	74.2	72.3	-[a]
Minutes 6-10	60.3	71.8	-
Minutes 11-15	54.7	62.8	-
Minutes 16-20	56.6	58.0	-
Minutes 46-50	46.9	38.3	-
Minutes 1-50	540.3	502.2	-

*Statistically significant ($p < 0.05$)
[a]Sacrificed prior to this time point.
**Statistically significant ($p < 0.01$)

6. Brain Measurements Both males and females in the high dose group had lower brain weights in the high dose group when compared to the control group. In addition, brain width was slightly less than the control group for the high dose males. However, the authors stated that these animals were sacrificed one week earlier than the other animals, and thus, the differences may reflect the lesser maturity of the rats rather than due to treatment with the chemical. The following tables, taken directly from the report, summarize the results.

Intergroup Comparison of Brain Parameters - Males

Observation	Dietary Concentration		
	0	4	8
Brain Weight (g)	1.99	1.97	1.85**
Brain Length (mm)	26.9	27.8	26.8
Brain Width (mm)	15.3	15.6	14.8*

*Statistically significant ($p < 0.05$).
**Statistically significant ($p < 0.01$).

Intergroup Comparison of Brain Parameters - Females

Observation	Dietary Concentration		
	0	4	8
Brain Weight (g)	1.79	1.78	1.73*
Brain Length (mm)	25.9	26.0	26.3
Brain Width (mm)	14.8	14.7	14.5

*Statistically significant ($p < 0.05$).
**Statistically significant ($p < 0.01$).

7. Neuropathology: In the high dose rats, there was "pronounced damage to the limbic system, characterized by neuronal cell necrosis of the hippocampal formation (CA1, CA3, CA4 and dentate gyrus), pyriform cortex, amygdaloid nuclei and olfactory tuberculum. The degree of necrosis was greatest in the hippocampal formation and pyriform cortex and least in the amygdaloid nuclei and olfactory tuberculum.

The spinal cord of the 8 ppm rats showed minimal/slight vacuolation/degeneration of ventral horn motor neurons (three males, one female).

In the peripheral nervous system there was minimal evidence of peripheral neuropathy, characterized by Wallerian-type degeneration of peripheral nerve, particularly sciatic in both control and 8 ppm trimethyltin chloride treated animals. Surprisingly, there was no Wallerian-type degeneration of the dorsal columns but there was minimal evidence in the 8 ppm trimethyltin chloride treated rats of degeneration in the sensory roots of a few rats. The degeneration was confined to the junction of the root with the spinal cord. No axonal swellings were seen." The following

table, taken directly from the report summarizes the findings.

Intergroup Comparison of Microscopic Findings

Observation	Dose Level (ppm) Males			Females		
	0	4	8	0	4	8
Animals on study	12	12	12	12	12	12
Animals completed	6	0	6	6	0	6
Brain (# Examined)	6	0	6	6	0	6
Neuronal cell necrosis:						
Amygdaloid nuclei	0	-	6	0	-	6
Pyriform cortex	0	-	6	0	-	6
Dentate gyrus	0	-	6	0	-	6
CA1 hippocampus	0	-	6	0	-	6
CA3/CA4 hyppocampus	0	-	6	0	-	6
Tuberculum olfactorium	0	-	6	0	-	6
Dorsal root ganglia lumbar (# Examined)	6	0	6	6	0	6
Occasional eccentric nucleus	0	-	1	0	-	0
Gasserian ganglia (# Examined)	6	0	6	6	0	6
Sensory root degeneration	0	-	1	0	-	0
Sciatic nerve (# Examined)	6	0	6	6	0	6
Nerve fiber degeneration	0	-	2	1	-	4
Sensory spinal root-cervical (# Examined)	4	0	6	5	0	5
Nerve fiber degeneration	0	-	1	0	-	1
Sensory spinal root-lumbar (# Examined)	5	0	6	5	0	6
Nerve fiber degeneration	0	-	0	0	-	1
Spinal cord (# Examined)	6	0	6	6	0	6
Ventral horn cell vacuolation/degeneration	0	-	3	0	-	1
Sural nerve (# Examined)	6	0	5	6	0	6
Nerve fiber degeneration	0	-	0	0	-	1
Axonal degeneration	1	-	0	0	-	0
Tibial nerve (# Examined)	6	0	6	5	0	6
Nerve fiber degeneration	1	0	0	1	-	2

8. Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability, homogeneity and achieved concentration of the test substance in the diet were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that clinical signs of neurotoxicity and neuropathological lesions may be observed in this test system with a known neurotoxicant and since the purpose of the study was

achieved, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that trimethyltin chloride induces neurotoxic effects in rats when administered in the diet for a period of 29 days.

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Health Effects Division

DATA EVALUATION RECORD

STUDY TYPE: Positive Control Study: Acrylamide Neurotoxicity Study in the Rat

ACCESSION NO./MRID NO.: 430133-05

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Acrylamide

STUDY NUMBER(S): PRO705

REPORT NUMBER: CTL/P/2226

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, Delaware

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

TITLE OF REPORT: Acrylamide: Neurotoxicity Study in Rats

AUTHOR(S): M. D. Stonard

REPORT ISSUED: 7/30/90

CONCLUSION: Acrylamide monomer (100%) was tested in Alpk:AP rats as a positive control in a neurotoxicity feeding study for 29 days. It was administered in the diet at 0, 250 or 500 ppm (0, 12.5 or 25 mg/kg/day). Clinical signs of toxicity, body weights, food consumption, motor activity, sensory function, muscle weakness, peripheral nerve function and neuropathology observations were measured and recorded.

At 250 ppm, the animals displayed severe clinical signs of toxicity which included tail erection, tiptoe gait, upward curvature of the spine, piloerection, pinched in sides, abnormal gait, reduced reflex responses, downward curvature of the spine and upward curvature of the spine. Other clinical signs were observed as well. In addition to these, decreases in body weight and body weight gain, food consumption and food efficiency, motor activity, possible motor and sensory nerve conduction velocities and motor and sensory nerve action potential amplitudes were observed. Increases in pull-up time were also observed. Microscopic examinations were not conducted at this dose level.

At 500 ppm, both sexes also showed severe signs of toxicity, although more severely than those mentioned above. Therefore, at this dose level, the test diet was removed after 3 weeks. In addition to the other effects mentioned above, there was "unequivocal histopathological evidence of a peripheral neuropathy characterized by axonal degeneration of Wallerian-type...Mild axonal degeneration of the dorsal columns was also seen at this dose level."

There were effects at both dose levels tested. Therefore, the LEL is 250 ppm based on clinical signs of neurotoxicity, decreased body weights and body weight gains and food consumption, decreases in motor activity and changes in the functional observational battery. In addition, at 500 ppm, there was microscopic evidence of neurotoxicity.

This study is acceptible as a positive control study for this particular laboratory.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Acrylamide monomer

Description: white solid
Batch #(s), Other #(s): Batch 95822; CTL YO0574/009/001
Purity: 100%
Source: Aldrich Chemical Company
Vehicle: N/A

2. Test Animals

Species and Strain (sexes): Male and female Alpk:AP rats
Age: 6 weeks old upon receipt.
Source(s): ICI Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

a. Dietary Preparation: The diets were prepared in 10 kg batches from 1000g premixes. It was not stated how the premixes were prepared. It is assumed that the appropriate amount of the test substance was weighed out and added to a measured amount of stock diet.

Frequency of preparation: Weekly.

Storage conditions: Not stated.

Stability Analyses: Not conducted.

Homogeneity Analyses: Not conducted.

Concentration Analyses: Not conducted.

b. Basis For Selection of Dose Levels: It was not stated on what basis the dose levels were selected. However, this particular chemical has extensive literature references.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 29 days male	female
Control	0	12	12
1	250	12	12
2	500	12	12

d. Clinical Signs of Toxicity and Mortality: All rats were examined prior to the start of the study (day -1) and immediately prior to feeding the experimental diets. Animals were observed at least once daily. Detailed clinical observations were conducted weekly by an observer who was blind with respect to the treatment of each group of animals.

e. Body Weight Determinations: Bodyweights were recorded weekly, starting on day -1, immediately before feeding the experimental diet and then on the same day of each week until termination.

f. Food and/or Water Consumption: Food consumption was recorded weekly.

g. Motor Activity: An automated activity recording apparatus was used to measure locomotor activity. The animals were tested on days -1 and 28 of the exposure period. The report stated that "each observation period was divided into fifty scans of one minute duration. Treatment groups were counter balanced across test times and across devices", and when the trials were repeated on day 28, each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed behind a screen minimize disturbances.

h. Sensory Function Test: Assessment of pain perception was made using the rat tail-flick test on days -1, 8, 15, 22 and 28.

i. Muscle Weakness: Muscle weakness was assessed for all animals on days -1, 8, 15, 22 and 29 using the pull-up test.

j. Peripheral Nerve Function: The report stated that "at the end of the exposure period, all animals had peripheral nerve conduction velocity and amplitude measured using electrophysiological techniques. Each animal was deeply anesthetised by an intraperitoneal injection of barbituate. Motor and sensory nerve conduction velocity and action potential amplitude of the caudal nerves of the tail were measured using needly electrodes connected to a Neurolog electrophysiological amplification and recording system using a method similar to Misumi, 1979."

k. Neuropathology:
All animals requiring euthanasia during the study were anesthetised with halothane and killed by exsanguination using cardiac puncture. These and all animals which were found dead, were given full post mortem examinations. The tissues listed below were processed for microscopic examination.

For those animals surviving to termination, the brains were weighed and the length and width were recorded. Following the assessment of peripheral nerve function and whilst they remained deeply anasthetized, they were killed by perfusion fixation. The tissues listed below were removed and processed for microscopic examination. Nine levels of brain were blocked in paraffin wax and transverse secitons from each block were stained with alum hematoxylin and eosin. In addition, represetative sections were stained with Palmgren's method for nerve fibers, Haltzer's method for glial fibers and Luxol Fast Blue for myelin. Microscopic examinations were limited to 6 male and 6 female rats in the control and 500 ppm dose groups.

The following tissues were removed and examined microscopically:

|x| Brain
|x| Spinal cord form cervical region and lumbar region
|x| Gasserian ganglia
|x| Spinal roots
|x| Dorsal root ganglea
|x| Gastrocnemius muscle
|x| Sciatic nerve
|x| Sural nerve
|x| Tibial nerve

j. Statistical Analyses: Statistical analyses included analysis of variance. Unbiased estimates of the treatment group means were provided by the least square means. Each treatment group mean was compared with the control group mean using a two-sided Student's t-test.

B. RESULTS:

1. Clinical Observations and Mortality: At 250 ppm, the animals displayed severe clinical signs of toxicity after 2 weeks of treatment. These included tail erection, tiptoe gait, upward curvature of the spine, piloerection and pinched in sides. These signs increased in number and severity throughout the course of the study. After 4 weeks, both sexes displayed abnormal gait and reduced reflex responses. Some animals displayed downward curvature of the spine and all animals displayed upward curvature of the spine. Other clinical signs of toxicity included dehydration, decreased activity, piloerection, pinched in sides, reduced stability and ungroomed appearance.

At 500 ppm, both sexes showed severe signs of toxicity. Most had moderate splayed and tiptoe gait, upward curvature of the spine, reduced stability, dehydration, piloerection, pinched in sides and tail erection. Several animals also had reduced righting and splay reflex responses. Therefore, at this dose level, the test diet was removed on day 18 (replicates 1 and 4 - the animals were started on the diets on different days), days 17 and 16 for replicates 2 and 5 and 3 and 6 respectively and replaced with control diet. The clinical condition of the animals continued to decline and 2 males and 1 female either had to be sacrificed in extremis, were found dead or died during observation. By the end of the study, the animals had begun to

recover. The following tables summarize the most pertinent observations during weeks 4 and 5, where the clinical signs were most prevalent.

Clinical Observations at Weeks 4 and 5 - Males

Observation	Dose Level (ppm)		
	0	250	500[a]
Activity decreased			
Week 4			3
Week 5		5	
Downward curvature of spine			
Week 4			4
Week 5		4	
Reduced righting reflex			
Week 4			6
Week 5		4	1
Splayed gait			
Week 4		7	12
Week 5		12	9
Sides pinched in			
Week 4		2	10
Week 5		3	1
Reduced splay reflex			
Week 4	1		
Week 5	1	1	2
Reduced stability			
Week 4		1	4
Week 5		4	
Tip toe gait			
Week 4		5	12
Week 5		11	10
Upward curvature of spine			
Week 4		10	12
Week 5		12	10

[a]Put on control diet, starting on days 16-18.

Clinical Observations at Weeks 4 and 5 - Females

Observation	Dose Level (ppm)		
	0	250	500[a]
Downward curvature of spine			
Week 4		1	2
Week 5		3	3
Reduced righting reflex			
Week 4			5
Week 5		8	2
Splayed gait			
Week 4		6	13
Week 5		12	9
Sides pinched in			
Week 4		5	7
Week 5		10	6
Reduced splay reflex			
Week 4	4	1	2
Week 5	4	1	3
Reduced stability			
Week 4			9
Week 5		7	2
Tip toe gait			
Week 4		11	12
Week 5		11	10
Upward curvature of spine			
Week 4		12	13
Week 5		12	11

[a]Put on control diet, starting on days 16-18.

2. Body Weight Determinations: Statistically significant decreases in bodyweight and bodyweight gain were observed in both sexes at both dose levels during the study. The following tables summarize bodyweight gain.

Bodyweight Gain (g) - Males

Week	Dose Level (ppm)		
	0	250	500
1	0.0	0.0	0.0
2	49.3	31.8**	7.8**
3	81.2	57.4**	0.1**
4	106.5	62.8**	14.3**
5	129.5	65.7**	47.1**

*Statistically significantly different from controls ($p < 0.05$)
**Statistically significantly different from controls ($p < 0.01$)

Week	Bodyweight Gain (g) - Females Dose Level (ppm)		
	0	250	500
1	0.0	0.0	0.0
2	22.9	11.0**	-5.0**
3	35.1	16.0**	-17.2**
4	50.7	13.1**	1.0**
5	56.8	13.0**	7.7**

*Statistically significantly different from controls ($p < 0.05$)
**Statistically significantly different from controls ($p < 0.01$)

3. Food and/or Water Consumption: Food consumption was significantly decreased in the high dose group in both sexes for weeks 1-3. At week 4, food consumption was still decreased for high dose females. For the 250 ppm dose groups, food consumption was significantly decreased in weeks 3 and 4 for males and all weeks for females. Food utilization was also significantly decreased for both treated groups in both sexes, although not at every time point. These tables will not be summarized here since this is a positive control study for neurotoxicity.

4. Pull-Up Test for Detection of Muscle Weakness: At 250 ppm, a statistically significant increase in pull-up time was observed by the end of the third week of treatment in both sexes. By the end of the fourth week, the pull-up time had increased in female rats but in male rats, although the pull-up time was increased, it was not statistically significantly increased when compared to the control group. At 500 ppm, a statistically significant increase in pull-up time was observed in both sexes by the end of 2 weeks. Although the test diet was subsequently withdrawn, a week later these animals took progressively more time to complete the test. Both sexes showed signs of recovery by week 5, but the times were still higher than the control groups. The following table taken directly from the report summarizes the results.

Intergroup Compararison of the Logarithm of Mean Pull-Up Time(s)

Day	Dietary Concentration (ppm)		
	0	250	500
Day -1			
Males	0.692	0.484	0.483
Females	0.371	0.435	0.593
Day 8			
Males	0.209	0.124	0.214
Females	0.108	0.067	0.074
Day 15			
Males	0.069	0.250	0.475**
Females	0.095	0.376	0.511**
Day 22			
Males	-0.120	0.255*	1.02**
Females	0.111	0.510*	1.01**
Day 29			
Males	-0.047	0.260	0.697**
Females	-0.059	0.711**	0.406*

*Statistically significant from control group mean ($p < 0.05$)
**Statistically significant from control group mean ($p < 0.01$)

5. Tail-Flick Test for Assessment of Sensory Perception: There was no evidence for an effect on tail-flick. In males, there was no difference between the treated and control group and in females, the response times were significantly reduced at several time points in the 250 and 500 ppm dose groups when compared to controls.

6. Motor Activity: At 250 ppm, there was a statistically significant decrease in motor activity in females at 2-5 minutes. At 500 ppm, there was a decrease in motor activity in males during the first minute. In females, the activity was reduced at the 16-20 and 46-50 minute intervals. The following table summarizes the results.

Intergroup Comparison of Mean Activity Monitoring Measurements

Period	Dose Level (ppm)					
	0		250		500	
	Day -1	Day 28	Day -1	Day 28	Day -1	Day 28
Period 1						
Males	598.1	479.3	946.7**	312.6	631.2	204.6*
Females	546.6	503.9	576.6	431.1	313.6	507.9
Period 2-5						
Males	104.4	88.1	90.9	93.2	95.8	81.7
Females	82.5	113.3	74.1	80.2*	86.6	84.9
Period 6-10						
Males	77.4	84.7	53.6	81.9	76.4	79.1
Females	63.9	94.9	64.8	79.2	66.3	70.7
Period 16-20						
Males	33.2	35.7	19.5	33.8	24.1	48.7
Females	20.8	63.6	32.9	61.3	29.7	24.9*
Period 46-50						
Males	13.9	20.8	13.8	8.7	3.8	4.4
Females	2.9	35.9	6.7	18.9	4.8	10.8*

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

7. Nerve Conduction Velocity Measurements: The report stated that "on day 30, motor and sensory nerve conduction velocities were statistically significantly reduced in males and females at both dose levels, when compared to control. There was, however, no evidence of a dose dependent effect. Similarly, both motor and sensory nerve action potential amplitudes were reduced in both sexes, although in only the sensory fibers was the reduction statistically significant." The following table summarizes the results.

Intergroup Comparison of Mean Nerve Conduction Measurements (Day 30)

Observation	Dietary Concentration (ppm)		
	0	250	500
	Males		
Motor Nerve Conduction Velocity (m/sec)	43.27	38.81*	37.20**
Motor Nerve Action Potential Amplitude (uv)	35.75	30.25	28.42
Sensory Nerve Conduction Velocity (m/sec)	42.04	31.00**	32.96**
Sensory Nerve Action Potential Amplitude (uv)	25.33	16.33**	15.26**

Intergroup Comparison of Mean Nerve Conduction Measurements (Day 30)

Observation	Dietary Concentration (ppm) 0	250	500
	Females		
Motor Nerve Conduction Velocity (m/sec)	42.43	35.63**	38.11*
Motor Nerve Action Potential Amplitude (uv)	38.67	35.58	31.48
Sensory Nerve Conduction Velocity (m/sec)	39.62	31.04**	31.99**
Sensory Nerve Action Potential Amplitude (uv)	22.00	15.42**	15.98**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

8. Brain Measurements: No treatment-related differences between the treated groups and the controls were observed.

9. Neuropathology: At 500 ppm, there was "primary axonal degeneration accompanied by myelin degeneration in a good proportion of fibers from both sural and tibial nerves. As expected in a distal axonopathy degenerative changes in the sciatic nerve were less pronounced than in the more distally distributed sural (sensory) and tibial (sensory-motor) nerves.

Peripheral neuropathy was characterized by axonal degeneration of Wallerian type. In some sections degenerating axons could be seen surrounded by an intact myelin sheath. However, in many sections axonal degeneration was accompanied by demyelination with macrophages evident in myelin ovoids. There was some degree of endoneural edema and fiber loss, especially in sural and tibial nerves...

There was a fairly mild degree of axonal degeneration of the dorsal columns from both cervical and lumbar regions of the spinal cord of treated rats.

Only minor changes were seen in the dorsal root ganglia of treated rats. The gasserian ganglia appeared histologically normal although it was possible to find an occasional chromatolytic neuronal cell body. As expected no degenerative changes were seen in the brain." The following table summarizes some of the pertinent results.

Intergroup Comparison of Microscopic Findings

Observation	Dose - ♂ (ppm)		Dose - ♀ (ppm)	
	0	500	0	500
Dorsal Root Ganglia - Cervical				
# examined	5	6	6	6
Occasional chromatolytic cell body	0	1	0	3
Satellite cell proliferation	0	1	0	0
Dorsal Root Ganglia - Lumbar				
# examined	5	6	-	-
Occasional chromatolytic cell body	0	3		
Satellite cell proliferation	0	1		
Gasserian Ganglia				
# examined	6	6	-	-
Occasional chromatolytic cell body	0	1		
Sciatic Nerve				
# examined	6	6	6	6
Peripheral neuropathy	0	4	0	3
Occasional degenerate fiber	0	2	1	3
Spinal Cord - Cervical				
# examined	5	5	6	5
Axonal degeneration of dorsal columns	0	5	0	5
Occasional degenerate fiber	1	0		
Spinal Cord - Lumbar				
# examined	5	6	6	4
Axonal degeneration of dersal columns	0	3	0	2
Occasional degenerate fiber in dorsal columns	0	3	0	1
Spinal Root - Lumbar				
# examined	5	6	6	6
Thinning of normal myelin	0	1		
Occasional degenerate fiber	0	1		
Peripheral neuropathy			0	1
Sural Nerve				
# examined	6	6	6	6
Occasional degenerate fiber	1	0		
Peripheral neuropathy	0	6	0	6
Tibial Nerve				
# examined	6	6	6	6
Peripheral neuropathy	0	6	0	6
Occasional degenerate fiber	1	0	1	0

10. Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability, homogeneity and achieved concentration of the test substance in the diet were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that clinical signs of neurotoxicity and neuropathological lesions may be observed in this test system with a known neurotoxicant and since the purpose of the study was achieved, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that acrylamide induces neurotoxic effects in rats when administered in the diet for a period of 29 days.