

US EPA ARCHIVE DOCUMENT



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

1-5 88
006542

JAN - 5 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Sulfosate; 2-Year Chronic Feeding/Oncogenicity
Study in Rats; 2-Year Chronic Feeding/Oncogenicity
Study in Mice; EPA File Symbol 476-EEEL, 476-EEEE
Accession No. 402140-07, Volumes 1-7 (Rat Study);
402140-06, Volumes 1-9 (Mouse Study)

Project No. 7-0751A
Caswell No. 893C
Record No. 196920/196921

FROM: William Dykstra *William Dykstra 12/29/87*
Toxicology Branch
Hazard Evaluation Division (TS-769C)

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

THRU: Edwin Budd, Section Head *Edwin Budd 12/30/87*
Review Section II, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Requested Action

Review 2-year combined chronic toxicity/oncogenicity
studies in rats and mice.

Conclusions and Recommendations

1. The 2-year rat feeding study 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.

1/3/88

006542

-2-

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

cc: Robert Jaeger, Section Head
Review Section I, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TOXR 006542

Page _____ is not included in this copy.

Pages 3 through 8 are not included in this copy.

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.

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

006542

DATA EVALUATION REPORT

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

006542

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

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.

<u>Dose Group</u>	<u>ppm ai</u>	<u>Number of Animals</u>	
		<u>Male</u>	<u>Female</u>
0	Control ^a	60	60
1	0 ^b	80	80
2	100	80	80
3	500	80	80
4	1000	90	90

^aBasal diet, no vehicle.

^bBasal 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 palpation 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 and 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:

- Aspartate 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 for external abnormalities, including palpable masses. Viscera and body cavities were also examined.

The sacrifice schedule is shown below.

Group	ppm	Sacrifice Interval (Months)			
		<u>6</u>	<u>12</u>	<u>18</u>	<u>24</u>
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	Nasal passage
Mammary gland	Paranasal sinus
Muscle-thigh	Nasopharynx
Tibiofemoral joint	Larynx
Sternum	Trachea
*Lungs	Urinary bladder
*Heart	*Testes (BG)
Aorta - ascending and thoracic	Epididymides (BG)
*Spleen	Prostate
Thymus	Seminal vesicles
Bone marrow - Sternal	Coagulating glands
Lymph nodes - mesenteric and mediastinal	*Ovaries (BG)
Salivary glands - parotid and mandibular	Vagina
Buccal/alveolar mucosa	Cervix
Tongue	*Uterus
Esophagus	*Pituitary (BG)
Stomach	Thyroids
Duodenum	Parathyroids
Jejunum	*Adrenals (BG)
Ileum	*Brain
Cecum	Spinal cord - cervical, thoracic and lumbar
Colon	Sciatic nerve
Rectum	Eyes (BG)
Pancreas	Harderian glands (BG)
*Liver	Zymbal's glands
*Kidneys	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.

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

<u>Nominal ppm ai</u>	<u>Males</u>	<u>Females</u>
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, chromocjacryorrhea, 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.

<u>Leukocytes ($10^3/\text{mm}^3$)</u>			
	<u>Males</u>	<u>3 Months</u>	<u>Females</u>
Control	---	Mean \pm S.D.	---
0	13.2 \pm 2.7		9.4 \pm 2.8
100	11.9 \pm 1.3		8.2 \pm 2.4
500	11.3* \pm 2.5		8.6 \pm 2.0
1000	11.3* \pm 2.4		7.9 \pm 2.6

*p < 0.05

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

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

The decrease in mean leukocyte values for males at 500 and 1000 ppm are not considered toxicologically significant since (1) mean values \pm SD were within control mean \pm 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 $17.5 \times 10^3/\text{mm}^3$). At 1000 ppm, the range was 4.0 to $14.3 \times 10^3/\text{mm}^3$. 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 $17.5 \times 10^3/\text{mm}^3$ for animal #1090. Also, as with male rats, the mean values \pm SD were within control mean \pm 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 ± 1 , 15 ± 1 , and 17 ± 1 , respectively, in comparison to the control value which was 19 ± 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 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.

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).

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

Sex	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		516 (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

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

* 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

Sex	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

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

* 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

Sex	Dosage Group (ppm)	6 Months	12 Months	18 Months	24 Months
		n = 10	n = 10	n = 10	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	0.6 (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

d n = 9

b n = 7

u n = 11

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

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

Sex	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

Sex	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)
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:

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>1000</u>
No. Examined	60	80	80	80	90
<u>Liver</u>					
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)

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>1000</u>
No. Examined	60	80	79	78	90
Chronic Inflam- mation	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)

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>1000</u>
No. Examined	60	80	80	80	90
Chronic Inflam- mation	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)

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>1000</u>
No. Examined	60	80	80	80	90
Cardiomyo- pathy	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

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

Female Mammary Gland - Adenomas/Carcinomas

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

Adrenal Pheochromocytoma - Benign and Malignant

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>1000</u>
<u>No. examined (both sexes)</u>	60	80	80	80	90
<u>No. of males with tumor</u>	18	18	13	14	13
<u>No. of females with tumor</u>	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.

006542

R:16869:Dykstra:C.Disk:KENCO:12/6/87:EE:SG:VO:CB
R:16892:Dykstra:C.Disk:KENCO:12/22/87:CB:VO:CB

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

DATA EVALUATION REPORT

Study Type: 83-5, Chronic Toxicity/Oncogenicity

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

Accession Number: 402140-06 (Vol. 1-9)

Test Material: Sulfosate

Synonyms: SC-0224

Study Number: T-11813

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 Mice

Authors: Pavkov, K.L.; Turnier, J.C.

Report Issued: April 3, 1987

Conclusions: [Tentative]

The oncogenic potential was negative at the maximum tolerated dose (MTD) of 8000 ppm (highest dose tested [HDT]). The systemic NOEL is 1000 ppm. Body weight and food consumption were significantly decreased in 8000 ppm male and female mice during the study. There were no compound-related effects in palpable masses, hematology, clinical chemistry, and organ weights. Amyloidosis was frequent but not compound-related. In males at 8000 ppm there was an increased incidence of white matter degeneration in the lumbar region of the spinal cord. In female mice, epithelial hyperplasia of the duodenum occurred at an increased incidence at 8000 ppm.

Classification: Core-Supplementary, because the tissue masses listed in Table I (clinical observations) and Table L (necropsy observations) were not clearly identified in the histopathology observations (Table N) as being histologically examined. This deficiency has to be resolved.

II. Two-Year Chronic Toxicity and Oncogenicity Dietary Study With SC-0224 in Mice (Stauffer Labs Report No. T-11813; April 3, 1987).

Test Material--Technical SC-0224 (Trimethylsulfonium carboxymethylaminomethylphosphonate, active ingredient); Lot No. EHC 0586-08 (WRC Lot No. JHC-8865-20-1). Clear aqueous solution containing 56.17% active ingredient (w/w).

Experimental Design--Randomized groups of male and female Charles River (Kensington, NY) CD-1 mice (Cr1:CD⁰-1(ICR)BR) were used in the study. The mice 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 22 months. The experimental design is shown below:

<u>Dose Level</u>	<u>Number of Animals</u>	
	<u>Male</u>	<u>Female</u>
ppm ai		
Control ^a	60	60
0 ^b	80	80
100	80	80
1000	80	80
8000	80	80

^a Basal diet without vehicle.

^b Basal diet plus 1% propylene glycol vehicle.

There were interim sacrifices of variable numbers of mice at 6, 12, and 18 months. The number of mice scheduled for the full 22-month study duration was 50/sex/group.

Mice were observed twice daily for toxic signs. A general physical examination was performed on all test animals once per week including palpation for nodules or tissue masses. Moribund mice were sacrificed to lessen the likelihood of unobserved death and subsequent tissue autolysis.

Individual body weights were recorded weekly for the first 13 weeks of the study and thereafter every other week. 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 the allocated feed for a 7-day interval minus the residual from the 7-day period. Feed efficiency was calculated at each interval. Weight loss occasionally resulted in negative values for feed efficiency.

Blood samples were drawn for hematologic analyses (listed below) from 10 fasted animals of each sex in each dose group and vehicle control (0 ppm) at 6, 12, and 18 months and the 22-month termination (the control basal diet group was evaluated only at 12 and 22 months). The mice were anesthetized with sodium pentobarbital and the blood drawn from the abdominal aorta using an 18 to 21 gauge needle and a pediatric Butterfly® catheter prior to exsanguination. 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)
- Partial thromboplastin time (PTT)

Samples for blood chemistry were obtained from 10 fasted mice/sex/dose group (same animals used for hematologic analyses) at 6, 12, 18, and 22 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)
- Alkaline phosphatase (Alk. Phos.)
- Total protein (T. Prot.)
- Albumin (Alb)
- Blood urea nitrogen (BUN)
- Glucose (Glu)
- Total cholesterol (Choles)
- Serum cholinesterase (S ChE)

Red blood cell cholinesterase (RBC ChE)
 Total bilirubin (T Bili)
 Creatinine (Creat)
 Triglycerides (Triglyc)
 Sodium (Na)
 Calcium (Ca)
 Potassium (K)
 Chloride (Cl)
 Gamma glutamyl transferase (GGT)
 Inorganic phosphorus (Phos)
 Creatinine phosphokinase (CPK)
 Lactate dehydrogenase (LDH)

The right or left half of the brain from five mice/sex/
 dose level was homogenized at the 6-, 12-, 18-, and
 22-month sacrifice to measure the cholinesterase activity.
 Activity was expressed per gram of protein (Lowry method
 for protein analysis) at 6 and 12 months and per gram of
 tissue at 18 and 22 months.

Urinalyses were performed for 10 fasted mice/sex/dose
 level (same animals mentioned above in hematology). The
 parameters evaluated included:

pH
 Protein (Prot)
 Glucose (Glu)
 Ketones (Ket)
 Occult blood (Occ Bl)
 Urobilinogen (U-blin)

All mice were necropsied by trained prospectors 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 for
 external abnormalities, including palpable masses.
 Viscera and body cavities were also examined.

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

*Heart	Eyes
Ascending aorta	Harderian glands
Thoracic aorta	Auditory sebaceous glands
Buccal/alveolar mucosa	Middle ear(s)
Mandibular salivary gland	Pituitary
Parotid salivary gland	Thyroid
Tongue	Parathyroids
Esophagus	*Adrenals
Stomach	Thymus
	*Spleen

Duodenum	Bone marrow/sternum
Jejunum	Mesenteric lymph node
Ileum	Mediastinal lymph node
Cecum	Skin and mammary gland
Colon	Skeletal muscle (thigh)
Rectum	Bone tibia/femur and
Pancreas	joint sternum
*Liver	*Kidneys
Gallbladder	Urinary bladder
*Brain	*Testes
Cervical spinal cord	Epididymides
Thoracic spinal cord	Prostate
Lumbar spinal cord	Seminal vesicles
Sciatic nerve	Coagulating glands
Nasal passage	*Ovaries
Paranasal sinus	Vagina
Nasopharynx	Cervix
Larynx	*Uterus
Trachea	Gross lesions (specified
*Lungs	by the pathologist)

Those organs marked (*) above were weighed for mice sacrificed at the interim and final terminations. The paired organs were weighed separately. All tissues on the above list were routinely processed for light microscopic examination for all animals. Remaining wet tissue specimens were kept in fixative within gas-tight plastic bags and stored in the EHC archives.

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 for hematology, clinical chemistry, and organ weights were compared to the 0 ppm (control) dose groups at 6 and 18 months and were compared to the basal diet (control) groups at 12 and 22 months. Quantal data, such as necropsy findings, clinical observations, and histopathologic findings were compared using Fisher's Exact Test (Siegel, 1956) with Bonferonni's correction (Ingelfinger, et al. 1983). 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:

The average concentrations of SC-0224 active ingredient (measured by separate anion and cation analysis) were within 15 percent (anion analysis) and 20 percent (cation analysis) of the nominal values measured at regular intervals during the study.

Mean body weight values of male mice at 8000 ppm were significantly decreased during the study in comparison to controls. At study week 1, the mean body weight of control, 0, 100, 1000, and 8000 ppm male mice were 34, 33, 33, and 32 g, respectively. At termination of the study in week 95, the body weight of male mice at control, 0, 100, 1000, and 8000 ppm were 44, 42, 45, 43, and 39 g, respectively. The mean body weight values of the 8000 ppm male mice were decreased by 3 to 11 percent during most of the study.

Similarly in female mice of the 8000 ppm group, the mean body weight values were decreased by 4 to 17 percent during most of the study. At study week 1, the mean body weight values of control, 0, 100, 1000, and 8000 ppm female mice were 25, 25, 26, 25, and 22 g, respectively. At termination of the study in week 95, the mean body weight values of female mice in the control, 0, 100, 1000, and 8000 ppm groups were 35, 35, 38, 35, and 29 g respectively. The significant decreases at 8000 ppm in male and female mean body weight values are considered sufficient evidence that an MTD dose was used in the study for both sexes.

Part of the decreased body weight values at 8000 ppm in both sexes were apparently due to decreased food consumption. The grand mean values (g/day) for food consumption for control, 0, 100, 1000, and 8000 ppm male mice were 4.8, 4.8, 4.8, 4.8, and 4.6, respectively. The decreased food consumption at 8000 ppm amounted to about 4 percent for male mice as determined by grand mean differences.

For female mice, there were similar decreases in grand mean food consumption values, amounting to an 8 percent decrease at 8000 ppm in comparison to controls. The grand mean values (g/day) for female mice at control, 0, 100, 1000, and 8000 ppm were 5.1, 5.0, 5.2, 4.9, and 4.7, respectively.

Compound intake for male mice (grand mean daily doses) were 11.7, 118, and 991 mg/kg/day for the 100, 1000, and 8000 ppm groups, respectively.

For female mice, the compound intake expressed as grand mean daily doses were 16.0, 159, and 1341 mg/kg/day for the 100, 1000, and 8000 ppm groups, respectively.

Grand mean values for feed efficiency for male mice through 22 months (20 months for 0 ppm group) were 0.8, 0.9, 0.9, 0.9, and 0.7 for control, 0, 100, 1000, and 8000, respectively. The grand mean values for feed efficiency for female mice through 22 months were 1.1, 1.0, 1.1, 1.0, and 0.7 for control, 0, 100, 1000, and 8000 ppm, respectively.

Survival of male mice was unaffected by treatment. Male mice of the 0 ppm group were sacrificed at week 89 when 12 (out of 50 at risk) had survived. The remaining groups of male mice were sacrificed at week 95 when survivors numbered 14, 21, 12, and 21 of the control, 100, 1000, and and 8000 ppm groups, respectively. At week 95, the surviving female mice numbered 17, 15, 14, 13, and 34 for the control, 0, 100, 1000, and 8000 ppm groups, respectively. Survival was apparently increased in 8000 ppm female mice in comparison to controls.

There were no compound-related toxic signs observed in male or female mice during the study.

The most frequent observations were abscesses, alopecia, chromodacryorrhea, chromorhinorrhea, conjunctivitis, dehydration, diarrhea, reduced activity, convulsions, distended abdomen, abrasions, hair loss, rough coat, scabs, and tremors.

There were no compound-related ophthalmoscopic findings at 6-, 12-, and 18-month interim examinations or at the 22-month terminal examination. The most frequent ocular observations were corneal opacity and cataracts.

There were no compound-related findings with respect to palpable masses for male and female mice.

Mean hematological values for male mice showed a statistically significant increase in hemoglobin of the 1000 ppm group at 18 months. This finding was not considered toxicologically significant since it was not dose related and control values (0 ppm) at 18 months were lower than control values at other intervals.

In female mice, the statistically significant increases at 18 months in hematocrit and hemoglobin of the 100 and 1000 ppm groups were not considered toxicologically significant since these findings were not dose-related

and were within the normal range of control and 0 ppm values at other intervals.

Similarly, increased erythrocyte values at 100, 1000, and 8000 ppm in female mice at 18 months were not considered toxicologically significant since these values were within the range of control and 0 ppm values at other intervals. Additionally, in male and female mice at all sampling intervals, there were no significant differences in monocytes, lymphocytes, mature neutrophils, immature neutrophils, eosinophils and basophils.

There were several statistically significant different clinical chemistry parameters in treated groups as compared to the control values at various intervals.

At 22 months, mean SGOT values of females of the 1000 ppm group were increased in comparison to controls (0 ppm = 75 IU/L; 1000 ppm = 125 IU/L) but this was not considered toxicologically significant since it was not dose related. Additionally, individual values for SGOT in females of the 1000 ppm group at 22 months ranged from 53 to 217 IU/L in comparison to a range of 46 to 144 IU/L for 0 ppm and 40 to 87 for control groups.

SGPT values, which would also be expected to be increased if SGOT values were toxicologically significantly increased, were comparable between controls and treated males and females at each sampling interval. Therefore, the increased SGOT values were not indicative of organ toxicity.

Alkaline phosphatase values in 8000 ppm females at 6 and 13 months were increased in comparison to control values, but these were only small increases. At 6 months, mean SAP values were: 0 ppm = 37 IU/L; 8000 ppm = 66 IU/L. At 18 months, mean SAP values were: 0 ppm = 37 IU/L; 8000 ppm = 55 IU/L. These increases were not dose-related and did not occur at 22 months. Therefore, they were not indicative of systemic or organ toxicity.

Mean values of serum total protein were decreased at 6 and 18 months in 8000 ppm females and decreased at 22 months in 8000 ppm males. Most of the decreased values were within the range of control values for male and female mice. At 6 months, range in females: 0 ppm = 4.1 to 4.8 g/dl; 8000 ppm = 3.7 to 4.4 g/dl. At 18 months, range in females: 0 ppm = 3.1 to 4.8 g/dl; 8000 ppm = 3.1 to 4.2 g/dl. At 22 months, range in males; control = 4.5 to 6.8 g/dl; 0 ppm = 4.0 to 7.5 g/dl; 8000 ppm = 3.8 to 4.6 g/dl.

Albumin values were decreased at 100 ppm females at 6 months and globulin values were decreased at 8000 ppm males at 18 months. The albumin decrease was not dose related. The decrease in globulin was not considered toxicologically significant since most individual values at 8000 ppm (range from 1.7 to 1.9 g/dl) were within 0 ppm range (1.8 to 3.1 g/dl).

The decreased differences in total protein, albumin, and globulin were not considered toxicologically significant. Small increases in BUN in females at 8000 ppm at 6 and 12 months were not considered toxicologically significant. Most values in 8000 ppm females at 6 months were 17 to 29 mg/dl with the exception of female animal 4158 which was 55 g/dl. These values were for the most part within the range of individual control values (13 to 21 mg/dl). At 12 months, the range of values for controls encompassed most individual values at 8000 ppm. In females, control range: 21 to 36 mg/dl; 0 ppm = 19 to 51 mg/dl; 8000 ppm = 36 to 74 mg/dl.

The decreases in serum glucose in 8000 ppm males at 6 months and 8000 ppm females at 18 months were not considered toxicologically significant. At 8000 ppm in males at 6 months, individual values were 54 to 98 mg/dl, as compared to the range of 77 to 113 mg/dl in 0 ppm males. These individual values are comparable. At 18 months in females, 0 ppm values ranged from 42 to 158 mg/dl as compared to 52 to 150 mg/dl at 8000 ppm. These individual values are comparable.

Differences in serum cholesterol and triglycerides were unaffected by treatment. Differences in serum sodium, calcium, potassium, phosphorus, chloride, gamma glutamyl transferase, creatinine phosphokinase, and lactic dehydrogenase were either not toxicologically significant or sufficient quantities were not available for analysis.

The increase in total bilirubin in 8000 ppm males at 12 months, decreases in 1000 ppm males at 18 months, and decreases in 8000 ppm males at 22 months were not dose-related and were not considered toxicologically significant.

The decreases in serum creatinine at 100 and 8000 ppm in males at 22 months and the increases in 100 and 1000 ppm in females at 22 months were not dose-related and were not considered toxicologically significant.

Therefore, in toto, clinical chemistry findings in male and female mice at each sampling interval did not reveal any compound-related or toxicologically significant differences.

Analysis of male and female brain, RBC, and serum cholinesterase activity did not reveal any toxicologically significant differences. Decreased mean values in brain cholinesterase in 100, 1000, and 8000 ppm female mice at 12 months were not dose-related. Similarly, the increased mean values of brain cholinesterase in 100 ppm females at 22 months were not dose-related and were not toxicologically significant. Most individual cholinesterase values in treated mice were within 25 percent of the control values and mean values were not dose related.

There were no compound-related effects in urinalysis values and findings.

At gross necropsy, the incidence of female mice exhibiting dehydration showed a dose-related increase (4, 9, 13, 18, and 19% in control, 0, 100, 1000, and 8000 ppm groups, respectively. There was also an increase in emaciation in female mice at 8000 ppm (9%) as compared to controls (3%).

In males at 8000 ppm, gross necropsy revealed an increased incidence of cysts in the kidney (9%) as compared to controls and other groups (2 to 4%). Microscopically, however, the incidence of renal cysts in the 8000 ppm males (24%) did not exceed the incidence in controls and other groups (22 to 33%).

There were no compound-related effects in absolute organ weight, organ weights relative to body weight, and organ weight relative to brain weight in male and female mice.

The statistically significant decreases in absolute heart, kidney, liver, spleen, and testes weights in males and absolute adrenal, brain, heart, kidney, liver, lungs, spleen, and uterus weights in females at 8000 ppm are considered due to decreased body weight of mice in these groups.

The increased relative brain weight of 8000 ppm male and female mice at all sampling intervals also appears to be due to the decreased body weight of these animals at these times. Similarly, the decreased organ to brain weight changes in male and female mice at 8000 ppm appears due to the diminished overall organ and body weights of these animals in comparison to controls.

The most frequent systemic non-neoplastic histologic lesion was amyloidosis. It occurred in both sexes and the most common grades of the lesion were moderate and severe. The occurrence of amyloidosis was not compound-related. The total numbers of male animals in which

amyloidosis was a major factor in the cause of death were 28, 26, 19, 30, and 13 for control, 0, 100, 1000, and 8000 ppm, respectively. The total numbers of female animals in which amyloidosis was a major factor in the cause of death were 22, 23, 22, 22, and 9 for control, 0, 100, 1000, and 8000 ppm, respectively.

There was slightly increased incidence of epithelial hyperplasia of the duodenum in male mice at 8000 ppm, which was not considered compound related since it was not statistically significant.

<u>Dose (ppm)</u>	<u>Duodenum (Males)</u>				
	<u>Control</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>8000</u>
No. Examined	51	75	73	72	73
Epithelial hyperplasia	7	9	6	8	12
Percent response	14	12	8	11	16

The grade of each lesion was slight.

The incidence of myocardial degeneration in male mice was 58, 49, 39, 34, and 50 percent for control, 0, 100, 1000, and 8000 ppm, respectively. The most frequent grade of the lesion was slight.

Glomerulonephropathy in male mice occurred in incidences of 3, 6, 6, 4, and 9 percent for control, 0, 100, 1000, and 8000 ppm, respectively. The most frequent grade of the lesion was slight. The occurrence of glomerulonephropathy was not considered compound-related.

Degeneration of the sciatic nerve occurred at an increased incidence in treated male mice. This lesion was not considered compound-related; however, it was not dose-related, statistically significant, and the grade of the lesion was slight. The incidences of sciatic nerve degeneration were 9, 15, 19, 21, and 21 percent in the control, 0, 100, 1000, and 8000 ppm groups, respectively.

The occurrence of lymphangiectasia of the skin occurred at increased frequency in male mice at 1000 and 8000 ppm.

<u>Skin (Males)</u>					
<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>8000</u>
No. Examined	59	80	79	78	77
Lymphangiectasia	0	1	2	4	6
Percent response	0	1	3	5	8

The grade of moderate for the lesion occurred at an incidence of 1, 1, 1, and 5 for the 0, 100, 1000, and 8000 ppm males. Additionally, the grade of moderate/severe occurred in two instances at the 1000 ppm level. Although it seems to be "dose-related," there were no other indications in any parameter in the entire study suggesting the lymphatic vessel system might be a primary target organ for this chemical. If anything at all, it might be a secondary effect resulting from the very widespread and severe amyloidosis observed in the study. Amyloidosis apparently caused general stasis in the blood and lymphatic circulation and drainage systems. Under these conditions, it is not at all surprising that some dilatation of the lymphatic vessels was observed in some animals.

Also occurring in a compound-related manner at the 8000 ppm level in male mice was an increased incidence of white matter degeneration of the lumbar region of the spinal cord.

<u>Spinal Cord - Lumbar (Males)</u>					
<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>8000</u>
No. Examined	59	80	79	80	79
Nerve-root degeneration	5	6	5	7	11
Percent response	9	8	8	9	14
White matter degeneration	1	2	3	3	6
Percent response	2	3	4	4	8

The incidence of nerve root degeneration was not statistically significantly increased at 8000 ppm (14 vs. 8%, 9%; 0 ppm and control) and is not considered compound-related. The incidence of white matter degeneration is statistically significantly increased (greater than 2.5X of control and 2X of 0 ppm) at 8000 ppm and is considered compound-related. Additionally, there was one instance of moderate grade of this lesion at 8000 ppm with the remainder of the grades of the lesion being slight.

White matter degeneration of the thoracic region of the spinal cord occurred at a high incidence in both control and treated male mice. The incidences were 70, 60, 53, 67, and 66 percent in the control, 0, 100, 1000, and 8000 ppm groups, respectively. The grades of the lesion were comparable among control and treated animals.

In female mice, the only non-neoplastic lesion which was considered compound-related was the increased incidence of epithelial hyperplasia of the duodenum at 8000 ppm.

Duodenum (Females)

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>8000</u>
No. Examined	52	70	77	71	75
Epithelial hyperplasia	5	9	12	11	18
Percent response	10	13	16	15	24

All grades of the lesion were slight.

Other non-neoplastic lesions in female mice occurred at comparable incidences and grades between control and treated animals.

There were no compound-related neoplastic lesions in male and female mice. Additionally, there was no decrease in latency. The most frequent neoplasms observed in male and female mice were in the liver and lungs.

The incidences of the most commonly found tumors in males are shown below.

Liver (Males)

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>8000</u>
No. Examined	58	79	79	78	78
Hepatocellular Adenoma (Percent)	7 12%	8 10%	6 8%	4 5%	2 3%
Hepatocellular Carcinoma (Percent)	3 5%	6 8%	5 6%	6 8%	1 1%

Lungs (Males)

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>8000</u>
No. Examined	59	80	79	79	80
Adenoma (Percent)	8 9%	4 5%	7 9%	9 11%	5 6%
Adeno- carcinoma (Percent)	3 5%	2 3%	3 4%	5 6%	1 1%

In female mice, there were 3/80 hepatocellular adenomas only at the 8000 ppm level and there were no hepatocellular carcinomas. In the lungs of female mice, the following incidences of pulmonary adenomas and adenocarcinomas were observed.

Lungs (Females)

<u>Dose (ppm)</u>	<u>Control</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>8000</u>
No. Examined	60	79	80	80	79
Adenoma (Percent)	2 3%	1 2%	5 6%	3 4%	0 0%
Adeno- carcinoma (Percent)	1 2%	1 2%	2 3%	3 4%	0 0%

Discussion

Mean body weight values of male and female mice were significantly decreased at 8000 ppm during the study. In male mice, the decreases were 3 to 11 percent and in female mice, the decreases were 4 to 17 percent. Part of the decreases in body weight were due to decreases in food consumption in male and female mice at 8000 ppm.

The significant decreases in male and female body weight at 8000 ppm are considered evidence that an MTD for both sexes was used in the study.

Survival of male mice was unaffected by treatment. High-dose (8000 ppm) females survived better than controls. There were no compound-related effects in toxic signs, clinical observations, palpable masses, ophthalmological findings, hematology, clinical chemistries, and organ weights.

The most frequent systemic non-neoplastic histologic lesion was amyloidosis. It occurred in both sexes and the most frequent grade was moderate/severe.

In male mice, the incidence of white matter degeneration was considered compound-related at 8000 ppm in the lumbar region of the spinal cord.

In female mice, epithelial hyperplasia of the duodenum occurred at an increased incidence at 8000 ppm and was considered compound related. The systemic NOEL was 1000 ppm.

The oncogenic potential was negative for male and female mice. There was no decrease in latency.

However, these are tentative conclusions since the study is Core-Supplementary.

16861:I/C:Dykstra:KENCO:12/15/87:DD:vo:dg:rw:
R:16892:Dykstra:KENCO:12/22/87:CB:VO:CB

006542