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

CASWELL FILE

JAN 18 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: SC-0224

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

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

FROM: Roland A. Gessert, DVM
Review Section #1
Toxicology Branch/HED (TS-769)

Roland A. Gessert 1/12/84

Applicant: Stauffer Chemical Company

Mr. Jack Wise, Manager, Regulatory Relations, Stauffer Chemical Company, wrote to confirm his meeting on September 1, 1983 with William Burnam and Bruce Jaeger concerning termination of the 2-year mouse study on SC-0224 which was started in July 1982.

Stauffer started an 18-month mouse study in accordance with EPA guidelines in April 1982.

Toxicology Branch acknowledges this information and looks forward to receipt of the complete data package.

TS-769:GESSERT:sll:X73710:1/11/84: card 6

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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: 402140-07 (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

3

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.

		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

4

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 for 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

6

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.

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.

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 <u>decreases</u> 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.

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).

13

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

006542

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

006542

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

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

	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

23

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

24

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.

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

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 tap water.

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.

Is this a [illegible]

Roland A. Gessert

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Toxicology Branch/HED (TS-769)

Mutagenicity Evaluation of R-50224 in Sex-Linked Recessive Lethal Test in Drosophila melanogaster.

Litton Bionetics, Inc. Project No. 22169, June 13, 1982

Procedure:

Drosophila Stock Used:

Males of the wild-type stock and females of the Muller-5 stock were used in this study. Newly hatched fruit flies (D. melanogaster) were maintained at 25°C in disposable vials containing the Carolina Biological Instant Drosophila Medium (Formula 4-24, without dyes). Flies were immobilized with filtered CO_2 for handling.

Range-Finding Study:

The toxicity test was performed by the adult feeding methods modified by Lewis and Bacher (1968). A piece of chemically inert glass filter paper lining a shell vial was saturated with 1.5 ml of 1% sucrose solution containing the test compound (0, 12.5, 25.0, and 50 mg/ml). Fifty adult male flies were fed in each vial for 24 hrs. Feeding behavior and toxic effect of the flies were monitored to ensure proper intake of the test compound. The test chemical was found to be non-toxic at the highest concentration tested (50 mg/ml Or 5% W/V).

The fertility results of the treated male flies at each of the selected concentrations are shown in the following table:

Dose	Number Treated	Number Survived	Number Fertile*	Percent Fertility
NC (1% sucrose)	50	48	31	65
12.5 mg/kg	50	47	-	-
25.0 mg/kg	50	48	30	63
50.0 mg/kg	50	43	28	65

* Sufficient numbers of progeny in treated groups were observed for genetic analysis when compared to the control.

Test Size:

The sex-linked recessive lethal test (SLRL) was conducted at two dose levels of the test compound (25 & 50 mg/kg) in conjunction with the negative (1% sucrose) and positive (0.005 M EMS) controls. At least 200 treated male flies were used for each dose level of the test compound. The number of chromosomes tested in the different germ-cell stages were estimated as follows:

	DAYS POST-TREATMENT		
	Brood I 1, 2, 3	Brood II 4,5,6,7	Total Chromosomes
NC (1% sucrose)	3750	3750	7500
PC (EMS)	150	150	300
2.5 mg/kg	3750	3750	7500
5.0 mg/kg	3750	3750	7500

P_1 Mating and Rearing of the F_1 Generation:

Following 24-hour waiting period after dosing, the treated males were mated individually to sequential groups of 3 virgin Basc females according to the designed breeding scheme (3-day sequence). The progenies of each male were kept separate and the data can be traced back to individual male used initially. The F_1 progenies of each culture were inspected to make certain the proper cross was made. The desired number of F_1 females was then pair-mated to their brothers. An equal number of F_1 females per treated males was tested to avoid biasing the data.

Scoring of the F_2 Generation:

The F_2 progenies were inspected for the occurance of sex-linked recessive lethals by the presence of males with yellow bodies. If this class of males is absent, the vial was marked as a potential lethal mutation occured on its X chromosome and was set aside for further examination. The following criteria were applied to cultures suspected of being lethal:

(a) If 20 or more progeny were present and there were not yellow-bodied males, the culture was considered to carry a lethal mutation on the treated chromosome and further testing was unnecessary.

(b) If there was less than 20 progeny, or if there was one yellow-bodied male, the culture was retested by mating three of the females heterozygous for the treated and Basc chromososmes to Basc males. The progeny of these crosses were scored for the presence of yellow-bodied males.

Data Analysis:

(a) The total number of X chromosomes tested equaled the sum of lethal and non-lethal cultures. The frequency of X-linked recessive lethal was calculated as:

$$\frac{\text{Number of Lethals}}{\text{No. of Lethals + No. of Non-Lethals}} \times 100 = \%\ \text{Lethal}$$

(b) Since the clusters of mutations among progeny from the same treated P_1 males may come from different origins, the cluster was counted as a single event to avoid biasing the interpretation of results.

Interpretation of Results:

A positive mutagenic effect is concluded if the difference in the number of sex-linked recessive lethals between a concurrent control group is statistically significant at the 5% level according to the Kastenbaum-Bowman test (1970). A test is considered negative if the two following criteria are met:

(a) The increase in the treated group over the control is less than 0.2% and the sample size is large enough to allow the detection of a statistically significant increase of 0.2% based on the Kastenbaum-Bowman test.

(b) If none of the broods analyzed shows a positive result, the increases in the treated group of at least two broods must be less than 0.4% and this sample size in each of the two broods must be large enough to allow the detection of a statistically significant increase of 0.4% based on the Kastenbaum-Bowman test.

Results:

Summarized Results in SLRL Test for the Compound R-50224

Dose	No. of F_2 Vials Scored		No. of Lethals	% Lethals
NC (1% sucrose)	Brood I	4033	7	0.17
	Brood II	4235	3	0.07
	Total	8268	10	0.12
25 mg/ml	Brood I	4124	4	0.10
	Brood II	4248	7	0.16
	Total	8372	11	0.13
50 mg/ml	Brood I	4381	5	0.11
	Brood II	4095	6	0.15
	Total	8476	11	0.13
*PC (0.005M EMS)	Brood I	214	101	47.20*
	Brood II	263	98	37.26*
	Total	477	199	41.72*

No significant difference in the percentage of sex-linked recessive lethals was observed in the R-50244 treated groups when compared to the negative control. The increase in the frequency of recessive lethals over the spontaneous frequency for the two broods of treated group (25 or 50 mg/kg) was only 0.01%. Based on the historical data table presented, 5667 flies should be sufficient to detect a significant increase of 0.35% (if the spontaneous background for the tester strain is 0.10%). The sample sizes of the treated groups (8372 to 8476 vials) and the negative control (8268 vials) used in this study were adequate to meet the criteria described in making mutagenicity evaluation of the test compound. The positive control (0.005M EMS) produced an approximately 400-fold increase in recessive lethals over the negative control. Therefore, the test compound, R-50224, was not mutagenic in the SLRL test in Drosophila at the dose levels tested.

Conclusions:

The SLRL test used in this study for detecting the sex-linked lethals in the developing fruit fly between the zygote and the adult stage appears to follow the general guidelines recommeded for the sex-linked recessive lethal test in *Drosphila melanogaster* (EPA 1982). However the following deficiencies in reporting of this study should be clarified:

1. The standard wild-type strain of the male Drosophila (Canton-S or Oregon-R) used in this study was not given and should be identified in the report.

2. The described procedure for scoring of the F_2 generation was incomplete, and should be clarified. The progeny in the F_2 generation should be scored to a fixed end point (generally, not more than 8 days) at 25° C in order to avoid the overlapping of F_3 generation.

3. The study may be up-graded with submission of these clarifications.

Reviewer: John H.S. Chen, DVM
Review Section #1
Toxicology Branch/HED (TS-769)

Mutagenicity Evaluation of SC-0224 in Bone Marrow Cytogenetic Analysis in Rats. Conducted by Jenness B. Majeska, Susan O'Lone, and James Tarca. Stauffer Chemical Co., In Vitro Toxicology Section, Environmental Health Center, Farmington, CT 06032. September 1982. Accession No. 249,802. Report No. T-10884. Caswell No. 893C.

Material Tested:

Trimethylsulfonium carboxymethylaminoethylphosphonate. SC-0224. Lot 6841-48-3. EHC-0293-21. T-10884. Purity 58.5%.

Positive Controls:

Cyclophosphamide in water, given by oral gavage at 30 mg/kg.

Negative and Solvent Controls:

Distilled water.

Animals:

Sprague Dawley adult male rats, Strain CD-CRL: COBS CD (SD)BR, 6 weeks old. Obtained from Charles River Breeding Laboratories.

Dose:

LD_{50} = 565 mg/kg. High dose was 1/3 the LD_{50} (188 mg/kg); intermediate dose was 1/9 the LD_{50} (63 mg/kg); low dose was 1/27 the LD_{50} (21 mg/kg).

Procedure:

In the ACUTE STUDY 128 rats were dosed with single doses of the chemical at 3 levels. In the SUBCHRONIC STUDY 32 rats were dosed with 5 consecutive doses at the same 3 levels. There were 8 POSITIVE CONTROLS. The rats were killed at 6, 12, 27, or 51 hours, or at 5 days after initial dosing.

Three hours before sacrifice the rats were injected intraperitoneally with 4 mg/kg colchicine. They were killed with carbon dioxide inhalation.

The soft tissue and epiphyses of both tibiae were removed and the marrow was aspirated or flushed from the bone, and placed in Hank's balanced salt solution, centrifuged and the pellet resuspended in 0.075 M KCl, centrifuged again and the pellet then resuspended in Carnoy's fixative. After 1/2 hour the fixative was changed and the cells left overnight at 4°C. Slides were prepared of the cells, were air-dried, and then stained with 10% Giemsa at pH 6.8.

If possible 100 spreads were read for each rat, scoring the cells for both structural and numerical aberrations. A mitotic index based on at least 500 cells was recorded.

Data Evaluation and Interpretation:

The testing laboratory describes the evaluation and interpretation of the data as follows:

> "Basically, an attempt is made to establish whether a substance or its metabolites can interact with chromosomes to produce gross lesions or changes in chromosome numbers and whether these are of a type which can survive more than one mitotic cycle of the cell. All aberration figures detected by this assay result from breaks in the chromatin which either fail to repair, or repair in atypical combinations. The cell transit time for bone marrow is normally 20 to 24 hours, so an indication of when in the cell cycle a substance is active can often be obtained based on the time of kill.
>
> ".... Gaps are not counted as significant aberrations unless they are present in a much higher than usual frequency. Open breaks are considered as indicators of genetic damage, as are configurations resulting from the repair of breaks. The latter includes translocations, multiradials, rings, multicentrics, etc.
>
> ".... Cells with more than one aberration are considered to indicate more genetic damage than those containing evidence of single events. Consistent variations from the euploid number are also considered in the evaluation of mutagenic potential."

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Results:

TOTAL NUMBERS OF STRUCTURAL ABERRATIONS OBSERVED

Dose	TIME OF SACRIFICE 6 hr.	12 hr.	24 hr.	48 hr.	5 day
Solvent/Vehicle Control	4	2	5	1	1
21 mg/kg	0	0	1	1	0
63 mg/kg	2	1	0	2	1
188 mg/kg	4	1	4	0	0
Positive Control	-	-	24	-	-

As indicated in the above table, SC-0224 did not induce chromosome aberrations in rat bone marrow cells. Compare with the solvent/vehicle controls. The positive controls, however, show a significantly greater number of abberrations ($p < 0.01$, student's t-test). SC-0224 is not mutagenic as determined by this test.

Conclusions:

The assay procedures of this study appears to follow the general guidelines recommended for the In-Vivo Mammalian Bone Marrow Cytogenetic Test (EPA 1982). However, the following inadequacies in reporting of this study must be clarified:

Although mentioned in the report, the described procedures were not adequate to assure the top quality of chromosome spread for aberration analysis.

1. The hypotonic treatment (0.075 M KCl at 37°C) is intended to cause swelling of the cells and spreading of the chromosomes. If the hypotonic treatment is too long or too short, chromosomes will not be spread properly.

2. Cell fixation is normally carried out in three changes of 3:1 methanol: glacial acetic acid, and refrigerated overnight at 4 C. The fixed cells were dispensed on chilled wet slides with subsequent flame warming before being stained in 10% Giemsa.

Mutagenicity Evaluation SC-0224 in Salmonella typhimurium (Lot No. 7269-10). Ames Salmonella/Microsome Mutagenesis Assay. Stauffer Chemical Co. In-Vitro Toxicology Sec. Report No. T-10847. Jan. 19, 1982.

Material Tested:

SC-0224, Lot No. 7269-10. Purity estimated at 90% by weight.

Positive Controls:

Sodium azide, Lot F8A. Eastman Kodak Co.

9-Aminoacridine, purity = 90%. Sigma Chemical Co., Lot. No. 117C-0119.

2-Nitrofluorene, purity = 98%. Aldrich Chemical Co. Lot. No. 112967.

2-Aminoanthracene. Aldrich Chemical Co., Lot. No. 061077.

Indicator Organisms:

Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 obtained from Dr. Bruce Ames, U. of Cal., Berkeley, Ca.

Metabolic Activation System Reaction mixture: NADP 4mM; Glucose-6-phosphate 5mM; sodium phosphate buffer 100mM; $MgCl_2$ 8mM; KCl 34mM; Homagenate S-9 fraction 100 ul/ml.

Procedure-Plate Test: (Taken from pg. 10 of study report)

Approximately 10^9 cells from an overnight culture of each indicator organism strain growing in nutrient broth were added to separate test tubes containing 2.0 ml molten agar supplemented with biotin and a trace of histidine. In addition, each tube received the designated concentration of test substance (dissolved in water) and either 0.5 ml of phosphate buffer (non-activation) or 0.5 ml of a reaction mixture containing the 9000 Xg liver homogenate supernatant (S-9 activation assay). The dose levels of the test compound in this study were 0.12, 0.37, 1.11, 3.33, and 10 mg/plate in the absence of the S-9 metabolic activation system; and 0.56, 1.11, 1.67, 3.33, 5.0, 10.0, and 15.0 mg/plate in the presence of the S-9 metabolic activation system. The contents of each tube were mixed and then poured onto the surface of selective agar plates and allowed to solidify. The plates were incubated for approximately 48 hours at 37°C and the colonies growing on each plate counted. At least 5 dose levels of the test substance and positive and negative controls were used in each assay.

Preparation of Liver S-9 Supernatant (9000 xg):

Sprague-Dawley male rats or $B6C3F_1$ male mice purchased from the Charles River Breeding Laboratories were used as the source of hepatic/S-9.

500 mg/kg Aroclor 1254 in corn oil was injected I.P. to 200 gram rats or 20 gram mice 5 days prior to sacrifice. They were deprived of food on the evening before sacrifice.

80 mg/kg phenobarbital in corn oil was injected I.P. daily for 5 consecutive days prior to sacrifice.

At sacrifice the livers of 7-10 rats or 20-50 mice were aseptically removed and transferred to sterile ice cold 0.15 M KCl. They were washed with 0.15 M KCl, weighed, minced and diluted with 3 ml of 0.15 M KCl per gram (wet weight) of liver, homogenized with a Potter-Elvehjem or polytron tissue homogenizer.

The homogenate from all animals was pooled prior to being centrifuged for 20 minutes at 9000 xg at 4°C. After centrifugation the S-9 supernatant was transferred by pipetting to storage vials, frozen, and retained at -80°C until used.

Data Evaluation:

Colonies are counted and presented as revertants per plate for each indicator strain. A dose-dependent increase in revertants is considered necessary to establish mutagenicity. If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data lose significance.

Results:

Lot. No. 7269-10. Tested directly without S-9 metabolic activation system. Solvent/Medium control gave averages of 4, 4, 5, 27, and 98/72 revertants per plate for the 5 strains of Salmonella. The test chemical gave similar numbers of revertants at the dose levels tested (0.12 through 10 mg/plate). The positive controls (sodium azide, 9-aminoacridine, and 2-nitrofluorene) gave averages of 444, 69, 300, 295, and 442/438 revertants per plate.

When tested with the S-9 metabolic activation system (rats and mice), no significant differences were observed) between the negative controls and the test chemical (0.56 through 15 mg/plate).

In summary SC-0224 (Lot No. 7269-10) demonstrated no mutagenic activity when tested in the Salmonella/mammalian microsome mutagenicity test.

Conclusion:

The in-vitro Samonella mutagenicity test used for detecting the mutagenic activity of the test compound, SC-0224 (Lot #7269-10) appears to follow the general procedures of Salmonella typhimurium histidine reversion assay described by Ames (Ames et al., 1975). However, the following inadequacies in reporting of this study must be clarified:

1. The described procedures for the preparation of media were unclear and must be clarified:

(a) The source of nutrient broth used in this study must be identified. The variation in the growth of Ames tester strains from the overnight incubation appears to result from variability in the nutrient quality of the medium.

(b) The selective medium should be Vogel Bonner Medium E with 2% glucose. The overlay soft agar should consist of 0.6% purified agar with 0.5 mM L-Histidine-HCl, 0.5 mM biotin, and 0.5% NaCl.

2. The interpretation of results was not clearly given. A test compound which produces neither a statistically significant dose-related increase in the number of revertants nor a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic.

Mutagenicity Evaluation SC-0224 in Salmonella typhimurium (Lot No. 7646-0901). Ames Salmonella/Microsome Mutagenesis Assay. Stauffer Chemical Co. In-Vitro Toxicology Sec. Report No. T-11070. Sept. 24, 1982.

Material Tested:

SC-0224, Lot No. 7646-0901. Purity: 19.2% by weight.

Positive Controls:

Sodium azide, Lot F8A. Eastman Kodak Co.

9-Aminoacridine, purity = 90%. Sigma Chemical Co., Lot. No. 117C-0119.

2-Nitrofluorene, purity = 98%. Aldrich Chemical Co. Lot. No. 112967.

2-Aminoanthracene. Aldrich Chemical Co., Lot. No. 061077.

Indicator Organisms:

Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 obtained from Dr. Bruce Ames, U. of Cal., Berkeley, Ca.

Metabolic activation System Reaction mixture: NADP 4mM; Glucose-6-phosphate 5mM; sodium phosphate buffer 100mM; $MgCL_2$ 8mM; KCl 34mM; Homagenate S-9 fraction 100 ul/ml.

Procedure-Plate Test: (Taken from pg. 10 of study report)

Approximately 10^9 cells from an overnight culture of each indicator organism strain growing in nutrient broth were added to separate test tubes containing 2.0 ml molten agar supplemented with biotin and a trace of histidine. In addition, each tube received the designated concentration of test substance and either 0.5 ml of phosphate buffer (non-activation) or 0.5 ml of a reaction mixture containing the 9000 Xg liver homogenate supernatant (S-9 activation assay). The dose levels of the test compound in this study were 0.005, 0.014, 0.041, 0.123, 0.370, 0.617, 1.111, 1.852, 3.333, 5.556, 10.0, 16.667, 25.0, and 50.0 ul/plate in the absence of the S-9 metabolic activation system; and the same concentrations in the presence of the S-9 metabolic activation system using phenobarbital-induced rat liver. Using Arochlor 1254 or phenobarbital-induced mouse liver, concentrations were 0.617, 1.852, 5.556, 16.667 or 50.0 ul/plate. The contents of each tube were mixed and then poured onto the surface of selective agar plates and allowed to solidify. The plates were incubated for approximately 48 hours at 37°C and the colonies growing on each plate counted. At least 5 dose levels of the test substance and positive and negative controls were used in each assay.

A6

Preparation of Liver S-9 Supernatant (9000 xg):

Sprague-Dawley male rats or $B6C3F_1$ male mice purchased from the Charles River Breeding Laboratories were used as the source of hepatic/S-9.

500 mg/kg Araclor 1254 in corn oil was injected I.P. to 200 gram rats or 20 gram mice 5 days prior to sacrifice. They were deprived of food on the evening before sacrifice.

80 mg/kg phenobarbital in corn oil was injected I.P. daily for 5 consecutive days prior to sacrifice.

At sacrifice the livers of 7-10 rats or 20-50 mice were aseptically removed and transferred to sterile ice cold 0.15 M KCl. They were washed with 0.15 M KCl, weighed, minced and diluted with 3 ml of 0.15 M KCl per gram (wet weight) of liver, homogenized with a Potter-Elvehjem or Polytron tissue homogenizer.

The homogenate from all animals was pooled prior to being centrifuged for 20 minutes at 9000 xg at 4°C. After centrifugation the S-9 supernatant was transferred by pipetting to storage vials, frozen, and retained at -80°C until used.

Data Evaluation:

Colonies are counted and presented as revertants per plate for each indicator strain. A dose-dependent increase in revertants is considered necessary to establish mutagenicity. If a chemical produces a response in a single test that connot be reproduced in one or more additional runs, the initial positive test data lose significance.

Results:

Lot No. 7646-0901. Tested directly without S-9 metabolic activation system. Solvent/Medium control gave averages of 13, 5,7, 38, and 163 revertants per plate for the 5 strains of Salmonella. The test chemical gave similar numbers of revertants at the dose levels tested. The positive controls (sodium azide, 9-aminoacridine, and 2-nitrofluorene) gave averages of 988, 144, 872, 662, and 767 revertants per plate.

When tested with the S-9 metabolic activation system (rats and mice), no significant differences were observed between the negative controls and the test chemical.

In summary SC-0224 (Lot No. 7646-0901) demonstrated no mutagenic activity when tested in the Salmonella/mammalian microsome mutagenicity test.

Conclusions:

The in-vitro Samonella mutagenicity test used for detecting the mutagenic activity of the test compound, SC-0224 (Lot #7646-0901) appears to follow the general procedures of Salmonella typhimurium histidine reversion assay described by Ames (Ames et al., 1975). However, the following inadequacies in reporting of this study must be clarified:

1. The described procedures for the preparation of media were unclear and must be clarified:

(a) The source of nutrient broth used in this study must be identified. The variation in the growth of Ames tester strains from the overnight incubation appears to result from variability in the nutrient quality of the medium.

(b) The selective medium should be Vogel Bonner Medium E with 2% glucose. The overlay soft agar should consist of 0.6% purified agar with 0.5 mM L-Histidine-HCl, 0.5 mM biotin, and 0.5% NaCl.

2. The interpretation of results was not clearly given. A test compound which produces neither a statistically significant dose-related increase in the number of revertants nor a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic.

SC-0224 Mutagenicity Evaluation in the Chinese Hamster Ovary Cytogenetic Assay. Lot #6841-48-3. Stauffer Chemical Co., In-Vitro Toxicology Section Report No. T-10875. July 6, 1982.

Procedure:

Chinese Hamster Ovary Cells were maintained in HAM's F-12 medium (modified by the omission of hypoxanthine) supplemented with 10% fetal calf serum, L-glutamine, penicillin, and streptomycin. Treatment medium is the above growth medium (nonactivation) or serum-free growth medium (activation). The assay is initiated by seeding 8 X 10^5 cells per 75 cm^2 flask in 10 ml of medium. The activation system consists of an S-9 fraction of liver homogenate and necessary cofactors.

In a nonactivation assay cultures are exposed to a test substance from 2-12 hours, colcemid being added to the medium or medium plus chemical 2 to 3 hours prior to fixing the cells. Without S-9 metabolic activation doses of 2, 4, and 6 mg/ml of test substance were used. In an activation assay cultures are exposed to test substances for 2 hours in a serum free medium in the presence of S-9 and cofactors. Cultures then are washed at least twice with phosphate buffer saline and fresh medium added. Colcemid is added 2-3 hours prior to fixing the cells. With S-9 metabolic activation doses of 2, 4, 6, 8, 10, and 12 mg test substance/ml were used.

In the aberration assay cells are treated as above, and 8 to 28 hours after initiating the treatment colcemid is added to the cultures and incubation continued for 2-3 hours.

For the sister chromatid exchange assay, bromodeoxyuridine (final concentration 10uM) is added to the cultures two hours after initiation of treatment. Incubation is continued for another 26 hours, and Colcemid is added 2-3 hours prior to harvest.

Cells are collected by mitotic-shake off, treated with hypotonic KCl, washed twice with methanol:glacial acetic acid fixative, dropped on slides, and air dried. The cells then are stained and are scored for aberrations, and 20 metaphases are scored for sister chromatid exchanges.

Results:

Aberrations:

In the non activation portion of the assay increased aberrations over controls were seen at 4 mg/ml ($p<0.05$).

In the activation portion, increased chromosomal aberrations were seen at 8, 10, and 12 mg/ml. $p<0.01$ at 10 and 12 mg/ml.

Sister chromatid exchange was not determined.

Conclusions:

The in-vitro cytogenetic assay in CHO cells used for this study appears to follow the general guidelines recommended for the in-vitro mammalian cytogenetics and in-vitro sister chromatid exchange assay (EPA 1982, OECD 1981). However, the following inadequacies in reporting of this study must be clarified:

1. Although mentioned in the report, the described procedures were not adequate to assure the top quality of chromosome spread for aberration or sister chromatid exchange analysis.

(a) The hypotonic treatment (0.075 M KCl at 37 C) is intended to cause swelling of the cells and spreading of the chromosomes. If the hypotonic treatment is too long or too short, chromosomes will not be spread properly.

(b) Cell fixation is normally carried out in three changes of 3:1 methanol:glacial acetic acid, and refrigerated overnight at 4 C. The fixed cells were dispensed on chilled wet slides with subsequent flame warming before being stained in 10% Giemsa.

2. In the Non-activation Assay treatment, it is stated that "Cultures are exposed to a test substance from 2 - 12 hours", and that the exposure time will be indicated on the raw data. Since the raw data were not provided, the exposure time should be included in the report.

SC-0224 Mutagenicity Evaluation in the Chinese Hamster Ovary Cytogenetic Assay. Lot #7466-18-01. Stauffer Chemical Co., In-Vitro Toxicology Section Report No. T-11019. July 22, 1982.

Procedure:

Chinese Hamster Ovary Cells were maintained in HAM's F-12 medium (modified by the omission of hypoxanthine) supplemented with 10% fetal calf serum, L-glutamine, penicillin, and streptomycin. Treatment medium is the above growth medium (nonactivation) or serum-free growth medium (activation). The assay is initiated by seeding 8 X 10^5 cells per 75 cm^2 flask in 10 ml of medium. The activation system consists of an S-9 fraction of liver homogenate and necessary cofactors.

In a nonactivation assay cultures are exposed to a test substance from 2-12 hours, colcemid being added to the medium or medium plus chemical 2 to 3 hours prior to fixing the cells. In an activation assay cultures are exposed to test substances for 2 hours in a serum free medium in the presence of S-9 and cofactors. Cultures then are washed at least twice with phosphate buffer saline and fresh medium added. Colcemid is added 2-3 hours prior to fixing the cells. With S-9 metabolic activation doses of 1, 2, 3, 4, 5, 6, 7, and 8 ul test substance/ml were used.

In the aberration assay cells are treated as above, and 8 to 28 hours after initiating the treatment colcemid is added to the cultures and incubation continued for 2-3 hours.

For the sister chromatid exchange assay, bromodeoxyuridine (final concentration 10uM) is added to the cultures two hours after initiation of treatment. Incubation is continued for another 26 hours, and colcemid is added 2-3 hours prior to harverst.

Cells are collected by mitotic shake-off, treated with hypotonic KCl, washed twice with methanol:glacial acetic acid fixative, dropped on slides, and air dried. The cells then are stained and are scored for aberrations, and 20 metaphases are scored for sister chromatid exchanges.

Results:

Aberrations:

In the activation assay, increased chromosomal aberrations were seen at 6, 7, and 8 ul/ml of the test substance ($p < 0.01$).

Sister Chromatid Exchange: No significant increase in sister chromatid exchanges at the dose levels tested (1 through 8 ul/ml) in the presence of S-9 metabolic activation system.

Conclusions:

The in-vitro cytogenetic assay in CHO cells used for this study appears to follow the general guidelines recommended for the in-vitro mammalian cytogenetics and in-vitro sister chromatid exchange assay (EPA 1982, OECD 1981). However, the following inadequacies in reporting of this study must be clarified:

1. SC-0224 induces chromosomal aberrations in Chinese hamster ovary cells in the presence of Aroclor 1254 induced rat liver metabolic activation.

2. Although mentioned in the report, the described procedures were not adequate to assure the top quality of chromosome spread for aberration or sister chromatid exchange analysis.

(a) The hypotonic treatment (0.075 M KCl at 37°C) is intended to cause swelling of the cells and spreading of the chromosomes. If the hypotonic treatment is too long or too short, chromosomes will not be spread properly.

(b) Cell fixation is normally carried out in three changes of 3:1 methanol:glacial acetic acid, and refrigerated overnight at 4°C. The fixed cells were dispensed on chilled wet slides with subsequent flame warming before being stained in 10% Giemsa.

3. In the Non-activation Assay treatment, it is stated that "Cultures are exposed to a test substance from 2 - 12 hours," and that the exposure time will be indicated on the raw data. Since the raw data were not provided, the exposure time should be included in the report.

SC-0224 Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test-Forward Mutation Assay. Study T-10848. Lot. Nos. 7269-10 and 6841-48-3. By Stauffer Chemical Company. In-Vitro Toxicology Section; Farmington, Ct. Feb. 8, 1982.

Procedure:

Indicator cells are from the L5178Y ($TK^{+/-}$) cell line from the 3.7.2 clone of Fischer L 5178Y cells furnished by Dr. Donald Clive, and are maintained until needed in frozen nitrogen.

For metabolic activation to the treatment medium are added S-9 homogenate, 240 ug NADP, and 450 ug isocitrate.

The test compound was tested for mutagenic effects on L5178Y ($TK^{+/-}$) mouse lymphoma cells in vitro. The investigations were performed with microsomal activities at concentrations of 0.375, 0.750, 1.50, 3.00, 6.00, 8.0, 8.5, 9.0, 9.5, and 10 mg/ml and without microsomal activation at concentrations of 0.375, 0.75, 1.50 3.0, 6.0, 7.0, 8.0, 9.0 and 10 mg/ml. The mutagenicity test was carried out by treating L5178Y ($TK^{+/-}$) cells with the selected concentrations at a cell density of 12 X 10^6 cells for 4 hours. Following exposure the substance is removed and the cells are resuspended in 40 ml of media. A portion of the cell suspension is used for the forward mutation assay described by Clive (Clive, et al. 1975 and 1979).

Evaluation:

Mutation Assay:

To be acceptable, the cloning efficiency of solvent/vehicle controls should be 75% or greater. Values greater than 100% are possible because of errors in cell counts and dilutions in cloning. Cloning efficiencies in the range of 50% to 70% may be conditionally acceptable. Generally in order to call a susbtance non-mutagenic it should be tested within a dose range which induces 80% to 90% toxicity at the higher limits unless prevented by insolubility.

$TK^{+/-}$ Mouse Lymphoma Mutation Assay: A substance is considered mutagenic if it produces a dose-dependent increase in mutation frequency over 3 doses to a level at least 2.5 times the solvent/vehicle control.

Results:

1. Toxicity was seen at doses exceeding 7 mg/ml.

2. In the direct assay, insufficient effect was seen to indicate a positive result.

3. With metabolic activation there was a significant reproducible increase in mutation frequency. This indicates mutagenicity at the thymidine kinase locus in L5178Y mouse lymphoma cells under S-9 rat liver metabolic activation.

Conclusions:

The assay used to evaluate the mutagenic activity of the test compound, SC-0224 (Lot #7269-10 and 6841-48-3) in the L5178Y/TK$^{+/-}$ mouse lymphoma cells with and without S9 metabolic activation system, appears to follow the procedures described by Clive (1975 and 1979). However, the data presented in Table 4 were confusing and must be corrected. These data presume to be the results of repeated study under activation test conditions instead of the results from non-activation assay.

SC-0224 Morphological Transformation of BALB/3T Cells. (Lot #729-10). Report T-10849. Stauffer Chemical Co., In Vitro Toxicology Section, Farmington, Ct. January 4, 1982.

Indicator Cells Used:

1-1 subclone of clone A-31 of BALB/3T3 mouse cells obtained from Dr. Takeo Kakunaga of NCI, or the subclone C-14 of clone CI-13.

Medium:

Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum or new born calf serum, glutamine (2mM), penicillin (100 units ml), and streptomycin (100 ug/ml).

Dose Selection:

Dishes are seeded 24 hours before dosing with 200 cells per dish. After 3 days exposure to the test substance the cells are washed and incubated in growth medium for an additional 6-10 days. When the colonies reach adequate size (3 to 7 mm), the plates are fixed, stained, and counted. Relative survival of each dose = $\frac{\text{no. of colonies surviving treatment}}{\text{no. of colonies in solvent/vehicle control dishes.}}$

Transformation Assay Procedure: Described by Kakunaga (1973).

Cells are plated at density of 1 X 10^4 cells/T-25 flask 24 hours prior to treatment. Fifteen flasks are treated for each of the 5 doses of the test chemical (0.313, 0.625, 1.25, 2.50, and 5.0 mg/ml), a positive control, and two negative (solvent/medium and medium) controls. After 3 days exposure, the cells are washed and refed. Flasks are refed twice weekly for 4-6 weeks. The assay is then fixed unless transformed foci have not been observed in positive control flasks. A concurrent toxicity assay for the solvent/vehicle control and treatment doses may be included with each transformation.

The results were presented as the number of foci per set of cultures for each dose level. The medium/solvent control and positive control, 3-methylcholanthrene, were run concurrently.

Results:

1. 3 mg/ml was the lowest toxic dose.

2. There was no increased number of transformed foci over controls in the treated Balb/3T3 cell culture when compared to the negative control.

Conclusions:

The assay which evaluates the test compound for its ability to induce malignant transformation of Balb/3T3 cells in vitro appears to follow the general procedures described by Kakunaga (Int. J. Cancer 12:463, 1973). However, the following inadequacies in reporting of this study must be clarified:

Two different culture vessels (dishes or T-25 flask) were mentioned in this report. If the dishes were employed in this study, CO_2 concentration, temperature and relative humidity are critical factors in maintaining the proper cell growth. The specific incubator for propagating the cell cultures should be identified.

Roland A. Gesset

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Veterinary Medical Officer
Review Section #1
Toxicology Branch/HED (TS-769)

TS-769:GESSERT:sll:X73710:9/26/83 card 5

Acute Oral Toxicity of SC-0224 4-LC Formulation in Male and Female Sprague-Dawley Rats. Conducted by S. Sorenson, B.J. Jones, D. Crume, and T.R. Billow. Stauffer Chemical Co. Richmond Toxicology Laboratory, Richmond, California. Report T-11189. November, 1982. Accession No. 249803, Caswell No. 893 C.

Material Tested:

SC-0224 4LC. Lot # 8014-42. Formulation contains 41.4% w/w trimethylsulfonium carboxymethylaminomethyl phosphonate, [redacted]

Procedure:

Male Rats:

After 16-18 hours fasting ten male rats per dose level were treated by oral gavage at the rate of 650, 800, 900, and 1000 mg/kg formulation suspended in corn oil. Twenty rats were dosed with water for negative controls. Mortality was 8/10, 6/10, 4/10, and 0/10 for doses of 1000 mg/kg, 900 mg/kg, 800 mg/kg, and 650 mg/kg, respectively. The oral LD_{50} for male rats was 846 mg/kg, with 95% confidence limits of 762-940 mg/kg.

Female Rats:

After 16-18 hours fasting, ten female rats per dose level were treated by oral gavage at the rate of 1000, 900, 800, 700, and 650 mg/kg formulation suspended in corn oil. Twenty rats were dosed with water for negative controls. Mortality was 8/10, 7/10, 7/10, 0/10, and 1/10 for doses of 1000 mg/kg, 900 mg/kg, 800 mg/kg, 700 mg/kg, and 650 mg/kg, respectively. The oral LD_{50} for female rats was 805 mg/kg, with 95% confidence limits of 726-892 mg/kg.

Clinical Signs:

All mortalities occurred within 24 hours. Clinical Signs were depression, hypopnea, ptosis, piloerection; ataxia, and occasionally tremors and prostration. Survivors appeared normal by days 5 or 6.

Necropsy findings in rats dying on test were darkened lungs, livers, and spleens and clear fluid in the GI tracts of rats on the high dose levels. There were no visible lesions in rats on the lower dose levels or in those surviving to 14 days.

INSERT INGREDIENT INFORMATION IS NOT INCLUDED

57

Twenty males and 20 females dosed with water appeared normal throughout the 14-day observation period and on necropsy at day 14.

The data meet Core-Minimum Standards.

Toxicity Category III.

Acute Dermal Toxicity of SC-0224 4-LC Formulation in Stauffland Albino Rabbits - Abraded Skin. Conducted by T. Billow, et al.; Stauffer Chemical Co.; Richmond Toxicology Laboratory; Richmond, California. Report T-11189. November, 1982. Accession No. 249803. Caswell No. 893 C.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Material Tested:

SC-0224 4LC. Lot #8014-42. Formulation contains 41.4% w/w trimethylsulfonium carboxymethylaminomethyl phosphonate, [redacted]

Procedure:

Four male and female Stauffland albino rabbits per dose level had the test material applied to the clipped abraded abdominal skin under a protective cover. Dose levels were 1200, 1000, 800, 650, 550, and 450 mg/kg body weight. Three male and 3 female rabbits served as zero dose controls. After 24 hours the binder and the test material were removed, the treatment sites were inspected for irritation, and the abdomens were rewrapped in a gauze binder. Three days later the gauze binder was removed and the rabbits were observed for 14 days following initial treatment. Necropsies were performed on all rabbits that died during the study and on all survivors at 14 days.

Results:

Four of 8 rabbits died at 1200 mg/kg; 4 of 8 at 1000 mg/kg; 3 of 8 at 800 mg/kg; 2 of 8 at 650 mg/kg; and 1 of 8 at 550 mg/kg. There was no mortality at 450 mg/kg or in the controls.

Necropsy Effects seen in rabbits that died during the study included pale lungs in 2 rabbits at 1000 mg/kg; a red and purple area between the right kidney fat and abdominal wall in 1 rabbit at 1000 mg/kg; darkened lungs in 1 rabbit at 800 mg/kg; and reddened saliva in 1 rabbit at 800 mg/kg. A red and purple area also was seen on the inside wall of the abdominal cavity in 1 rabbit on 550 mg/kg. No other findings were seen on necropsy.

Clinical Signs were mild to severe depression in some rabbits at all treatment levels. Salivation, prostration, and tremors also were seen in some rabbits, with no particular regard to dose level.

Local Dermal Effects was a mild to moderate erythema and mild to moderate edema after 24 hours exposure, but without any relation to dose level.

No clinical, necropsy, or dermal effects were seen in the controls.

LD_{50} (abraded skin) = 1061 mg/kg - male and female rabbits combined. 95% confidence limits = 759 - 1483 mg/kg.

Acute Dermal Toxicity of SC-0224 4LC Formulation in Stauffland Albino Rabbits - Intact Skin.

This portion of the study was conducted in the same manner as for the abraded skin. Doses of 2000, 1850, 1700, 1500, 1200, 1000, and 800 mg/kg were used. Mortality was 8/8 at 2000 mg/kg; 6/8 at 1850 mg/kg; 4/8 at 1700, 1500, and 1200 mg/kg; 2/8 at 1000 and 800 mg/kg.

The acute dermal LD_{50} in intact skin was 1316 mg/kg, with 95% confidence limits of 1007-1722 mg/kg.

Clinical signs and necropsy findings in rabbits treated on intact skin were similar to those with abraded skin.

Combined LD_{50}, intact and abraded skin = 1279 mg/kg, 95% confidence limits for LD_{50} = 999-1639 mg/kg. Differences between intact skin and abraded skin rabbits were not statistically significant at $p > 0.05$ (by method of Litchfield and Wilcoxon).

Conclusions:

1. Acute dermal LD_{50} in male and female rabbits (intact and abraded skin) = 1279 mg/kg. 95% confidence limits for LD_{50} = 999-1639 mg/kg. Dermally, the formulation is slightly more acutely toxic than the technical chemical.

2. Toxicity Category II.

3. Study meets Core Minimum Requirements.

Primary Skin Irritation of SC-0224 - 4LC Formulation in Stauffland Albino Rabbits. Conducted by T.R. Billow and D. Crume. Stauffer Chemical Co. Richmond Toxicology Laboratory; Richmond, California. Report T-11189. November 1982. Accession No. 249,803. Caswell No. 893C.

Material Tested:

SC-0224 4LC. Lot#8014-42, Formulation contains 41.4% w/w trimethylsulfonium carboxymethylamino methylphosphonate; [redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Procedure:

Twenty-four Hour Exposure:

Six Stauffland albino rabbits were used in the study. One-half ml of SC-0224 4LC formulation was placed on an abraded site and an intact site and covered with a one-inch square gauze patch. This was secured by adhesive tape and wrapped with rubberized damming for 24 hours. After 24 hours the coverings and test material were removed and the reactions scored. The site reactions were also scored after 72 hours.

Results of 24-Hour Exposure:

At 24 hours mild to moderate erythema and edema were seen in the intact and abraded skin of the 6 rabbits. At 72 hours mild edema and scar tissue were seen in all rabbits. The Primary Irritation Score (Draize) was 2.92.

Four-Hour Exposure:

The previous procedure was also conducted with an exposure time of 4 hours and observations and scoring being made after 4 hours, 24 hours, and 72 hours.

After 4 hours mild to moderate erythema in 5 rabbits and cracked skin in 1 rabbit were seen in the intact and abraded skin. At 72 hours, mild erythema was seen in 4 rabbits and moderate erythema in 1 rabbit. One rabbit was found dead prior to the 4-hour reading. The primary irritation score was 1.27.

Conclusions:

1. SC-0224 4LC Formulation is a moderate dermal irritant.

2. Toxicity Category III.

3. Data are Core Minimum.

Primary Eye Irritation of SC-0224 4LC Formulation in Stauffland Albino Rabbits. Conducted by T.R. Billow and D. Crume. Stauffer Chemical Company. Richmond Toxicology Laboratory. Richmond, California. Report T-11189. November, 1982. Accession No. 249,803. Caswell No. 893C.

Material Tested:

SC-0224 4LC. Lot #8014-42. Formulation contains 41.4% w/w trimethylsulfonium carboxymethylaminomethylphosphonate; [redacted]

Procedure:

Nine rabbits were treated by placing 0.1 ml of the formulation inside the lower lid of one eye of each rabbit. In 3 of the rabbits the eye was washed with water 20-30 seconds after exposure, and in the remaining 6 rabbits the eye was not washed. Untreated eyes served as negative controls. The cornea, iris, and conjunctivae were observed at 24, 48, and 72 hours, and at 4, 7, 10, 14, 16, 17, 21, and 24 days after treatment. Scoring was according to the method of Draize.

Results:

In the six rabbits whose eyes were not washed mild to severe corneal opacity, moderate iritis, and moderate to severe conjunctival irritation were seen. This persisted through 10 and 14 days, with moderate to severe corneal opacity and mild conjunctivitis still observed in 2 rabbits at 24 days.

In the three rabbits whose eyes were washed, mild corneal opacity and mild iritis were produced in 2 rabbits, with mild to severe conjunctivitis seen in all 3 rabbits. By day 24 the only observation in these rabbits was mild conjunctivitis in 2 rabbits.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Conclusions:

1. SC-0224 4LC formulation is very corrosive to the eye, producing corneal opacity, iritis, and severe conjunctivitis. This is in contrast to the technical chemical which is only mildly irritating to the eye.

2. The irritation and corrosiveness is reduced somewhat by washing the eyes.

3. Toxicity Category I.

4. The data are Core Minimum.

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DCR-44903:Gessert:12/13/83:HED-05:del. 12/29/83:LM
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64