



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

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MAY 30 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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

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

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

THRU: Robert B. Jaeger, Section Head
Review Section #1
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APPLICANT: Stauffer Chemical Company
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Brian Dementi, 5/23/86

DLR hnd of 5-29-86

W. L. H. 5/29/86

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

Summary of Results:

- 1) SC-0224 Two-Generation Reproduction Study in Rats (T-11051)

** I don't think*
Overall Reproductive NOEL = < 150 ppm (F2B male, weanlings, *the weanlings*
relative spleen weight reduction)

Overall Clinical NOEL = 150 ppm (platelet count increase, *relative spleen*
combined male and female adult F2B generation) *wt reduction. Should*

Core: supplementary

- 2) Acute Inhalation Study, Rat with SC-0224 (T-11728)

LC₅₀ > 0.81 mg/L

Core = guideline

- 3) Acute Inhalation Study with SC-0224 4LC (T-11870)

LC₅₀ = 1.30 mg/L (male); LC₅₀ = 1.56 mg/L (female)

Core: guideline

reproduction effect. Only
fertility, gestation, live
birth and lactation
indices should be
used to set the
reproduction end point
i. f. b. d.

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- 4) Dermal Sensitization Test with SC-0224 4LC (T-11420)
NOEL = 3% SC-0224 4LC
Core: guideline
- 5) Dermal Sensitization Test with SC-0224 (T-11269)
NOEL = 3% SC-0224
Core = Guideline
- 6) Metabolism, Tissue Residue and Balance Studies of Orally Administered
[Methyl ¹⁴C] Trimethylsulfonium carboxymethylaminomethylphosphonate
(SC-0224) in Rats (PMS-148)
Quality of study acceptable
- 7) Fertility Screen with SC-0224 in Rats (T-10896)
Quality of study acceptable

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

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Laboratory: Environmental Health Center
Stauffer Chemical Company
Farmington, Connecticut

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

Accession No.: 258398 (Appendix 8), 258399

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

Animals: Rat [CrI CD (SD)Br]

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

Materials and Methods: (as paraphrased from Study Procedure)

Husbandry: Standard GLP

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

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

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

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

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

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

B. In-Life Observations

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

1. During the Growth Phases

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

2. During the Mating Phases

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

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

3. During the Gestational Phases

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

4. During the Perinatal Phases

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

5. During the Lactational Phases

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

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

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

C. Terminal Procedures and Observations

"1. Scheduled Terminations

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

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

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| <u>Organ System</u> | <u>Tissue</u> | <u>Fixative*</u> |
|-----------------------|--------------------|------------------|
| Digestive (cont'd) | Rectum | NBF |
| | Pancreas | NBF |
| | Liver | NBF |
| Urogenital | Kidneys | NBF |
| | Urinary bladder | NBF |
| | Testes | BG |
| | Epididymides | BG |
| | Prostate | NBF |
| | Seminal vesicles | NBF |
| | Coagulating glands | NBF |
| | Ovaries | BG |
| | Vagina | NBF |
| | Cervix | NBF |
| | Uterus | NBF |
| Endocrine | Pituitary | BG |
| | Thyroids | NBF |
| | Parathyroids | NBF |
| | Adrenals | BG |
| Nervous | Brain | NBF |
| | Sciatic nerve | NBF |
| Special Senses | Eyes | BG |
| | Harderian glands | BG |

Gross lesions (as specified
by the pathologist)

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

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

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

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

Hematology

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

Blood Chemistry

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

- d. Histopathology: A single set of slides were prepared for the tissues in the collection list for five animals/sex/dose group for the P0, P1, and Adult F2b animals. The slides were sent to the Japanese collaborators for histological evaluation. Their findings are not included in this report."

"2. Unscheduled Terminations

- a. Parental Animals: Moribund animals which were sacrificed and animals which were found dead were necropsied under the supervision of a Veterinary Pathologist.
- b. Prewaning Pups: Prewaning F1b and F2b pups which died and stillborn pups were necropsied under the supervision of the Study Director. The heads of these pups were fixed in Bouin's fixative and examined according to the method of Wilson (1965). The thoracic and abdominal viscera were examined by the technique of Staples (1974)."

Data Interpretation

"1. Parameters

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

"2. Statistical Analysis

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

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

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

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

Results:

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

I. CLINICAL OBSERVATIONS ON PARENTAL ANIMALS

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

A. General Effects in Parental Animals

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

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

B. Body Weights, Feed Intake

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

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

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

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

C. Feed Efficiencies

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

D. SC-0224 Intake

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

E. Necropsy - P0, P1

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

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

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

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

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

F. Reproductive Effects

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

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

G. Food Intake During Gestation

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

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

H. Dam Weights During Lactation

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

I. Dam Food Intake During Lactation

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

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

II. LITTER AND PUP PARAMETERS

A. Litter Size

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

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

B. Mean Pup Weights

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

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

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

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

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

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

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

C. Pup Survival and Development

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

D. Macroscopic Findings in Pups

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

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

Additional abnormalities worthy of notation are tabulated below.

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

()* - Litter ID Number.

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

E. Absolute Organ Weights, (F1B) Weanlings

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

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

F. Absolute Organ Weights (F2B) Weanlings

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

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

G. Relative Organ Weights, F1B and F2B Weanlings

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

Relative Spleen Weight (%), F2B Generation Weanlings

| | <u>Control</u> | <u>150 ppm</u> | <u>800 ppm</u> | <u>2000 ppm</u> |
|--------|----------------|----------------|----------------|-----------------|
| Male | 0.676 | 0.499* | 0.504* | 0.434* |
| Female | 0.534 | 0.473 | 0.470 | 0.423* |

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

III. GENERAL FINDINGS IN F2B ADULT MALES

A. Clinical Observations

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

B. Body Weights

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

C. Feed Intake

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

D. Findings at Necropsy

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

E. Organ Weights of F2B Adult Males

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

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

F. Clinical Test Results, F2B Males

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

IV. GENERAL FINDINGS ON F2B ADULT FEMALES

A. Clinical Observations

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

B. Body Weights

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

C. Feed Intake

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

D. Findings at Necropsy

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

E. Organ Weights

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

F. Clinical Findings

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

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

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

Summary of Findings

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

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

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

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

Evaluations on F2B adult rats revealed the following:

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

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

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

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

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

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

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

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

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

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

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

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

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

Core Rating = Supplementary

Repairability = Nonrepairable

Reference:

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

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

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Study: Acute Inhalation Study, Rat with SC-0224

Laboratory: Environmental Health Center Inhalation Facility
Stauffer Chemical Company
Farmington, Connecticut

Study No. and Date: T-11728, May 6, 1983

Accession No.: 258398, Appendix 1

Material Tested: SC-0224 (56.2%)

Animals: Sprague-Dawley Rat

The purpose of this study was to determine the toxicity of the test material in rats when administered in a 4-hour acute inhalation exposure.

Materials and Methods:

Husbandry: Standard GLP

Details of the exposure system are provided diagrammatically in an appendix to the test procedure. The test material was aerosolized using a Barbington Nebulizer, wherein heated (temperature not indicated) corn oil was circulated in order to maintain the test material at suitably low viscosity to be aerosolized. The exposure chambers, constructed of stainless steel and glass, enclosed a volume of 447 liters. The chamber air temperature and relative humidity were monitored and recorded periodically. Chamber flows were maintained at 13.7 air changes/hour and percent oxygen was monitored to assure that atmospheric oxygen exceeded 19 percent.

Procedure:

Ten male and ten female rats were exposed simultaneously to a regulated atmosphere of the aerosolized test material for a period of 4 hours, according to standard Guideline protocol. The animals were exposed to a mean chamber air concentration calculated to be 0.81 mg/L. Rats were observed frequently during the exposure period and then twice daily during the subsequent 14-day total observation period.

Ten rats of each sex, constituting the control groups, were similarly tested with the exception that SC-0224 was not administered. The study authors describe this as a sham exposure.

Animal body weights were recorded on day 0 (day of initial exposure) and on days 2, 7, and 14. After 14 days of observation, and following overnight fasting animals were

anesthetized with sodium pentobarbital and sacrificed by exsanguination. The following tissues were examined at necropsy, and portions retained in formalin for future reference: trachea, larynx, bronchi, nasal passages, lungs, liver, spleen, kidneys, adrenals, heart and any tissues appearing abnormal.

During the period of 4-hour exposure to the test material, atmospheric aerosol concentrations were determined periodically, at 30-minute intervals. In addition, particle size analyses were performed at the 55- and 170-minute time points during the exposure period. This determination involved the use of low-volume cascade impactors.

The nominal concentration of test material in the chamber test atmosphere, indicated above to be 0.81 mg/L, is a calculated figure derived from the ratio of amount of test material used during the exposure to the total chamber air flow during this period.

Results:

As indicated previously, the mean concentration \pm S.D. of the test material in the exposure chamber as determined by gravimetric means was 0.81 ± 0.42 mg/L (0.45 mg ai/L, as calculated based on 56.2% purity).

Measurement of aerosol particle size as determined at the 55- and 170-minute exposure time points were, in terms of mass median aerodynamic diameter (MMADar) and geometric standard deviation (σ), 2.5 ± 2.75 μ m and 2.0 ± 2.36 μ m, respectively.

Conclusion: At the highest achievable concentration of 0.81 mg/L of SC-0024, there were no deaths recorded. Only a few transient clinical signs were observed.

Core: Guideline.

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Study: EPA Acute Inhalation Study with SC-0224 4LC

Laboratory: Environmental Health Center Inhalation Facility
Stauffer Chemical Company
Farmington, Connecticut

Study No. and Date: T-11870, May 9, 1984

Accession No.: 258398, Appendix 3

Material Tested: SC-0224 4LC (41.2%)

Animals: Sprague-Dawley Rats

The purpose of this study was to determine the toxicity of the test material in rats when administered in a 4-hour acute inhalation exposure.

Materials and Methods:

Husbandry: Standard GLP

Details of the exposure system are provided diagrammatically in Appendix III of the test procedure. The test material was aerosolized using a Barbrington Nebulizer. The exposure chambers, constructed of stainless steel and glass, enclosed a volume of 447 liters. The chamber air temperature and relative humidity were monitored and recorded periodically. Chamber flows were maintained at 11 to 14 air changes/hour and percent oxygen was monitored to assure that atmospheric oxygen exceeded 19 percent.

Procedure (Standard Guidelines Protocol):

The generation of aerosol, once initiated, reached 99 percent of final concentration of the test material in a particular study within 19 to 24 minutes of initial exposure. Chamber flow rate was 11 to 14 air changes/hour. The animals were exposed for 4 hours. The mean concentration of SC-0224 4LC in the series of exposures, as determined by gravimetric analyses will be presented subsequently. Following the exposure period, animals remained in the exposure chamber for an additional 1-hour period during which the exposure chamber was purged with air at a flow rate > 26 air changes/hour to remove test material from animal's pelts. Rats were then transferred to holding cages in an inhalation holding room. Rats were observed frequently during the exposure period and then twice daily during the 14-day total observation period.

Ten rats of each sex, constituting the control group, were similarly tested with the exception that SC-0224 4LC was not administered. The study author described this as a

sham exposure. (Would be curious to know whether anything such as water or emulsifier was or should have been used.)

Animal body weights were recorded on day 0 (day of initial exposure) and on days 2, 7, and 13. The body weights of all nonscheduled deaths were obtained immediately before necropsy. After 14 days of observation, animals were anesthetized with sodium pentobarbital and sacrificed by exsanguination.

During the 4-hour exposure periods to the test material, atmospheric aerosol concentrations were determined periodically, at 30-minute intervals, except for the high-dose group which was sampled more frequently. In addition, particle size analyses were performed at approximately 1 and 3 hours into the experiments. The determination involved the use of low-volume cascade impactors.

The nominal concentrations of test material in the chamber test atmosphere in the various studies were calculated values derived from data obtained gravimetrically. In particular, under each set of exposure conditions a known volume of chamber air was filtered, residue weighed and concentration of test material in the atmosphere calculated and reported as grams/liter.

Results:

The calculated concentrations of SC-0224 4LC in the various studies were reported as 0.61, 1.30, 1.58, and 1.60 mg/L. The study author notes that "The test material in the generator reservoir became too foamy to use after a short period and had to be replaced several times during each exposure. This made the nominal concentrations artificially high and they had no relationship to actual measured exposure levels" (Page 5).

The mass median aerodynamic diameters (MMADar) in the various studies ranged from 1.68 μm to 3.10 μm , with geometric standard deviations ranging 1.90 μm to 2.22 μm . A stable particle size was reportedly achieved in each study.

General Animal Observations:

As observed during the exposure, the animals were described as "Lethargic with stained muzzles, some tearing and signs of labored breathing" (p. 6). Mortality was high, particularly among males, following exposure. Most deaths occurred during the first 2 days postexposure. Mortality for males was 80 percent in the two high-dose groups, 50 percent in the next to lowest dose group and 0 percent in the low-dose group, the LC_{50} being 1.30 mg/L. Mortality for females in the various dose groups was from high to low dose 60, 50, 10 and 0 percent. LC_{50} = 1.56 mg/L for females.

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Toxic signs in addition to death observed during the 14-day posttreatment observation period most notably included rough coat, red stains on face and body, stains on muzzle and ventral neck, labored breathing, reduced activity, prostration, and dehydration. These phenomena were most commonly observed in the 1.30 to 1.60 mg/L dose groups.

Body Weight:

There was evidence of weight loss on day 2 for both male and female rats in the 1.60 and 1.30 mg/L dose groups. Depression of weight gain was evident on day 7 for males in the 1.30 mg/L group and for females in the 1.60 mg/L group. Significant weight gains were seen by day 7 for males in the low dose group and controls. Significant weight gains were reported for females by day 7 in all but the high-dose group. Thus, in general, weight gain was adversely affected as a result of treatment, with males being more markedly affected than females. Signs of toxicity were: pulmonary edema and redness.

Conclusions:

Dosages employed were sufficiently high to cause mortality; male LC_{50} = 1.30 mg/L, female LC_{50} = 1.56 mg/L.

Edema and redness observed at various sites.

Core Rating: Guideline.

Study: Dermal Sensitization Test with SC-0224 4LC

Laboratory: Richmond Toxicology Laboratory
Stauffer Chemical Company
deGuigne Technical Center
Richmond, California

Study No. and Date: T-11420, October 22, 1984

Accession No.: 258398, Appendix 4

Material Tested: SC-0224 4LC (Lot No. WCH-2304). Purity is reported to be 41.2% (p. 26).

Animals: Guinea Pigs, Male (Hartley strain)

The purpose of this study was to evaluate the potential for SC-0224 4LC to cause dermal sensitization.

Materials and Methods:

Husbandry: Standard GLP

The open epicutaneous test (OET) procedure, described in Appendix I, was followed.

This particular procedure, developed in accordance with EPA Guidelines, includes a primary irritation phase, a 26-day induction phase and two challenge phases, days 29 to 31 and 44 to 46. The material was applied topically and left uncovered.

Further details of the test procedure are quoted as follows:

"During the induction phase, the materials were applied in a volume of 100 ul to an approximate area of 2 cm². The quantities used for primary irritation and challenge phases were applied to a smaller area, 1 cm², in a volume of 25 ul. During the induction phase, each animal was exposed daily, 5 days per week for 4 weeks, to a single concentration of material. The material was applied to the right flank. Each animal was then challenged on the left flank with concurrent applications of several concentrations of the material. To minimize variations in response due to flank location, the various concentrations of test material were rotated among different application sites (Appendix II). The skin reactions were evaluated for erythema (redness) and edema (swelling) according to an 8-point scoring system (Table 2) (2). These evaluations were made 24 hours after application in the primary irritation test, at weekly intervals during induction, and daily for the 3 days following challenge and rechallenge applications."

Table 1 reproduced from the study (p. 7) summarizes the exposures the various groups of eight animals each received, including the induction challenge and rechallenge phases.

Table 1

| Group | Concentrations Applied (%) | | |
|-------------|----------------------------|--------------------------|---------------|
| | Induction | Challenge | Rechallenge |
| SC-0224 4LC | | | |
| I | 10 | 10,3,1,d.w. ^a | 10,3,1,d.w. |
| II | 3 | 10,3,1,d.w. | 10,3,1,d.w. |
| III | 1 | 10,3,1,d.w. | 10,3,1,d.w. |
| IV | 0.3 | 10,3,1,d.w. | 10,3,1,d.w. |
| V | 0.1 | 10,3,1,d.w. | 10,3,1,d.w. |
| VI | d.w. | 10,3,1,d.w. | 10,3,1,d.w. |
| Controls | | | |
| VII | 3-HCHO ^b | 1,3 HCHO d.w. | 1,3 HCHO d.w. |
| VIII | d.w | 1,3 HCHO d.w. | 1,3 HCHO d.w. |

^a d.w. = Distilled Water

^b HCHO = Formaldehyde

Results:

In the primary irritation study, the full strength (100%) test material produced mild irritation in all eight animals tested. At 30 percent concentration the test material produced generally somewhat milder irritation overall, but all animals were affected. Applications of 10 percent and 3 percent test material did not produce any remarkable effects (Table, p. 10).

Induction (Table, p. 11)

By day 5 there was seen a mild erythema response in 6 of 8 animals of the high-dose group. By day 12 the frequency of response declined in the high-dose group to 2/8, but the average score for the two animals was somewhat higher (2.5).

By day 19 there was erythema in the high-dose group. Lower doses of SC-0224 4LC did not elicit any remarkable effects.

Formaldehyde (3%) elicited a positive erythema response (score 1.7 to 2.3) in 6/8 animals during the 26-day induction phase.

In summary, during the induction phase, 10 percent SC-0224 4LC gave a positive skin response in terms of erythema. Lower doses were ineffective in this respect.

Challenge (Table p. 12)

When induced animals were challenged with 10 percent, 3 percent, 1 percent, 0.3 percent, 0.1 percent SC-0224 4LC and vehicle control (water), a mild erythema response (score 1.5) was seen in the high-dose group in approximately 50 percent of the animals challenged with the high dose. A milder response (score 1.0 to 1.5) was seen in the 3 percent group challenged with the high dose, and, as in the former challenge, approximately 50 percent of the animals responded. There were no other remarkable findings observed in the challenge phase. A positive response was obtained with formaldehyde.

In summary, dermal sensitization studies in which guinea pigs were induced with SC-0224 4LC and subsequently challenged with the same material, it was demonstrated that animals induced by the two highest concentrations (10% and 3%) were sensitized and responded to the high concentration (10%) challenge.

Rechallenge (Table p. 13)

When induced animals were rechallenged with 10 percent, 3 percent, and 1 percent SC-0224 4LC and vehicle control (water), a response similar to that seen in the challenge study was obtained. The magnitude of response (score approximately 2) was a little higher than in the challenge, but the frequency of response was less than 50 percent.

In summary, in this dermal sensitization study it was demonstrated that animals induced by the two highest concentrations (10% and 3%) were sensitized, as evidenced by a positive response upon rechallenge with the highest concentration (10%) of those used.

Additional Comments

Appendix III (pp. 31 to 38) covering individual animal responses shows that there were no remarkable findings with respect to edema in the challenge and rechallenge studies.

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Animal weight data over the 46-day trial interval revealed a tendency by the high-dose (10%) group to gain less weight. Weight gain in this group was 59.3 percent as compared to 87 percent and 72.6 percent for vehicle control groups.

Conclusions:

Results indicate that SC-0224 4LC is a mild sensitizer, with the most pronounced erythema scores observed at the high dose not exceeding 2 in the challenge and rechallenge phases of the study. As a point of definition, according to the study scoring system, a score of 2 on a scale of 0 to 4 is characterized as slight (but well-defined) erythema (pp. 8 and 17). Edema was not observed as a characteristic or complication of this study.

Conclusion: SC-0224 4LC is a mild sensitizer
NOEL = 3% SC-0224 4LC for challenge
and rechallenge.

Core: Guideline.

Study: Dermal Sensitization Test with SC-0224 Technical

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Laboratory: Richmond Toxicology Laboratory
Stauffer Chemical Company
deGuigne Technical Center
Richmond, California

Study No. and Date: T-11269, October 12, 1984

Accession No.: 258398, Appendix 2

Material Tested: SC-0224 Technical (56.3%)

Animals: Guinea Pigs, Male (Hartley strain)

The purpose of this study was to evaluate the potential for SC-0224 to cause dermal sensitization.

Materials and Methods:

Husbandry: Standard GLP

The open epicutaneous test (OET) procedure, described in Appendix I, was followed.

This particular procedure, developed in accordance with EPA Guidelines, includes a primary irritation phase, a 26-day induction phase and two challenge phases, days 29 to 31 and 44 to 46. The material was applied topically and left uncovered.

Further details of the test procedure are quoted as follows:

"During the induction phase, the material was applied in a volume (liquid) or mass (petrolatum) of 100 ul or mg, respectively, to an approximate area of 2 cm². The primary irritation and challenge applications were made to smaller area, 1 cm², in a volume or mass of 25 ul or mg. During the induction phase, each animal was exposed daily, 5 days per week for 4 weeks, to a single concentration of material. The material was applied to the right flank. Each animal was then challenged with concurrent applications of several concentrations of the material applied to the left flank. To minimize variations in response due to flank location, the various concentrations of test material were rotated among different application sites. The skin reactions were evaluated for erythema (redness) and edema (swelling) according to an 8-point scoring system. These evaluations were made 24 hours after application in the primary irritation test, at weekly intervals during induction, and daily for the 3 days following challenge and rechallenge applications.

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"Known sensitizers, formaldehyde and 2-mercaptobenzothiazole (2-MBT), were used as positive controls (3). Deionized water was used as the vehicle for SC-0224 Technical and formaldehyde while petrolatum (Plough, Inc.) was used as the vehicle for 2-MBT). Control and test solutions were prepared at weekly intervals. Negative control groups (animals induced with vehicle and challenged with chemical) were included for each positive control material and SC-0224 Technical. In addition, each animal served as its own control because a vehicle site was included at challenge and rechallenge.

"A group was considered to have a positive response if one or more animals exhibited an erythema score of 2 or greater. The material was considered to be a sensitizer if the challenge reaction was positive and greater than the irritation reaction. Irritation was determined from the primary irritation results and the challenge response of the appropriate vehicle group." (pp 1-2)

Table 1 reproduced from the study (p. 8) summarizes the exposures the various groups of 8 animals each received, including the induction, challenge and rechallenge phases.

Table 1

| Group | Concentrations Applied (%) | | |
|-------------------|----------------------------|-----------------------------|--------------|
| | Induction | Challenge | Rechallenge |
| SC-0224 Technical | | | |
| I | 100 | 30,10,3,1,d.w. ^a | 30,10,3,d.w. |
| II | 30 | 30,10,3,1,d.w. ^a | 30,10,3,d.w. |
| III | 10 | 30,10,3,1,d.w. ^a | 30,10,3,d.w. |
| IV | 3 | 30,10,3,1,d.w. ^a | 30,10,3,d.w. |
| V | 1 | 30,10,3,1,d.w. ^a | 30,10,3,d.w. |
| VI | 0.3 | 30,10,3,1,d.w. ^a | 30,10,3,d.w. |
| VII | d.w. | 30,10,3,1,d.w. ^a | 30,10,3,d.w. |

Table 1 (cont'd)

| | | | |
|----------|-----------------------|---------------|---------------|
| Controls | | | |
| VIII | 1% HCHO ^b | 1% HCHO, d.w. | 1% HCHO, d.w. |
| IX | d.w. | 1% HCHO, d.w. | 1% HCHO, d.w. |
| X | 3% 2-MBT ^c | 3% 2-MBT, Pet | 3% 2-MBT, Pet |
| XI | Pet ^d | 3% 2-MBT, Pet | 3% 2-MBT, Pet |

a d.w. = Deionized Water

b HCHO = Formaldehyde

c 2-MBT = 2-Mercaptobenzothiazole

d Pet = Petrolatum

Results:

Induction (Table p. 12)

With respect to the total 26-day induction phase, it was found that by day 12 there was a positive response, in terms of erythema in all 8 animals of Group I (high dose), where the average score was 2.1. Erythema was also observed in approximately 50 percent of the Group II animals during days 12 to 26 (score 1.5 to 1.8). One animal of 8 in Group III exhibited a positive reaction. There were no responses to SC-0224 reported in any other dose groups.

Formaldehyde (1%) at days 12 to 19 elicited a positive erythema response (score 1.3 to 1.9) in 7/8 of the animals treated, with the incidence declining by day 26 to 3/8. 2-MBT (3%) yielded a response (score 2.0) in only 1 of 8 animals by day 12. Surprisingly, the vehicle for 2-MBT yielded a positive response which increased in frequency from 3/8 on day 5 to 8/8 on day 19, then decreasing to 4/8 by day 26, scores ranged 1.6 to 2.0.

In summary, during the induction phase, 100 percent and 30 percent SC-0224, 1 percent formaldehyde and petrolatum vehicle (for 2-MBT) gave positive skin responses. Responses to 10 percent SC-0224 and 3 percent 2-MBT were equivocal.

Challenge (Table, p. 13)

When induced animals were challenged with 30 percent, 10 percent, 3 percent, and 1 percent SC-0224 and vehicle control (water), a dose response for erythema was observed in

terms of frequency of response (but not magnitude which remained around 2) both as a function of challenge and induction doses. In most cases the response frequency was highest at 24 hours postchallenge, declining (exhibiting progressive decline) at the 48- and 72-hour observation times. A very definite response was seen in the formaldehyde challenge of formaldehyde induced guinea pigs. Also, it should be noted that the magnitude of the response to the vehicle (water) challenge in the formaldehyde case was surprisingly high. A meaningful challenge response was not seen in the case of 2-MBT (2%).

In summary, dermal sensitization studies in which guinea pigs were induced with SC-0224 and subsequently challenged with the same material, it was demonstrated that animals induced by the five highest doses were sensitized in a dose dependent manner and responded to challenge concentrations of SC-0224 of as low as 10 percent.

Rechallenge (Table 7)

When induced animals were rechallenged with 30 percent, 10 percent, 3 percent, and 1 percent SC-0224 and vehicle control (water), a dose response in terms of erythema (both frequency and magnitude of response) was observed as a function of the induction and rechallenge doses. In most cases, the response frequency was highest at 24-hour postrechallenge, as was true in the case of challenges. Also, as before, a very definite response was seen in the case of formaldehyde rechallenge. A striking response was not seen with 2-MBT (3%) rechallenge.

In summary, the rechallenge findings were essentially the same as those of the original challenge study, with the exception that the magnitude of the erythema response tended to decline from about 2.0 at high induction doses to 1.0 for the lower induction doses.

Additional Comments

Appendix III of the study submitted, skin response data for individual animals, shows that edema was not observed in any of the challenge or rechallenge tests, and, hence, does not constitute a positive finding in this study of SC-0224.

Weight gain of the guinea pigs over the 46-day period of study did not reveal any remarkable compound-related effects. It should be noted, perhaps, that there may have been a slight tendency for the higher dosed animals, those exposed to 30 to 100 percent test material, to gain less weight.

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Conclusions:

Results indicate that SC-0224 is a mild sensitizer. The most pronounced responses, generally seen at the higher doses, were, in terms of erythema scoring, approximately 2 in the extreme case. As a point of definition, according to the study scoring system, a score of 2 on a scale of 0 to 4 is characterized as slight (but well-defined) erythema (p. 9). Edema was not observed as a characteristic or complication of this study.

The LOEL in terms of induction in the guinea pig was observed resultant to application of the 10 percent concentration sample.

In terms of response to challenge, the LOEL was 10 percent with respect to challenge concentration, eliciting a response in the group sensitized by 1 percent technical.

In terms of response to rechallenge, the LOEL was 10 percent also, but in this case with respect to the group sensitized by 10 percent technical.

Conclusion: SC-0224 is a mild sensitizer
NOEL = 3% SC-0224 for challenge and
rechallenge.

Core: Guideline.

Study: Metabolism, Tissue Residue and Balance Studies of Orally Administered [methyl ^{14}C -]trimethylsulfonium carboxymethyl amino methylphosphonate (SC-0224) in Rats.

Laboratory: Stauffer Chemical Company
Mtn. View Research Center
Pesticide Metabolism Section

Study No. and Date: Report Number PMS-148
February 4, 1985

Accession No.: 258398 (Appendix 5)

Material Tested: Analytical Grade SC-0224. (methyl ^{14}C -]trimethylsulfonium carboxymethyl amino methylphosphonate (20 mCi/mmol) was prepared by Stauffer Chemical Company.

Animals: Sprague-Dawley Rat

The purpose of this study was to evaluate the absorption, tissue distribution, and excretion of the radiolabeled SC-0224.

Methods: (As paraphrased from pages 6 to 8 of submittal)

SC-0224, ^{14}C -radiolabeled on the cation portion of the molecule (i.e., on the trimethylsulfonium portion), was administered orally to three male and three female rats at each of the doses, 35 mg/kg or 350 mg/kg. The test compound was dissolved in distilled water before administration to the animals. Urine and feces were collected at 6, 12, and 24 hours and subsequently at 24-hour intervals until termination at 120 hours. Samples of urine and feces were stored frozen.

At 120 hours into the study, rats were sacrificed by exsanguination under ether anesthesia. Tissue samples were removed, weighed and stored frozen pending combustion analysis. Tissues assayed for ^{14}C included: adrenals, bladder, blood, brain, fat (mesenteric), gonads, small intestine, large intestine, kidney, liver, lung, skeletal muscle, spleen, stomach, thymus, thyroid, heart, and hide. The remaining carcass was homogenized.

Results:

Rats showed signs of toxicity within 1 hour of administration of the high dose. The toxic signs included lethargy, ataxic movements, slow and labored breathing, salivation and occasional tremors. These signs were more noticeable in females than in males. Symptoms were markedly reduced by 12 hours following dosing.

Within 24 hours of dosing the compound was largely excreted. By this time point, the low dose males and females had eliminated 96.3 percent and 89.1 percent, respectively, and high dose males and females had eliminated 83.9 percent and 86.1 percent, respectively. Most of the recovered dose was in the urine: low dose, male 98.5 percent, female 93.1 percent; high dose, male 94.9 percent, female 91.4 percent.

Data generated showed that the radiolabeled portion of SC-0224, trimethylsulfonium ion (TMS), was excreted essentially unmetabolized in both urine and feces.

Necropsy revealed that radiolabeled compound was distributed throughout the tissues and organs. Organs in which radiolabel was most concentrated included the following (Tables 12 to 15, pp. 29 to 32):

| <u>35 mg/kg</u> | | <u>350 mg/kg</u> | |
|-----------------|---------------|------------------|---------------|
| <u>Male</u> | <u>Female</u> | <u>Male</u> | <u>Female</u> |
| Adrenals | Adrenals | Adrenals | Adrenals |
| Kidney | Bladder | Bladder | Bladder |
| Liver | Hide | Kidney | Kidneys |
| Thyroid | | Liver | Liver |
| Hide | | Stomach* | Stomach |
| | | Thyroid | Thyroid |

*Very high level.

When tissue residues are compared in terms of ratios of residue levels at the high to low dose and for males as compared to females, it is apparent that, firstly, a tenfold increase in dose results in more than a tenfold increase in tissue residues and, secondly, that this trend is much more pronounced in females (Table 20, p. 37). This suggests that elimination mechanisms become relatively saturated at the higher dose allowing more residue than expected to accumulate in tissues. Furthermore, this suggests that at high doses, females tend to develop higher tissue residues. This is borne out by inspection of Tables 14 to 15, pp. 31 to 32. Thus, to the extent that SC-0224 manifests toxicity in a given tissue, females would be more vulnerable as dose increases.

Conclusions:

When [methyl ¹⁴C] trimethylsulfonium carboxymethylamino-methylphosphonate is administered to rats, radiolabeled trimethylsulfonium ion is rapidly excreted, unmetabolized, in urine and feces (predominantly in urine, > 90%).

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Furthermore, results show that the principal sites for localization of trimethylsulfonium ion are the adrenals, kidney, bladder, liver, thyroid, and stomach.

At high dose, elimination mechanisms appear to become saturated as evidenced by accumulation in tissues exceeding the proportionate increase in dose. This phenomenon is more pronounced in females, suggesting that as dose increases, at some dose level females will tend to begin accumulating more residue in various tissues. This would also indicate increased vulnerability of females to toxic manifestations of the cation beyond the dose at which elimination mechanisms become strained.

It should be noted that the parent compound is a water-soluble salt consisting of the trimethylsulfonium ion (cation) and the carboxymethylaminomethylphosphonate ion (anion). Only the disposition of trimethylsulfonium ion is evaluated in this study, as only this portion of the dissociable parent is labeled. A second radiolabel metabolism study in which the anionic portion of the molecule is tagged will be necessary in order to properly evaluate metabolism and distribution of the parent molecule, SC-0224.

Quality of study acceptable

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Study: Fertility Screen with SC-0224 in Rats

Accession No.: 258398, Appendix 7

Study Date: January 6, 1983

Since this study is not required by EPA Guidelines, this review is limited to the development of a summary of the intent and findings.

The purpose of this study was to determine whether the test substance, administered via the drinking water, has an effect on fertility in rats as indicated by fertility index (pregnant females/mated females) and litter data. Concentrations of test material in drinking water were 0, 100, 500, and 1000 ppm.

During the course of the study it was observed that with increasing dose, there was a decrease in water consumption, hence, the ingestion of test compound did not increase in the proportions anticipated.

Results show that there was no compound-related mortality or remarkable compound-related clinical observations. Body weight gain was significantly reduced in the high-dose group. As evidenced by pregnancy index, there was no adverse effect on pregnancy. Furthermore, no dose-related adverse effects were observed in terms of mean number of pups born, survival index over 21 days or lactation index. Neither mean pup weight nor pup weight gain were remarkably altered under the influence of test material for 21 days postpartum. In summary, SC-0224 as tested did not have an adverse effect on rat fertility or pup survival.

Quality of study acceptable.