7-29-87

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

006337

JUL 29 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: SULFOSATE (SC-0224); Request for EXPEDITE Review from Mr. E. Tinsworth, Director RD (Memo of 6/9/87)

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

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

The attached review from Dr. J. Chen for the one-year dog chronic feeding study and several mutagenicity studies partially fulfills the EXPEDITE request (6/9/87). As noted in our 6/17/87 memo to John Melone (attached), the data package for Sulfosate was divided between Review Sections 1 and 2 in Toxicology Branch in order to provide the turnaround time requested. The long-term bioassays in mice and rats are presently in Section #2 for review. Therefore, the attached review does not constitute a completed action until the rodent bioassay reviews are completed. For information regarding the status of those reviews please contact Mr. E. Budd (TB).

cc: A. Barton (HED)
T. Farber (TB)
E. Budd (TB)

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83-1 - Dog - Chronic Toxicity

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Reviewed by: John H.S. Chen John H.S. Chen 7/15/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger
Section I, Toxicology Branch (TS-769C) R.B.J. 7/16/87

DATA EVALUATION REPORT

Study Type: One-Year Dog Chronic Toxicity

TOX. CHEM. No.: 853C

Accession No.:

MRID No.: 402140-05

Test Material: SC-0224 (EHC No. 0469-15; WRC No. 8108-24-1; 56.2% Purity)

Study Number(s): T-11075

Sponsor: Stauffer Chemical Company

Test Facility: Environmental Health Centers, Stauffer Chemical Co.

Title of Report: One-Year Chronic Oral Toxicity Study with SC-0224 in Beagle Dogs

Author(s): H.F. Knapp and R.W. Thomassen

Report Issued: April 3, 1987

Conclusions:

Chronic Toxicity NOEL = 10 mg/kg/day

Chronic Toxicity LEL = 50 mg/kg/day
(decreased lactate dehydrogenase values)

Dose levels tested: 2, 10, and 50 mg/kg/day

Classification of Data: Supplementary

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

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Title of Report: One-Year Chronic Oral Toxicity Study with SC-0224 in Beagle Dogs

I. Procedures

The method used to evaluate the chronic oral toxicity of SC-0224 during a one-year study is described below:

1. Test Animals

The study consisted of 40 young adult beagles (4 to 6 months of age). The dogs were acclimated at EHC for at least 3 weeks prior to study initiation. Animals were singly housed in kennels providing at least 0.74 m^2 floor area and separated by galvanized steel wire mesh. Feed (Purina Certified Canine Diet #5007) and tap water were provided continuously during the quarantine and study periods. Number of animals in each group is given below:

Dose Group mg/kg/day	Conc. of SC-0224 (mg/ml)	Number of Animals	
		Males	Females
0	0	5	5
2	4	5	5
10	20	5	5
50	100	5	5
Total		20	20

2. Test Compound Preparation and Administration

The test compound, SC-0224 (Trimethylsulfonium Carboxymethyl Aminoethylphosphonate, 56.2% Purity) which was provided by Stauffer Chemical Co., was administered orally to animal by gavage. The dogs were dosed no earlier than 2 hours following feeding. Each daily dose level was administered in a volume of 0.5 ml/kg. The administered volume of test compound was adjusted weekly, based on the most recent body weight determination, to maintain a constant level in mg/kg/day. The control group received tap water (vehicle) in the same manner as the SC-0224 dose group.

3. Statistical Analysis

Body weights, hematology, blood chemistry, and cholinesterase analysis and relative and absolute organ weights were compared between the dose groups and the control group by Bartlett's test for homogeneity of variance, a one-way analysis of variance, and Dunnett's t-test. Selected clinical observations were analyzed by Fisher's Exact Probability Test. The probability of Type I error was set at 0.05.

II. Methods and Results

1. Clinical Observation

All animals were observed twice daily for general appearance, behavior, signs of toxicity and mortality.

Results: All dogs survived throughout the study. There were no treatment-related effects on clinical observations noted in the dosed animals during the study. However, transient salivation was observed in 1 and 5 female dogs at the 10 and 50 mg/kg/day dose levels respectively. Single episodes of emesis were also noted in 3 of 5 females at the 50 mg/kg/day dose level. Emesis was not observed in the remainder of the female dogs nor in any of the male dogs. No treatment-related effects on rectal temperature, pulse or respiration rate were observed in the treated animals during the study.

2. Body Weight

Body weight of each animal was recorded prior to initiation of the study then weekly thereafter for the 53-week study.

Results:

Dose Level mg/kg/day	Males WK-0 Wt.(g)	Males WK-53 Wt.(g)	Males % Change	Females WK-0 Wt.(g)	Females WK-53 Wt.(g)	Females % Change
0	9.6	12.3	28	7.7	10.0	30
2	9.1	12.6	38	8.0	10.9	36
10	9.0	12.7	41	7.5	10.0	33
50	9.6	12.8	33	7.8	10.3	32

There were no treatment-realted effects on body weight noted in the dosed animals during the study. The percent body weight gain was comparable between the control and the SC-0224-treated animals at the termination of this study.

3. Food Consumption

Food consumption of each animal was recorded quarterly for the 52-week study.

Results: Food Consumption (g/day/dog)

Study WK	Males(mg/kg) 0	2	10	50	Females(mg/kg) 0	2	10	50
0	267	205	235	225	250	225	206	200
25	340	320	330	330	263	295	320	269
52	350	310	340	345	285	350	320	290

There were no treatment-related changes in the mean food consumption values observed from the treated animal groups during the 52-week study.

4. Ophthalmic Examinations

All animals were examined before initiation of the treatment phase and at 26 and 52 weeks.

Results: No treatment-related ocular abnormalities were observed in any of the dogs.

5. Blood, fecal, and urine samples were collected from all animals during the pretest quarantine period and at 3, 6, and 12 months.

A. Hematology - Parameters (X) were examined.

*(X) Hematocrit (HCT)
*(X) Hemoglobin (HGB)
*(X) Erythrocyte Count (RBC)
*(X) Leukocyte Count (Total and Differential)
*(X) Platelet Count
(X) Reticulocyte Count (if HCT is less than 35%)

* Recommended by the Pesticide Assessment Guidelines Hazard Evaluation Series 82-1.

Results: There were no hematological values considered to be significantly different between the control and the treated animal groups during this study. All hematology parameters were unaffected by SC-0224 administration.

B. Blood Chemistry - Parameters (X) were examined.

*(X) Calcium
*(X) Potassium
*(X) Phosphorous
*(X) Chloride
*(X) Sodium
*(X) Aspartate aminotransferase (SGOT)
*(X) Alanine aminotransferase (SGPT)
(X) Gamma glutamyl-transferase (GG)
(X) Lactate dehydrogenase (LDH)
(X) Alkaline phosphatase
(X) Plasma cholinesterase
(X) Red blood cell cholinesterase
(X) Brain cholinesterase (termination only)
(X) Creatine phosphokinase (CPK)
*(X) Blood urea nitrogen
*(X) cholesterol
*(X) Total bilirubin
*(X) Total protein
*(X) Albumin
(X) Globulin
*(X) Glucose
*(X) Creatinine

* Recommended by the Pesticide Assessment Guidelines Hazard Evalution Series 82-1.

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Significant Mean Values for Clinical Chemistry Parameters (1-12 Month)

Month	Males (mg/kg) 0	2	10	50	Females (mg/kg) 0	2	10	50
SGOT (U/l)								
3	20	21	28*	25*	17	21	24	19
6	21	24	24	20	21	26	30*	23
12	23	21	24	21	25	24	26	20
ALP (U/l)								
3	50	31*	43	30*	41	44	47	50
6	34	29	30	32	34	38	45	33
12	24	19	18	18	22	28	32	25
Plasma Cholinesterase (U/l)								
3	2121	2221	2453	2151	2185	2384	2127	2339
6	2097	2200	2474	2101	2618	2545	2300	2557
12	1946	2504	2512	2465	2380	2653	2428	2704
RBC Cholinesterase (U/l)								
3	7072	7720	8820*	8692*	6755	9120*	8644*	7684
6	7760	8008	7816	8036	9344	10032	9872	10436
12	7212	7850	7324	7512	8016	8432	7208	7824
Brain Cholinesterase (U/l)								
12	35.1	31.2	33.8	34.9	43.8	30.2*	32.8	39.3
LDH (U/l)								
3	43	26	22	33	64	42	39	29
6	40	62	49	36	46	53	57	25
12	66	53	42	26	53	41	32	15*
Phosphorous (mg/dl)								
3	4.6	4.2	5.2	5.2	4.1	4.3	4.0	5.6*
6	4.4	4.5	4.5	4.6	3.6	4.5	3.8	4.7*
12	3.8	3.8	3.9	4.2	3.8	4.0	3.6	4.6
Albumin (g/dl)								
3	3.3	3.3	3.4	3.7	3.3	3.1	3.2	3.2
6	3.4	3.4	3.3	3.4	3.6	3.6	3.4	3.3*
12	3.5	3.5	3.4	3.5	3.6	3.5	3.7	3.7

* Significantly different from control value, $P<0.05$.

Results: Significant changes in the mean values of SGOT, ALP, RBC cholinesterase, brain cholinesterase, phosphorous, and albumin found in the treated animal groups during this study were either transient in nature or did not exhibit a treatment-related effect. However, a significant reduction in the mean values of lactate dehydrogenase (LDH) was observed in the high dose female group at the 12-month interval. There were no other clinical chemistry values considered to be significantly different between the control and the treated groups during this study.

C. Urinalysis - Parameters (X) were examined.

*(X) Color appearance	*(X) Ketones	*(?) Volume
*(X) Specific gravity	*(X) Bilirubin	
(X) pH	(X) Urobilinogen	
*(X) Protein	*(X) Sediment	
*(X) Glucose	*(X) Occult blood	

* Recommended by the Pesticide Assessment Guidelines Hazard Evaluation Series 82-1.

Results: All urinalysis parameters were comparable between the control and the treated groups during this study. However, the volume of urine for all animals at the pretest period and at 3, 6, and 12 months was not included in this study report.

D. Fecal Analysis - Parameters (X) were examined.

(X) Occult blood (X) Parasites

Results: All fecal parameters were comparable between the control and the treated groups during this study.

6. Sacrifice and Pathology

All animals were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

*(X) Skin	*(X) Duodenum	(X) Vagina
*(X) Mammary gland	*(X) Jejunum	*(XX) Brain
*(X) Semimembranosus muscle	*(X) Ileum	(X) Cervical
*(X) Femur	*(X) Caecum	* (X) Thoracic and lumbar spinal cord
(X) Nasal passage	*(X) Colon	* (X) Sciatic nerve
*(X) Trachea	*(X) Rectum	*(XX) Ovaries
*(X) Lungs	*(X) Gallbladder	*(XX) Testes
*(XX) Heart	*(X) Pancreas	(XX) Epididymides
*(X) Thoracic marrow	*(XX) Liver	*(XX) Pituitary
*(X) Salivary gland	*(XX) Kidneys	*(XX) Thyroids
*(X) Esophagus	*(X) Urinary bladder	*(XX) Adrenals
*(X) Stomach	(XX) Prostate	*(X) Eye
*(X) Thymus	*(X) Uterus	*(X) Aorta
*(X) Lymph nodes	*(X) Spleen	

* Recommended by the pesticide Assessment Guidelines Hazard Evaluation Series 82-1.

Results:

A. Organ Weight (Mean Values)

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Absolute Wt.								
Terminal Body Wt. (g)	12340	12040	12100	11775	10000	10330	9370	10350
Addrenals	1.322	1.203	1.218	1.362	1.243	1.229	1.457	1.275
Brain	81.59	78.13	76.02	83.61	75.49	73.17	73.51	73.80
Gonads	18.97	15.47	19.17	21.16	1.745	1.540	1.151	1.368
Heart	104.1	97.44	99.68	99.63	82.47	80.66	82.36	87.14
Kidneys	59.99	69.63	64.73	61.51	48.71	48.50	47.71	48.90
Liver	324.7	311.4	295.3	319.7	256.2	285.4	247.9	266.7
Pituitary	0.078	0.075	0.069	0.070	0.074	0.067	0.076	0.068
Prostate	11.38	11.34	12.13	15.74	-	-	-	-
Thyroids	0.993	0.921	1.063	1.258	0.976	0.951	0.898	0.801
Relative Wt.(%)								
Addrenals	0.011	0.010	0.010	0.012	0.013	0.012	0.016	0.013
Brain	0.644	0.651	0.631	0.719	0.771	0.717	0.797	0.700
Gonads	0.153	0.129	0.159	0.172	0.018	0.015	0.012	0.013
Heart	0.847	0.810	0.824	0.823	0.835	0.786	0.891	0.883
Kidneys	0.486	0.575	0.530	0.507	0.490	0.473	0.511	0.492
Liver	2.649	2.591	2.440	2.618	2.617	2.753	2.677	2.639
Pituitary	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Prostate	0.092	0.094	0.104	0.130	-	-	-	-
Thyroids	0.008	0.008	0.009	0.011	0.010	0.009	0.010	0.008

* Significantly different from control value, P<0.05.

Summary of Findings: There were no significant differences in absolute organ weights and organ/body weight ratios observed between the control and treated animal groups in this study.

B. Necropsy Observations

	Incidences of Gross Pathology Findings							
	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
No. of Animals Examined	5	5	5	5	5	5	5	5
Whole Animal								
Tail-Bent	0	2	0	1	0	0	0	0
Alopecia	3	3	2	0	2	3	2	4
Rough Hair	0	1	1	0	0	0	0	0
Umbilical Hernia	0	0	0	0	0	0	0	1

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3. Necropsy Observations - continued

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Skin								
Discoloration-General-Red	0	0	2	0	1	2	0	0
Dry	1	0	0	0	0	1	0	0
Focus-Raised	0	0	0	1	0	1	0	0
Abrasion	0	0	0	0	0	0	1	0
Mass (es)	0	0	0	0	0	0	0	1
Heart								
Rav. Valve-Cyst(s)-Red	1	0	0	0	1	2	0	2
Thymus								
Discoloration-Focal-Red	0	0	0	0	0	0	0	1
Lymph Nodes								
Discoloration-Focal-Red	0	0	0	1	0	0	0	0
Enlarged	0	0	0	1	0	0	0	0
Kidneys								
Cyst(s)	0	0	1	0	0	0	0	0
Medulla(AZ)-Discoloration-General-Tan	0	0	0	1	0	2	2	1
Discoloration-General-Tan	0	0	0	0	0	0	0	1
Corticomedullary Junction-Discoloration - General-Tan	0	0	0	0	0	3	2	0
Testes								
Discoloration-Focal-Red	0	0	0	1	-	-	-	-
Eyes								
Moist by Tears	0	0	0	1	0	0	0	0
Chromodacryorrhea	0	0	0	0	0	1	1	0
Vagina								
Discoloration-General-Red	-	-	-	-	0	0	1	0
Mucosa-Discoloration-General-Red	-	-	-	-	0	0	0	1

* Significantly different from control value, P<0.05.

Summary of Findings: Although there were no dose-related gross lesions observed in the treated animal groups, the increased incidences of a tan discoloration in the outer medullary or corticomedullary zones of the kidneys were found in 11 females and 1 male dogs treated with SC-0224. Five of the females were in the 2 mg/kg dose group, 4 in the 10 mg/kg group, and 2 in the 50 mg/kg group. The male was in the 50 mg/kg dose group. No other gross lesions considered to be significantly different between the control and the treated animal groups in this study.

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C. Histopathology

(a) Incidences of Non-Neoplastic Lesions

Organ	Males (mg/kg/day) 0	2	10	50	Females (mg/kg/day) 0	2	10	50
Skin								
No. Examined	5	5	5	5	5	5	5	5
No lesion	4	4	3	5	3	3	4	5
Folliculitis	1	1	2	0	0	0	0	0
Dermatitis	0	0	0	0	2	2	1	0
Lung								
No. Examined	5	5	5	5	5	5	5	5
No lesion	2	2	3	4	2	4	0	4
Bronchiolitis	0	0	1	0	1	1	0	0
Emphysema	0	1	0	0	0	0	0	0
Inflammation, Granulomatous	1	1	0	0	2	1	2	0
Inflammation, Interstitial	2	1	2	1	1	1	5	1
Parasitism	1	2	1	0	1	0	1	0
Fibrosis	0	0	0	0	0	0	1	0
Spleen								
No. Examined	5	5	5	5	5	5	5	5
No lesion	4	3	3	5	3	3	5	3
Fibrosiderosis	1	1	2	0	2	0	0	2
Hyperplasia, Nodular	0	1	0	0	0	0	0	0
Congestion	0	0	0	0	0	1	0	0
Hemosiderosis	0	0	0	0	0	1	0	0
Liver								
No. Examined	5	5	5	5	5	5	5	5
No lesion	1	2	2	4	0	3	3	2
Capsulitis	0	0	0	1	0	0	0	0
Cholangitis, Peri	0	0	1	0	0	0	0	0
Inflammation	2	3	0	1	2	1	2	0
Inflammation, Granulomatous	0	0	1	0	0	0	0	0
Inflammation w/ necrosis	1	0	0	0	0	1	0	0
Microgranuloma	1	0	2	0	3	1	1	2
Vacuolation, Cytoplasmic	0	0	0	0	0	1	0	0
Kidney								
No. Examined	5	5	5	5	5	5	5	5
No lesion	2	1	2	2	1	0	0	0
Cyst	0	0	1	0	0	0	0	0
Inflammation, Interstitial	1	0	0	0	0	0	0	0
Mineralization	0	4	2	1	2	1	0	1
Proximal Tubule-Vacuolation, Cytoplasmic	2	2	1	2	3	5	5	5
Testis								
No. Examined	5	5	5	5	-	-	-	-
No lesion	5	5	3	4	-	-	-	-
Degeneration	0	0	2	0	-	-	-	-
Hemorrhage/Congestion	0	0	0	1	-	-	-	-

(a) Incidences of Non-Neoplastic Lesions

Organ	Males (mg/kg/day) 0	2	10	50	Females (mg/kg/day) 0	2	10	50
Epididymis								
No. Examined	5	5	5	5	-	-	-	-
No lesion	2	2	4	2	-	-	-	-
Cele, Spermato	0	1	0	0	-	-	-	-
Inflammation	1	0	0	2	-	-	-	-
Inflammation, Granulomatous	0	1	0	2	-	-	-	-
Inflammation, Interstitial	2	2	1	1	-	-	-	-
Prostate								
No. Examined	5	5	5	5	-	-	-	-
No lesion	5	5	5	4	-	-	-	-
Inflammation	0	0	0	1	-	-	-	-
Brain								
No. Examined	5	5	5	5	5	5	5	5
No lesion	5	2	5	4	5	5	3	5
Cuffing, Perivascular	0	2	0	0	0	0	1	0
Gliosis	0	1	0	0	0	0	1	0
Hydrocephalus	0	0	0	1	0	0	1	0
Pituitary								
No. Examined	5	5	5	5	5	5	5	5
No lesion	5	5	5	5	5	4	3	3
Cele, Muco	0	0	0	0	0	1	2	2
Pinna								
No. Examined	2	1	1	1	1	0	0	1
Dermatitis	2	0	0	0	0	0	0	0
Folliculitis	0	0	1	0	1	0	0	0

* Significantly different from control value, $P<0.05$.

Summary of Findings: The non-neoplastic lesions were sporadically distributed among all the control and dosed animal groups. There were no treatment-related lesions observed in various organs of dosed animal groups in this study. Although all dosed and 3 of 5 control females exhibited some degree of tubular lipidosis, this study indicates that tubular lipidosis is a normal physiologic phenomenon in the adult beagle, more evident in females than males, and is not a toxic effect in animals exposed to SC-0224. However, the increased incidences of pathologic swelling in the pituitary of the dosed females cannot be evaluated properly without the historical control data of the beagle dogs.

C. Histopathology - continued

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(b) Incidences of Neoplastic Lesions

Organ	Males (mg/kg/day) 0	2	10	50	Females (mg/kg/day) 0	2	10	50
Pinna								
No. Examined	2	1	1	1	1	0	0	1
Neoplasm(s)-Benigh	0	1	0	1	0	0	0	
Hamartoma, Dermal	0	1	0	0	0	0	0	0
Histiocytoma, Canine Cutaneous	0	0	0	1	0	0	0	1

* Significantly different [illegible] value, P<0.05.

Summary of Findings: There [illegible] treatment-related neoplastic lesions observed in the pinna of dosed animals in this study.

III. Conclusions

1. There were no adverse effects on mortality, clinical signs, body weight, food consumption, ophthalmic normality, hematology, clinical chemistry, urinalysis and organ weight observed in the dosed animal groups during the course of this study attributable to the administration of SC-0224.

2. Although there were no dose-related gross lesions observed in the dosed animal groups, the increased incidences of a tan discoloration in the outer medullary or corticomedullary zones of the kidneys from the dosed animal groups cannot be evaluated properly without the historical control data of the beagle dogs.

3. The frequently observed non-neoplastic lesions were sporadically distributed among the control and the dosed animal groups. These incidences of non-neoplastic lesions did not display a dose-related positive trend. However, the increased incidences in the abnormal protrusion of pituitary from the dosed female groups (i.e. 0 in control; 1 in 2 mg/kg; 2 in 10 mg/kg; 2 in 50 mg/kg) cannot be evaluated properly without the historical control data of the beagle dogs.

4. Historical controls are also needed to evaluate the incidences of hamartoma and dermal histiocytoma of pinna described in this study.

5. There are no subchronic oral non-rodent studies which can be examined to assess the selection of dose levels in this one-year study. It is, therefore, difficult to assess whether or not they were properly selected. The pesticide Assessment Guidelines Hazard Evaluation Series 83-1 recommend a high dose which produces some signs of toxicity without causing excessive lethality. This was not achieved. Since there are insufficient data upon which to draw a conclusion regarding the high dose, Toxicology Branch must consider significant changes in LDH and phosphorous at 50 mg/kg/day as suggestive of toxicity, even though there were no observable morphological effects. Accordingly, the NOEL in this study is 10 mg/kg/day, unless significant data are provided to demonstrate beagle dogs can tolerate doses greater than 10 mg/kg/day for one year without adverse effects.

6. Classification of Data: Supplementary

Chronic Toxicity NOEL = 10 mg/kg/day
Chronic Toxicity LEL = 50 mg/kg/day
(decreased lactate dehydrogenase values)

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Request for Expedite Review of Sulfosate (SC-0224); Memo from E. Tinsworth, Director RD (6/9/87)

FROM: R. B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) 6/17/87

THRU: Theodore Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769)

TO: John W. Melone, Director
Hazard Evaluation Division (TS-769)

The referenced memorandum from Mr. E. Tinsworth is a request for an expedited review of Sulfosate in accordance with the specific directions of Mr. Campt. Sulfosate has been routinely handled by Tox Review Section #1. Toxicology Branch recently received additional data on this chemical which includes three long-term studies (1 yr dog; 2 yr rat; 2 yr mouse), plus some new mutagenicity data. In order for Toxicology Branch to comply with this request the data have been divided between Tox Review Sections #1 and #2. Nonetheless there is a substantial amount of new data and the earliest possible delivery date, after secondary and tertiary review, is August-September 1987 time-frame. These data are presently being reviewed within these respective Sections.

cc: A. Barton
E. Budd
W. Dykstra
J-H. Chen

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Sulfosate (SC-0224) 476-EEEL/476-EEEA: Review and and Assessment of the Toxicological Studies with SC-0224. Caswell No. 893C. MRID Nos. 402140-01, 402140-02, 402140-03, 402140-04, and 402140-05.

From: John H.S. Chen, D.V.M. John H.S. Chen 7/10/87
Review Section No. I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

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

Thru: Robert B. Jaeger, Section Head 7/16/87
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Petitioner:

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

Actions Requested:

1. Review and Assessment of the One-Year Chronic Oral Toxicity Study with SC-0224 in Beagle Dogs and the Mouse Micronucleus Test with SC-0224.

2. Review of the Registrant's Response to the Previous Toxicology Branch Review Comments (TB MEMO 9/10/86 Brian Dementi) Concerning the Mutagenicity Studies with SC-0224.

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Toxicology Branch Recommendation:

1. The Registrant should be apprised of the following deficiencies noted in the following studies which are identified in the detailed review:

A. One-Year Chronic Oral Toxicity Study with SC-0224 in Beagle Dogs. April 3, 1987. MRID No. 402140-05.

B. Mutagenicity Evaluation in Mouse Micronucleus Test with SC-0224. April 3, 1987. MRID No. 402140-04.

2. Registrant's responses to the reporting deficiencies cited in the previous Toxicology Branch Review of the following mutagenicity studies are considered reasonable and acceptable. The following mutagenicity studies with SC-0224 are upgraded to be acceptable:

A. Mouse Lymphoma Mutation Assay with SC-0224. EHC Report No. T-12661. December 19, 1985.

B. Cytogenetic Assays (Chromosomal Aberration and Sister Chromatid Exchange) with SC-0224 in the Mouse Lymphoma (L5178Y) Cell Systems. EHC Report No. T-12662. December 19, 1985.

C. Cytogenetic Assays (Chromosomal Aberration and Sister Chromatid Exchange) with SC-0224 in the Chinese Hamster Ovary Cell Systems. EHC Report No. T-12663. December 18, 1985.

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84-1 - Mouse - Micronucleus Test

Reviewed by: John H.S. Chen 7/10/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger 7/10/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Mouse Micronucleus Test

TOX. CHEM. No.: 693C

Accession No.:

MRID No.: 402140-04

Test Material: SC-0224 (EHC-0701-25; Lot No. JHC 8865-20-1; 55.3% Purity)

Study Number(s): T-1[illegible]89

Sponsor: Stauffer Chemical Company

Test Facility: Environmental Health Center, Stauffer Chemical Co.

Title of Report: Mutagenicity Evaluation in Bone Marrow Micronucleus

Author(s): J.B. Majeska and D.W. Matheson

Report Issued: April 23, 1987

Conclusions:

Failed to induce any significant increase in the number of PCE containing micronuclei from animals dosed with SC-0224.

Dose levels tested: 700, 900, and 1100 mg/kg for males and 400, 600, and 800 mg/kg for females

Classification of Data: Unacceptable

(Detailed range-finding test results were missing in the study report)

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Title of Report: Mutagenicity Evaluation in Mouse Bone Marrow Micronucleus

I. Procedures

1. Test Animals

The test compound, SC-0224, dissolved in distilled water, was administered once via oral gavage to groups of 15 male and 15 female mice (Charles River D-1 strain; 6-7 weeks old; 21-28 grams) at 3 predetermined dose concentrations (i.e., 700, 900, and 1100 mg/kg for the males; 400, 600, and 800 mg/kg for the females) in the first trial. In a second micronucleus assay, the same procedure was followed using only female mice. Concurrently, fifteen female and 15 male vehicle control mice were treated with distilled water and five female and 5 male positive control mice treated with cyclophosphamide (150-200 mg/kg). At the end of specific intervals (i.e., 24, 48, and 72 hours after treatment), animals were sacrificed and the tibia and femur of each animals were removed according to the following post-treatment sampling times.

Treatment	30 Hours		48 Hours		72 Hours	
1st Trial	M	F	M	F	M	F
Vehicle control	5	-	5	-	5	-
SC-0224, 700 mg/kg	5	-	5	-	5	-
" 900 "	5	-	5	-	5	-
" 1100 "	5	-	5	-	5	-
CPA, 100 "	-	-	5	-	-	-
" 150 "	-	-	5	-	-	-
Vehicle control	-	5	-	5	-	5
SC-0224, 400 mg/kg	-	5	-	5	-	5
" 600 "	-	5	-	5	-	5
" 800 "	-	5	-	5	-	5
CPA, 150 "	-	-	-	5	-	-
" 200 "	-	-	-	5	-	-
2nd Trial						
Vehicle control	-	5	-	5	-	5
SC-0224, 400 mg/kg	-	5	-	5	-	5
" 600 "	-	5	-	5	-	5
" 800 "	-	5	-	5	-	5
CPA, 150 "	-	-	-	5	-	-
" 200 "	-	-	-	5	-	-
Total	20	40	30	60	20	40

2. Slide Preparation for the Bone Marrow Cells

Bone marrow cells were flushed from the femurs into centrifuge tubes containing newborn calfserum. Following centrifugation to pellet the cells, the bone marrow was resuspended in 0.2 ml of serum. This suspension was placed on a clean microscope slide and spread with a second slide. Slides were fixed in absolute methanol and stained with 2%

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2. Slide Preparation - continued

Giemsa in phosphate buffer (pH 6.8). One thousand polychromatic erythrocytes (PCE) were scored from each mouse for the presence of micronuclei.

3. Statistical Analysis

The Kastenbaum-Bowman tables (1975) were used to determine statistical significance, considering the number of micronuclei and PCE's evaluated at each dose level and time point. A substance is positive if it induces a response that reaches the $P<0.01$ level of significance compared to solvent controls and shows dose and/or time related pattern of activity.

II. Results

1. In range-finding test (data not shown), there were deaths in male mice at a dose level greater than or equal to 1400 mg/kg. Although no death or clinical signs were noted in the dose range of 200-700 mg/kg, a reduction of PCE frequency was observed in the 700 mg/kg dose males. Therefore, the doses of 700, 900, and 1100 mg/kg were chosen for the male mice in this study. Female mice were found to be more sensitive to the exposure of SC-0224, and there were deaths at a dose level greater or equal to 1000 mg/kg. Therefore, the doses chosen for the micronucleus assay were 400, 600, and 800 mg/kg for the females.

2. Summary of Micronucleus Data (Mean Values)

Treatment	Time (hrs)	No. of Animals	No. of PCE	No. of Micronuclei	No. of Micronuclei/ Animal	Ave. No. PCE/1000 Erythrocytes
1st Trial - Males						
Vehicle control	24	5	5000	3	0.6	151
	48	5	5000	3	0.6	165
	72	5	5000	3	0.6	185
SC-0224, 700 mg/kg	24	5	5000	0	0	344
	48	4	4000	5	1.3	237
	72	5	5000	1	0.2	159
" 900 "	24	5	5000	6	1.2	247
	48	5	5000	3	0.6	170
	72	5	5000	0	0	161
" 1100 "	24	5	5000	1	0.2	183
	48	5	5000	3	0.6	119
	72	5	5000	1	0.2	121
CPA, 100 mg/kg	48	5	5000	14**	2.8	72
150 "	48	5	5000	16**	3.2	20

2. Summary of Micronucleus Data - continued

Treatment	Time (hrs)	No. of Animals	No. of PCE	No. of Micronuclei	No. of Micronuclei/ Animal	Ave. No. PCE/1000 Erythrocytes
1st Trial - Females						
Vehicle control	24	5	5000	9	1.8	282
	48	5	5000	25	5.0	332
	72	5	5000	1	0.2	247
SC-0224, 400 mg/kg	24	5	5000	2	0.4	276
	48	5	5000	26	5.2	222
	72	5	5000	5	1.0	279
" 600 "	24	5	5000	12	2.4	269
	48	5	5000	22	4.4	234
	72	5	5000	3	0.6	250
" 800 "	24	5	5000	15	3.0	255
	48	5	5000	25	5.0	276
	72	5	5000	1	0.2	254
CPA, 150 mg/kg	48	5	7500	78*	10.4	57
200 "	48	5	5000	7	1.4	27
2nd Trial - Females						
Vehicle control	24	5	5000	2	0.4	281
	48	5	5000	0	0	420
	72	5	5000	6	1.2	372
SC-0224, 400 mg/kg	24	5	5000	4	0.8	286
	48	5	5000	0	0	348
	72	5	5000	13	2.6	320
" 600 "	24	5	5000	5	1.0	225
	48	5	5000	4	0.8	247
	72	5	5000	6	1.2	351
" 800 "	24	5	5000	7	1.4	271
	48	5	5000	1	0.2	301
	72	5	5000	3	0.6	292
CPA, 150 mg/kg	48	5	4629	47**	10.1	34
200 "	48	5	3215	62*	19.3	25

* Significantly greater than vehicle control value, $P<0.05$.
** Significantly greater than vehicle control value, $P<0.01$.
CPA = Cyclophosphamide

Summary of Findings:

i. The spontaneous rates of micronuclei in the PCE found from the vehicle control groups (Male mice: 0.06%; Female mice: 0.02-0.5%) were considered within the normal range in the region of less than 0.6% (J.A. Heddle et al (1983)., A Report of U.S.E.P.A. Gene-Tox Program).

ii. The positive control compound, CPA, apparently induced marked increase of the PCE with micronuclei which indicated the sensitivity of the assay system.

iii. The test compound, SC-0224, did not induce any significant increase in the number of PCE containing micronuclei from animals dosed with SC-0224 (400 through 1100 mg/kg) at all the time intervals evaluated.

III. Conclusion

Since the detailed range-finding test results were not included in this study report, it is unclear that the highest tolerated dose of the test compound was actually used in this study. According to the current EPA Health Effects Test Guidelines in performing the mouse micronucleus test (EPA 560/6/83-001), the highest tolerated dose level should produce some indication of cytotoxicity of the test compound in the bone marrow of dosed animals (i.e., The ratio of polychromatic to normochromatic erythrocytes should be clearly below that of the vehicle control animals). Therefore, the submitted report is incomplete and unacceptable in the present form. However, the study may be upgraded on resolution of the reporting deficiency.

Review of the Registrant's Response to the Previous Toxicology Branch Review Comments Concerning the Following Mutagenicity Studies with SC-0224 (Toxicology Branch Memorandum 9/10/86 Brian Dementi)

I. Mouse Lymphoma Mutation Assay with SC-0224, EHC Report No. T-12661, December 19, 1985 and II. Cytogenetic Assay (chromosomal aberration and sister chromatid exchange) with SC-0224 in the Mouse Lymphoma (L5178Y) Cultured Cell System, EHC Report No. T-12662, December 19, 1985 Accession No 260966

Registrant's Response:

"1. The positive control compounds were tested at the same pH as the solvent control in all experiments. The culture medium used in these studies contains a color indicator that permits the pH to be visually monitored throughout an experiment. In these studies the culture medium was adjusted to approximately pH 7.4 and the solvent, positive control substance or SC-0224 was added to form 10X stock solutions. When necessary, the pH of the stock was readjusted to approximately pH 7.4 by the addition of NaOH. The final pH after readjustment was measured by pH meter. Since the solvent control and positive control compounds did not shift the color of the pH indicator, the pH was not readjusted or further measured. We have subsequently made measurements on the positive control compounds EMS and DMN and verified that they do not shift the pH of the medium significantly. Furthermore, it is well documented that L5178Y cells are responsive to chemicals at physiological pH's and if they were not the assay would not be very useful. Clive routinely adjusts his test media to the 7.2 to 7.4 range. Since the cells responded to EMS and DMN at neutral pH, the difference between the results of the two experiments can not be due to a pH induced change in the competence of the cells, but rather to some change in the chemical's activity. Also, because we are determining the effect of pH on the mutagenicity of SC-0224, the proper control for this experiment is SC-0224 at unadjusted pH levels."

Reviewer's Comments:

The provided supplemental information concerning the culture conditions (pH and osmolality values) for all the positive control compounds used in these studies are considered adequate. The submitted explanation for confirming the adequacy of the L5178Y cell systems under the adjusted acidic test conditions (pH 7.4) from these studies is also considered reasonable. Because the recent published studies (1, 2, and 3) clearly indicate that false positive responses in the cultured L5178Y cells can be produced either by low pH treatment conditions or by high osmotic levels alone in the culture media, Toxicology Branch agrees that the treatment conditions (pH and osmolality values) should be considered for the interpretation of test results.

"2. Solubility and cytotoxicity are the factors usually used to establish the highest dose in an experiment. Doses for SC-0224 under the adjusted conditions were chosen by the convention that for freely soluble compounds an upper level of 5 ul/ml is usually considered sufficient and therefore 10 ul/ml

is more than adequate. Furthermore, any further increase in dose raised the osmolality of the medium to near or above 400 mOsm, established as a critical level and readjusting the pH to neutrality would have raised it even higher. The reviewer notes that only slight toxicity occurred under pH adjusted conditions. To determine the significance of this comment, mutation frequencies for pH unadjusted and pH adjusted conditions should be compared at near equivalent toxicities. The lowest cell survived levels for non-activation and activation studies that could be obtained under pH adjusted conditions at 10 ul/ml SC-0224 and 400 mOsm were approximately 30% and 77% respectively. No significant increase in mutation frequencies was observed. At the closest comparable toxicities under non-adjusted conditions mutation frequencies were approximately twice background. These observations demonstrate an apparent dissociation between mutagenicity and toxicity under these test conditions."

Reviewer's Comments:

The provided rationale for selecting 10 ul/ml of SC-0224 as the MTD in these stuides under the adjusted acidic test conditions (pH 7.4) is considered to be justified.

Recommendation:

The test compound, SC-0224 was not a clastogenic agent and did not induce any increase of mutant frequency in the cultured L5178Y mouse lymphoma cell system with and without metabolic activation under the pH adjusted test conditions (pH 7.4) at the concentrations tested (4 through 10 ul/ml). The positive responses of SC-0224 that were observed in these studies under the pH unadjusted test conditions (pH 5.62-7.07) were primarily associated with either reduced pH or increased ion concentrations in the culture media during the treatment periods and cannot be used for the interpretation of test results in these studies. These studies are upgraded to be acceptable.

References:

1. Cifone, M.A., Fisher J., and Myhr, B.(1984): Evidence for pH Effect in the L5178Y TK^{+}/$^{-}$Mouse Lymphoma Forward Mutation Assay. Environ. Mutagen 6:423.

2. Cifone, M.A.(1985): Relationship Between Increases in the Mutant Frequency in L5178Y TK+/- Mouse Lymphoma Cells at Low pH and Metabolic Activation. Environ. Mutagen 7: (Suppl3);27.

3. Brusick, D. (1986): Genotoxic Effects in Cultured Mammalian Cells Produced by Low pH Treatment Conditions and Increased Ion Concentrations. Enviro. Mutagen 8: 879-886.

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III. Cytogenetic Assays (Chromosomal Aberration and Sister Chromatid Exchange) with SC-0224 in the Chinese Hamster Ovary Cell System, EHC Report No. T-12663, December 18, 1985 Accession No 260962

Registrant's Response:

"1. My copy of the guidelines referenced for the in vitro chromsomes proceudres states: "the highest test substance concentration tested with and without metabolic activation should show evidence of cytotoxicity or reduced mitotic activity." "Relatively insoluble substances should be tested up to the limits of solubility." "For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis." No mention was made of suppressing mitotic activity 50%. The guidelines for in vitro sister chromatid exchanges paraphase the same requirements. For the study in question, T-12663, several factors went into determining the highest dose. First, 5 ul/ml is generally accepted by the scientific community as a reasonable upper limit for most soluble test chemicals. Second, as indicated in the report summary, page 3, higher doses of SC-0224 adjusted to pH approximately 7.4 would have increased the osmolality above the 400 mOsm level reported by other authors to be a level at which artifacts may occur; third, studies previously submitted (T10875) had defined the response under non-adjusted pH (acid) conditions.

2. The literature supports the position that CHO cells are responsive to test chemicals under physiological (pH 7.4) conditions. Our culture media includes a pH indicator that did not indicate a shift in pH upon addition of the positive control compounds. This visual observation has been verified by pH measurements. Furthermore, the media for the positive control compounds, the solvent controls and the test chemicals once adjusted, were maintained at near equilibrium by the buffer system in the medium and the CO_2 in the incubators.

3. The response of CHO cells to SC-0224 under "standard test conditions" has been reported previously (T10875). Furthermore, there is really no basis for considering this assay to be non-standard. The guidelines cite only that "appropriate culture media and incubation conditions ...," should be used. Since the test substance response is being compared to the solvent control, it does not seem inappropriate to have them both at the same pH initially.

4. Reference to the guidelines shows "For established cell lines and strains, multiple harvest times are recommended." "If the test chemical changes the cell cycle length, the fixation intervals should be changed accordingly." For sister chromatid exchanges "A single harvest time, one that yields an optimal percentage of second division metaphases is recommended." "If there is reason to suspect that this is not a representative sampling time ..., then additional harvest times should be selected." The sister chromatid exchange assay provides a convenient means for monitoring population doubling time that reflects changes in the length of the cell cycle. Table 1, in report T-12663 provides this information. After 20 hours in culture the number of cells in treated cultures was similar to the number in control cultures. The average relative staining index shows that at

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least 91% of the cells had progressed through two cell divisions by the time of harvest for all doses. These two measurements show that it is unnecessary to use multiple harvest times for the cytogenetic assay or to extend harvest times for the sister chromatid exchange assay. I did not find reference to a requirement for using "at least three harvest times."

I disagree with the reviewer's conclusion that this study (T-12663) is inconclusive. I would hope that our response to his concerns will remove any ambiguities and enable him to reclassify the results as acceptable."

Reviewer's Comments:

The submitted rationale for selecting 10 ul/ml of SC-0224 as the MTD in these studies under the adjusted pH test conditions (pH 7.4) is considered reasonable. Toxicology Branch agrees that the treatment conditions (pH and osmolality values) should be considered in the context of dose level selection for these cytogenetic studies in the CHO cell system (Brusick, Environ. Mutagen 8: 879-886, 1986). Furthermore, the submitted addendum provides adequate information for the clarification of reporting deficiencies cited in the previous Toxicology Branch review of thiese studies. The request for the test results of these studies under the unadjusted pH test conditions is unnecessary (See reasons given in the recommendation for T-12661 and T-12662). However, it may be appropriate to point out that under normal test condition, the highest test substance concentration selected should show a cytotoxic effect but allows sufficient metaphases for a reliable analysis (generally, a 50% reduction in mitotic index as compared to the solvent control is acceptable).

Recommendation:

The test compound, SC-0224, was not a clastogenic agent in the cultured CHO cell system with and without metabolic activation at the concentrations tested (4 through 10 ul/ml). These studies are upgraded to be acceptable.