



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

CASWELL FILE

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 *RFJ 6/17/87*  
Review Section #1  
Toxicology Branch/HED (TS-769)

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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JUL 29 1987

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Sulfosate (SC-0224) 476-EEEL/476-EEEE: 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 *RBJ 7/16/87*  
Review Section I  
Toxicology Branch  
Hazard Evaluation Division (TS-769C) *W. J. Bunn*

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.

2

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.

84-1 - Mouse - Micronucleus Test

Reviewed by: John H.S. Chen  
Section I, Toxicology Branch (TS-769C)

Secondary reviewer: R.B. Jaeger  
Section I, Toxicology Branch (TS-769C)

*John H.S. Chen 7/10/87*

*R.B. Jaeger 7/10/87*

DATA EVALUATION REPORT

Study Type: Mouse Micronucleus Test

TOX. CHEM. No.: 8930

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-12689

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)

A

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.

<u>Treatment</u> <u>1st Trial</u>	<u>30 Hours</u>		<u>48 Hours</u>		<u>72 Hours</u>	
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
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	-	-
<u>2nd Trial</u>						
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%

5

## 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 Micro- nuclei	No. of Micro- nuclei/ Animal	Ave. No. PCE/1000 Erythro- cytes
<u>1st Trial - Males</u>						
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	5000	16**	3.2	20

6

## 2. Summary of Micronucleus Data - continued

Treatment	Time (hrs)	No. of Animals	No. of PCE	No. of Micro- nuclei	No. of Micro- nuclei/ Animal	Ave. No. PCE/1000 Erythro- cytes
<u>1st Trial - Females</u>						
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 "	5	5000	7	1.4	27
<u>2nd Trial - Females</u>						
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 "	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:

1. 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).

77

- 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.

8



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

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. Glive 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

9

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 studies 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<sup>+</sup>/Mouse Lymphoma Forward Mutation Assay. Environ. Mutagen 6:423.
2. Cifone, M.A.(1985): Relationship Between Increases in the Mutant Frequency in L5178Y TK<sup>+</sup>/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.

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

Registrant's Response:

"1. My copy of the guidelines referenced for the in vitro chromosomes procedures 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 paraphrase 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 a near equilibrium by the buffer system in the medium and the CO<sub>2</sub> 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

11

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 these 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.