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

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SEP 10 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 476-EEEL/476-EEEA

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

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

DQR for RBJ 9-8-86

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

Brian Dementi 9/8/86

E.R. 9/9/86

9/10/86

The following studies submitted by Stauffer Chemical Company in support of registration of SC-0224 concentrate and SC-0224 4-LC nonselective foliar systemic herbicides (Ref: Letter of Ralph L. Riggs to Mr. Robert J. Taylor, January 13, 1986) have been reviewed and are herewith submitted to your office.

1. MUTAGENICITY EVALUATION of SC-0224 in SALMONELLA TYPHIMURIUM. Report No. T-12660, Septmeber 25, 1985, Accession No. 260966.

Evaluation: The test compound, SC-0224 (55.6% purity), was considered non-mutagenic in the Ames Test at the concentration tested. The study is acceptable.

2. MOUSE LYMPHOMA MUTATION ASSAY with SC-0224. Report No. T-12661, December 19, 1985. Accession No. 260966.

Evaluation: The test compound, SC-0224, was considered mutagenic in this assay with and without metabolic activation under the normal test conditions and concentrations tested. The study is acceptable.

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3. CYTOGENETIC ASSAYS (CHROMOSOMAL ABERRATION and SISTER CHROMATID EXCHANCE) with SC-0224 in the MOUSE LYMPHOMA (L5178Y) CULTURED CELL SYSTEM. Report No. T-12662, December 19, 1985, Accession No. 260966.

Evaluation: The test compound, SC-0224, was considered clastogenic in mouse lymphoma cells with and without metabolic activation at the dose levels tested. SC-0224 was also positive for sister chromatid exchanges in lymphoma cells with and without metabolic activation. Both studies are acceptable.

4. CYTOGENETIC ASSAYS (CHROMOSOMAL ABERRATION and SISTER CHROMATID EXCHANGE) with SC-0224 in the CHINESE HAMSTER OVARY CELL SYSTEM. Report No. T-12663, December 18, 1985, Accession No. 260966.

Evaluation: The two studies in question are considered inadequate due essentially to lack of conformity to procedures for in vitro cytogenetic and sister chromatid exchange assays as recommended by EPA (EPA HEALTH EFFECTS TEST GUIDELINES 560/6-83-001). See reviews for further clarification.

5. A TERATOLOGY STUDY in NEW ZELAND WHITE RABBITS with SC-0224. Report No. T-11052, June 21, 1983, Accession No. 260966 (Appendix 1).

Evaluation: Findings are summarized as follows:

a) Increased maternal mortality
LOEL = 100 mg/kg/day, NOEL = 40 mg/kg/day

b) Increased spontaneous abortion
LOEL = 100 mg/kg/day, NOEL = 40 mg/kg/day

c) Maternal Toxicity
LOEL = 10 mg/kg/day, NOEL = undetermined

d) Reduced feed intake during days 7-20.
LOEL = 40 mg/kg/day, NOEL = 10 mg/kg/day

Core Rating: Guideline

TS-769:DEMESTI:sll:473T10:9/5/86

Study: A Teratology Study in New Zeland White Rabbits with SC-0224.

Laboratory: Environmental Health Center
Stauffer Chemical Company
Farmington, Connecticut.

Study Number and Date: T-11052, June 21, 1983.

Accession Number: 260966 (Appendix 1)

Material Tested: SC-0224, 56.2% Pure

Animals: New Zeland White Rabbit [Dla: (NZW)SPF]

Procedure (As paraphrased or quoted from the study protocol):

Following a 45-day quarantine period, 71 virgin females ranging in age 185-213 days were cohabited with 6 males to obtain a total of 56 successfully mated females which were then assigned to the study.

Fifteen mated females were assigned to each of 4 dosage groups, with 6 additional animals being assigned to the highest dosage group. The date of mating was designated as day 0 of gestation. These females subsequently received 13 consecutive daily doses of 0, 10, 40 or 100 mg/kg of SC-0224 in tap water on days 7 through 19 of gestation. Aqueous solutions used for administration of the test compound were prepared to contain 0, 5.0, 20.0 and 50.0 mg/ml. Analytical determinations were made to assure these levels.

Animals were observed daily for clinical evidence of change. Body weights were determined on days 0, 7, 14, 21 and 30. Feed intakes were recorded for gestational intervals 0-7, 7-14, 14-21 and 21-30. The females were sacrificed on day 30 of gestation and necropsied. The liver, kidneys, spleen, ovaries, and heart were weighed and preserved in 10% neutral buffered formalin or 2.5% buffered glutaraldehyde. Paired organs were weighed separately. Placentas were weighed collectively for live fetuses, individually for dead fetuses, or attached for resorptions. Uteri without conceptuses and ovaries were weighed.

Ovaries were examined to determine the number of corpora lutea. The uterus was opened and examined for the number and distribution of fetuses and resorptions. Resorption sites were noted as early, if there was no fetal tissue present; as mid, if fetal tissue was present, but without recognizable features; or as late, if the conceptuses showed either external degenerative changes or an arrested state of development. Placenta and associated fluids were inspected for any unusual appearances. Obvious malformations in late resorptions were described, when present, but not examined further or included

in statistical analyses of structural deviations.

Fully developed fetuses were classified as dead if reflexes were absent when the neck was pressed at the time it was removed from the uterus. Dead fetuses were subsequently examined for anomalies, but not included with live fetuses for statistical analyses of structural malformations.

All fetuses were weighed and examined for external malformations. Pups within a litter which weighed less than three-fourths of the litter mean were designated as calculated runts. Live pups were sacrificed by intrathoracic injection of sodium pentobarbital. Identity of the pups was maintained throughout the examination process.

Each fetus was examined for both soft-tissue and skeletal anomalies. The head of each fetus was removed and fixed in Bouin's fixative for examination of the organ structure by a modification of the Wilson (1965) serial cross-section technique. The trunk of each fetus was examined internally by a modification of the Staples (1974) fresh dissection technique. The sex of each fetus was determined at this time. Following the internal examination, the fetus was eviscerated and processed by a modification of the Kimmel (1981) procedure for skeletal examinations" p.3.

Principle findings are presented as follows:

1. Among the control and three dose groups of females, deaths occured during the observation period only in the high dose group where mortality was 38%, a statistically significant finding. In addition, fertility was numerically reduced in the high dose group (81%) as compared with controls (93%), but the reduction was not statistically significant.

2. Four females (36%) of the high dose group aborted, an increase which was statistically significant.

3. Of 14 surviving pregnant females of the low dose group on gravid day 30, seven were observed to exhibit clinical signs of toxicity including diarrhea, head tilt, nasal discharge, wet stains (chin), scab (mouth), red urine and red stains on cage pad. These clinical findings constituted a significant increase for the low dose group with respect to the control group. Toxic signs were essentally absent in the control group. Some of the same toxic signs were observed in the middle and high dose groups, but were not reported as statistically significant

in the study itself. However, in the high dose group 10 of 17 pregnant females were apparently eliminated from gravid day 30 observations due to unscheduled sacrifice (Table 2, p. 14). There was much evidence of toxicity among the ten gravid females which underwent unscheduled sacrifice. Notable findings included red foci of the lungs and reddened trachea/laryrx (Table 3, p.16).

Necropsy findings for terminally sacrificed animals, as revealed in Table 4 (p. 17), suggest increased numbers of lesions in the low dose group, but probably is not statistically significant. Necropsy evaluations on ten rabbits (unscheduled sacrifice, Table 3, p. 15) of the high dose group certainly indicate toxic effects of SC-0224.

Maternal Body Weights and Feed Intakes

Mean body weight determinations on pregnant survivors (14 rabbits each from control, low dose and middle dose groups and 7 rabbits from the high dose group) did not reveal any adverse effects related to dosing.

Feed intake was significantly reduced in the high dose group during days 7-14 and 14-21, but numerically (not signigicant) increased on days 21-30. Total feed intake for days 7-30 was significantly reduced for this group. Feed intake was essentially unaltered in the other groups excepting a significant reduction during days 7-14 in the 40 mg/kg dose. Similarly feed intake on a per kg body weight basis was significantly reduced in the high dose group, with consistency (Table 5, p. 19).

Maternal Organ Weights

Absolute organ weights measured of pregnant survivors were unaltered by dosing. There was a numerical reduction in mean reproductive tract weight of the high dose group (379 ± 108 gms vs. control 434 ± 80), but was not a statistically significant change. Similarly, relative organ weight data did not reveal any compound related adverse effect. (Table 6 p. 20).

Intrauterine Data

Intrauterine findings as disclosed on the basis of a variety of expressions, eg. corpora lutea/dam, implants/dam, live fetuses, dead fetuses, implants/corpora lutea, % viable implants, % resorptions, fetuses/dam, live fetuses/implants, mean placental weight/live fetus, etc. did not disclose any

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meaningful compound related effects. (Perhaps it should be noted that live fetuses/dam and live fetuses/implants (-) were numerically reduced in the high dose group with respect to the control group (5.4 ± 1.5 vs. 7.4 ± 2.0 and 78 ± 20 vs. 88 ± 13, respectively) (Table 7, p. 21).

External Anomalies

External examinations revealed numerically more anomalies among the yet smaller number of fetuses in the high dose group. Identified anomalies included domed cranium w/wo prominent vasculature, meningocele, prominent eye, enlarged fontanels, dark distended abdomen, arthrogryposis of forelimb. This increased number of anomalies was not reported as statistically significant, but may indeed reflect adverse effects of SC-0224 at the highest dose. There were no meaningful findings at the lower doses. (Table 8, p. 22).

Soft Tissue Anomalies

Table 9 (p. 23) reveals the number of male and female fetuses in each group. Percentages of males in each group are: group 1 (45.6%), group 2 (43%), group 3 (45%) and group 4 (39.5%). We do not consider that the numerically smaller percentage of males in the high dose group to be significant.

The number of specific anomalous findings in the various groups does not suggest an adverse effect of SC-0224. Examples of anomalies identified include: head (hydrocephalus, cleft palate, dilated 4th ventricle, etc.); thorax (fluid-filled pericardium, reddened lungs); abdomen (necrotic focus on liver, small pear shaped gallbladder, pale spleen, etc.). The tal number of rats in the highest dose group with anomalies, expressed as % of fetuses examined, is suggestive of an effect of SC-0224 at the highest dose. The percentage of fetuses having anomalies ranked 3 and 4 are 10.5% and 7.9%, respectively. The comparable percentages for the control group are both 2%. The numerically higher percentage of more severe anomalies in group 4 is not indicated as significant. There appear to be no specific anomalies accounting for this difference as tabulated.

Skeletal Anomalies

No remarkable findings at any dose with respect to skeletal anomalies were reported. Table 10, p. 25.

Summary

A. Statistically Significant Findings

1. Increased maternal mortality, LOEL = 100 mg/kg/day
NOEL = 40 mg/kg/day

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2. Increased spontaneous abortion, LOEL = 100 mg/kg/day
   NOEL = 40 mg/kg/day

3. Clinical evidence of maternal toxicity, LOEL = 10 mg/kg/day
   NOEL = undetermined

4. Reduced feed intake during days 7-30, LOEL = 40 mg/kg/day
   NOEL = 10 mg/kg/day

Developmental Toxicity Index*

$$\text{A/D Ratio} = \frac{\text{LOEL, maternal toxicity}}{\text{LOEL, fetotoxicity}}$$

$$= \frac{10 \text{ mg/kg/day}}{> 100 \text{ mg/kg/day}}$$

$$= < 0.1$$

Core Rating: Guideline

* Chitlik, et al. (1985) HED/SEP Teratology Studies. EPA-540/9-84-018.

Study: Mutagenicity Evaluaton of SC-0224 in Salmonella typhimurium Environmental Health Center Report No. T-12660, September 25, 1985 (Author: J. B. Majeska), Accession No. 260966.

Procudure:

The mutagenic activity of SC-0224 (Lot No. JHC 2865-20-1; 55.6% Purity) dissolved in water at five concentrations (2.5, 5, 10, 20, and 40 ul/ml) was evaluated by the plate incorporation procedures and the pre-incubation procedures of Ames test in the presence and absence of metabolic activation (Mutation Res. 31:347-364, 1975). The histidine-requiring strains of Salmonella typhimurium (TA1535, TA1537, TA98, and TA100) were used in this study. The in-vitro mammalian metabolic activation system consisted of male rat or male mouse liver induced by Aroclor 1254 and cofactor solution described by Ames. Mutations were quantified on triplicate plates for each strain by counting his+ revertant colonies after 48 hours of incubation at 37 C on a histidine deficient agar. If the compound is mutagenic, it would demonstrate at least 3-fold increase over the control value and also exhibited a dose-related increase in the number of histidine independent colonies. Positive controls and solvent control run concurrently with the test compound in this study.

Results:

(A) Standard Plate Assay Results - Mean number of his+ revertant colonies per plate.

Treatment Per Plate	TA1535			TA1537			TA98			TA100		
	-S9	+S9a	+S9b	-S9	+S9a	+S9b	-S9	+S9a	+S9b	-S9	+S9a	+S9b
Solvent Control (Water, 50 ul)	28	29	23	12	13	14	48	52	55	148	166	171
Positive Control												
SA, 10 ug	1155*	-	-	-	-	-	-	-	-	1004*	-	-
9-AA, 50 ug	-	-	-	251*	-	-	-	-	-	-	-	-
2-NF, 10 ug	-	-	-	-	-	-	509*	-	-	-	-	-
2-AA, 5 ug	-	309*	350*	-	260*	378*	-	1375*	1847*	-	1892*	2238*
SC-0224												
2.5 ul	29	27	32	10	16	13	46	54	54	139	150	172
5.0 ul	28	22	23	11	11	9	49	45	52	128	166	172
10.0 ul	27	26	27	10	14	9	40	56	50	115	142	149
20.0 ul	11	20	16	10	10	9	32	42	35	69	91	119
40.0 ul	0	16	19	0	7	2	0	41	41	12	10	81

* Significantly different from the solvent control: greater than 3-fold increase over the solvent control value.
SA = Sodium Azide; 9-AA = 9-Aminoacridine; 2-NF = 2-Nitrofluorene; 2-AA = 2-Amino-anthracene; +S9a = Aroclor 1254 induced rat liver S9; +S9b = Aroclor 1254 induced mouse liver S9.

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(B) Pre-incubation Assay Results - Mean number of his+ revertant colonies per plate

Per Plate	TA1535			TA1537			TA98			TA100		
	-S9	+S9a	+S9b	-S9	+S9a	+S9b	-S9	+S9a	+S9b	-S9	+S9a	+S9b
Solvent Control (Water, 50 ul)	21	25	20	12	12	14	36	39	38	149	174	150
Positive Control												
SA, 10 ug	995*	-	-	-	-	-	-	-	-	664*	-	-
9-AA, 50 ug	-	-	-	65*	-	-	-	-	-	-	-	-
2-NF, 10 ug	-	-	-	-	-	-	663*	-	-	-	-	-
2-AA, 5 ug	-	308*	291*	-	522*	456*	-	2167*	2251*	-	2460*	2388*
SC-0224												
2.5 ul	23	19	22	9	8	11	30	37	27	132	161	139
5.0 ul	15	19	13	7	12	11	32	31	29	63	143	149
10.0 ul	11	14	16	4	7	5	26	35	31	13	11	114
20.0 ul	0	9	12	0	3	5	0	23	20	0	14	37
40.0 ul	0	0	0	0	7	0	0	0	0	0	0	0

* Significantly different from the solvent control: greater than 3-fold increase over the solvent control value.

Summary of Findings:

a. Because there were clearly toxic effects observed in the concentration of 40 ul/plate SC-0224 in the standard plate assay as well as in the pre-incubation assay under the activation and nonactivation systems, the concentration range of SC-0224 selected for this study was from 2.5 to 40 ul/plate.

b. The spontaneous revertant colonies for each of these four strains of Salmonella typhimurium were found within the normal range of his+ revertant colonies recommended by the Ames test (i.e., W/O S9:TA1535, 10-50; TA1537, 5-19; TA98, 15-45; TA100, 100-200. W/S9: TA1535, 4-26; TA1537, 5-19; TA1538, 13-39; TA98, 15-50; TA100, 100-200).

c. The strain specific control compounds (i.e., sodium azide, 9-aminoacridine, and 2-nitrofluorene) and the positive control compound (2-aminoanthracene) to ensure the efficiency of the activation system have given the expected positive responses in the standard plate assay and the pre-incubation assay.

d. No significant increases in the number of revertant colonies for any test strain in either the standard plate assay or the pre-incubation assay were observed following exposure to the test compound (2.5 through 40 ul/plate) with or without the metabolic activation.

Evaluation:

Under the test conditions reported, the assay was conducted in a manner to generate valid results. The test compound, SC-0224 (55.6% Purity), was considered non-mutagenic in the Ames test at the concentrations tested. This study is acceptable.

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Study: Mouse Lymphoma Mutation Assay with SC-0224 Environmental Health Center Report No. T-12661, December 19, 1985 (Authors: K. Hertzel and J.B. Majeska). Accession No. 260966

Procedure:

The L5178Y (TK+/-) mouse lymphoma cell line used in this assay was derived from the 3.7.2 clone of Fischer L5178Y cells provided by Dr. Donald Clive, Research Triangle Park, North Carolina. The procedures used for this study were primarily based on the method of Clive et al. (Mutation Res. 31: 17-29, 1975).

The L5178Y cells, approximately 6 X 10^5 cells/ml were treated with the SC-0224 dissolved in Fischer's medium at predetermined doses (W/O S9: 1-5 ul/ml; W/S9: 2.5-5.4 ul/ml) in the presence and absence of S9 in activation to 4 hours at 37 °C. At the end of exposure, the treated cells were washed twice with medium to remove the treated compound and resuspended in the growth medium (3 X 10^5 cells/ml) for 48 hours to allow recovery, growth and expression of the induced $TK^{-/-}$ phenotype. At the end of the expression period the treated cells from each treatment were seeded in soft agar plates with the selective medium (cloning medium) containing 4 ug/ml of Trifluorothymidine (TFT) and resistant (mutant) colonies were counted after 11 days incubation in a humidified CO_2 (5%) incubator at 37 °C.

The number of colonies in each plate were counted in a new Brunswick Biotran II counter. The ratio of mutant colony count to the viable colony count was considered to be the mutant frequency. The test compound is considered to be mutagenic in this system, if the mutation frequency of treated cells exceeds that of the solvent control by a factor more than 2.5-fold and exhibits a dose-related manner.

The in-vitro metabolic activation system consisted of 1 ml of S-9, 2.4 mg NADP and 4.5 mg isocitrate per 10 ml of treatment volume. Aroclor-induced rat liver homogenates (S-9) were prepared according to the procedures described by Ames et al. 1975.

Results:

1. Preliminary Cytotoxicity Assay Results (Range-Finding)

	Nonactivation (-S9)		Activation (+S9)	
	Viable Cell Count (x 10^5/ml)	% Relative Growth (20 Hrs)	Viable Cell Count (x 10^5/ml)	% Relative Growth (20 Hrs)
Trial 1 - Unadjusted acidic Cond.				
Solvent control	11.6	100	11.8	100
SC-0224				
0.313 ul/ml	12.6	105	12.4	104
0.625 ul/ml	12.4	103	12.2	103
1.250 ul/ml	11.7	97	11.7	98
2.500 ul/ml	10.5	87	11.4	95
5.000 ul/ml	7.8	64	8.8	74
10.000* ul/ml	0*	0	2.8*	24
20.000* ul/ml	0*	0	0*	0
Trial 2 - Adjusted acidic Cond.				
Solvent control	8.3	100	10.1	100
SC-0224				
0.48 ul/ml	12.4	143	9.3	93
0.97 ul/ml	10.0	115	9.3	93
1.94 ul/ml	10.5	121	9.1	92
2.91 ul/ml	8.9	102	9.2	93
3.88 ul/ml	10.1	116	8.5	86
4.84 ul/ml	8.3	95	9.0	91
5.81 ul/ml	8.6	99	8.6	87
6.78 ul/ml	8.1	93	8.3	84
7.75 ul/ml	10.8	124	7.5	75
Trial 3 - Adjusted acidic Cond.				
Solvent control	11.7	100	9.4	100
	8.0	100	9.2	100
SC-0224				
6.00 ul/ml	10.7	108	9.2	98
7.00* ul/ml	5.1*	52	8.4	89
8.00* ul/ml	6.2*	63	8.1	86
9.00 ul/ml	9.3	94	8.0	85
10.00* ul/ml	6.3*	64	8.2	87
11.00 ul/ml	7.8	79	8.1	86
12.00* ul/ml	6.4*	65	8.0	85

1. Cytotoxicity Results (cont'd)

	Nonactivation (-S9) Assay		Activation (+S9) Assa	
	Viable Cell Count (x 10^5/ml)	% Relative Growth (20 Hrs)	Viable Cell Count (x 10^5/ml)	% Relat Growt (20 Hr
13.00* ul/ml	4.8*	49	7.8	83
14.00* ul/ml	4.5*	46	7.4	78
15.00* ul/ml	1.9*	19	7.3	77
16.50* ul/ml	2.2*	22	6.9*	73

* Toxic effect on viable cell count.
% Relative Growth - Relative suspension growth x relative cloning efficiency/10
Adjusted acidic Cond. - Adjusted the pH of medium to 7.2-7.5 by the addition of NaOH (equal to control value).

Summary of Findings:

1. SC-0224 was toxic at doses greater than 5 ul/ml in the presence or absence of metabolic activation system under the normal test conditions (unadjusted acidic condition).

2. Under the adjusted acidic condition, the toxicity of SC-0224 was reduced and there was no reduction in cell growth at concentrations less than 7.0 ul/ml in the nonactivation assay and no substantial reduction in cell growth at dose levels less than 15 ul/ml in the presence of activation system.

2. L5178Y Mutation Assay Results:

Test	S-9	pH	Osmolality	Average Mutant Clones	Average Viable Clones	Average % Relative Growth	Average Mutant Freque x 10-5
Trial 1 - Normal - Test Cond.	-						
Solvent control	-	7.64	304	55	6.42	100	17
EMS, 0.4 ul/ml	-	-	-	687	319	36	431*
SC-0224							
1 ul/ml	-	7.07	303	51	457	56	22
2 ul/ml	-	6.68	306	54	431	47	25
3 ul/ml	-	6.37	311	64	493	50	27
4 ul/ml	-	6.00	318	59	369	29	32
5 ul/ml	-	5.82	320	163	186	3	175*

2. Mutation Assay Results (cont'd)

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Test	S-9	pH	Osmolality	Average Mutant Clones	Average Viable Clones	Average % Viable Clones	Average Mutant Frequency x 10^{-6}
Trial 2 - Normal	-						
Test Cond.	-						
Solvent control	-	7.64	304	39	573	100	14
EMS, 0.4 ul/ml	-	-	-	585	183	12	639*
SC-0224							
2.5 ul/ml	-	6.44	310	43	360	49	24
3.0 ul/ml	-	6.37	311	50	351	41	29
3.5 ul/ml	-	6.11	-	65	378	38	35*
4.0 ul/ml	-	6.00	318	43	258	27	33
4.4 ul/ml	-	5.96	318	47	296	26	31
4.8 ul/ml	-	5.90	318	60	256	21	47*
5.0 ul/ml	-	5.82	320	61	283	20	43*
Trial 3 - Normal	+						
Test Cond.							
Solvent control	+	6.58	-	167	550	100	63
DMN, 0.02 ul/ml	+	-	-	288	226	29	255*
SC-0224							
3.5 ul/ml	+	6.19	334	316	378	42	175*
4.0 ul/ml	+	6.13	338	334	440	35	151*
4.4 ul/ml	+	6.06	339	482	280	23	346*
4.8 ul/ml	+	6.01	340	443	274	20	325*
5.0 ul/ml	+	5.98	340	425	213	14	398*
5.2 ul/ml	+	-	-	476	263	18	377*
5.4 ul/ml	+	-	-	335	240	16	283*
Trial 4 - Normal	+						
Test Cond.							
Solvent control	+	6.58	-	78	413	100	37
DMN, 0.02 ul/ml	+	-	-	104	61	8	341*
SC-0224							
3.0 ul/ml	+	6.27	331	136	258	40	105*
4.0 ul/ml	+	6.13	338	116	205	22	113*
5.0 ul/ml	+	5.98	340	71	61	2	237*
Trial 5 - Normal	+						
Test Cond.							
Solvent control	+	6.58	-	107	309	100	70
DMN, 0.025 ul/ml	+	-	-	61	36	6	339*
SC-0224							
2.5 ul/ml	+	6.36	-	225	311	66	145
3.0 ul/ml	+	6.27	-	201	235	40	175*
3.5 ul/ml	+	6.19	334	230	191	33	243*
4.0 ul/ml	+	6.13	338	208	228	31	207*

2. Mutation Assay Results (cont'd)

Test	S-9	pH	Osmolality	Average Mutant Clones	Average Viable Clones	Average % Viable Clones	Average Mutant Frequency x 10^{-6}
4.4 ul/ml	+	6.06	339	190	120	14	378*
4.8 ul/ml	+	6.01	340	92	60	4	331*
5.0 ul/ml	+	5.98	340	110	55	3	403*
Trial 6 - Adjusted acidic Cond.	+						
Solvent control	+			71	240	100	59
DMN, 0.02 ul/ml	+			122	75	17	325*
SC-0224							
5.0 ul/ml	+			60	227	79	70
6.0 ul/ml	+			98	251	82	82
7.0 ul/ml	+			104	257	86	81
8.0 ul/ml	+			107	246	79	86
9.0 ul/ml	+			122	327	107	74
10.0 ul/ml	+			101	250	76	81
Trial 7 - Adjusted acidic Cond.	-						
Solvent control	-			47	501	100	19
EMS, 0.4 ul/ml	-			590	267	20	442*
SC-0224							
4.0 ul/ml	-			62	466	61	27
5.0 ul/ml	-			74	449	58	33
6.0 ul/ml	-			66	492	58	27
7.0 ul/ml	-			39	317	37	25
8.0 ul/ml	-			56	388	43	29
9.0 ul/ml	-			46	350	35	26
10.0 ul/ml	-			49	339	30	29

* Significant difference from the solvent control; greater than 2.5-fold increase over the solvent control value.
Solvent control - Fisher's medium (0.1 ml/ml); Adjusted acidic condition - Adjusted the pH of medium to 7.2 to 7.5 by the addition of NaOH (Equal to control value); DMN - N-nitrosodimethylamine (positive control); EMS - Ethyl methanesulfonate (positive control).

Summary of Findings:

1. Under the normal test conditions reported, the test compound, SC-0224, induced a reproducible increase in mutant frequency at the dose levels from 3.5 to 5.0 ul/ml when tested directly and from 3.0 to 5.0 ul/ml in the presence of activation system.

   The positive mutagenic responses at these dose levels were accompanied by a decrease in pH value and an increase in osmolality from the test media (Trials 1, 2, 3, 4, and 5).

2. Under the adjusted acidic test conditions, the toxicity of the test compound was reduced and there was no increase in mutant frequency at the dose levels tested either in the presence or absence of metabolic activation system (Trials 6 and 7).

3. The positive control compounds (Ethyl methansulfonate and N-nitrosodimethylamine) yielded expected mutation frequencies that were greatly in excess of the background. These results demonstrated the sensitivity of the assay and the metabolic activity of the S-9 mix under the normal test conditions (standard procedure of the assay). However, the pH and osmolality values of the test medium under the adjusted acidic test conditions were not given in the report.

Evaluation and Conclusion:

This study, which was conducted in accordance with the method of mouse lymphoma assay described by Olive et al. (Mutation Res. 31:17-29, 1975; Mutation Res. 49:61-108, 1979), appears adequate to generate valid results. The test compound, SC-0224, was considered mutagenic in this assay with and without the metabolic activation system under the normal test condition at the concentrations tested (3.5 through 5.0 ul/ml under the nonactivation system; 3.0 through 5.0 ul/ml under the activation system). The study is acceptable.

However, the interpretation of the results obtained under the adjusted acidic test conditions (pH 7.2 to 7.5) in this study was not considered to be adequate because of the following deficiencies:

1. The culture conditions (pH and osmolality values) for the positive control compounds (EMS and NMN) in all the seven test trials were not given. The positive control assay should be conducted to demonstrate that the test systems (either in normal or adjusted acidic test condition) are functional with these two known mutagens.

2. Since the toxicity (percent relative suspension growth) is primarily used to establish whether the mutagenic activity is related to an increase in effective treatment, the highest concentration of the test compound should demonstrate a low level of survival in this study (i.e., 5 to 10% relative suspension growth). (See also the acceptable procedure for the mouse lymphoma assay recommended by the EPA Health Effect Test Guidelines 560/6-83-001.) Therefore, the negative results obtained might be questionable because the test cultures were not treated with the maximum dose level of the test compound under the adjusted acidic test conditions (Trial 6 - 76%; Trial 7 - 30% relative suspension growth).

88005:Little:C.Disk:KENCO:8/7/86:TAR:VO
R:88011:Little:C.Disk:KENCO:8/20/86:TAR:VO

Study: Cytogenetic Assays (Chromosomal Aberration and Sister Chromatid Exchange) with SC-0224 in the Mouse Lymphoma (L5178Y) Cultured Cell System; Environmental Health Center Report No. T-12662, December 19, 1985 (Authors: K. Hartzel, J. Tarca, S. O'lone, N. Malick and J. Majeska) Accession No. 260966

Procedure:

1. Indicator Cells

The mouse lymphoma cell line used in these studies is identical to that described previously in the Environmental Health Center Report No. T-12661. Laboratory cultures were maintained at a density less than or equal to 1 x 10^6 cells/mL and were routinely exposed to methotrexate which selects against spontaneously arising $TK^{-}/^{-}$ cells to maintain a low background frequency of Trifluorothymidine resistant cells. Growth medium consisted of Fisher's medium, horse serum (heat inactivated, 10%), glutamin (1.9 mM), penicillin-streto-mycin (95 units/mL, 95 ug/mL), sodium pyruvate (210 ug/mL) and pluronic (476 ug/mL). Treatment medium was growth medium with serum reduced to 5 percent.

2. Metabolic Activation System

The in vitro metabolic activation system contained rat liver enzymes and an energy-producing system necessary for their function (i.e., 1 mL S-9, 2.4 mg NADP and 4.5 mg isocitrate per 10 mL of treatment volume). The preparation of S-9 fraction from male rats treated previously with Aroclor 1254 was based on the method described by Ames et al. (Mutation Res. 31:347-364, 1975).

3. Preliminary Toxicity Test (Range-finding)

The mouse lymphoma cells at a density of 6 x 10^5 cells/mL in 10 mL were exposed to concentrations of SC-0224 (0.313, 0.625, 1.25, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0 uL/mL) for 4 hours in the presence and absence of metabolic activation system. At the end of 4-hour exposure period, cells were washed with culture medium, resuspended in 20 mL of growth medium and incubated at 37 °C for approximately 20 hours. Cell numbers were determined by counting with a Coulter counter. Relative suspension growth calculated from these data is indicative of toxicity as an effect of treatment. The highest dose selected for these studies was expected to produce cytotoxicity of 80 to 90 percent.

4. Chromosomal Aberration Assay

The mouse lymphoma cells (6 x 10^5 cells/mL) were exposed to SC-0224 at predetermined concentrations (1 through 5 uL/mL) with and without metabolic activation for 4 hours. After the exposure period, the test compound was immediately washed out from the cell suspension and resuspended in fresh growth medium at 3 x 10^5 cell/mL. These cells were incubated with agitation for approximately 24 hours at 37 °C in a 5% CO_2 incubator. The harvest procedure for the mitotic cells was adopted from the assay reported by Lebowitz et al. (8th Annual Meeting of the Environmental Mutagen Society, Colorado Springs, CO, February 13-17, 1977). The cells were then fixed with Carnoy's fixative (3 parts methanol:1 part glacial acetic acid) and incubated at 4 °C overnight. The swollen fixed cells were dropped into microscope slides at room temperature and air dried. These chromosome slides were stained in 10% Giemsa for 5 to 10 minutes, rinsed in distilled water and air dried. A total of 100 metaphases were scored for each data point. Structural aberrations were analyzed on a per cell basis using Student's T-test ($P < 0.01$).

5. Sister Chromatid Exchange Assay

The general procedures used in the SCE assay were similar to that described in the chromosomal aberration assay except the method of treatment and cell staining for SCE analysis. The treated cells were specifically resuspended in growth medium (3 x 10^5 cells/mL) with the addition of 5'-bromodeoxyuridine (0.3 x 10^{-4} to 10^{-4} M). Cells stained for SCE analysis were stained according to the procedure of Perry and Wolff (Nature 251:156-158, 1974). The significant difference in SCE frequency of the treated groups compared to that of the solvent control was assessed by the Student's T-test ($P < 0.001$).

Results:

(1) Summary of Mouse Lymphoma Cell Toxicity Study

Treatment	S-9	Viable Cell Count (x 10^5 Cells/mL)	% Relative Growth (20 hrs)
Solvent control (Fisher's Med)	-	11.6	100
	-	12.5	100

(1) Summary of Mouse Lymphoma Cell Toxicity Study (Cont'd)

Treatment	S-9	Viable Cell Count (x 10^5 Cells/mL)	% Relative Growth (20 hrs)
SC-0224			
0.313 uL/mL	-	12.6	105
0.625 "	-	12.4	103
1.250 "	-	11.7	97
2.500 "	-	10.5	87
5.000 "	-	7.8*	64*
10.000 "	-	3.1*	25*
20.000 "	-	3.1*	26*
30.000 "	-	2.9*	24*
40.000 "	-	2.7*	22*
50.000 "	-	2.3*	19*
Solvent control (Fisher's Med)	+	11.8	100
	+	12.1	100
SC-0224			
0.313 uL/mL	+	12.4	104
0.625 "	+	12.2	103
1.250 "	+	11.7	98
2.500 "	+	11.4	95
5.000 "	+	8.8*	74*
10.000 "	+	2.8*	24*
20.000 "	+	0*	0*
30.000 "	+	0*	0*
40.000 "	+	0*	0*
50.000 "	+	0*	0*

*Relative growth rate below 75%

Summary of Findings:

The test compound, SC-0224, was found to reduce cell growth at doses greater or equal to 5 uL/mL with no surviving cells at doses greater than or equal to 10 uL/mL in the presence and absence of metabolic activation.

(2) Summary of Chromosomal Aberrations in L5178Y cells

Treatment	pH	Osmolality	Total No. of Cells Examined	No. of Aberrations Observed	No. of Aberrations Per Cell	Mitotic Index (%)
Nonactivation						
Trial 1: Normal Cond.						
Solvent control	7.64	3.04	50	2 (O)	0.04	16.8
			50	0	0	19.8
EMS, 0.4 uL/mL	?	?	50	4 (E,F)**	0.08**	15.8
SC-0224						
1.0 uL/mL	7.07	303	50	4 (O.P)**	0.08**	12.0
"			50	1 (O)	0.02	20.0
2.0 uL/mL	6.68	306	50	3 (O)*	0.06*	10.2
"			50	8 (O,P)**	0.16*	16.2
3.0 "	6.37	311	50	0	0	17.0
"			50	8 (O)**	0.16**	16.2
4.0 "	6.00	318	50	19 (A,D,O, P,R) *	0.38**	18.2
4.0 "			50	8 (O)**	0.16**	13.6
5.0 "	5.82	320	50	24 (A,E,F,G, I,K,Q)**	0.48**	8.8
5.0 "			50	17 (A,E,F,G,N, O,Q,R)**	0.33**	13.0
Trial 2: Normal Cond.						
Solvent control	7.64	304	50	2 (O,P)	0.04	8.2
			50	2 (O)	0.04	8.8
EMS, 0.4 uL/mL	?	?	50	9 (A,F,R)**	0.18**	13.8
SC-0224						
3.0 uL/mL			50	3 (O)	0.06	8.6
"			50	3 (O)	0.06	9.0
3.5 uL/mL	6.11	310	50	4 (O,R)	0.08	9.2
"			50	5 (E,O,P)	0.10	7.0
4.0 "			50	4 (O,P,R)	0.08	10.0
"			50	7 (O,R)**	0.14**	9.4
4.4 "	5.96	318	50	7 (A,O,P,R)	0.14	7.4
"			50	8 (A,O,R)	0.16	8.4
4.8 "	5.90	318	50	7 (A,O,P)	0.14	8.4
"			50	6 (F,O,P,R)	0.12	10.0
5.0 "			50	8 (A,O,R)*	0.16*	10.4
"			50	12 (O,Q)**	0.24**	7.4

(2) Summary of Chromosomal Aberrations in L5178Y cells (Cont'd)

Treatment	pH	Osmolality	Total No. of Cells Examined	No. of Aberrations Observed	No. of Aberrations Per Cell	Mitotic Index (%)
Activation Trial 3: Normal Cond.						
Solvent control	6.58	326	50	4 (O,Q)	0.08	16.3
			50	2 (O,P)	0.04	15.6
DMN, 0.02 uL/mL	?	?	50	12 (A,D,E,F, G,J,O,Q)**	0.24**	10.2
SC-0224						
3.0 uL/mL	6.27	331	50	16 (A,O,P,Q)**	0.32**	18.0
"			50	15 (N,O,P,Q)**	0.30**	16.6
4.0 "	6.13	338	50	25 (A,D,E,F, J,O,P,Q)**	0.50**	13.6
"			50	24 (A,B,D,E, F,G,O,Q,R)**	0.48**	22.4
5.0 "	5.98	3.40	50	39 (A,D,E,F, G,I,J,N)**	0.78**	9.0
"			50	44 (A,C,D,E,F, G,H,J,N,O, P,Q,R)**	0.88**	10.0
Activation Trial 4: Normal Cond.						
Solvent control	6.58	326	50	1 (O)	0.02	17.4
			50	1 (O)	0.02	20.8
DMN, 0.025 uL/mL	?	?	50	12 (A,E,F,G, J,O,R)**	0.24**	19.2
SC-0224						
3.0 uL/mL			50	17 (B,E,F,L, O,Q)**	0.34**	17.6
"			50	11 (G,O,Q)**	0.22**	19.2
3.5 "	6.19	334	50	11 (D,E,F,J, N,O,P,Q)**	0.22**	20.4
"			50	10 (A,E,F,G, O,Q)**	0.20**	20.8
4.0 "			50	26 (A,D,E,F, O,P,Q)**	0.52**	20.2
"			50	23 (A,D,E,F, G,O,Q)**	0.46**	23.8
4.4 "	6.06	339	50	36 (A,D,E,F, G,O,Q,R)**	0.72**	21.8
"			50	26 (A,E,F,G, J,O,Q)**	0.52**	18.2

(2) Summary of Chromosomal Aberrations in L5178Y cells (Cont'd)

Treatment	pH	Osmolality	Total No. of Cells Examined	No. of Aberrations Observed	No. of Aberrations Per Cell	Mitotic Index (%)
Trial 4: (Cont'd)						
SC-0224						
4.8 uL/mL	6.01	340	50	36 (D,E,F,G, J,L,O,Q)**	0.72**	19.6
"			50	47 (A,D,E,F, G,J,L,O,Q)**	0.94**	16.4
5.0 "			50	39 (A,D,E,F,G, J,O,Q,R)**	0.78**	14.6
"			50	38 (A,D,E,F,G, J,O,P,Q)**	0.76**	12.6
Nonactivation						
Trial 5: Adjusted Acidic Cond.						
Solvent control		310	50	1 (D)	0.02	6.8
			50	1 (O)	0.02	8.6
EMS, 0.4 uL/mL	?	?	50	3 (D,F,L,P)**	0.06**	13.0
SC-0224						
4.0 uL/mL		333	50	4 (P,R)	0.08	5.8
"			50	1 (O)	0.02	6.8
5.0 "		342	50	0	0	6.6
"			50	3 (D,O)	0.06	9.2
6.0 "		349	50	3 (O)	0.06	4.6
"			50	2 (P,R)	0.04	6.6
7.0 "		353	50	4 (D,E,P,R)	0.08	6.3
"			50	4 (E,O,P)	0.08	5.8
8.0 "		356	50	2 (O)	0.04	5.6
"			50	5 (A,E,O,R)	0.10	5.2
9.0 "		364	50	3 (O,P)	0.06	4.2
"			50	3 (O)	0.06	4.4
10.0 "		368	50	3 (O,P)	0.06	8.4
"			50	3 (O)	0.06	8.4
Activation						
Trial 6: Adjusted Acidic Cond.						
Solvent control		334	50	0	0	7.8
			50	1 (R)	0.02	7.4
DMN, 0.02 uL/mL	?	?	50	9 (A,B,D,E, F,J)**	0.18**	9.8

(2) Summary of Chromosomal Aberrations in L5178Y cells (Cont'd)

Treatment	pH	Osmolality	Total No. of Cells Examined	No. of Aberrations Observed	No. of Aberrations Per Cell	Mitotic Index (%)
Trial 6: (Cont'd)						
SC-0224						
3.0 uL/mL		357	50	2 (D,O)	0.04	12.2
4.0	"	338	50	0	0	11.0
	"		50	0	0	14.2
5.0	"	340	50	0	0	10.8
	"		50	1 (A)	0.02	9.8
6.0	"	375	50	1 (D)	0.02	11.4
	"		50	1 (O)	0.02	13.0
7.0	"	382	50	3 (D,R)**	0.06**	10.8
	"		50	0	0	10.0
8.0	"	387	50	0	0	11.2
	"		50	2 (P,R)	0.04	10.0
9.0	"	396	50	1 (D)	0.02	12.2
	"		50	2 (R)	0.04	10.4
10.0	"	402	50	0	0	10.6
	"		50	1 (D,R)	0.02	11.4

*Significantly greater than solvent control $P < 0.05$;
**Significantly greater than solvent control $P < 0.01$;
Adjusted Acidic Condition = Adjusted the pH of cultures to 7.2 -7.5 by the addition of NaOH; (A) = Chromatid break; (B) = Chromosome break; (C) = Chromotid deletion; (D) = Fragments; (E) = Translocation; (F) = Triradial; (G) = Quardriradial; (H) = Pulverized chromosome; (I) = Pulverized cell; (J) = Rearrangement; (K) = Ring; (L) = Dicentric; (M) = Minute; (N) = Greater than 10 aberrations; (O) = Polyploidy; (P) = Aneuploidy; (Q) = Endoreduplication; (R) = Acentric Fragment.

Summary of Findings:

1. Under the normal test conditions reported, the test compound, SC-0224, induced significant increase of chromosomal aberrations at 5.0 uL/mL in the absence of activation system and at doses greater than 3.0 uL/mL with activation. The positive control compounds (Ethyl methanesulfonate and N-nitrosodimethylamine) used in the evaluation of the sensitivity of the in vitro L5178Y cell assay system under the normal test conditions (nonactivation and activation) have demonstrated the positive responses as expected. In addition, the positive clastogenic responses at these dose levels of SC-0224 were accompanied by a decrease in pH value and an increase in osmolality from the test cultures (Trial 1, 2, 3, and 4).

2. Under the adjusted acidic test conditions (Trials 5 and 6), the toxicity of the test compound was reduced and there was no substantial reduction in cell growth at doses up to 10 uL/mL either with or without metabolic activation. There was also no significant increase of chromosome aberrations observed in the treated cultures either in the presence or absence of metabolic activation (dose range: 3.0 - 10.0 uL/mL). However, the pH and osmolality values of the cultures treated with the positive test compounds under the adjusted test conditions were missing in the report.

(3) Summary of Sister Chromatid Exchanges in L5178Y Cells

Treatment	Total Cells Scored	No. of Chromosomes	No. of SCE's	No. of SCE/Chrom.	No. of SCE/Cell
Nonactivation					
Trial 1: Normal Cond.					
Solvent control	50	1956	514	0.26	10.3
	50	1951	565	0.29	11.3
EMS, 0.4 uL/mL	50	1965	2091	1.06	41.8**
SC-0224					
1.0 uL/mL	50	1929	558	0.29	11.2
"	50	1952	531	0.27	10.6
2.0 "	50	1953	574	0.29	11.5
"	50	1939	559	0.29	11.2
3.0 "	50	1935	640	0.33	12.8**
"	50	1938	518	0.27	10.4
4.0 "	43	1669	517	0.31	12.0*
"	50	1936	522	0.27	10.4
5.0 "	50	1946	739	0.38	14.8***
"	50	1968	660	0.34	13.2***
Trial 2: Normal Cond.					
Solvent control	50	1948	402	0.21	8.0
	50	1945	412	0.21	8.2
EMS, 0.4 uL/mL	50	1928	2747	1.42	54.9***
SC-0224					
3.0 uL/mL	50	1933	414	0.21	8.3
"	50	1951	424	0.22	8.5
3.5 "	50	1948	390	0.20	7.8
"	50	1929	437	0.23	8.7
4.0 "	50	1941	455	0.23	9.1
"	50	1941	435	0.22	8.7
4.4 "	50	1952	458	0.23	9.2*
"	50	1950	437	0.22	8.7
4.8 "	50	1927	455	0.24	9.1
"	50	1967	451	0.23	9.0
5.0 "	50	1957	467	0.24	9.3*
"	50	1950	464	0.24	9.3

(3) Summary of Sister Chromatid Exchanges in L5178Y Cells

Treatment	Total Cells Scored	No. of Chromosomes	No. of SCE's	No. of SCE/Chrom.	No. of SCE/Cell
Activation					
Trial 3: Normal Cond.					
Solvent control	50	1956	947	0.48	18.9
DMN, 0.02 uL/mL	50	1942	2267	1.17	45.3***
SC-0224					
3.0 uL/mL	50	1941	1429	0.74	28.6***
"	28	1085	726	0.67	25.9***
4.0 "	17	654	496	0.76	29.2***
"	5	190	147	0.77	29.4***
Trial 4: Normal Cond.					
Solvent control	50	1935	684	0.35	13.7
	50	1954	632	0.32	12.6
DMN, 0.025 uL/mL	19	737	785	1.07	41.3***
SC-0224					
3.0 uL/mL	50	1946	1270	0.65	25.4***
"	50	1960	1116	0.57	22.3***
3.5 "	50	1948	1312	0.67	26.2***
"	50	1941	1376	0.71	27.5***
4.0 "	50	1960	1425	0.73	28.5***
"	46	1792	1160	0.65	25.2***
4.4 "	9	346	210	0.60	23.3***
"	50	1937	1401	0.72	28.0***
4.8 "	23	897	692	0.77	30.1***
"	9	355	205	0.58	22.8***
5.0 "	5	193	156	0.81	31.2***
Nonactivation					
Trial 5 - Adjusted Acidic Cond.					
Solvent control	50	1946	382	0.20	7.6
	50	1963	415	0.21	8.3
EMS, 0.4 uL/mL	50	1953	1880	0.96	37.6***
SC-0224					
4.0 uL/mL	50	1952	418	0.21	8.4
"	50	1918	383	0.20	7.7
5.0 "	50	1951	368	0.19	7.4
"	50	1924	360	0.19	7.2
6.0 "	50	1950	408	0.21	8.2
"	50	1966	410	0.21	8.2
7.0 "	18	698	160	0.23	8.9
"	34	1308	237	0.18	7.0
8.0 "	14	539	98	0.18	7.0
"	50	1938	401	0.21	8.0
9.0 "	50	1955	430	0.22	8.8
"	50	1956	392	0.20	7.8
10.0 "	44	1705	367	0.22	8.3
"	50	1935	432	0.22	8.6

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(3) Summary of Sister Chromatid Exchanges in L5178Y Cells

Treatment	Total Cells Scored	No. of Chromosomes	No. of SCE's	No. of SCE/Chrom.	No. of SCE/Cell
Activation					
Trial 6 - Adjusted Acidic Cond.					
Solvent control	50	1990	513	0.26	10.3
DMN, 0.02 uL/mL	20	798	911	1.14	45.6***
SC-0224					
4.0 uL/mL	50	1990	551	0.28	11.0
"	50	1997	575	0.29	11.5*
5.0 "	34	1349	339	0.25	10.0
"	50	1983	511	0.26	10.2
6.0 "	50	1987	524	0.26	10.5
"	50	1997	555	0.28	11.1
7.0 "	50	1999	508	0.25	10.2
"	50	1999	551	0.28	11.0
8.0 "	50	1993	589	0.30	11.8*
"	50	1992	566	0.28	11.3
9.0 "	50	1994	525	0.26	10.5
"	50	1990	579	0.29	11.6*
10.0 "	50	1990	581	0.28	11.6*
"	50	1996	632	0.32	12.6*

* Significantly greater than solvent control $P < 0.05$;
** Significantly greater than solvent control $P < 0.01$;
*** Significantly greater than solvent control $P < 0.001$;
Adjusted Acidic Condition - Adjusted the pH of cultures to 7.2-7.5 by the addition of NaOH.

Summary of Findings:

Under the normal test conditions reported, there was a significant increase in SCE at 5.0 uL/mL in the direct assay and at cell doses greater than or equal to 3.0 uL/mL in the activation assay (Trials 1, 2, 3, and 4).

(3) Summary of Sister Chromatid Exchanges in L5178Y Cells

Summary of Findings:

1. The positive control compounds (Ethyl methanesulfonate and N-nitrosodimethylamine) demonstrated significant increase of the number of SCE's per cell in comparison with the corresponding solvent control value as expected. In addition, the positive responses at these dose levels of SC-0224 were accompanied by a decrease in pH value and an increase in osmolality from the test cultures.

2. Under the adjusted acidic test conditions (Trials 5 and 6), the toxicity of the test compound was reduced and there was no substantial reduction in cell growth at doses up to 10 uL/mL either with or without metabolic activation. In the groups treated with the various doses of SC-0224 (4, 5, 6, 7, 8, 9, 10 uL/mL) no significant difference of the number of SCEs per cell was found in comparison with the negative control (solvent) value except the highest dose level (10 uL/mL) under the activation system. However, the pH and osmolality values of the cultures treated with the positive test compounds under the adjusted acidic test condition were not given in the report.

Evaluation and Conclusion:

These studies, which have been conducted in accordance with the method of chromosomal aberration assay described by Lebowitz (8th Annual Meeting of EMS, February 13-17, 1977) and the procedure of Perry and Wolff for performing the SCE assay (Nature 251:156-158, 1974) appear adequate to generate valid results. The test compound, SC-0224, was considered clastogenic in the mouse lymphoma cells with and without the metabolic activation system at the dose levels tested (Activation, 3 through 5 uL/mL; Nonactivation, 5 uL/mL). The test compound was also considered positive for inducing sister chromatid exchanges in the lymphoma cells under both the metabolic activation and nonactivation conditions of this assay (Activation, 3 through 5 uL/mL; Nonactivation, 5 uL/mL). These two studies are acceptable.

However, the interpretation of the results obtained under the adjusted acidic test condition (pH 7.2-7.5) for these two studies is not considered adequate because of the following deficiencies:

1. The culture conditions (pH and osmolality values) for all the positive control compounds (EMS, DMN) in all of the two studies were not given. The positive control reference compounds should demonstrate that the test systems under the adjusted acidic condition are functioning as predicated.

2. According to the acceptable In Vitro Cytogenetic Assay and In Vitro Sister Chromatid Exchange Assay recommended by EPA (EPA Health Effects Test Guidelines 560/6-83-001), the highest concentration selected for these studies should demonstrate a significant toxic effect such as suppressing mitotic activity by approximately 50 percent or decreasing in growth potential of the cells. It appears that the negative results obtained under the adjusted acidic condition might be questionable because the maximum dose level of the test compound was not used in these two studies.

Study: Cytogenetic Assays (Chromosomal Aberration and Sister Chromatid Exchange) with SC-0224 in the Chinese Hamster Ovary Cell System; Environmental Health Center Report No. T-12663, December 18, 1985 (Authors: S. O'Lone and J. Majeska). Accession No. 260966

Procedure:

1. Indicator Cells

The Chinese hamster ovary (CHO) cell line was originally obtained from the American Type Culture Collection (Strain CCL61). Laboratory cultures were maintained in William's medium E supplemented with 5% fetal bovine serum, 2 mM Lglutamine, 100 units of penicillin, and 100 ug/mL streptomycin in a humidified incubator with 5% CO_2 at 37 °C. Treatment medium was the above growth medium (nonactivation) or serum-free medium (activation).

2. The in vitro metabolic activation system contained rat liver enzymes and an energy-producing system necessary for their function. The final concentration of the activation system components with the cells were: 1.44 mg NADP/mL, 2.7 mg isocitric acid/mL, and 15 uL S-9/mL. The preparation of S-9 fraction from male rats treated previously with Aroclor 1254 was based on the method described by Ames et al. (Mutation Res. 31:347-364, 1975).

3. Preliminary Toxicity Test

Seven concentrations of SC-0224 were assayed (4, 5, 6, 7, 8, 9, and 10 uL/mL) according to the same method used for the main assay.

4. Chromosomal Aberration Assay

The CHO cells (1-1.2 x 10^6 cells/flask) were exposed to 7 concentrations of SC-0224 (4, 5, 6, 7, 8, 9, and 10 uL/mL) with and without metabolic activation for 4 hours at 37 °C. After the exposure period, the treated cells were washed with PBS, resuspended in fresh growth medium and incubated for 10 hours at 37 °C. The mitotic cells were harvested by the metaphase shake-off method, swelled by hypotonic solution and fixed in the Carnoy's fixative (3 parts methanol:1 part glacial acetic acid) Chromosome slides were prepared by dropping cells on clean, wet glass slides to produce metaphase chromosome

spread. The slides were stained in 10% Giemsa and rinsed in distilled water and air-dried. A total of 100 metaphases were scored for each data point. Structural aberrations were analyzed on per cell basis using Student's t-test ($P < 0.01$).

5. Sister Chromatid Exchange Assay

The general procedures were similar to that described in the chromosomal aberration assay except the method of treatment and cell staining for SCE analysis. Specifically, the treated cells were resuspended in growth medium with the addition of 5'-bromodeoxyuridine (10 mM) and incubated for additional 24 hours. Cells were stained for SCE according to the procedure of Perry and Wolff (Nature 251:156-158, 1974). The significant difference in SCE frequency of the treated groups compared to that of the solvent control was assessed by the Student's t-test ($P < 0.05$).

Results:

(1) Summary of CHO Cell Cytotoxicity Results

Treatment	Cells x 10^5/flask (20 hours)	% Relative Growth	Average % M_1	Average Relative Staining Index
Nonactivation				
Trial 1 - Adjusted Acidic Cond.				
Solvent control	25.3	100	3	100
	28.3	100	-	-
Mito, 2.5 ug/mL	17.5	65	-	-
0.025 "	26.8	100	9	94
SC-0224				
4.0 uL/mL	20.0	75	4	99
5.0 "	26.7	100	2	101
6.0 "	25.1	94	1	102
7.0 "	29.5	110	2	101
8.0 "	24.9	93	5	98
9.0 "	25.5	95	11	91
10.0 "	26.2	98	11	92
Activation				
Trial 2 - Adjusted Acidic Cond.				
Solvent control	44.0	100	3	100
CPA, 70 ug/mL	21.0	48	-	-
1 "	38.2	87	30	72

Results: (Cont'd)

(1) Summary of CHO Cell Cytotoxicity Results

Treatment	Cells x 10^5/flask (20 hours)	% Relative Growth	Average % M_1	Average Relative Staining Index
Trail 2 - (Cont'd)				
SC-0224				
4.0 uL/mL	38.8	89	3	99
5.0 "	42.5	97	3	99
6.0 "	38.7	88	3	99
7.0 "	44.1	101	2	101
8.0 "	38.5	88	1	102
9.0 "	41.5	95	3	98
10.0 "	46.6	107	4	99

M_1 = Cells have completed one S-cycle in BrdU;
ARSI = Obtained by dividing the sum of M_2 and M_3 cells of each treatment group by the similar sum of the means of the solvent controls and multiplying the quotient by 100.
Note: High % M_1, low relative staining index - Failure to complete two cycles within the expected time;
Mito = Mitomycin C; CPA = Cyclophosphamide.

Summary of Findings:

Under the adjusted acidic test condition, SC-0224 was not toxic to the cells at the dose range tested (4 through 10 uL/mL).

(2) Summary of Chromosomal Aberrations in CHO Cells

Treatment	Total No. of Cells Examined	No. of Aberrations Observed	No. of Aberrations Per Cell
Nonactivation			
Trial 1 - Adjusted Acidic Cond.	50	1 (O)	0.02
Solvent control	50	3 (H,O)	0.04
Mito, 2.5 ug/mL	50	18 (A,E,F,G,J. K,N)**	0.36**
SC-0224			
4.0 uL/mL	50	1 (O)	0.02
"	50	3 (N,O)	0.06
5.0 "	50	1 (O)	0.02
"	50	4 (L,N,O,P)	0.08

(2) Summary of Chromosomal Aberrations in CHO Cells

Treatment	Total No. of Cells Examined	No. of Aberrations Observed	No. of Aberrations Per Cell
Trail 1 - (Cont'd)			
6.0 uL/mL	50	2 (H,L)	0.04
"	50	0	0
7.0 "	50	1 (F)	0.02
"	50	1 (K)	0.02
8.0 "	50	4 (A,I,K)	0.08
"	50	3 (A,O)	0.06
9.0 "	50	2 (F,K)	0.04
"	50	0	0
10.0 "	50	1 (O)	0.02
"	50	1 (O)	0.02
Activation			
Trial 2 - Adjusted Acidic Cond.	50	0	0
Solvent control	50	0	0
CPA, 70 ug/mL	50	161 (A,D,E,F,G, I,J,N)**	0.32**
SC-0224			
4.0 uL/mL	50	1 (O)	0.02
	50	4 (K,O)	0.08
5.0 "	50	1 (Q)	0.02
"	50	1 (O)	0.02
6.0 "	50	2 (K,O)	0.04
"	50	6 (E,L,P,Q)	0.12
7.0 "	50	2 (O)	0.04
"	50	4 (K,L,O)	0.08
8.0 "	50	3 (G,O)	0.06
	50	1 (I,Q)	0.02
9.0 "	50	2 (Q,O)	0.04
"	50	1 (Q)	0.02
10.0 "	50	0	0
"	50	0	0

* Significantly greater than solvent control $P < 0.01$; Adjusted Acidic Cond. = Adjust the pH of dosing solution to pH 7.4 to 7.6 prior to treatment of the cells; (A) = Chromatid break; (B) = Chromosome break; (C) = Chromatid deletion; (D) = Fragments; (E) = Translocation; (F) = Triradial; (G) = Quardriadial; (H) = Pulverized chromosome; (I) = Pulverized cell; (J) = Rearrangement; (K) = Ring; (L) = Dicentric; (M) = Minute; (N) = Greater than 10 aberrations; (O) = Polyploidy; (P) = Aneuploidy; (Q) = Endoreduplication; (R) = Acentric fragment.

Results: Chromosomal Aberrations in CHO Cells

Summary of Findings:

Under the adjusted acidic test conditions (pH 7.4 to 7.6), the test compound, SC-0224, did not induce any statistically significant increase in the number of aberrations per cell at the dose levels tested (4 through 10 uL/mL) either in the presence or absence of metabolic activation. However, the pH values of the positive control compounds (Mitomycin C and Cyclophosphamide) prior to treatment of the cells were not given in the report.

Results:

(3) Summary of Sister Chromatid Exchanges in CHO Cells

Treatment	Total Cells Scored	No. of Chromosomes	No. of SCEs	No. of SCE/Chrom.	No. of SCE/Cell
Nonactivation					
Trial 1 - Adjusted Acidic Cond.					
Solvent control	50	997	454	0.46	9.1
	50	1012	442	0.44	8.8
Mito, 0.025 ug/mL	21	420	458	1.09	21.8***
SC-0224					
4.0 uL/mL	50	1010	418	0.41	8.4
"	50	1009	412	0.41	8.2
5.0 "	50	1004	437	0.44	8.7
"	32	650	299	0.46	9.3
6.0 "	50	1004	451	0.45	9.0
"	50	1022	438	0.43	8.8
7.0 "	50	1013	455	0.45	9.1
"	50	1003	437	0.44	8.7
8.0 "	50	1021	472	0.46	9.4
"	50	1009	466	0.46	9.3
9.0 "	50	1014	446	0.44	8.9
"	50	997	461	0.46	9.2
10.0 "	50	1009	429	0.43	8.6
"	46	937	427	0.46	9.3

*** Significantly greater than solvent control $P < 0.001$;
Adjusted Acidic Cond. = Adjusted the pH of dosing solution to pH 7.4 to 7.6 prior to treatment of the cells.

Results: (Cont'd)

(3) Summary of Sister Chromatid Exchanges in CHO Cells

Treatment	Total Cell Scored	No of Chromosomes	No. of SCEs	No. of SCE/Chrom.	No. of SCE/Cell
Activation					
Trial 2 - Adjusted Acidic Cond.					
Solvent control	50	996	492	0.49	9.8
	50	1025	513	0.50	10.3
CPA, 1 ug/mL	4	80	88	1.10	22.0***
SC-0224					
4.0 uL/mL	41	816	408	0.50	10.0
"	50	1011	502	0.50	10.0
5.0 "	45	910	394	0.43	8.8
"	50	1010	515	0.51	10.3
6.0 "	41	834	403	0.48	9.8
"	43	857	390	0.46	9.1
7.0 "	50	1018	472	0.46	9.4
"	50	999	515	0.52	10.3
8.0 "	50	1005	485	0.48	9.7
"	50	1006	488	0.49	9.8
9.0 "	37	743	335	0.45	9.1
"	21	422	186	0.44	8.9
10.0 "	13	260	115	0.44	8.8
"	50	1017	492	0.48	9.8

*** Significantly greater than solvent control $P < 0.001$;
Adjusted Acidic Cond. = Adjusted the pH of dosing solution to pH 7.4 to 7.6 prior to treatment of the cells.

Summary of Findings:

Under the adjusted acidic test conditions (pH 7.4 - 7.6), no statistically significant difference in the number of SCE's per cell was found between the SC-0224-treated cultures and the control culture at the dose levels tested either in the presence or absence of metabolic activation (4 through 10 uL/mL). However, the p^H values of the positive control compounds (Mitomycin C and Cyclophosphamide) prior to treatment of cells were not given in the report.

Evaluation and Conclusion:

These studies, which were not conducted in accordance with the acceptable procedures for the in vitro cytogenetic assay and the in vitro sister chromatid exchange assay recommended by EPA

(EPA Health Effects Test Guidelines 560/6-83-001), are not considered adequate because of the following deficiencies:

1. According to the acceptable procedures for performing the in vitro cytogenetic assays (EPA Health Effects Test Guidelines), the highest concentration selected for these studies should demonstrate a significantly toxic effect to the CHO cells such as suppressing mitotic activity by approximately 50 percent or decreasing in growth potential of the cells. Since there was no toxic effect to the CHO cells at the dose levels tested (4 through 10 uL/mL), and the mitotic index of the CHO cells from each treated and untreated cultures was not determined, no evidence has been shown that the maximum tolerated dose of the test compound was used for these studies. Rationale for dose selection in these studies should be given.

2. Since the p^H values of the positive control compounds (Mito. and CPA) prior to the treatment of cells were not determined for all the studies, it is questionable that the test systems under the adjusted acidic condition were functioning as predicated.

3. Results obtained from these studies under the standard test condition (unadjusted acidic condition) were missing in this report.

4. Because many known clastogens inhibited progression of cells through the cell cycle and damaged cells which recover enough to progress to mitosis may do so much more slowly than normal cells (i.e., mitotic delay), single treatment followed by at least three harvest times is strongly recommended in order to avoid missing a peak of aberration yield.

Since the submitted report is inconclusive, these studies are considered unacceptable in the present form.

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