

FINAL REPORT  
In Vitro Chromosomal Aberration Assay  
on SC-19129

Bioassay Systems Corporation  
Project Number: 12159

Prepared for:

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March 20, 1985  
Date

S.A. 2464

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TITLE: In Vitro Chromosomal Aberration Assay on SC-19129

Author: Marybeth Thomas, B.A.  
(Bioassay Systems Corporation, Woburn, MA)

Study Number: S.A. 2464

Date: March 20, 1985

Type of Report: Final

Summary:

SC-19129 was investigated for the potential to induce chromosomal aberrations in Chinese Hamster Ovary (CHO) cells in the presence and absence of a rat liver homogenate metabolic activation system. The intended test concentrations that were analyzed for inductions of aberrations were 2.0, 1.76, and 1.5 mg/ml (the three highest test levels). The actual test concentrations based on analysis of the stock solution were 94.5% of the target concentrations. There were no significant increases in chromosomal aberrations observed. The responses obtained from the negative and positive controls demonstrated that the test system was capable of detecting chemical mutagens.

These results lead to the conclusion that SC-19129 does not have the potential to cause chromosomal aberrations under the conditions of the assay employed.

## 1.0 INTRODUCTION

### 1.1 Objective of the study

The purpose of this study was to assess the ability of SC-19129 to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells. Aliquots of the sample were received 10/19/84 and 10/31/84. The range-finding experiments were initiated 11/14/84. The in vitro Chromosomal Aberration assay was initiated 12/5/84 and completed 12/28/84. All assays were performed according to FDA Good Laboratory Practice Regulations (21 CFR 58.1-58.219, 1979).

### 1.2 Principles of the assay

To detect chromosomal damage, cells are arrested at the first metaphase following administration of the chemical; and chromosomes are analyzed for damage which includes breaks, translocations, deletions, ring chromosomes, triradials, quadriradials or other complex rearrangements. The formation of breaks in chromosomes is generally considered to have arisen from primary DNA damage caused by the chemical (for example, alkylation of the nucleotides or DNA strand scission). Those breaks which are not repaired would then be detected as either chromatid or chromosomal breaks at the first metaphase. The formation of more complex aberrations (rings, triradials, or quadriradials) presumably results from exchanges that take place between chromosomes at the break points.

## 2.0 MATERIALS

### 2.1 Test Substance

Name:	SC-19129
BSC No.:	84-1226A, 84-1226B
Lot No.:	84K-047-101
Physical State:	solid; powder
Color:	white
Purity:	greater than 99%
Composition:	determined by Sponsor
Stability:	determined by Sponsor
Stability of Formulations:	determined by Sponsor
Solubility:	greater than 100 mg/ml in dimethylsulfoxide
Storage Conditions:	ambient, protect from light
Safety Precautions:	routine

The test sample was assayed in solution using dimethylsulfoxide as a vehicle. A stock solution with a target concentration of 200 mg/ml was prepared for the activated and nonactivated assays. Concentration analysis (Appendix C) of the test article mixture confirmed the stock concentration (94.5%). All further dilutions were made in dimethylsulfoxide. Aliquot 84-1226A was used for the range-finding experiments and for the in vitro Chromosomal Aberration Assays.

The Sponsor assumed responsibility for determining the identity, strength, purity, composition and stability of the test sample and the stability of the formulations.

### 2.2 Negative Control Substance

Name:	Dimethylsulfoxide (DMSO)
Supplier:	J.T. Baker Chemical Co.
Lot No.:	327701
Physical State:	liquid
Color:	clear
Composition:	on file with manufacturer
Purity:	reagent grade
Stability:	indefinite
Storage Conditions:	room temperature
Safety precautions:	Avoid topical and respiratory contact

### 2.3 Positive Control Substances

	Activated Assay	Non-activated Assay
Name:	Cyclophosphamide (CP)	Mitomycin C
Supplier:	Sigma Chem. Co.	Sigma Chem. Co.
Lot No.:	33F-0157	123F-0463
Physical State:	solid	powder

Color:	white	slightly blue
Purity:	reagent grade	reagent grade
Stability:		
	(solid) indefinite	indefinite
	(solution) at least 4 mo. at 4°C	6 mo. at 4°C
Solubility:	DMSO, water	water
Composition:	on file with manufacturer	
Storage Conditions:	4°C	4°C
Safety Precautions:	avoid topical and respiratory contact	

Stock solutions of the positive controls were prepared and aliquots added to the exposure medium. A 0.5 mg/ml stock solution of Mitomycin C in deionized water was used to provide a final concentration of 5 ug/ml. A 10 mg/ml stock solution of Cyclophosphamide in deionized water was used to provide a final concentration of 50 ug/ml. Stock solutions of the positive controls are stored at 4°C.

#### 2.4 CHO Cell Culture

Cells used in this assay were obtained from Dr. Sheila Galloway at Litton Bionetics, Kensington, MD.

Master vials are stored in liquid nitrogen or in a freezer at -70°C at passage 7. All frozen cultures have been prescreened for mycoplasma contamination. Working cultures are maintained in cell culture incubators in McCoy's 5A medium plus 10% fetal calf serum. The cells were used at passage 13 for the aberration assay.

#### 2.5 Microsomal Activation System.

An S9 microsome fraction prepared from the liver of Aroclor 1254 induced (500 mg/kg) Sprague-Dawley rats was used in the activated assays. The S9 fraction was combined with a solution of cofactors and culture medium to form the metabolic activation system. A mixture of 10% 10x Isocitrate Cofactors and 90% serum-free McCoy's 5A medium was prepared and used as the exposure medium. Following the addition of the test sample, an aliquot of the S9 fraction was added to each flask. The final concentrations in each flask were 4.5 mg/ml Isocitric acid (trisodium salt), 2.4 mg/ml NADP and 20 ul/ml S9 fraction. The cofactor/medium mixture was prepared just prior to use and the S9 fraction was maintained on ice until use.

The following S9 fraction was used in the study:

Source:	Microbiological Associates, Bethesda, MD.
Lot/Batch No.:	179
Storage Conditions:	-80°C

## 2.6 Identification of Test System

All the experimental vessels were labeled with the last three digits of the project number, assay number and a code number. The key to both the assay and the code numbers are given in the raw data sheets.

## 3.0 EXPERIMENTAL DESIGN

### 3.1 Range-finder and Chromosomal Aberration Assay

The effect of SC-19129 on the cell cycle of CHO cells was determined by growing CHO cells, exposed to various test sample concentrations, for approximately 28 hours in medium containing BrdU ( $1 \times 10^{-5}$  M). In the activated assay cells were exposed to the test sample for approximately two hours in serum free medium followed by a 28 hour growth period in medium containing serum and BrdU. In the nonactivated assay cells were exposed to the test sample in medium containing serum. Two hours later an aliquot of BrdU was added to the exposure medium and the cells were incubated an additional 28 hours. Cells were harvested and stained with Hoechst and Giemsa to obtain differentiated sister chromatids. The percentage of first and second division cells was determined by counting 100 metaphase cells. The concentrations tested in the chromosomal aberration assays were based on the solubility limit of the test sample (200 mg/ml in DMSO yielding a 2.0 mg/ml test concentration) since no cell cycle delay was observed.

#### Nonactivated Assay

CHO cells were seeded at a density of  $1.5 \times 10^6$  in plastic T75 tissue culture flasks. The next day the cells were exposed in McCoy's 5A medium supplemented with 10% fetal calf serum, 20 mM HEPES buffer and 1% penicillin-streptomycin to eight test sample concentrations and the positive and negative controls. All test sample concentrations and the positive control were performed in duplicate flasks. Two sets of duplicate flasks were used for the negative control. The length of the exposure period was 8 hours. An aliquot of vinblastine sulfate (final concentration 0.26 ug/ml) was added to the exposure medium 2-2½ hours before the cells were harvested.

At the end of the incubation period, metaphase cells were collected by treatment with trypsin and concentrated by centrifugation with a table top centrifuge. For processing the cells were suspended in hypotonic solution (0.03 M KCl and 0.01 M sodium citrate) for 12 minutes at 37°C and fixed 3 times in 3:1 methanol: acetic acid. Drops of the concentrated cell suspension were placed on glass slides, and air dried and stained in 5% Giemsa for approximately 5 minutes at room temperature.

### Activated Assay

The methods used in the activated assays followed that of the nonactivated assays with the following changes. The exposure time was 2 hours in serum-free medium followed by a growth period of 8 hours in the medium supplemented with serum. Cells were harvested at the end of the growth period as outlined above.

### Chromosomal Analysis

Metaphase cells were analyzed for chromosomal aberrations through a microscope using a 100x objective. The mitotic index was determined by counting a minimum of 500 total cells. One hundred metaphase cells from each of the duplicate flasks of the three highest test sample concentrations and the positive controls and one set of negative control duplicates were analyzed for the following types of chromosomal aberrations.

chromatid gap	triradial
chromatid break	dicentric chromosome
chromosome gap	pulverized chromosome
chromosome break	cell with greater than one
chromatid deletion	pulverized chromosome
fragment	pulverized cell
acentric fragment	complex rearrangements
translocation	ring
quadriradial	double minute chromosomes
interstitial deletions	uncoiled chromosomes

Endoreduplication was recorded but was not included in the aberration frequency. Coordinates of positive cells were recorded.

$$\% \text{ Cells with aberrations} = \frac{\# \text{ cells with aberrations}}{\text{Total } \# \text{ cells analyzed}} \times 100$$

$$\text{Number of Aberrations Per Cell} = \frac{\text{Total } \# \text{ aberrations}}{\text{Total } \# \text{ cells analyzed}}$$

$$\text{Mitotic Index} = \frac{\# \text{ metaphase cells}}{\text{Total } \# \text{ cells}}$$

## 4.0 RESULTS

### Range-finding Experiments

The effects of SC-19129 on the cell cycle of CHO cells in the presence and absence of a metabolic activation system are presented in Table 1. The test sample did not cause cell cycle delay under activated or nonactivated test conditions. Since no toxicity was observed, the concentrations used in the *in vitro* chromosomal aberration assays were based on the solubility limit of the test sample.



### Nonactivated Assay

The effects of SC-19129 on the induction of chromosomal aberrations in CHO cells in the absence of the metabolic activation system are presented in Table 2. Eight test sample concentrations ranging from 0.10-2.00 mg/ml were tested. The three highest test sample concentrations (1.50, 1.76 and 2.00 mg/ml) and the positive and negative controls were analyzed. Data obtained from the duplicate flasks are presented separately. The aberration frequencies of the 1% DMSO controls were 0.00 and 0.01 aberrations/cell. None of the test sample concentrations resulted in aberration frequencies different from the negative controls. The positive control, Mitomycin C (5 ug/ml), induced significant increases in aberration frequency (greater than 0.57 and greater than 0.54 aberrations/cell).

### Activated Assay

The test sample, SC-19129, was also tested in the presence of a metabolic activation system. Eight concentrations ranging from 0.10-2.00 mg/ml were tested. The three highest concentrations (1.50, 1.76 and 2.00 mg/ml) and the positive and negative controls were analyzed. The aberration frequencies of the 1% DMSO +S9 controls were 0.00 and 0.00 aberrations/cell. None of the test sample concentrations resulted in aberration frequencies different from the negative controls. The positive control, Cyclophosphamide (50 ug/ml) +S9, induced significant increases in aberration frequency (greater than 0.24 and greater than 0.26 aberrations/cell).

## 5.0 CONCLUSION

SC-19129 was assayed for its ability to induce chromosomal aberrations in CHO cells in the presence and absence of a metabolic activation system. Under the conditions of the assay employed, significant increases in chromosomal aberrations were not observed under activated or nonactivated conditions.

TABLE 1  
The effects of SC-19129  
on the Cell Cycle of CHO Cells

Range Finding Experiments

Activated Assay

Conc. (mg/ml)	Cell Cycle <sup>a</sup>	
	M1	M2
SC-19129 +S9		
1	1	99
0.3	13	87
0.1	5	95
0.03	13	87
0.01	0	100
0.003	0	100
0.001	1	99
0.0003	3	97
1% DMSO +S9	9	91
1% DMSO +S9	3	97

Nonactivated Assay

Conc. (mg/ml)	Cell Cycle <sup>a</sup>	
	M1	M2
SC-19129		
1	18	82
0.3	33	67
0.1	2	98
0.03	24	76
0.01	10	90
0.003	41	59
0.001	13	87
0.0003	45	55
1% DMSO	19	81
1% DMSO	6	94

M1, first division metaphase cells

M2, second division metaphase cells

<sup>a</sup>The values given represent the percentage of cells found in each division stage.

TAI 2  
The Effect of SC-19129 on the  
Induction of Chromosomal Aberrations in  
Chinese Hamster Ovary Cells

Nonactivated Assay, 8 hr. Exposure

Conc. (mg/ml)	# Cells Scored	Mitotic Index	Number and Type of Aberrations <sup>a</sup>											Total No. of Aberr.	No. of Aberra- tions per cell	% Cells with Aber- rations	% Cells with > 1 Aber- rations
			TB	SB	TD	F	AF	TR	QR	CR	ID	R	PU				
SC-19129																	
2.00	100	0.110	0	0	0	0	1	0	0	0	0	0	0	0	0.01	1	0
	100	0.104	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0
1.76	100	0.098	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0
	100	0.092	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0
1.50	100	0.118	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0
		0.132	0	0	0	0	0	0	0	0	0	1	0	0	0.01	1	0
1% DMSO	100	0.082	0	0	0	1	0	0	0	0	0	0	0	0	0.01	1	0
	100	0.117	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0
Mitomycin C																	
0.005	100	0.033	17	5	2	1	2	8	5	3	0	1	3	1	> 0.57	33	14
	100	0.020	14	7	0	7	5	7	3	4	4	0	3	0	> 0.54	36	12

<sup>a</sup>Code for identifying chromosomal aberrations presented in Appendix B.

The Effect of SC-19129 on the  
Induction of Chromosomal Aberrations in  
Chinese Hamster Ovary Cells

Activated Assay, 2 hr. Exposure + 8 hr. growth period

Conc. (mg/ml)	# Cells Scored	Mitotic Index	Number and Type of Aberrations <sup>a</sup>													Total No. of Aberr.	No. of Aberra- tions per cell	% Cells with Aber- rations	% Cells with >1 Aber- rations
			TB	SB	TD	F	AF	TR	QR	CR	ID	R	PU	PC	P+				
SC-19129 +S9																			
2.00	100	0.053	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
	100	0.083	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
1.76	100	0.086	0	0	0	0	0	0	0	0	0	0	0	1	0	0.01	1	1	
	100	0.090	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
1.50	100	0.097	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
		0.116	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
1% DMSO +S9	100	0.089	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
	100	0.096	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
Cyclophosphamide +S9																			
0.005	100 <sup>b</sup>	0.006	14	1	0	0	0	1	3	0	1	0	2	1	1	>0.24	19	6	
	100	0.010	8	1	2	1	1	8	2	0	1	0	0	1	1	>0.26	25	4	

<sup>a</sup>Code for identifying chromosomal aberrations presented in Appendix B.

<sup>b</sup>One cell observed with endoreduplication.

BIOASSAY SYSTEMS CORPORATION

Quality Assurance Report

Study Title: In Vitro Chromosomal Aberration Assay on SC-19129

Sponsor: G.D. Searle & Co.

BSC Project No.: 12159

BSC Sample No.: 84-1226 A

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<u>Date(s) of Inspection</u>	<u>Date Findings Reported to Study Director</u>	<u>Date Findings Reported To Management</u>
10/31/84	10/31/84	11/5/84
12/5/84	12/5/84	12/10/84
12/7/84	12/7/84	12/10/84
1/23/85	1/23/85	1/29/85
3/14/85	3/14/85	3/20/85

Date: 3/20/85

Quality Assurance Officer: Susan M. O'Connor

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APPENDIX A

## APPENDIX A

### 1. Supervisory Personnel

Kenneth S. Loveday, Ph.D., Director of Genetic Toxicology  
Marybeth Thomas, B.A., Study Director  
Susan M. O'Connor, B.S., Manager, Quality Assurance

### 2. Storage Location Information

Raw Data: BSC Archives  
Final Report: BSC Archives

**APPENDIX B**



## APPENDIX B

### Code for identifying Chromosomal Aberrations

TB -	Chromatid Break
SB -	Chromosome Break
TD -	Chromatid Deletion
F -	Fragment
AF -	Acentric Fragment
TR -	Triradial
QR -	Quadriradial
CR -	Complex rearrangement
ID -	Interstitial Deletion
R -	Ring
UC -	Uncoiled chromosome
PU -	Pulverized chromosome <sup>a</sup>
PC -	Pulverized cell <sup>a</sup>
P+ -	Cell w/greater than 1 pulverized chromosome <sup>a</sup>
> -	greater than 10 aberrations <sup>b</sup>

<sup>a</sup>Counted as greater than one aberration.

<sup>b</sup>Counted as greater than ten aberrations.

)

APPENDIX C

## REPORT OF ANALYSIS

BSC PROJECT NO(s): 12159

DATE OF ANALYSIS: 12/5-6/84

SPONSOR: G. D. Searle

### TEST SAMPLE IDENTIFICATION

Sponsor Identification: SC19129 B-APM

BSC Sample No.: 84-1226A

CHEMICAL VEHICLE: Dimethyl sulfoxide (DMSO)  
Source: Baker Chemical Co.  
Lot No.: 327701

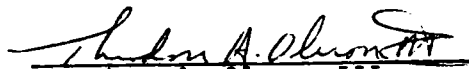
METHOD OF ANALYSIS: BSC Standard Operating Procedure # 103-146

### SUMMARY OF RESULTS:

1. System Suitability: Mean Standard Peak Area: 33.7894  
Relative Standard Deviation (RSD): 2.9%  
Number of Injections: 6
2. Standard Check - Percent of Theory: 102.0%
3. Control Sample - Percent Recovery: 93.3%
4. Test Samples - Concentrations Measured/% Recovery
  - a. Formulation Lot No. 12-5-84: 189.1 mg/ml DMSO  
94.5% Recovery

### REVIEW OF ANALYSIS

The results reported above have been reviewed and found to accurately represent the data collected during this analysis.

  
Theodore A. Olsson III  
Manager, Chemistry

Date 3-18-85

SA2464

# REPORT OF ANALYSIS

BSC PROJECT NO(s): 12159

DATE OF ANALYSIS: 12/5-6/84

## SUMMARY OF DATA

Sample/Standard Identification	Preparation of Sample/Standard <sup>c</sup>	Injection No.	Peak Area	Mean Peak Area
Standard	0.100g to 100 ml with mobile phase	1 (a)	34.5254	33.7894 (2.9% RSD)
		2 (a)	35.0809	
		3 (a)	33.3606	
		4 (a)	32.8844	30.6060
		5 (a)	32.6080	
		6 (a)	34.2771	
		1 (b)	31.2076	
		2 (b)	37.3094*	
		9 (b)	30.0044	
Standard Check	0.1000g to 100 ml with mobile phase	3 (b)	30.5881	30.0013
		4 (b)	29.4144	
Control Sample	0.1000g +1ml DMSO to 100 ml with mobile phase	5 (b)	27.5852	28.5561
		6 (b)	29.5270	
Test Sample (Form. Lot # 12-5-84)	Diluted 1:1 with DMSO, then 1 ml to 100 ml with mobile phase <sup>d</sup>	7 (b)	28.3776	28.9376
		8 (b)	29.4976	

(a) System suitability check-prior to analysis of test samples

(b) Standard and sample chromatographic runs for analysis of test sample

(c) All standards and samples were diluted 1.0 ml to 10.0 ml with mobile phase after the preparations noted below.

(d) Due to technician error, 1.0 ml DMSO was also added to the first 1.0 ml to 100ml dilution of the sample. Although this resulted in twice the concentration of DMSO in the test sample dilution, no effect was observed upon the quantitation of the test sample.

\* Not used - this standard vial previously used for "system suitability" - evaporation suspected.

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# REPORT OF ANALYSIS

BSC PROJECT NO(s).: 12159

DATE OF ANALYSIS: 12/5-6/84

## SUMMARY OF CALCULATIONS:

### 1. Standard Check - Percent of Theory

$$\% \text{ STD} = \frac{R \text{ STD}}{R \text{ STDCK}} \times \frac{W \text{ STD CK}}{W \text{ STD}} \times 100\%$$

$$= \frac{30.6060}{30.0013} \times \frac{100.0 \text{ mg}}{100.0 \text{ mg}} \times 100\%$$

$$= 102.0\%$$

### 2. Control Sample - Percent Recovery

$$\% \text{ Recovery} = \frac{R \text{ CS}}{R \text{ STD}} \times \frac{\text{Conc. STD}}{\text{Conc. CS}} \times 100\%$$

$$= \frac{28.5561}{30.6060} \times \frac{1.000 \text{ mg/ml}}{1.000 \text{ mg/ml}} \times 100\%$$

$$= 93.3\%$$

### 3. Concentrations of Test Sample

$$\text{Conc. Test Sample} = \frac{R \text{X}}{R \text{STD}} \times \text{Conc. STD} \times \text{dilution factor}$$

(SC19129 mg/ml DMSO)

a) Test Sample Form. Lot No. 12-5-84

$$\text{Conc. Test Sample} = \frac{28.9376}{30.6060} \times 1.000 \text{ mg/ml} \times 200$$

$$= 189.1 \text{ mg/ml}$$

$$\text{Expected Conc.} = 200 \text{ mg/ml}$$

$$\% \text{ Recovery} = 94.5\%$$

Note: R STD = mean peak height or area for Standard  
 R STD CK = mean peak height or area for Standard Check  
 W STD = weight of test sample in Standard (mg)  
 W STD CK = weight of test sample in Standard Check (mg)  
 R CS = mean peak height or area for Control Sample  
 Conc STD = concentration of test sample in Standard (mg/ml equivalent DMSO)  
 Conc CS = concentration of test sample in Control Sample (mg/ml DMSO)

SA2464

## 1.0 Study Title

In Vitro Chromosomal Aberration Assay on SC-19129

## 2.0 Purpose of Study

The purpose of this study is to assess the ability of the test substance to induce chromosomal breaks and aberrations in cultured Chinese hamster ovary (CHO) cells.

## 3.0 Management of Study

### 3.1 Sponsor's Name and Address:

G.D. Searle & Co.  
4901 Searle Parkway  
Skokie, IL 60077

### 3.2 Sponsor's Study Coordinator:

Charles E. Piper, Ph.D.

### 3.3 Testing Laboratory's Name and Address:

Bioassay Systems Corporation  
225 Wildwood Avenue  
Woburn, MA 01801

BSC Project Number: 12159

### 3.4 Supervisory Personnel:

Director of Genetic Toxicology: Kenneth S. Loveday, Ph.D.

Study Director: Marybeth Thomas, B.A.

### 3.5 Manager, Quality Assurance: Susan O'Connor, B.S.

### 3.6 Proposed Study Schedule:

3.6.1 Test Substance Received:	10/19/84
3.6.2 Study Initiated:	11/12/84
3.6.3 Study Completed:	1/11/86

#### 4.0 Test Material Data

##### 4.1 Test Sample Description:

	Test Sample
4.1.1 Identification:	SC-19129
4.1.2 BSC No.:	To be specified in final report
4.1.3 Lot No.:	84K-047-101
4.1.4 Physical State:	Solid; powder
4.1.5 Color:	White
4.1.6 Density:	Not applicable
4.1.7 Purity:	>99%
4.1.8 Composition:	To be determined by Sponsor
4.1.8 Stability of Bulk Compound:	To be determined by Sponsor
4.1.9 Stability of Formulations:	To be determined by Sponsor
4.1.10 Solubility:	Dimethylsulfoxide ( 100 mg/ml)
4.1.11 Storage Conditions:	Ambient, protect from light
4.1.12 Safety Precautions:	Routine

The Sponsor will determine the identity, strength, purity, composition and stability of the test article. Bioassay Systems will determine the concentration of the test article in the carrier. The Sponsor will analyze the test article for identity (at a minimum) upon return from the testing laboratory.

#### 4.2 Positive Control Material Characterization (Activated Assay)

4.2.1 Name: Cyclophosphamide  
4.2.2 Supplier: Sigma  
4.2.3 Lot/Batch No.: to be supplied in final report  
4.2.4 Physical State: solid  
4.2.5 Color: white  
4.2.6 Purity: reagent grade  
4.2.7 Composition: on file with manufacturer  
4.2.8 Stability: indefinite (solid)  
24 hours (solution)  
4.2.9 Solubility: DMSO, water  
4.2.10 Storage  
Conditions: 4°C  
4.2.11 Safety  
Precautions: avoid topical and respiratory contact

#### 4.3 Positive Control Material Characterization (Nonactivated Assay)

4.3.1 Name: Mitomycin C.  
4.3.2 Supplier: Sigma  
4.3.3 Lot/Batch No.: to be specified in final report  
4.3.4 Physical State: solid  
4.3.5 Color: slightly blue  
4.3.6 Purity: reagent grade  
4.3.7 Composition: on file with manufacturer  
4.3.8 Stability: indefinite (solid)  
6 months (solution)  
4.3.9 Solubility: water  
4.3.10 Storage  
Conditions: 4°C  
4.3.11 Safety  
Precautions: avoid topical and respiratory contact

#### 4.4 Negative Control Material Characterization

4.4.1 Name: Dimethylsulfoxide (DMSO)  
4.4.2 Supplier: J.T. Baker Chemical Co.  
4.4.3 Lot No.: To be specified in final report  
4.4.4 Physical State: liquid  
4.4.5 Color: clear  
4.4.6 Purity: reagent grade  
4.4.7 Composition: on file with manufacturer  
4.4.8 Stability: indefinite  
4.4.9 Storage  
Conditions: room temperature  
4.4.10 Safety  
Precautions: Avoid topical and respiratory contact.



## 5.0 Description of Test Systems

### 5.1 Cell Line:

Chinese hamster ovary cells (CHO) were obtained from Dr. Sheila Galloway at Litton Bionetics, Kensington, MD.

### 5.2 Storage:

Master vials are stored in liquid nitrogen or in a -70°C freezer. Working cultures are maintained in cell culture incubators in McCoy's medium plus 10% fetal calf serum. Routine cell culture methods, preparation of media and reagents, and quality control measures are detailed in BSC standard operating procedures.

### 5.3 Justification for Selection:

The CHO cell line is recommended by the EPA's Gene-Tox panel, is easily grown in culture and has been used extensively for in vitro cytogenetic testing.

### 5.4 Identification of Test System:

All the experimental vessels will be labeled with last three digits of project no., assay no., and a code no. The key to both assay and the code numbers will be given in raw data sheets.

## 6.0 Experimental Design

### 6.1 Test Concentrations:

Concentrations of the test substance to be used in the chromosomal aberration assay are based on toxicity limits. When test concentrations are determined, concentrations of test sample and controls and the method of formulation will be listed in the form of a protocol amendment.

### 6.2 Exposure Periods:

The exposure period will be determined from the effect of the compound on the cell cycle of CHO cells. Cells exposed to various concentrations of the test compound are grown for approximately 28 hours (2 cell cycles), in medium containing BrdU ( $1 \times 10^{-5}$  M). Approximately two hours before cell harvest, vinblastine is added to the flasks as described below. Metaphase cells are stained using Hoechst and Giemsa to obtain differentiated sister chromatids. The percent of first division and second division metaphase cells will be determined based on counting 100 metaphase cells. In the absence of cell cycle delay, the

in vitro chromosomal aberration assay will use a growth and exposure period of 8 hours for the nonactivated assay. In the activated assay, the chemical exposure period will be two hours followed by a growth period of 8 hours.

If significant cell cycle delay is seen, then the in vitro chromosomal aberration assay will use an overnight growth period (approximately 16-18 hours). The use of this longer time ensures the harvest of mitotic cells which have been exposed to the compound during DNA synthesis phase even when a compound causes cell cycle delay.

### 6.3 Chromosomal Aberration Assay

#### 6.3.1 Nonactivated Assay

CHO cells are seeded at a density of  $1 - 1.5 \times 10^6$  in T75 tissue culture flasks and incubated overnight at 37°C. The next day a minimum of three test sample concentrations and control articles are added to the appropriate flasks. All concentrations are performed in duplicate flasks; the exposure is in medium containing serum. An aliquot of vinblastine sulfate (20 ug/ml) is added to each flask 2-2½ hours before the end of the exposure period. At the end of exposure period the flasks are removed from the incubator and the cells are harvested for the preparation of slides.

#### 6.3.2 Activated Assay

Procedures for the activated assay follow those outlined for the nonactivated assay except the exposure time is two hours in serum free medium. A predetermined aliquot of the S9 metabolic activation mixture is added to each flask immediately after the addition of the test or control substances. At the end of the exposure period, the cells are washed twice and incubated in medium containing serum until the harvest time. Vinblastine sulfate is added 2-2½ hours before harvesting as in the nonactivated assay.

#### 6.3.3 Cell harvest

Harvesting of cells and slide preparation follow standard cytogenetic procedures which are outlined in BSC standard operating procedures.

#### 6.3.4 Chromosomal analysis

Metaphase cells will be analyzed for aberrations through a microscope using a 100x objective. The mitotic index will be determined for each concentration by counting a minimum of 500 total cells. One hundred metaphase cells from each flask will be analyzed for the following types of aberrations.

chromatid gap	dicentric chromosome
chromatid break	endoreduplication
chromosome gap	pulverized chromosome
chromosome break	cell with 1 pulverized chromosome
chromatid deletion	pulverized cell
fragment	complex rearrangement
acentric fragment	ring
translocation	double minute chromosomes
quadriradial	

Coordinates for positive cells will be recorded.

#### 6.3.5 Data Evaluation

The number and type of aberration will be listed for each concentration. The percent of cells with aberrations and the number of aberrations per cell will be recorded. Chromatid and chromosomal gaps will not be used in this analysis. Analyses of results will be based on the paper by Margolin et al Environmental Mutagenesis 5: 705-716, 1983.

### 7.0 Report

At the termination of the study, a draft and a final report will be prepared describing or containing: purpose of study, experimental design, sample properties and preparation, tabular and textual presentation of data, statistical analysis of data where appropriate, summary of results, conclusions, and quality assurance information (final report only).

### 8.0 Records to be Retained

All original data and a copy of the final report will be retained for not less than five years after completion of the study and stored in the Bioassay Systems Corporation archives. This material will be made available for inspection upon request or by permission of authorized representatives of the sponsor. The sponsor will be notified before final disposition of these items. The test sample will be returned to sponsor upon completion of testing.

#### 9.0 Quality Assurance

This study will be monitored under provisions of the BSC Quality Assurance Program and the final report will be reviewed by BSC Quality Assurance Unit personnel. This study will be conducted in accordance with FDA Good Laboratory Practice Regulations (21 CFR 58.1-58.219, 1979).

#### 10.0 Alterations of Study Design

All changes in or revisions of an approved protocol and the reasons therefore will be documented, signed by the Study Director, the Study Coordinator and a BSC Quality Assurance officer, dated, and maintained with the protocol.

# 11.0 Protocol Approval

## 11.1 Bioassay Systems Corp.

By: Marybeth Thomas  
 Title: Study Director  
 Date: 10/31/84

## 11.2 Bioassay Systems Corporation Quality Assurance Unit

### Content Approval:

By: Susan M. O'Connor  
 Title: Mgr. Quality Assurance  
 Date: 10/31/84

## 11.3 G. D. Searle & Co.

Charles E. Piper  
 Charles E. Piper, Ph.D.  
 Diplomate, A.B.T.  
 Study Coordinator  
 Product Safety Assessment

11/2/84  
 Date

Frank N. Kotsonis  
 Frank N. Kotsonis, Ph.D.  
 Diplomate, A.B.T.  
 Director of Toxicology  
 Product Safety Assessment

11/2/84  
 Date

Fred E. Kohn  
 Fred E. Kohn, Ph.D.  
 Senior Director  
 Product Safety Assessment

11/2/84  
 Date

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G.D. Seale & Co  
BSC Project Number: 12159 BSC Sample Number: 84-1226  
Study Title: In vitro Chromosomal Aberrations  
Protocol Amendment Number: #1

Section 3.6 Proposed Study Schedule

The initiation date of the range-finding  
experiment for the in vitro Chromosomal  
Aberration Assay is 11/14/84.

The initiation date of the in vitro  
Chromosomal Aberration Assay will  
be documented in a future amendment

	Date
Study Director Signature: <u>Margaret Thomas</u>	<u>11/20/84</u>
BSC Quality Assurance Officer Signature: <u>Susan M. Coleman</u>	<u>11/20/84</u>
Sponsor Representative Signature (if applicable): <u>Charles S. Papp</u>	<u>12/5/84</u>
Telephone Authorization of Sponsor (if applicable): <u>N/A</u>	

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G.D. Searle & Co  
BSC Project Number: 12159 BSC Sample Number: 84-1226  
Study Title: In vitro Chromosomal Aberrations  
Protocol Amendment Number: #2

## Section 3.6 Proposed Study Schedule

The initiation date for the in vitro  
Chromosomal Aberration assay is 12/4/84.

## Section 6.1 Test Concentrations

The following test sample and control  
concentrations will be performed:

⊕ S9

Test sample: 0.01, 0.51, 0.75, 0.99, 1.50, 2.01, 2.49, 3.00 mg/ml

Positive Control: 50 µg/ml Cyclophosphamide

Negative Control: 10% DMSO + S9

⊖ S9

Test sample: 0.01, 0.51, 0.75, 0.99, 1.50, 2.01, 2.49, 3.00 mg/ml

Positive Control: 5 µg/ml Mitomycin C

Negative Control: 10% DMSO

A 300 mg/ml stock solution of the test sample  
will be prepared in 100% DMSO. All dilutions  
of the test sample will be made in 100% DMSO.  
The initial stock solution will be used up to 72 hours.  
Dilutions will only be used on day prepared.

Study Director Signature:	<u>Marybeth Thomas</u>	Date	<u>12/4/84</u>
BSC Quality Assurance Officer Signature:	<u>Larry Perkins</u>		<u>12/4/84</u>
Sponsor Representative Signature (if applicable):	<u>Charles E. Phipps</u>		<u>12/10/84</u>
Telephone Authorization of Sponsor (if applicable):	<u>N/A</u>		

Bioassay Systems CorporationProtocol Amendment FormSponsor Name: G.D. Searle & Co.BSC Project Number: 12159 BSC Sample Number: 94-1226Study Title: In vitro Chromosomal AberrationsProtocol Amendment Number: #3Section 3.6 Proposed Study Schedule

The initiation date of the in vitro Chromosomal Aberration Assay is 12/5/84 not 12/4/84 as specified in Protocol Amendment # 2 because the stock solution prepared 12/4/84 (300 mg/ml) solidified & could not be used. A 200 mg/ml stock solution in DMSO was prepared prior to use on 12/5/84.

Section 6.1 Test Concentrations

The following test sample & control concentrations were used - instead of those listed in Amendment # 2 - as a result of the solidification of the 300 mg/ml stock solution:

⊕ S9 Test sample: 0.10, 0.50, 0.76, 1.00, 1.26, 1.50, 1.76, 2.00 mg/ml

Positive Control: 50 µg/ml Cyclophosphamide + S9

Negative Control: 1% DMSO + S9

⊖ S9 Test sample: 0.10, 0.50, 0.76, 1.00, 1.26, 1.50, 1.76, 2.00 mg/ml

Positive Control: 5 µg/ml Mitomycin C

Negative Control: 1% DMSO

A 200 mg/ml stock solution was prepared in 100% DMSO. All dilutions of the test sample were made in 100% DMSO & used only on the day prepared.

Section 4.0 Test Material Data

4.1.10 Solubility: Dimethylsulfoxide (> 100 mg/ml) - to reflect actual solubility.

Date

Study Director Signature:

Marjorie Thomas12/6/84

BSC Quality Assurance Officer Signature:

Dorothy Quinn12/6/84

Sponsor Representative Signature (if applicable):

Charles E. Piper12/18/84

Telephone Authorization of Sponsor (if applicable):

Charles E. Piper, Ph.D.12/5/84



Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G.D. Searle & Co  
 BSC Project Number: 12159 BSC Sample Number: 84-384 84-1226  
 Study Title: in vitro Chromosomal Aberrations  
 Protocol Amendment Number: #4

Section 3.6 Proposed Study Schedule  
 3.6.3 Study Completed 12/28/84

Section 4.1 Test Sample Description  
 Bioassay Systems determined the concentration of the test article in the carrier according to SOP Series 103, No. 146.

Section 4.2 Positive Control Material Characterization (Activated Assay)  
 4.2.8 Stability: at least 4 months (solution) at 4°C

Section 6.3.4  
 Metaphase cells were also analyzed for the following types of aberrations:  
 - interstitial deletions  
 - triradials  
 - cell with greater than one pulverized chromosome  
 - uncoiled chromosomes  
 Endoreduplication was recorded, but not included in the aberration frequency.

Study Director Signature: <u>Marybeth Thomas</u>	Date: <u>1/10/85</u>
BSC Quality Assurance Officer Signature: <u>Nancy G. Lewis</u>	<u>1/11/85</u>
Sponsor Representative Signature (if applicable): <u>Charles R. Piper</u>	<u>1/17/85</u>
Telephone Authorization of Sponsor (if applicable): <u>Dr. James Shaw</u>	<u>1/26/84</u> (C)

(C) MBT 1/10/85 authorization applies to authorization of methodology used in chemical analysis

MBT 1/10/85 - misc

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G.D. Searle & Co  
 BSC Project Number: 12159 BSC Sample Number: 8N-1226  
 Study Title: in vitro Chromosomal Aberrations  
 Protocol Amendment Number: # 5

Section 6.2

In the activated range-finding experiment the exposure period was two hours in serum free medium followed by a growth period of 28 hours in medium supplemented with serum and BrdU ( $1 \times 10^{-5} M$ ). In the nonactivated assay the cells are exposed to the test sample for a total of 30 hours in medium containing serum. Two hours after chemical addition an aliquot of BrdU (final conc.  $1 \times 10^{-5} M$ ) is added to the exposure medium.  
 In both assays the cells are grown for 28 hours in medium containing BrdU and harvested after a total of 30 hours.

Section 6.3.2<sup>14</sup> Chromosomal Analysis

One hundred cells from each of the duplicate flasks from a minimum of three test sample concentrations and the positive and the negative controls were analyzed for chromosomal aberrations.

Section 6.3.5 Data Evaluation

The percent of cells with greater than 1 aberration/cell is also reported.

@MBT 1/24/85 Wrong section of protocol

Study Director Signature: Marybeth Thomas Date: 1/25/85  
 BSC Quality Assurance Officer Signature: Steven M. O'Connell 1/25/85  
 Sponsor Representative Signature (if applicable): Charles E. Piper 1/30/85  
 Telephone Authorization of Sponsor (if applicable): \_\_\_\_\_

Bioassay Systems CorporationProtocol Amendment FormSponsor Name: G.D. Searle & Co.BSC Project Number: 12159BSC Sample Number: 84-1226A,BStudy Title: in vitro Chromosomal AberrationsProtocol Amendment Number: #6

Section 3.6.1  
 Test Substance Received: 10/19/84 & 10/31/84  
 - Two aliquots, A+B, respectively, were received.

Section 3.6 Changes made to section 3.6 in  
 Amendment #'s 1,2,3,4 were made to  
 reflect the actual initiation & completion  
 dates of the study.

Section 6.1 Additions made to section 6.1 in  
 Amendment #'s 2 & 3 were made to  
 complete information specified in the protocol

Section 4.1 Additions made to section 4.1 in  
 Amendment #4 <sup>②</sup> were made to clarify  
 the methodology used in analysis of  
 the test article in the carrier.

Section 4.2.8. The changes made in Amendment  
 #4 were made to clarify current stability  
 information.

Section 6.3.4 The changes made in Amendment  
 #4 clarified the complete list of aberrations  
 used in Chromosomal Analysis of CHO  
 cells. The additions listed were additions from  
 the original protocol.

Study Director Signature: Marybeth Thomas Date: 3/14/85BSC Quality Assurance Officer Signature: James M. O'Connor 3/16/85Sponsor Representative Signature (if applicable): Charles S. Viper 3-1-85Telephone Authorization of Sponsor (if applicable): N/A

② MBT 3/14/85 misentry

Bioassay Systems CorporationProtocol Amendment FormSponsor Name: G.D. Searle & CoBSC Project Number: 12159BSC Sample Number: 84-1226A,BStudy Title: in vitro Chromosomal AberrationsProtocol Amendment Number: #6 (con't)

(Amendment #5)

## Section 6.2

The additions made to section 6.2 were made to clarify the procedures used in the range-finding experiment.

## Section 6.3.4

The additions made to section 6.3.4 in Amendment #5 were made to clarify the number of cells to be counted from each of the duplicate flasks for the test samples & positive & negative controls.

## Section 6.3.5

The additions made to section 6.3.5 in Amendment #5 were made to clarify the results to be reported in the final report.

Study Director Signature: Marybeth Thomas Date: 3/14/85BSC Quality Assurance Officer Signature: Susan M. P. Connor 3/16/85Sponsor Representative Signature (if applicable): Charles E. Piper 3-21-85Telephone Authorization of Sponsor (if applicable): N/A

R&D PRODUCT DEVELOPMENT FUNCTION  
REPORT REVIEW AND RELEASE

DEPARTMENT: Product Development Analytical

DOCUMENT NUMBER: F-319-034-07

TITLE OF REPORT: SC-19129

TYPE OF REPORT: Analytical Summary in Support of Product Safety  
Assessment Study Number 2464

AUTHOR(S): Charles Demarest Charles Demarest 2-12-85

TECHNICAL WRITER: Michele Newcomb Michele Newcomb 3/14/85

APPROVAL: James Jiu James Jiu 14-March-85

REVIEWED BY:	<u>Reviewer</u>	<u>Signature</u>	<u>Date</u>
	Daniel Sweeney	<u>Daniel Sweeney</u>	<u>3-12-85</u>
	Kathy Klimovitz	<u>Kathryn P. Klimovitz</u>	<u>3-12-85</u>

APPROVAL FOR RELEASE:

<u>R. Baum</u> R. Baum, Director Analytical Development	<u>3/19/85</u> Date	<u>Larry Hansen</u> L. Hansen, Senior Director Product Development	<u>3/19/85</u> Date
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NORTH AMERICAN PRECLINICAL RESEARCH AND DEVELOPMENT  
SEARLE PHARMACEUTICALS AND CONSUMER PRODUCTS  
SKOKIE, ILLINOIS

ANALYTICAL SUMMARY  
Product Development Analytical Department

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Subject: SC-19129

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Summary Number: F-319-034-07

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Applicable to SA Study Number: 2464

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Test Article Characterization and Stability

Lot 84K-047-101 was analyzed using the release methods of testing, released against the current specifications (NS-S84-015-A), and given a re-evaluation period of one year prior to use in this study.

A summary of the significant results used to characterize the SC-19129 is presented in Table 1.

Table 1

	Pre-Study	Post-Study	
Analysis Report #	84N1058	85N0093	85N0094
Completion Date	10/16/84	02/15/85	02/15/85
Identity (HPLC)	Conforms to Standard	Conforms to Standard	Conforms to Standard
Assay (HPLC)	100.0% n = 3 s = 0.2	98.9% n = 3 s = 0.8	99.1% n = 3 s = 0.5
Water	9.8%	8.7%	8.4%

These results and all other results, coupled with the use of lot 84K-047-101 within its re-evaluation period, indicate that lot 84K-047-101 of SC-19129 was suitable for use in this study.

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Subject: SC-19129

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Summary Number: F-319-034-07

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Applicable to SA Study Number: 2464

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### Stability of Test Article in Carrier

The stability of SC-19129 in 100 mg/mL solutions of dimethyl sulfoxide was determined using a stability indicating HPLC method (M84-046-A). The samples were stored at ambient conditions and sampled at times 0, 1, 2, 4, 8, 24, 48, and 72 hours. The results of the analysis are presented in Table 2. The statistics, using data at  $t = 0$  as reference, are based on the percent relative recovery values.

The results of the linear regression analysis (MINITAB, Reference 1) gave a  $t$  value less than the table value (Reference 2, Table A-4), indicating no significant downward trend. The correlation between the observed recovery values and the predicted values exhibited a normal probability plot (References 1 and 3). Since the slope of the regression line showed no significant downward trend, and the differences between the observed recovery values and the predicted values were normally distributed, the SC-19129 in solutions of dimethyl sulfoxide at 100 mg/mL is considered stable for at least 72 hours at ambient conditions.

Notebook Reference: K. Klimovitz PDAD61 pp. 160-176

ANALYTICAL SUMMARY  
Product Development Analytical Department

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Subject: SC-19129

Summary Number: F-319-034-07

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Table 2

Stability of Test Article in Carrier

100 mg of SC-19129/mL of Dimethyl Sulfoxide

Report of Analysis # 84-2365

Time (Hours)	% SC-19129 Recovered	% Relative Recovery
0	100.8 101.2 100.8 99.7	Reference $\bar{X} = 100.6$
1	100.4 102.3	99.8 101.7
2	103.9 100.8	103.3 100.2
4	101.6 100.4	101.0 99.8
8	101.5 100.5	100.9 99.9
24	100.5 100.2	99.9 99.6
48	99.1 99.4	98.5 98.8
72	101.0 100.6	100.4 100.0
Intercept		100.7
Slope		- 0.020
t-Ratio		- 1.69
t(0.95, 12 df)		1.789
Correlation Coefficient Predicted vs Observed		0.945



ANALYTICAL SUMMARY  
Product Development Analytical Department

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Subject: SC-19129

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Summary Number: F-319-034-07

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Applicable to SA Study Number: 2464

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References:

1. Ryan, Jr., T. A., Joiner, B. L., and Ryan, B. F., "MINITAB Student Handbook", 1976, Wadsworth Publishing Co., Inc.
2. Natrella, M. G., "Experimental Statistics, National Bureau of Standards Handbook 91", 1963, US Government Printing Office
3. Filliben, J., Technometrics, 17 (1), 111 (1975)

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Product Development Analytical Department

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GLP Compliance Statement

To the best of our knowledge, the support activities provided by the Product Development Analytical Department for this study were conducted in compliance with the Good Laboratory Practices Regulations, as set forth in part 58, 21 CFR.



IN VITRO CHROMOSOMAL ABERRATION ASSAY OF SC-19129

Report Amendment Number 1

S.A. 2464

Change: The attached pages are an amendment to the original report.

Reason for Change: The concentration of Cyclophosphamide was incorrectly listed as 0.005 mg/ml and was corrected to 0.050 mg/ml.

This amendment does not affect the conclusions of the original report.

APPROVAL:

Charles E. Piper 7-18-85  
Charles E. Piper, Ph.D. Date  
Diplomate, A.B.T.  
Study Coordinator  
Product Safety Assessment

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Revised: 6/18/85, See Appendix D

S.A. 2464

TABLE 3  
The Effect of 5-19129 on the  
Induction of Chromosomal Aberrations in  
Chinese Hamster Ovary Cells

Activated Assay, 2 hr. Exposure + 8 hr. growth period

Conc. (mg/ml)	# Cells Scored	Mitotic Index	Number and Type of Aberrations <sup>a</sup>													Total No. of Aberr.	No. of Aberra- tions per cell	% Cells with Aber- rations	% Cells with >1 Aber- rations
			TB	SB	TD	F	AF	TR	QR	CR	ID	R	PU	PC	P+				
SC-19129 +S9																			
2.00	100	0.053	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
	100	0.083	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
1.76	100	0.086	0	0	0	0	0	0	0	0	0	0	1	0	1	0.01	1	1	
	100	0.090	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
1.50	100	0.097	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
		0.116	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
1% DMSO +S9	100	0.089	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
	100	0.096	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0	0	
Cyclophosphamide +S9																			
0.050	100 <sup>b</sup>	0.006	14	1	0	0	0	1	3	0	1	0	2	1	1	>24	>0.24	19	6
	100 <sup>b</sup>	0.010	8	1	2	1	1	8	2	0	1	0	0	1	1	>26	>0.26	25	4

<sup>a</sup>Code for identifying chromosomal aberrations presented in Appendix B.

<sup>b</sup>One cell observed with endoreduplication.

Revised: 6/18/85, See Appendix D

## APPENDIX D

### Amendment to the Final Report

1. Section Changed: Table 3, page 10, concentration of Cyclophosphamide.  
Reason for Change: The concentration of Cyclophosphamide was incorrectly listed as 0.005 mg/ml and was corrected to 0.050 mg/ml.
2. Section Changed: Table of Contents, page i  
Reason for Change: To reflect the addition of Appendix D.

Marybeth Thomas 6/18/85  
Study Director Date

Susan M. O'Connor 6/18/85  
Quality Assurance Officer Date