

FINAL REPORT

Micronucleus Assay with SC-19129

Prepared for:

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Prepared by:

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Director, Genetic Toxicology

6/7/85  
Date

S.A. 2504

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Revised: 6/14/85, See Appendix C

**TITLE:** Micronucleus Assay with SC-19129

**Author:** Maria H. Lugo, Ph.D. (Bioassay Systems Corporation, Woburn, MA)

**Study Number:** S.A. 2504

**Date:** 6/7/85

**Type of Report:** Final

**Summary:**

SC-19129 was investigated for its ability to cause micronuclei in mouse bone marrow cells. The three intended test concentrations that were analyzed for the induction of micronuclei were 500, 750 and 1000 mg/kg. Based on chemical analysis of the stock solution, the actual test concentrations were 102.3% of the target concentrations. No evidence of a significant increase in micronuclei formation was seen. The responses obtained from the negative and positive controls demonstrated that the test system was capable of detecting chemically induced micronuclei. These results lead to the conclusion that SC-19129 is not capable of inducing micronuclei in mouse bone marrow cells.

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Revised: 6/14/85, See Appendix C

## 1. Introduction

### 1.1 Objective of the Study

The objective of the study was to assess the ability of SC-19129 to cause micronuclei in mouse bone marrow cells. The test sample was received January 25, 1985. The study was initiated February 12, 1985 when animals were received for quarantine and technical work was completed April 19, 1985.

### 1.2 Principles of the Assay

The induction of micronuclei in bone marrow erythrocytes as a result of chemical treatment is recognized as a sensitive indicator of genotoxic potential.

Micronuclei are cytoplasmic bodies with the appearance of small nuclei in the cell. These entities arise from chromosomal lagging at anaphase, from acentric chromosomal fragments, or from spindle malformation during cell division. In mouse bone marrow, the cell population tested consists of erythroblasts undergoing their final mitosis before expulsion of the nucleus. The frequency of occurrence of micronuclei in the polychromatic erythrocyte cells of treated animals provides an indicator of in vivo cytogenetic damage.

## 2. Materials

### 2.1 Test Substance

|                            |                              |
|----------------------------|------------------------------|
| Identification:            | SC-19129                     |
| BSC No.:                   | 84-1226C                     |
| Lot/Batch No.:             | 84K-047-101                  |
| Physical State:            | Solid; powder                |
| Color:                     | White                        |
| Purity:                    | 99%                          |
| Stability:                 | Determined by the Sponsor    |
| Stability of Formulations: | Determined by the Sponsor    |
| Solubility:                | Dimethylsulfoxide, 100 mg/ml |
| Storage Conditions:        | Ambient, protect from light  |
| Safety Precautions:        | Routine                      |

The test chemical was assayed in solution using dimethylsulfoxide (DMSO) as a vehicle. A stock solution with a target concentration of 100 mg/ml was prepared. Concentration analysis (Appendix B) of the test chemical mixture confirmed that the stock solution was 102.3% of the target concentration.

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Two dilutions of the 100 mg/ml stock solution were made in DMSO to prepare solutions of 75 and 50 mg/ml. These solutions of 50, 75 and 100 mg/ml were prepared to provide doses of 500, 750 and 1000 mg/kg, respectively.

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

## 2.2 Positive Control Substance

Name: Cyclophosphamide  
Supplier: Sigma  
Lot/Batch No.: 33F-0157  
Physical State: Solid  
Color: White  
Purity: Reagent grade  
Composition: On file with manufacturer  
Stability: At least 1 year (solid), at least 4 months at 4°C (solution).  
Solubility: Water  
Storage Conditions: 4°C  
Safety Precautions: Avoid topical and respiratory contact.

A stock solution of 6 mg/ml cyclophosphamide in deionized water was prepared on the day of use to provide a dose of 60 mg/kg.

## 2.3 Negative Control Substance

Name: Dimethylsulfoxide (DMSO)  
Supplier: J.T. Baker Chemical Co.  
Lot No.: 144601  
Physical State: Liquid  
Color: Clear  
Purity: Reagent grade  
Composition: On file with manufacturer  
Storage Condition: Room temperature  
Stability: Indefinite  
Safety Precautions: Avoid topical and respiratory contact

## 2.4 Animals

7-week old male and female CD-1 mice (Mus musculus) were obtained from Charles River Breeding Labs, Wilmington, MA and quarantined at Bioassay Systems for 7 days. Mice were 56 days old at the time of dosing.

Each mouse was identified by ear punch. Animals were randomly assigned to dose groups using computer generated random numbers.

Mice were housed five per cage, and all animals in a cage received the same treatment. Mice were fed on Agway Prolab R-MH 3000, and untreated municipal water was supplied ad libitum.

The animal room had 12 hr light/dark cycle (lights on 7 a.m. to 7 p.m.), with temperature range of  $74^{\circ}\text{F} \pm 5^{\circ}\text{F}$ , and humidity range of  $50\% \pm 15\%$ . On the 5th day of quarantine the humidity was 5% below the specified range. This deviation does not affect the validity of the study.

Once a day during quarantine and test period, animals were examined for clinical disorders by physical observation. Quarantine observations for females were not recorded for the fifth day (2/16/85) of quarantine. On the first day of treatment all animals were weighed. Animal weight ranged from 23.3-34.6 grams.

### 3. Experimental Design

3.1 Test Groups: Animals for each test chemical were divided into groups as follows:

| Treatment        | Male | Female | Total |
|------------------|------|--------|-------|
| 1000 mg/kg       | 5    | 5      | 10    |
| 750 mg/kg        | 5    | 5      | 10    |
| 500 mg/kg        | 5    | 5      | 10    |
| Positive Control | 5    | 5      | 10    |
| Negative Control | 5    | 5      | 10    |
| Total            | 25   | 25     | 50    |

Two doses, 24 hours apart for each dose of test chemical, negative and positive controls, were administered to five males and five females by gavage. Dosing volumes for each animal were determined from the weight of the animal and the final dose desired (in mg/kg). Dosing volumes of 10 ml/kg were used.

### 3.2 Bone Marrow Extraction

Approximately 24 hours after the second dosing, animals were sacrificed by cervical dislocation. After cutting the skin, the distal end of a femur was cut open, and bone marrow cells were aspirated into a syringe containing approximately 1 ml fetal calf

serum using a 25 g x 5/8" needle. The bone marrow was transferred to a centrifuge tube containing approximately 1 ml fetal calf serum and gently mixed. The second femur was processed similarly using the same centrifuge tube, fetal calf serum and syringe as used to process the first femur.

Due to an error in time observation, positive control groups were sacrificed 1 hr 45 minutes early, negative control groups were sacrificed 30 minutes late. The rest of the study groups were sacrificed within 10-15 min. of the correct time. This deviation does not affect the validity of the study.

### 3.3. Slide Preparation and Staining

The pooled suspension from the two femurs was centrifuged for about 5 minutes at approximately 1000 rpm, and the supernatant was removed leaving a very small drop of serum on the pellet. The pellet was gently mixed to ensure a homogeneous mixture. A small drop of suspension mixture was placed near the frosted end of 4 glass microscope slides and spread in a thin smear on the slide. Slides were labelled with the animal number and A-D. Slides were allowed to air dry overnight. A and B slides were stained in Camco Quick stain (methyl alcohol: Wright-Giemsa stain combination) for 15 seconds. Slides C and D were stored unstained in case needed. After drying, slides were mounted with coverslips using Pro-Texx.

### 3.4 Slide Analysis and Data Collection:

Since animals were randomly assigned to test groups, the animal number was used as a coding number. Slide A was analyzed first and slide B was analyzed if needed. For each animal, 1000 polychromatic erythrocytes (PCEs) were counted for the presence of micronuclei. The frequency of micronucleated cells per animal was expressed as the number of micronucleated PCEs per 1000 PCEs counted. The ratio of polychromatic erythrocytes to normochromatic erythrocytes i.e., PCE/NCE in 200 erythrocytes for each animal was also recorded.

### 3.5 Data Evaluation

The Student's t-test was performed using the number of micronucleated cells/1000 PCEs/animal. A chemical is considered positive if the "t" value is greater than the critical t value at the 0.05 level of significance. Data from each sex were analyzed separately. The means and standard deviations for the number of micronucleated cells were calculated.

#### 4.0 Results

Male and female mice were dosed with 500, 750, and 1000 mg/kg of SC-19129. The results of micronucleus induction in each animal are presented in Tables 1 and 2. These results are summarized in Table 3. The average number of micronucleated PCEs ranged from 1.2 to 3.0 per 1000 PCEs for the animals dosed with SC-19129. The average numbers of micronucleated PCEs found in the male and female negative control animals were 2.4 and 1.0 per 1000 PCEs, respectively (Table 3). A Student's t-test analysis comparing each SC-19129 dosage to the negative control showed no significant effect of the test chemical for micronucleus induction in either sex. The positive control, cyclophosphamide (60 mg/kg), induced significant increases in the number of micronucleated PCEs in both sexes. The average number of micronucleated PCEs found in the male and female positive control animals were 42.0 and 43.6 per 1000 PCEs, respectively (Table 3).

Data for the percent PCE are also included in Table 1 and Table 2. The percent PCE at each SC-19129 dose level was similar to that of the negative controls. There was a slight reduction of the mean percent PCEs in the positive control animals.

#### 5.0 Conclusion

Under the conditions of the assay employed, SC-19129 did not cause any significant increase in the induction of micronuclei in the bone marrow cells of either male or female mice.



# Micronucleus Assay with SC-19129

**TABLE 1**

## Individual Data

| Dose       | Sex | Animal #        | %PCE <sup>a</sup> | MN-PCE <sup>b</sup> |
|------------|-----|-----------------|-------------------|---------------------|
| 500 mg/kg  | M   | 13              | 57.0              | 0                   |
|            |     | 25              | 60.5              | 0                   |
|            |     | 28              | 56.5              | 3                   |
|            |     | 31              | 47.0              | 0                   |
|            |     | 36              | 54.5              | 3                   |
|            | F   | 43              | 55.5              | 3                   |
|            |     | 47              | 51.0              | 2                   |
|            |     | 50              | 47.5              | 0                   |
|            |     | 58              | 41.0              | 4                   |
|            |     | 61              | 48.0              | 6                   |
| 750 mg/kg  | M   | 15              | 40.5              | 3                   |
|            |     | 18              | 55.5              | 1                   |
|            |     | 23              | 52.5              | 3                   |
|            |     | 27              | 55.5              | 0                   |
|            |     | 33              | 51.5              | 1                   |
|            | F   | 41              | 53.0              | 5                   |
|            |     | 65              | 23.5              | 0                   |
|            |     | 66              | 57.5              | 3                   |
|            |     | 67 <sup>c</sup> | -                 | -                   |
|            |     | 79              | 70.0              | 0                   |
| 1000 mg/kg | M   | 7               | 48.5              | 0                   |
|            |     | 12              | 37.5              | 5                   |
|            |     | 14              | 41.5              | 1                   |
|            |     | 26              | 45.0              | 1                   |
|            |     | 32              | 48.5              | 1                   |
|            | F   | 38              | 43.0              | 3                   |
|            |     | 56              | 31.0              | 2                   |
|            |     | 60              | 37.0              | 4                   |
|            |     | 62              | 47.0              | 0                   |
|            |     | 63              | 39.5              | 4                   |

<sup>a</sup> % PCE = 100 X PCE/(NCE + PCE). Values are based on 200 erythrocytes per animal.

<sup>b</sup> MN - PCE - Micronucleated Polychromatic Erythrocytes. Values are based on 1000 PCEs per animal.

<sup>c</sup> Slide was not used due to poor distinction between PCEs & NCEs.

# Micronucleus Assay with SC-19129

**TABLE 2**

## Individual Data

| Dose  | Sex | Animal #        | %PCE <sup>a</sup> | MN-PCE <sup>b</sup> |
|---|-----|-----------------|-------------------|---------------------|
| Positive<br>Control<br>Cyclophosphamide<br>60 mg/kg | M   | 9               | 30.5              | 29                  |
|   |     | 10              | 11.5              | 32                  |
|   |     | 11              | 18.5              | 61                  |
|   |     | 19 <sup>c</sup> | -                 | -                   |
|   |     | 35              | 15.0              | 46                  |
|   | F   | 40              | 17.5              | 43                  |
|   |     | 69              | 38.0              | 53                  |
|   |     | 74              | 14.0              | 38                  |
|   |     | 77              | 18.0              | 43                  |
|   |     | 78              | 11.5              | 41                  |
| Negative<br>Control<br>DMSO 100%<br>10ml/kg         | M   | 6               | 37.5              | 3                   |
|   |     | 22              | 45.0              | 1                   |
|   |     | 24              | 61.5              | 5                   |
|   |     | 30              | 56.5              | 1                   |
|   |     | 34              | 57.5              | 2                   |
|   | F   | 48              | 45.5              | 2                   |
|   |     | 64              | 75.5              | 1                   |
|   |     | 68              | 45.0              | 0                   |
|   |     | 70              | 45.5              | 2                   |
|   |     | 71              | 41.0              | 0                   |

<sup>a</sup>% PCE =  $100 \times \text{PCE} / (\text{NCE} + \text{PCE})$ . Values are based on 200 erythrocytes per animal.

<sup>b</sup>MN - PCE - Micronucleated Polychromatic Erythrocytes. Values are based on 1000 PCEs per animal.

<sup>c</sup>Slide was not used due to poor distinction between PCEs & NCEs.

# Micronucleus Assay with SC-19129

**TABLE 3**

## Summary Data

| Dose           | Sex | No. of Animals | MN-PCE <sup>a,b</sup><br>Mean $\pm$ S.D. |
|----------------|-----|----------------|--|
| CP, 60 mg/kg   | M   | 4              | 42.0 $\pm$ 14.7 <sup>c</sup>             |
|                | F   | 5              | 43.6 $\pm$ 5.64 <sup>c</sup>             |
| DMSO, 10 ml/kg | M   | 5              | 2.4 $\pm$ 1.67                           |
|                | F   | 5              | 1.0 $\pm$ 1.0                            |
| 500 mg/kg      | M   | 5              | 1.2 $\pm$ 1.64                           |
|                | F   | 5              | 3.0 $\pm$ 2.24                           |
| 750 mg/kg      | M   | 5              | 1.6 $\pm$ 1.34                           |
|                | F   | 4              | 2.0 $\pm$ 2.45                           |
| 1000 mg/kg     | M   | 5              | 1.6 $\pm$ 1.95                           |
|                | F   | 5              | 2.6 $\pm$ 1.67                           |

<sup>a</sup>MN - PCE - Micronucleated Polychromatic Erythrocytes. Values are based on 1000 PCEs per animal.

<sup>b</sup>Student's t-test analysis showed no significant increase in micronucleus induction (t less than critical t value at the 0.05 level of significance) with the test chemical treatment in either male or female mice.

<sup>c</sup>Statistically significant (t greater than critical t value at the 0.05 level of significance) compared to negative control (DMSO).

BIOASSAY SYSTEMS CORPORATION

Quality Assurance Report

Study Title: Micronucleus Assay with SC-19129  
Sponsor: G.D. Searle & Co.  
BSC Project No.: 850011  
BSC Sample No.: 84-1226C

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| <u>Date(s) of Inspection</u> | <u>Date Findings<br/>Reported<br/>to Study Director</u> | <u>Date Findings<br/>Reported<br/>To Management</u> |
|------------------------------|---|---|
| 1/29/85                      | 1/29/85   | 2/7/85  |
| 2/15/85                      | 2/15/85   | 2/22/85   |
| 2/21/85                      | 2/21/85   | 2/22/85   |
| 5/2/85                       | 5/2/85  | 6/7/85  |

Date: 6/7/85

Quality Assurance Officer: Susan M. O'Connor

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## APPENDIX A

### 1. Supervisory Personnel

Kenneth S. Loveday, Ph.D., Director of Genetic Toxicology  
Maria H. Lugo, Ph.D., 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  
REPORT OF ANALYSIS

BSC PROJECT NO(s): 850011

DATE OF ANALYSIS: 2/19-21/85

SPONSOR: G. D. Searle

TEST SAMPLE IDENTIFICATION

Sponsor Identification: SC19129 B-APM

BSC Sample No.: 84-1226C

CHEMICAL VEHICLE: Dimethyl sulfoxide (DMSO)  
Source: Baker Chemical Co.  
Lot No.: 144601

METHOD OF ANALYSIS: BSC Standard Operating Procedure # 103-146 R-1

SUMMARY OF RESULTS:

1. System Suitability: Mean Standard Peak Area: 2225601.7  
Relative Standard Deviation (RSD): 1.1%  
Number of Injections: 6
2. Standard Check - Percent of Theory: 98.1%
3. Control Sample - Percent Recovery: 109.2% (mean % recovery of two control samples)
4. Test Samples - Concentrations Measured/% Recovery
  - a. Formulation Lot No. 2-19: 102.4 mg/ml  
Theoretical Concentration: 100 mg/ml  
102.4% 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

# REPORT OF ANALYSIS

BSC PROJECT NO(s): 850011

DATE OF ANALYSIS: 2/19-21/85<sup>d</sup>

## SUMMARY OF DATA

| Sample/Standard Identification              | Preparation of Sample/Standard <sup>c</sup>                   | Injection No. | Peak Area <sup>e</sup> | Mean Peak Area          |
|---|---|---------------|------------------------|-------------------------|
| Standard                                    | 0.1000g to 100 ml with mobile phase                           | 1(a)          | 2188118                | 2225601.7<br>(1.1% RSD) |
|   |   | 2(a)          | 2234459                |                         |
|   |   | 3(a)          | 2201184                |                         |
|   |   | 4(a)          | 2246750                |                         |
|   |   | 5(a)          | 2245450                |                         |
|   |   | 6(a)          | 2237649                |                         |
|   |   | 7(b)          | 1916736                | 1887660<br>(3.4% RSD)   |
|   |   | 8(b)          | 1949995                |                         |
|   |   | 15(b)         | 1932111                |                         |
|   |   | 16(b)         | 1831709                |                         |
|   |   | 21(b)         | 1466246*               |                         |
|   |   | 22(b)         | 1807749                |                         |
| Standard Check                              | 0.1000g to 100 ml with mobile phase                           | 9(b)          | 1953352                | 1924059.5               |
|   |   | 10(b)         | 1894767                |                         |
| Control # 1<br>Sample (prepared on 2/19/85) | 0.1000g +1ml DMSO to 100 ml with mobile phase                 | 11(b)         | 2096112                | 1956358.5               |
|   |   | 12(b)         | 1816605                |                         |
|   | 0.1001g +1ml DMSO to 100 ml with mobile phase                 | 13(b)         | 2188061                | 2168287                 |
|   |   | 14(b)         | 2148513                |                         |
| Test Sample (Form. Lot # 2-19)              | Trial 1 (sampled 2/19/85)<br>1 ml to 100 ml with mobile phase | 17(b)         | 1925010                | 1920004                 |
|   |   | 18(b)         | 1914998                |                         |
|   | Trial 2 (sampled 2/20/85)<br>1 ml to 100 ml with mobile phase | 19(b)         | 2003261                | 1943646                 |
|   |   | 20(b)         | 1884031                |                         |

- (a) System suitability check-prior to analysis of test samples
- (b) Standard and sample chromatographic runs for analysis of test sample
- (c) All samples and standards were diluted 1.0 ml to 10.0 ml with mobile phase after the preparations noted below.
- (d) Results are from chromatographic runs performed 2-21-85. Chromatography was also run on 2-20-85, but results not used due to reproducibility problems thought to be the result of an increase in room temperature during the runs.
- (e) Peak area numbers from Perkin Elmer LCI-100 Laboratory Computing Integrator.

\* Not used - rejected as outlier, greater than 2x standard deviation from mean.

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# REPORT OF ANALYSIS

BSC PROJECT NO(s): 850011

DATE OF ANALYSIS: 2/19-21/85

## SUMMARY OF CALCULATIONS:

### 1. Standard Check - Percent of Theory

$$\begin{aligned} \% \text{ STD} &= \frac{R \text{ STD}}{R \text{ STDCK}} \times \frac{W \text{ STD CK}}{W \text{ STD}} \times 100\% \\ &= \frac{1887660}{1924059.5} \times \frac{100.0 \text{ mg}}{100.0 \text{ mg}} \times 100 \\ &= 98.1\% \end{aligned}$$

### 2. Control Sample - Percent Recovery

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

$$\begin{aligned} \text{CS\#1 \% Recovery} &= \frac{1956358.5}{1887660} \times \frac{1.000}{1.000} \times 100 \\ &= 103.6\% \end{aligned}$$

$$\begin{aligned} \text{CS\#2 \% Recovery} &= \frac{2168287}{1887660} \times \frac{1.000}{1.001} \times 100 \\ &= 114.8\% \end{aligned}$$

$$\text{Average \% Recovery} = 109.2\%$$

### 3. Concentration of Test Sample

$$\text{Conc. Test Sample} = \frac{R_x}{R_{STD}} \times \text{Conc. STD} \times \text{dilution factor}$$

(SC19129 mg/ml DMSO)

a) Test Sample Form. Lot No. 2-19, Trial 1

$$\text{Conc. Test Sample} = \frac{1920004}{1887660} \times 1.000 \text{ mg/ml} \times 100 = 101.7 \text{ mg/ml}$$

b) Test Sample Form. Lot No. 2-19, Trial 2

$$\text{Conc. Test Sample} = \frac{1943646}{1887660} \times 1.000 \text{ mg/ml} \times 100 = 103.0 \text{ mg/ml}$$

$$\text{Mean Conc. Test Sample Form. Lot No. 2-19} = 102.4 \text{ mg/ml}$$

$$\begin{aligned} \text{Expected Conc.} &= 100 \text{ mg/ml} \\ \% \text{ Recovery} &= 102.4\% \end{aligned}$$

SEE NEXT PAGE FOR KEY TO ABBREVIATIONS



NOTE: R STD = mean peak area for 5 standards (bracketed standards for % Recovery and Concentration of Sample Calculations).

R STD CK = Mean peak area for Standard Check

W STD = Weight of test material (BSC No. 84-1226C) in Standard

W STD CK = Weight of test material in Standard Check

R CS = Mean peak area of Control Sample

Rx = Mean peak area of Sample

Conc.STD = Concentration of test material in Standard (mg/ml equivalent DMSO)

Conc.CS = Concentration of test material in Control Sample (mg/ml DMSO)

## Appendix C

### Amendment to the Final Report

1. Section Changed: Table of Contents, page i  
Change Made: Addition of Appendix C  
Reason for Change: To reflect the addition of the Amendment to the final report.
2. Section Changed: Table of Contents, page i  
Change Made: Appendix B page numbers changed to 12-15  
Reason for Change: Page numbers were incorrect in final report.
3. Section Changed: Summary, page 1  
Change Made: Addition of final report date, 6/7/85  
Reason for Change: Date was inadvertently omitted in final report.
4. Section Changed: Section 2.1, page 2  
Change Made: "ABC No." was changed to "BSC No."  
Reason for Change: To clarify testing laboratory identification of the test sample.

Maria H. Lugo 6/14/85  
Study Director/Date

Susan M. O. Cramer 6/14/85  
Quality Assurance Officer/Date

BIOASSAY SYSTEMS CORPORATION

1.0 Study Title

Micronucleus Assay with SC-19129

2.0 Purpose of Study:

The purpose of this study is to assess the ability of the test substance to cause chromosome breaks and spindle malformation in mouse bone marrow 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: 850011

3.4 Supervisory Personnel:

Study Director: Maria Lugo, Ph.D.

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

3.5 Manager, Quality Assurance: Susan O'Connor

3.6 Proposed Study Schedule:

- 3.6.1 Test Substance Received: January 25, 1985
- 3.6.2 Study Initiated: week of Feb. 11, 1985
- 3.6.3 Study Completed: week of April 22, 1985
- 3.6.4 Final Report to Sponsor: May, 1985

#### 4.0 Test Material Data:

##### 4.1 Test Sample Description

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

|        |                            |                                 |
|--------|----------------------------|---------------------------------|
| 4.1.1  | Identification:            | SC-19129                        |
| 4.1.2  | BSC No.:                   | 84-1226                         |
| 4.1.3  | Lot/Batch No.:             | 84K-047-I01                     |
| 4.1.4  | Physical State:            | Solid; powder                   |
| 4.1.5  | Color:                     | White                           |
| 4.1.6  | Purity:                    | 99%                             |
| 4.1.7  | Stability:                 | To be determined by the Sponsor |
| 4.1.8  | Stability of Formulations: | To be determined by the Sponsor |
| 4.1.9  | Solubility:                | Dimethylsulfoxide 100 mg/ml     |
| 4.1.10 | Storage Condition:         | Ambient, protect from light     |
| 4.1.11 | Safety Precautions:        | Routine                         |

##### 4.2 Positive Control material Characterization

|        |                     |   |
|--------|---------------------|---|
| 4.2.1  | Name:               | Cyclophosphamide  |
| 4.2.2  | Supplier:           | Sigma   |
| 4.2.3  | Lot/Batch No.:      | To be specified 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           | At least 1 year (solid),<br>at least 4 months at 4°C<br>(solution). |
| 4.2.9  | Solubility:         | Water   |
| 4.2.10 | Storage conditions: | 4°C   |
| 4.2.11 | Safety Precautions: | Avoid topical and respiratory<br>contact.                           |

##### 4.3 Negative Control:

|        |                     |                                 |
|--------|---------------------|---------------------------------|
| 4.3.1  | Name:               | Dimethylsulfoxide (DMSO)        |
| 4.3.2  | Supplier:           | J.T. Baker Chemical Co.         |
| 4.3.3  | Lot No.:            | To be specified in final report |
| 4.3.4  | Physical state:     | Liquid                          |
| 4.3.5  | Color:              | Clear                           |
| 4.3.6  | Purity:             | Reagent grade                   |
| 4.3.7  | Composition:        | On file at manufacturer         |
| 4.3.8  | Storage condition:  | Room temperature                |
| 4.3.9  | Stability:          | Indefinite                      |
| 4.3.10 | Safety Precautions: | Avoid topical contact.          |

## 5.0 Description of Test System

### 5.1 Animals

- 5.1.1 Species: Mus musculus
- 5.1.2 Strain: CD-1
- 5.1.3 Supplier: Charles River Breeding Labs, Wilmington, MA
- 5.1.4 Age at start of study: 7-12 weeks
- 5.1.5 Weight at start of study 20 to 45 grams
- 5.1.6 Number and sex: 25 males and 25 females.
- 5.1.7 Justification of test system: This strain of mice has been used extensively in in vivo cytogenetics and has been shown to be sensitive to the effects of a variety of chemicals.

### 5.2 Identification

All animals are given a number upon receipt from the supplier. The number is placed on an animal ID card. Each animal is identified according to the S.O.P. entitled "Individual Identification of Rats, Mice and Guinea Pigs". Animals are randomly assigned to groups according to computer generated random numbers table as specified in Animal Toxicology SOP entitled "Cage and Treatment Assignment Using Computer Generated Random Numbers".

### 5.3 Husbandry

- 5.3.1 Housing: Five mice per cage
- 5.3.2 Food: Agway Prolab R-M-H 3000  
There are no contaminants that are reasonably expected to be present in the food at levels that are known to be capable of interfering with the purpose or conduct of the study.
- 5.3.3 Water: Untreated from municipal water supply ad libitum. There are no contaminants that are reasonably expected to be present in the water at levels that are known to be capable of interfering with the purpose or conduct of the study.
- 5.3.4 Light: 12-hour light/dark cycle (lights on 7am to 7pm)
- 5.3.5 Therapeutic agents: none
- 5.3.6 Temperature: 74°F ± 5°F
- 5.3.7 Humidity: 50% ± 15%
- 5.3.8 Air flow: 12-16 complete changes of 100% fresh air hourly.

### 5.4 Quarantine Period: Minimum of five days

- 5.5 Animal Selection: Once a day during quarantine and test period, animals will be examined for clinical disorders by physical observation. On the first day of treatment, all animals are weighed.

## 6.0 Experimental Design:

6.1 Test Groups: Animals for each test chemical are divided into groups as follows:

| Treatment        | Male | Female | Total |
|------------------|------|--------|-------|
| 1000 mg/kg       | 5    | 5      | 10    |
| 750 mg/kg        | 5    | 5      | 10    |
| 500 mg/kg        | 5    | 5      | 10    |
| Positive Control | 5    | 5      | 10    |
| Negative Control | 5    | 5      | 10    |
| Total            | 25   | 25     | 50    |

### 6.2 Dose Selection:

Doses provided by sponsor are 1000, 750 and 500 mg/kg.

6.3 Route of Administration: gavage as specified by Sponsor.

6.4 Frequency of Administration: Two, 24 hours apart for each dose of test substance, and negative and positive controls.

6.5 Absorption of the chemical into tissues of the animals will not be measured.

### 6.6 Dosage Formulation:

The concentrations will be formulated as solutions in DMSO for test chemical and in deionized water for cyclophosphamide, according to BSC standard operating procedure "General Procedures for the Preparation of Test Chemical Solutions."

### 6.7 Test Procedure:

6.7.1 Dosing volumes for each animal are determined from the weight of the animal and the final dose desired (in mg/kg). Dosing volumes of 5-10 ml/kg are used.

- 6.7.2 The negative control will be administered by gavage at 5-10 ml/kg
- 6.7.3 The positive control, cyclophosphamide, will be dosed twice, at a dose of 60 mg/kg at each time in the same volumes as the test chemical.
- 6.7.4 Approximately 24 hours after the second dosing, animals will be sacrificed by cervical dislocation. After cutting the skin, the distal end of a femur is cut open carefully with a pair of scissors until an opening to the marrow canal becomes visible.
- 6.7.5 A needle fitted on a syringe containing approximately 1 ml fetal calf serum, is inserted into the bone. The bone marrow is gently aspirated into syringe and transferred into a centrifuge tube containing approximately 2 ml fetal calf serum. The suspension is mixed gently.
- 6.7.6 The suspension is centrifuged for approximately 5 minutes at approximately 1000 rpm, and the supernatant is removed leaving a very small drop of serum on the pellet. The pellet is gently mixed to ensure a homogeneous mixture.
- 6.7.7 A small drop of suspension mixture is placed near the frosted end of a glass microscope slide, pulled behind slightly with a clean slide at 45° angle and pushed forward resulting in a thin smear on the slide. Slides are allowed to air dry overnight, or at 37°C, for one to two hours.
- 6.7.8 Slide Staining and Mounting.
- Slides are stained either in direct Giemsa, or in May Grunwald-Giemsa combination according to the procedures outlined in BSC Standard Operating Procedures "Processing bone marrow cells, and preparing slides for mouse bone marrow micronucleus assay". After drying, slides are coverslipped with Pro-Texx, or Permount.

#### 6.7.9 Slide Analysis and Data Collection.

One thousand (1000) polychromatic erythrocytes (PCE) are counted for the presence of micronuclei. Ratio of Polychromatic erythrocytes to normochromatic erythrocytes (NCE) i.e., PCE/NCE in 200 erythrocytes is also recorded.

#### 6.7.10 Data Evaluation:

The frequency of micronucleated cells is expressed as number of micronucleated PCEs per 1000 PCEs counted. Results are analyzed using a t-test at the 0.05 level of significance. An increasing dose-response is not necessary to obtain a positive result.

### 7.0 Report

At the termination of the study, a draft and final report will be prepared describing the 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 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 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 the 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 Corporation

By: Maria H. Lugo  
Title: Study Director  
Date: 1/31/85

11.2 Bioassay Systems Corporation Quality Assurance Unit

Content Approval

By: Susan M. O'Connor  
Title: Manager, Q.A.  
Date: 1/31/85

11.3 G.D. Searle & Co.

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

2-5-85  
Date

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

2/5/85  
Date

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

2/5/85  
Date

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G.D. Searle  
BSC Project Number: 850011 BSC Sample Number: 84-1226C  
Study Title: Micronucleus Assay with SC-19129 <sup>cep 7/4/85</sup>  
Protocol Amendment Number: 1

Section 6.7.5

Instead of a total of 3 ml of FCS  
(1 ml in syringe + 2 ml in 15 ml tube)  
we will have 2 ml of FCS total (1 ml  
in syringe + 1 ml in 15 ml tube) to  
minimize loss of material.

① Study Title completed by CE. Piper.  
<sup>cep 7/4/85</sup>

Study Director Signature: Maria H. Lugo Date: 2/21/85  
BSC Quality Assurance Officer Signature: Susan M. O'Connor <sup>2/21/85</sup>  
Sponsor Representative Signature (if applicable): Charles S. Piper <sup>3/4/85</sup>  
Telephone Authorization of Sponsor (if applicable): \_\_\_\_\_

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G. D. Searle & Co.  
BSC Project Number: 850011 BSC Sample Number: 84-1226C  
Study Title: Micronucleus Assay with SC-19129  
Protocol Amendment Number: 2

PROTOCOL SECTION 2.0 Purpose of the Study:

The purpose of the study is to assess the ability of the test substance to cause micronuclei in mouse bone marrow cells.

REASON FOR CHANGE: The protocol purpose was changed to better reflect the endpoint of the assay. Micronuclei formation is the end product of the micronucleus assay. Chromosome breaks and spindle malformation are the events during cell division which lead to the formation of micronuclei.

Study Director Signature: Maria H. Lugo Date: 5/3/85  
BSC Quality Assurance Officer Signature: Simon M. O'Connor 5/3/85  
Sponsor Representative Signature (if applicable): Charles E. Piper 5/14/85  
Telephone Authorization of Sponsor (if applicable): \_\_\_\_\_

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G.D. Searle  
BSC Project Number: 850011 BSC Sample Number: 84-1226C  
Study Title: Micronucleus Assay with SC-19129  
Protocol Amendment Number: 3

Section 4.1: Bioassay Systems will determine concentration of the test article in carrier according to SOP Series 103 No. 146-R1, and original.

Reason for change: To clarify method of chemical analysis.

Study Director Signature: Maria H. Lago Date: 5/10/85  
BSC Quality Assurance Officer Signature: Marky Herrero 5/10/85  
Sponsor Representative Signature (if applicable): Charles E. Piper 5/14/85  
Telephone Authorization of Sponsor (if applicable): \_\_\_\_\_

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G.D. Searle & Co.  
BSC Project Number: 850011 BSC Sample Number: 84-12266  
Study Title: Micronucleus Assay with SC-19129  
Protocol Amendment Number: 4

PROTOCOL SECTION 4.1.3 Lot/Batch No.:

84K-047-101

REASON FOR CHANGE: Error in protocol.

Study Director Signature: Maria H. Lugo Date: 5/29/85  
BSC Quality Assurance Officer Signature: James H. Oden 6/5/85  
Sponsor Representative Signature (if applicable): Charles E. Piper 6-5-85  
Telephone Authorization of Sponsor (if applicable): \_\_\_\_\_

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G. D. Searle & Co.  
BSC Project Number: 850011 BSC Sample Number: 84-1226C  
Study Title: Micronucleus Assay with SC-19129  
Protocol Amendment Number: 5

PROTOCOL SECTION 3.6 Proposed Study Schedule

The purpose of this amendment is  
to document actual study dates:

- Study was initiated February 1<sup>st</sup> <sup>2<sup>nd</sup> 6/5/85</sup>, 1985
- Technical work was completed April 19, 1985

Study Director Signature: Maria H. Lago Date: 5/29/85  
BSC Quality Assurance Officer Signature: James M. O'Connor 6/5/85  
Sponsor Representative Signature (if applicable): Charles E. Piper 6-5-85  
Telephone Authorization of Sponsor (if applicable): \_\_\_\_\_

Bioassay Systems CorporationProtocol Amendment Form

Sponsor Name: G. D. Searle & Co.  
BSC Project Number: 850011 BSC Sample Number: 84-1226C  
Study Title: Micronucleus with SC-19129  
Protocol Amendment Number: 6

PROTOCOL SECTION 5.1.5

Weight at start of dosing: 20-45 grams

REASON FOR CHANGE:

"Weight At start of study" was changed to read  
"Weight AT start of dosing" in order to reflect  
the actual time the animals are  
weighed as specified by Section 5.5  
of protocol.

Study Director Signature: Maria H. Hugo Date: 6/6/85  
BSC Quality Assurance Officer Signature: James H. O. G. 6/6/85  
Sponsor Representative Signature (if applicable): Charles E. Pugh 6/6/85  
Telephone Authorization of Sponsor (if applicable): \_\_\_\_\_

R&D PRODUCT DEVELOPMENT FUNCTION  
REPORT REVIEW AND RELEASE

Page 1 of 6

DEPARTMENT: Product Development Analytical

DOCUMENT NUMBER: F-327-034-10

TITLE OF REPORT: SC-19129

TYPE OF REPORT: Analytical Summary in Support of Product Safety  
Assessment Study Number 2504

| AUTHOR(S):                | DATE           | REVIEWER(S):             | DATE           |
|---------------------------|----------------|--------------------------|----------------|
| <u>Charles W. Darnest</u> | <u>5-24-85</u> | <u>Mary E. Nepe</u>      | <u>5/24/85</u> |
|                           |                | <u>Pollyam Gu (D.S.)</u> | <u>5/28/85</u> |
|                           |                |                          |                |

TECHNICAL WRITER:

Michele Newcomb Michele Newcomb

| APPROVAL:        | DATE              |
|------------------|-------------------|
| <u>James Jui</u> | <u>28-May-'85</u> |
|                  |                   |

APPROVAL FOR RELEASE:

|                        |                |                     |                |
|------------------------|----------------|---------------------|----------------|
| <u>R. Baum</u>         | <u>5/29/85</u> | <u>L. Hansen</u>    | <u>5/31/85</u> |
| R. Baum, Director      | Date           | L. Hansen,          | Date           |
| Analytical Development |                | Senior Director     |                |
|                        |                | Product Development |                |

NORTH AMERICAN PRECLINICAL RESEARCH AND DEVELOPMENT  
SEARLE PHARMACEUTICALS AND CONSUMER PRODUCTS  
SKOKIE, ILLINOIS



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Subject: SC-19129

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Summary Number: F-327-034-10

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Applicable to SA Study Number: 2504

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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                |
|--------------------|----------------------------|---------------------------|
| Report of Analysis | 84N1058                    | 85N0442                   |
| Completion Date    | 10/16/84                   | 05/16/85                  |
| Identity (HPLC)    | Conforms to<br>Standard    | Conforms to<br>Standard   |
| Assay (HPLC)       | 100.0%<br>n = 3<br>s = 0.2 | 99.8%<br>n = 3<br>s = 0.4 |
| Water              | 9.8%                       | 8.9%                      |

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-327-034-10

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Applicable to SA Study Number: 2504

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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

Subject: SC-19129

Summary Number: F-327-034-10

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

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Subject: SC-19129

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Summary Number: F-327-034-10

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Applicable to SA Study Number: 2504

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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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Subject: SC-19129

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Summary Number: F-327-034-10

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Applicable to SA Study Number: 2504

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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.