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FINAL REPORT

Test Facility Study No. 9601568

SM-102
In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes

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12 January 2017

TABLE OF CONTENTS

LIST OF TABLES 4
LIST OF APPENDICES 5
COMPLIANCE STATEMENT 6
QUALITY ASSURANCE STATEMENT 7
1. RESPONSIBLE PERSONNEL 8
1.1. Test Facility 8
1.2. Individual Scientists (IS) at Test Facility 8
2. SUMMARY 9
3. INTRODUCTION 10
4. MATERIALS AND METHODS 10
4.1. Test and Reference Items 10
4.1.1. Test Item 10
4.1.2. Reference Items 10
4.2. Test Item Characterization 11
4.3. Analysis of Test Item 11
4.4. Test Item Inventory and Disposition 11
4.5. Dose Formulation and Analysis 11
4.5.1. Preparation of Reference Items 11
4.5.2. Preparation of Test Item 12
4.5.3. Sample Collection and Analysis 12
4.6. Test System 12
4.6.1. Justification for Dose Level Selection 13
4.6.2. Blood Sampling 13
4.6.3. Culture Medium 13
4.6.4. Lymphocyte Culture 13
4.7. S9 Mix 13
4.8. Experimental Design 14
4.9. Treatment 14
4.10. Medium Change (Wash) and Cytochalasin B (Cyt B) Treatment 14
4.11. Harvesting and Staining 15
4.12. Slide Examination 15
4.12.1. Cytokinesis-Block Proliferation Index (CBPI) 15
4.12.2. Selection of Slides for Micronucleus Assessment 16
4.12.3. Microscopic Analysis of Micronucleus Frequencies 16
5. COMPUTERIZED SYSTEMS 17
6. EVALUATION AND INTERPRETATION OF RESULTS 18
6.1. Assay Acceptance Criteria: 18
6.2. Statistical Analysis 18
6.3. Interpretation of Results 18

7. RETENTION OF RECORDS 19
8. RESULTS 20
8.1. Dose Formulation Analyses 20
8.2. Dose Selection 20
8.3. Micronucleus Assessment 20
8.4. Incidental Observations 20
9. CONCLUSION 21

LIST OF TABLES

Table 1 SM-102 - Summary Results and Statistical Analysis 22

LIST OF APPENDICES

Appendix 1 Study Plan, Amendment and Deviations 23
Appendix 2 Test Item Characterization 61
Appendix 3 Results for Individual Cultures 64
Appendix 4 Dose Formulation Analysis Report 68
Appendix 5 Historical Control Results 86

COMPLIANCE STATEMENT

The study was performed in accordance with the OECD Principles of Good Laboratory Practice and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA), Japan (MHLW), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions from the above regulations are listed below.

- Characterization of the Test Item was performed by the Sponsor subcontractor according to established SOPs, controls and approved test methodologies to ensure integrity and validity of the results generated; these analyses will not be conducted in compliance with the GLP or GMP regulations.
- Stability testing of the supplied Test Item was not determined in this study. It will be performed by the Sponsor subcontractor at a laboratory that follows FDA Good Manufacturing Practice (GMP) regulations.

This study was conducted in accordance with the procedures described herein. All deviations authorized/acknowledged by the Study Director are documented in the Study Records. The report represents an accurate and complete record of the results obtained.

There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

[redacted] _____ Date: [redacted]

[redacted] MSc

Study Director

QUALITY ASSURANCE STATEMENT

Study Number: 9601568

This Study has been audited by Quality Assurance in accordance with the applicable Good Laboratory Practice regulations. Reports were submitted in accordance with SOPs as follows:

QA INSPECTION DATES

		Dates Findings Submitted to:	
Date(s) of Audit	Phase(s) Audited	Study Director	Study Director Management
12-Sep-2016	Final Study Plan	12-Sep-2016	12-Sep-2016
12-Sep-2016	Dose Preparation	12-Sep-2016	12-Sep-2016
18-Nov-2016 21-Nov-2016 - 22-Nov-2016	Data Review - In Vitro Sciences	07-Dec-2016	07-Dec-2016
21-Nov-2016 - 22-Nov-2016	Data Review - Analytical Chemistry	23-Nov-2016	23-Nov-2016
21-Nov-2016 - 22-Nov-2016	Final Report	07-Dec-2016	07-Dec-2016
22-Nov-2016	Final Phase Report - Dose Formulation Analysis	23-Nov-2016	23-Nov-2016
09-Jan-2017	Study Plan Amendment 1	09-Jan-2017	09-Jan-2017

In addition to the above-mentioned audits, process-based and/or routine facility inspections were also conducted during the course of this study. Inspection findings, if any, specific to this study were reported by Quality Assurance to the Study Director and Management and listed as a Phase Audit on this Quality Assurance Statement.

The Final Report has been reviewed to assure that it accurately describes the materials and methods, and that the reported results accurately reflect the raw data.

[redacted]
Quality Assurance Auditor

[redacted]
Date

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director	[redacted]	MSc
Test Facility Management	[redacted]	PhD, DABT

1.2. Individual Scientists (IS) at Test Facility

Dose Formulation Analysis	[redacted]	BSc

2. SUMMARY

The objective of this study was to determine the potential genotoxicity of SM-102, using an in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes.

The experimental design was as follows:

Text Table 1
Main Test

Dose No.	Formulation Conc. (µg/mL)[a]	Final Conc. (µg/mL)	Number of Cultures		
			4 Hours (0S9)	4 Hours (+S9)	24 Hours (0S9)
Negative Control	-	-	2	2	2
1/ SM-102	325	3.25	2	2	2
2/ SM-102	568	5.68	2	2	2
3/ SM-102	995	9.95	2	2	2
4/ SM-102	1740	17.4	2	2	2
5/ SM-102	3050	30.5	2	2	2
6/ SM-102	5330	53.3	2	2	2
7/ SM-102	9330	93.3	2	2	2
8/ SM-102	16300	163	2	2	2
9/ SM-102	28600	286	2	2	2
10/ SM-102	50000	500	2	2	2
NOC	25	0.25	2	-	-
	30	0.30	2	-	-
CP	1000	10	-	2	-
	1500	15	-	2	-
MMC	10	0.10	-	-	2
	20	0.20	-	-	2

a Theoretical concentrations; actual concentrations may differ slightly due to the limitations of the instruments used.
b Where the high level = 0.5 mg/mL

Human peripheral blood lymphocytes were treated in the absence and presence of an Aroclor-induced S9 activation system for 4 hours and continuously for 24 hours. The positive controls caused statistically significant increases in the incidence of micronucleated binucleate cells in each regime of the study, confirming the sensitivity of the test system and the effectiveness of the S9 mix.

Cultures treated with SM-102 at levels up to 500 µg/mL, did not show any statistically significant increases in the incidence of micronucleated binucleate cells. Precipitation was observed at the end of treatment at the highest dose level tested, in the 4 hour regime and the 24 hour regime in the absence of S9 mix. No precipitate was observed in the presence of S9 mix. Cloudy media was observed in the 4 hour regime in the absence of S9 mix at dose levels ≥ 93.3 µg/mL, in the 4 hour regime in the presence of S9 mix at the highest dose level and in the 24 hour regime at dose levels ≥ 286 µg/mL. No cytotoxicity was observed in the assay.

It is concluded that SM-102 did not show any evidence of genotoxic activity in this in vitro test for induction of micronuclei in human peripheral blood lymphocytes, when tested in accordance with regulatory guidelines.

3. INTRODUCTION

The objective of this study was to determine the potential genotoxicity of SM-102, using an in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes.

The design of this study was based on OECD Guideline 487 and ICH Guideline S2(R1).

The Study Director signed the Study Plan on 08 Sep 2016, and dosing was initiated on 15 Sep 2016. The experimental start date was 12 Sep 2016, and the experimental completion date was 26 Oct 2016. The study was completed on the date of the Study Director approval of this report (refer to the appropriate signature page). The Study Plan, last Study Plan amendment, and deviations are presented in Appendix 1.

4. MATERIALS AND METHODS

4.1. Test and Reference Items

4.1.1. Test Item

Identification:	SM-102
Batch (Lot) No.:	RL-100-211-1
Retest Date:	27 Oct 2017
Molecular Weight:	710.18
Purity:	95.72% (All concentrations and dose levels throughout this report were corrected for purity using a purity of 95.3%.)
Storage Conditions:	Kept in a freezer set to maintain -20°C

4.1.2. Reference Items

4.1.2.1. Negative Control

Identification:	Ethanol
Supplier:	Commercial Alcohols
Batch /Lot No.:	020612
Expiration Date:	Jun 2017
Storage Conditions:	Kept at ambient room temperature

4.1.2.2. Positive Controls

In the Absence of S9 Mix

Identification:	Mitomycin C (MMC)
CAS No.:	50-07-7
Identity:	Nocodazole (NOC)
CAS No.:	31430-18-9

In the Presence of S9 Mix

Identification: Cyclophosphamide monohydrate (cyclophosphamide, CP)

CAS No.: 6055-19-2

Full details of positive controls including supplier, storage, expiry date and formulation are retained as part of the Test Facility records (MMC, NOC) or study records (CP). Copies of certificates of analysis are retained as raw data.

4.2. Test Item Characterization

The Sponsor provided to the Test Facility documentation of the identity, strength, purity, composition, and stability for the Test Item. A Certificate of Analysis was provided to the Test Facility and is presented in Appendix 2.

4.3. Analysis of Test Item

A Certificate of Analysis was provided by the Sponsor and is presented in Appendix 2.

4.4. Test Item Inventory and Disposition

Records of the receipt, distribution, and storage of Test Item were maintained with the study raw data. All unused Test Item was returned to the Sponsor following completion of the experimental phase of the study. Any remaining Reference Items (negative and positive controls) will be retained at the Test Facility or discarded upon expiry.

4.5. Dose Formulation and Analysis

4.5.1. Preparation of Reference Items

4.5.1.1. Preparation of Negative Control

An adequate amount of the Negative Control, ethanol, was dispensed into a vial for administration to control cultures. The aliquot was stored in a refrigerator set to maintain 4°C until use. Any residual volumes were discarded before issuance of the Final Report.

4.5.1.2. Preparation of Positive Controls

Mitomycin C and Nocodazole were prepared the day of use or up to 6 months prior to use; adequate amounts were dispensed into vials, and stored in a freezer set to maintain -80°C (MMC) or -20°C (NOC), protected from light, until use. The aliquots were removed from the freezer and allowed to warm to ambient room temperature before dosing. Cyclophosphamide was prepared on the day of use. Any residual volumes were discarded after completion of dosing.

4.5.2. Preparation of Test Item

The Test Item was prepared as a stock solution (50 mg/mL) in the chosen vehicle (ethanol) and all lower level formulations were made by serial dilution. The formulations were prepared 3 days prior to use and were stored in a refrigerator set to maintain 4°C until use. The formulations were removed from the refrigerator and allowed to warm to room temperature for at least 30 minutes before dosing. Any residual volumes were discarded before issuance of the Final Report.

4.5.3. Sample Collection and Analysis

Samples from dose formulations 1 to 10 were collected for concentration analysis only. Homogeneity, density and stability were not determined on these samples. The positive control formulations were not subjected to analysis for safety reasons and because the biological response of the test system is considered to be the best measure of the appropriateness of the formulations.

Samples to be analyzed were transferred at ambient room temperature, to the Analytical Chemistry department at the Test Facility on the date prepared. Any residual/retained analytical samples were discarded before issuance of the Final Report.

4.5.3.1. Analytical Method

Analyses were performed by HPLC, using a validated analytical procedure (Test Facility Study Number 1801841).

4.5.3.2. Concentration Analysis

Duplicate 1 mL samples for dose numbers 1 and 2 and duplicate 0.5 mL samples for dose numbers 3 to 10 were taken and sent to the analytical laboratory for analysis. Additional duplicate 1 mL samples for dose numbers 1 and 2 and duplicate 0.5 mL samples for dose numbers 3 to 10 were taken and retained at the Test Facility as backup samples. Concentration results were considered acceptable if sample concentration results were within ±10% of nominal for the stock solution and ±15% of nominal for lower level solutions. After acceptance of the analytical results, backup samples were discarded.

4.5.3.3. Stability Analysis

Stability analyses performed previously at the Test Facility under Study Number 1801841 demonstrated that the Test Item is stable in the vehicle when prepared and stored under the same conditions at concentrations bracketing those used in the present study. Stability data have been retained in the study records for Study No. 1801841.

4.6. Test System

Primary cultures of human peripheral lymphocytes are recommended because of their low and stable background frequency of micronucleus formation. In addition, human cells are generally the most relevant for risk assessment.

4.6.1. Justification for Dose Level Selection

Typically, the Test Item is dosed at a range of concentrations, but is only assessed at the highest levels below the toxic level or, if non-toxic, at levels up to the standard limit of 0.5 mg/mL or 1 mM, whichever is lower (ICH S2(R1) guideline). Compounds with limited aqueous solubility are tested up to a level expected to show visible precipitation in the culture medium at the end of treatment.

4.6.2. Blood Sampling

A peripheral blood sample was taken by venipuncture from two young (approximately 18-35 years of age), healthy, non-smoking, male donors with no known recent exposures to genotoxic chemicals or radiation. The blood samples were collected directly into tubes containing sodium heparin and then held at room temperature for less than 2 hours prior to blood addition to the culture medium. Blood from both donors was pooled in the culture medium prior to culture initiation.

4.6.3. Culture Medium

Complete RPMI 1640 medium was prepared by supplementing RPMI 1640 medium with the following filter-sterilized components: 10% (v/v) fetal calf serum, 50 µg gentamycin per mL, and 4 units heparin per mL.

4.6.4. Lymphocyte Culture

Whole blood was mixed with medium (0.4 mL blood per 4 mL medium) and phytohemagglutinin (1 mL per 49 mL diluted blood) was added to stimulate lymphocyte division. Aliquots of 5 mL of cell suspension were dispensed into flat-sided culture tubes and then placed in an incubator set to maintain 37°C with 5% CO_2 in a humidified atmosphere.

4.7. S9 Mix

The S9 mix, used as a model of intact mammalian metabolism, was prepared on the day of use and contained 10% v/v S9 fraction (Aroclor 1254 induced male rat liver fraction supplied by Moltox) and the following sterile cofactors: 8 mM $MgCl_2$, 33 mM KCl, 100 mM sodium phosphate buffer pH 7.4, 5 mM glucose-6-phosphate, and 4 mM NADP. The S9 mix was stored in a refrigerator set to maintain 4°C or on ice until required. A copy of the manufacturer's quality control certificate for the S9 fraction is retained as raw data.

4.8. Experimental Design

Text Table 2
Main Test

Dose No.	Formulation Conc. (µg/mL)[a]	Final Conc. (µg/mL)	Number of Cultures		
			4 Hours (0S9)	4 Hours (+S9)	24 Hours (0S9)
Negative Control	-	-	2	2	2
1/ SM-102	325	3.25	2	2	2
2/ SM-102	568	5.68	2	2	2
3/ SM-102	995	9.95	2	2	2
4/ SM-102	1740	17.4	2	2	2
5/ SM-102	3050	30.5	2	2	2
6/ SM-102	5330	53.3	2	2	2
7/ SM-102	9330	93.3	2	2	2
8/ SM-102	16300	163	2	2	2
9/ SM-102	28600	286	2	2	2
10/ SM-102	50000	500	2	2	2
NOC	25	0.25	2	-	-
	30	0.30	2	-	-
CP	1000	10	-	2	-
	1500	15	-	2	-
MMC	10	0.10	-	-	2
	20	0.20	-	-	2

a = Theoretical concentrations; actual concentrations may differ slightly due to the limitations of the instruments used. b = Where the high level = 0.5 mg/mL.

4.9. Treatment

Treatments were performed approximately 48 hours (44-48 hours) after culture initiation. Appropriate dilutions of the Test Item and Positive Control formulations were prepared so as to reach the final concentrations indicated in the experimental design (see Text Table 2). Cultures tested in the absence of S9 mix were treated as indicated in the experimental design then returned to the incubator for 4 or 24 hours as appropriate. For cultures tested in the presence of S9 mix, 1 mL of S9 mix was added immediately prior to treatment, then the cultures were returned to the incubator for 4 hours. A standard dose volume of 10 µL Test Item, Negative Control or Positive Control per mL of culture was used throughout.

The Test Item was tested over a wide range of dose levels (3.25 to 500 µg/mL) using all treatment regimes (4-hour treatment period in the absence and presence of S9 mix and a 24-hour treatment period in the absence of S9 mix) so that analyzable cells would be available for at least three dose levels for each regime. Duplicate cultures were treated at each experimental point.

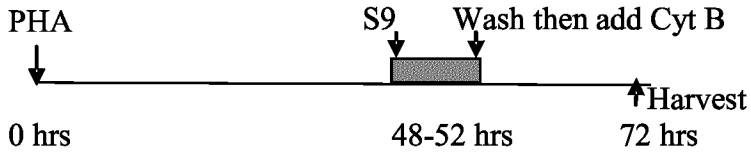
4.10. Medium Change (Wash) and Cytochalasin B (Cyt B) Treatment

After the 4-hour treatments, cultures were centrifuged, and the supernatant replaced with fresh complete medium containing 6 µg Cytochalasin B per mL. Incubation was continued for a further 20 hours prior to harvesting.

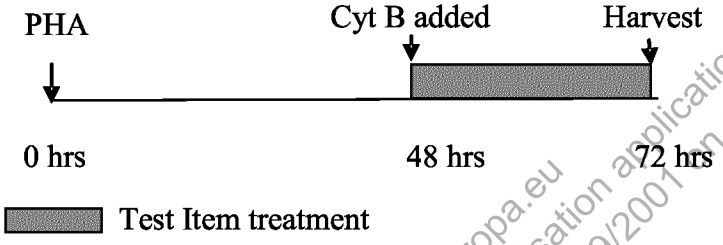
For the 24-hour treatment, the medium added to the cultures just prior to dosing contained 6 µg Cytochalasin B per mL of culture medium. The 24-hour treatment cultures were harvested at the end of the treatment period.

Text Figure 1

4-hour treatment in the absence or presence of S9 mix (+S9)



24-hour treatment in the absence of S9 (0S9)



4.11. Harvesting and Staining

Cultures were harvested by centrifugation. The cell pellet was resuspended in hypotonic solution (0.075 M KCl) at ambient temperature. Fixative (24:1 v/v, methanol:acetic acid) was mixed with the suspended cells and, following another centrifugation, the cells were treated with 3 changes of fixative. After the third change of fixative, the cell pellet was resuspended in fixative at an appropriate density for slide preparation. The fixed cells were dropped onto clean slides and air-dried before staining. Two or three slides were prepared from each culture. Fixed cells not used for slide preparation were discarded after completion of the experimental phase of study. Cells were stained with the fluorescent metachromatic dye, acridine orange. Stained fixed slides were kept for potential retrospective examination until study finalization.

4.12. Slide Examination

4.12.1. Cytokinesis-Block Proliferation Index (CBPI)

Slides were examined visually for toxicity and slides from a minimum of 3 concentrations up to the toxic concentration, or the lowest concentration which results in a visible precipitate in the culture medium at the end of treatment, whichever is least (or top concentration if there is no cytotoxicity or precipitate limitations) was selected for CBPI determination. Selected slides were randomized using Microsoft® Excel then encoded to minimize potential operator bias.

The CBPI was determined by examination of at least 500 cells (if available) per culture. Lymphocyte toxicity is normally indicated by a decreased CBPI compared to the concurrent negative control group.

$$CBPI = \frac{((\text{No. mononucleate cells}) + (2 \times \text{No. binucleate cells}) + (3 \times \text{No. multinucleate cells}))}{\text{Total number of cells}}$$

$$\% \text{ Cytotoxicity} = 100\text{-}100((CBPI_T\text{-}1) \div (CBPI_C\text{-}1))$$

T = Test Item treatment group

C = Negative Control group

4.12.2. Selection of Slides for Micronucleus Assessment

Justification for selection of dose levels for examination is presented in the results section of the report. Routinely, slides for examination for micronuclei are selected primarily on the basis of the CBPI results. Normally, the highest level examined is the lowest concentration which results in approximately 55% toxicity, based on CBPI, with a sufficient number of scorable binucleate cells or the lowest concentration which causes precipitation at the end of treatment, whichever is least; in the absence of toxicity or precipitation, it is the highest dose tested. In addition, two lower dose levels, the Negative Control, and one dose level of the Positive Control for each regime are included in the detailed analysis.

4.12.3. Microscopic Analysis of Micronucleus Frequencies

Slides selected for analysis of micronucleus frequencies were examined by fluorescence microscopy using a blue excitation filter and a yellow barrier filter, and (where practical) a total of 2000 binucleate cells per experimental point (1000 per culture) were examined for the presence of micronuclei using oil-immersion optics.

Readable binucleate cells are identified by the following criteria:

- The cell must have two main nuclei.
- The two main nuclei must each have an intact and well-defined membrane.
- The two main nuclei must be contained within the cytoplasm.
- The cell must be visible in its entirety in the field.
- The area around the cell must not contain micronucleus-like debris.
- The cytoplasmic boundary should be intact and distinguishable from the boundaries of adjacent cells.

Micronuclei (MN) are identified by the following criteria:

- The diameter of the MN must not exceed 1/3rd of each of the two main nuclei diameter.
- The micronuclei can touch but must not overlap the two main nuclei.
- Micronuclei should be large enough to discern morphological characteristics.
- Micronuclei should possess a generally rounded shape with a clearly defined outline.

- Micronuclei should be similar in color to the nuclei.
- Should lie in the same focal plane as the cell.
- Micronuclei must not be linked to the nuclei by a nucleoplasmic bridge.
- Micronuclei must be within cytoplasmic boundary.
- Micronuclei must be non-refractive (staining).

The location (Vernier readings) of the first nine micronucleated binucleate cells (MBC) was recorded for potential peer review. No peer-review was required for this study.

The % micronucleated binucleate cells (% MBC) is the proportion of micronucleated binucleate cells over the total number of binucleate cells evaluated. Slides from cultures not subjected to analysis were discarded after completion of the experimental phase of study.

5. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study are listed below or presented in the appropriate Phase Report. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 3
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
Microsoft® Excel	2007	Slide randomization and arithmetic calculations of mean values, etc, for presentation in reports.
SAS	9.2	Statistical analyses of number of micronucleated cells
Provantis	8	Statistical analyses of the number of micronucleated cells where less than 2000 binucleated cells were examined per experimental point
Mesa Laboratories AmegaView CMS	v3.0 Build 1208.8	Continuous Monitoring System. Monitoring of standalone fridges, freezers, incubators, and selected laboratories to measure temperature, relative humidity, and CO_2, as appropriate
Johnson Controls Metasys	MVE 5.4 (M5)	Building Automation System. Control of HVAC and other building systems, as well as temperature/humidity control and trending in selected laboratories and animal rooms
Empower 3 (Waters Corporation)	Build 3471 SR1	Dose formulation analyses using HPLC.
Empower 3 and Microsoft Excel	Build 3471 SR1/ 2007	Regression analysis and descriptive statistics for dose formulation analytical data.

6. EVALUATION AND INTERPRETATION OF RESULTS

6.1. Assay Acceptance Criteria:

- *Acceptable Negative Control:* The incidence of micronucleated binucleate cells in the Negative Control should be considered acceptable for addition to the negative control data base (should be within the 95% control limits of the distribution of the Negative Control database or, where the incidence of micronucleated binucleate cells falls outside of the limits, should not be an extreme outlier and there is evidence that the test system is under control).
- *Acceptable Positive Control:* The number of micronucleated binucleate cells in the Positive Control cultures should be compatible with those generated in the historical Positive Control database and produce a statistically significant increase compared to the concurrent Negative Control.
- *Acceptable Cell Proliferation*: Cell proliferation, as measured by the CPBI, should indicate that the treatments are conducted at appropriate levels of cytotoxicity.
- *Experimental Conditions:* All three treatment regimes are to be used in the assay unless a positive result is obtained in one of the regimes.
- *Acceptable Number of Cells and Analyzable Concentrations:* An acceptable number of cells (see section 15) and at least three test concentrations are obtained.
- *Selection of Top Concentration:* The criteria for the selection of the top concentration are consistent with section 15.2.

In the event that the controls fall slightly outside the normal range (historical or Study Plan), the Study Director will be allowed discretion in accepting the results of the experiment as valid based on the biological significance.

6.2. Statistical Analysis

The results obtained for each treatment group will be compared with the results obtained for the concurrent vehicle control group from the same treatment regime using the Fisher's Exact Test. For the statistical analysis, results from replicate cultures will be combined to facilitate interpretation and maximize the power of statistical analysis.

6.3. Interpretation of Results

A Test Item is considered clearly **negative** if:

1. none of the Test Item concentrations selected for micronuclei scoring exhibit a statistically significant increase in the incidence of micronucleated binucleate cells compared to the concurrent Negative Control,
2. all results are inside the distribution of the historical negative control data (e.g. 95% control limits)

The Test Item is then considered unable to induce chromosome breaks and/or gain or loss in this test system.

A Test Item is considered to be clearly **positive** if:

1. at least one of the Test Item concentrations selected for detailed chromosome analysis exhibit a statistically significant increase in the incidence of micronucleated binucleate cells compared with the concurrent negative control ($p \leq 0.05$) at a concentration that does not greatly exceed a 50% cytoxicity level,
2. the increase is dose-related when evaluated with an appropriate trend test (a trend test will be performed when the incidence of micronucleated binucleate cells falls outside the distribution of the historical negative control database (e.g. 95% control limits)),
3. and the increase is outside the distribution of the historical negative control database (e.g. 95% control limits).

The Test Item is then considered able chromosome breaks and/or gain or loss in this test system.

An equivocal result is concluded if no definite judgment can be made to fit the above criteria. An equivocal result indicates that a definitive conclusion cannot be made by performing the in vitro micronucleus test under the conditions described in this Study Plan. Alternate testing conditions may be performed as an aid in evaluating the test results. Any additional testing or analysis will be approved by the sponsor and will be documented by Study Plan amendment.

7. RETENTION OF RECORDS

All study-specific raw data, documentation, Study Plan and Final Report from this study were archived at the Test Facility by no later than the date of Final Report issue, unless otherwise specified in the Study Plan. One year after issue of the unaudited Draft Report, the Sponsor will be contacted to determine the disposition of materials associated with the study.

Electronic data generated by the Test Facility were archived as noted above, except the reporting files stored on SDMS, which were archived at the Charles River Laboratories facility location in Wilmington, MA

8. RESULTS

8.1. Dose Formulation Analyses

Results of the dose formulation analysis are presented in Appendix 4.

All doses met acceptance criteria, with chemical analysis indicating achieved concentrations within ±10% of the theoretical concentration for the stock solution and ±15% for lower level solutions.

8.2. Dose Selection

The highest dose level tested was 500 µg/mL, the maximum dose level recommended by the ICH S2(R1) guideline.

In the absence of overt toxicity, the highest dose level of the Test Item selected for micronuclei scoring in each regime was the highest dose level tested (500 µg/mL). In addition, the next two lower dose levels were also subjected to examination (see Table 1).

8.3. Micronucleus Assessment

SM-102 did not cause any statistically significant increases in the incidence of micronucleated binucleate cells compared to the concurrent Negative Control at any experimental point (see Table 1 for summary and Appendix 3 for detailed results). In addition, the incidence of micronucleated binucleate cells for all Negative Control and Test Item groups was within the laboratory negative historical control range (see Appendix 5 for laboratory negative and positive historical control results).

The Positive Controls caused statistically significant increases in frequency of micronuclei in each regime of the study, confirming the sensitivity of the test system and the effectiveness of the S9 mix (see Appendix 5).

8.4. Incidental Observations

Precipitation was observed at the end of treatment at 500 µg/mL, in both the 4 hour regime and the 24 hour regime in the absence of S9 mix. No precipitation was observed at the end of treatment in the presence of S9 mix. Cloudy media was observed in the 4 hour regime in the absence of S9 mix at dose levels ≥ 93.3 µg/mL, in the 4 hour regime in the presence of S9 mix at the highest dose level and in the 24 hour regime at dose levels ≥ 286 µg/mL. No cytotoxicity was observed in the assay.

9. CONCLUSION

It is concluded that SM-102 did not show any evidence of genotoxic activity in this in vitro test for induction of micronuclei in human peripheral blood lymphocytes, when tested in accordance with regulatory guidelines.

Table 1 SM-102 - Summary Results and Statistical Analysis

Treatment	Conc. (µg/mL)	Average CBPI	(%) [a]Cytotoxicity	Total No. of BC examined	% MBC
4 hours treatment in the absence of S9 (0S9)					
Ethanol	-	1.8	0	2000	0.2
SM-102	163	1.9	-8	2000	0.5
	286	1.9	-8	2000	0.6
	500[ppt]	1.8	0	2000	0.1
NOC	0.25	1.5	43	1482	4.4**
4 hours treatment in the presence of S9 (+S9)					
Ethanol	-	1.8	0	2000	0.4
SM-102	163	1.8	-3	2000	0.5
	286	1.8	-1	2000	0.6
	500	1.8	1	2000	0.3
CP	10	1.4	46	2000	2.2**
24 hours treatment in the absence of S9 (0S9)					
Ethanol	-	1.7	0	2000	0.4
SM-102	163	1.7	-8	2000	0.2
	286	1.7	-2	2000	0.2
	500[ppt]	1.7	1	2000	0.3
MMC	0.10	1.6	3	2000	2.0**

CBPI = Cytokinesis-Block Proliferation Index.
a = Relative to the vehicle control.
BC = Binucleated cells.
MBC = Micronucleated binucleated cells.
NOC = Nocodazole
CP = Cyclophosphamide
MMC = Mitomycin C
* $p \leq 0.05$, ** $p \leq 0.01$ otherwise $p > 0.05$ Fisher's exact test with single-sided probabilities.
ppt = Precipitate visible in the culture medium at the end of treatment