

# MICROBIO



## Appendix B



財團法人生物技術開發中心  
DEVELOPMENT CENTER FOR BIOTECHNOLOGY

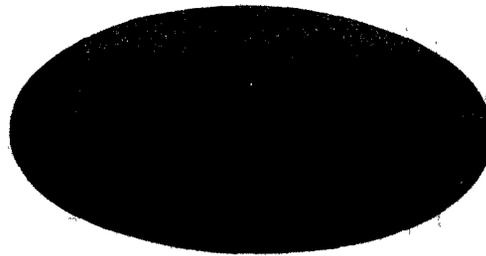
SERIAL NO: DV-TR-MN00019E
PROJECT CODE: DV-TA00199
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0302

# MICRONUCLEUS ASSAY IN MICE PRODUCT CODE MicSoy-20 (MS-20)

## FINAL REPORT



DEVELOPMENT CENTER FOR BIOTECHNOLOGY  
DRUG DEVELOPMENT DIVISION



## Signature Page

### Study Director:

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Juan Judy Liu Dec 1 26 12001  
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## QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit (QAU) has inspected the conduct of different phases of the study according to a predetermined testing schedule. To the best of our knowledge, there were no deviations from the protocol and standard operating procedures that would affect the integrity of this study.

This report has been audited by the QAU in accordance with the appropriate standard operating procedures of Drug Development Division, DCB. The report is considered to describe the methods and procedures used in the study, and the reported results accurately reflect the raw data generated during this study.

The Genetic Toxicology Laboratory of Drug Development Division, DCB has been recognized by the Council of Chinese National Laboratory Accreditation (CNLA) as an accredited laboratory, and *In Vivo* Cell Micronucleus assay is one of the registered testing item within the field of Biological Testing. We now had the CNLA logo attached on the cover page of this report for compliance.

Listed below are the phases in this study that were audited by the QAU and the dates the audits were performed and findings reported to management.

<u>Audit Date</u>	<u>Phase Audited</u>	<u>Date Reported to Study Director</u>	<u>Date Reported to Management</u>
Oct. 23, 2001	Protocol	Oct. 26, 2001	Oct. 26, 2001
Oct. 29, 2001	Test article preparation and oral administration	Oct. 29, 2001	Oct. 30, 2001
Nov. 01, 2001	Blood sampling and percentage of reticulocytes measurement	Nov. 02, 2001	Nov. 06, 2001
Nov. 23, 2001	Raw data; study records	Nov. 23, 2001	-----
Dec. 24, 2001	Final report	Dec. 24, 2001	Dec. 26, 2001

Jiun-Min Lai  
Jiun-Min Lai  
Quality Assurance Officer

Dec. 26, 2001  
Date



## TESTING FACILITY

Name: Genetic Toxicology Laboratory, Drug Development Division, Development Center for Biotechnology

Address: 103, Lane 169, Kang-Ning St., Hsi-Chih City, Taipei County 221, Taiwan, R.O.C.

## SPONSOR

Name: MICROBIO Co., Ltd.

Address: No. 81, Gauyang N. Road, Lung Tan Shiang, Tao Yuan, Taiwan, R.O.C.

Representative: Lu, William

## TEST ARTICLE (To be supplied by the sponsor prior to study initiation)

A. Name/Identification: Product code MicrSoy-20 (MS-20)

B. Receiving Date: Oct. 05, 2001

C. Batch/Lot Number: 20010209

D. DCB Code: DV00199-1

E. Ingredients: The components are very complicated. Until now, its effective components are still unable to determine.

F. Storage Conditions: Room temperature and protect from light

G. Expiration Date: Feb. 09, 2004

H. Physical Appearance: Dark-brown liquid with prune juice odor

### Statements:

1. The test article is a proprietary product of the sponsor, therefore the sponsor will be responsible for the requirements listed under "Test Article" of the GLP regulation.
2. The testing result is effective for submitted sample only, and shall not be excerpted from the contents of this report without the written approval of the testing facility.
3. The testing result and report are generated by DCB for the test article submitted by the sponsor, and are intended for petition to government agency for product registration.

## TEST SCHEDULE

A. Dates of Dosing: Oct. 29, 2001 and Oct. 30, 2001

B. Blood Sampling and Slides Preparation: Nov. 01, 2001

C. Date of Reticulocyte Ratios Analysis: Nov. 01, 2001

D. Period for Microscopic Observation of Slides: Nov. 05 ~ 07, 2001

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## MICRONUCLEUS ASSAY IN MICE – Product Code MicroSoy-20 (MS-20)

### SUMMARY

Micronucleus (MN) assay in mouse peripheral blood of MicroSoy-20 was studied in ICR mice. The assay was performed in two phases. The first phase, the preliminary toxicity assay, was used to establish the dose range for the micronucleus assay. The second phase, the micronucleus assay, was used to evaluate the clastogenic potential of the test article *in vivo*.

Injection water was used as the vehicle for preparing the test article based on the solubility of test article and its compatibility with the animals. The origin liquid sample of MicroSoy-20 from MICROBIO Co., Ltd. was administered to mice once per day for two consecutive days via gavage.

The preliminary test was done to determine the dose-range and sex-difference for micronucleus assay. The results of short-term oral toxicity test showed that there was no toxicity observed at 20 ml/kg with origin liquid both in male and female mice. Therefore, only male mice were used for micronucleus induction assay with the top dose set as 20 ml/kg of origin liquid.

In the micronucleus assay, the top dose of origin liquid, two following doses, 1/2X origin liquid and 1/4X origin liquid with volume of 20 ml/kg, as well as vehicle control and positive control were used to treat male mice, 5 animals per dose group. The presence of micronuclei was examined in reticulocytes at 36 to 48 h after the last test article administration. The positive control (1 mg/kg mitomycin C) was dosed i.p. once and blood was collected approximately 48 h after dosing. The results showed that there was no significant induction of micronucleus in all dosage groups. The animals in positive control group gave significant increase in the frequency of micronucleus compared to the vehicle control group. The results of percentages of reticulocytes to total erythrocytes in treated animals showed no significant decrease compared to vehicle control animals indicate that the test article had no effect on erythropoiesis.

Under the conditions of this study, MicroSoy-20 was concluded to be negative in the micronucleus assay with mouse peripheral blood.



## INTRODUCTION

The objective of this study was to evaluate the clastogenic potential of the test article MicrSoy-20 as measured by its ability to induce micronucleated reticulocytes in mouse peripheral blood.

## MATERIALS AND METHODS

### A. Test Animals

1. Species: Mouse
2. Strain: ICR
3. Source: National Laboratory Animal Breeding and Research Center, Taipei, Taiwan
4. Age at initiation of study: 8-9 weeks old
5. Body weights at start of study: 32 ~39 g. Actual weights were recorded in the data sheet of study (Appendix E). The weights of all animals were within the variance of 20% of the mean value within the same study.
6. Method of identification: Each animal was individually identified by ear notch, cage and sex.
7. Number on study: 3 male/female per dose group in the preliminary study; 5 males /dose group in definitive micronucleus assay.
8. Animal housing and acclimation

Animals were quarantined and housed in the AAALAC-accredited facility with a controlled environment of  $50 \pm 20\%$  relative humidity and  $21 \pm 2$  °C with a 12-hour light/dark cycle. Healthy mice of the same sex were randomly assigned to groups up to five per cage in polycarbonate autoclavable cages. Animals had free access to laboratory autoclavable rodent diet 5010 and to drinking water except the feed was removed during the fasting period. Animals were acclimated for 6 days prior to dose administration.

### B. Test Article Preparation and Administration

Injection water was used as the vehicle to prepare the test article solution. The dosing solutions were prepared by the test article in the vehicle on the days of administration.

Test article was administered to animals by gavage since it is the expected route of consumption in human. On the days of administration, solutions of the test article were prepared at concentrations providing 20 ml/kg volume. Animals were fasted about 4 h before dosing. Each dose was administered by gavage using a 1 ml plastic disposable syringe accessed to 20-gauge gavage needle. Each animal of vehicle control and dosage



groups was administered once per day for two consecutive days. The dosing volume was based upon the body weight of mice recorded immediately before administration. Positive control article of micronucleus assay was administered once by i.p. injection on the last administration day at dose volume providing 20 ml/kg.

#### C. Preliminary Dose-range Finding Test

A modified short-term toxicity study of MicrSoy-20 was performed to determine the values of maximum tolerance dose (MTD) and sex-related difference in ICR mice. This dose-range finding test was done using a vehicle control group and 20 ml/kg with origin liquid group of the test article. Due to the limit amount of test article and anticipated low toxicity of the test article, the dose group consisted of three animals per sex. The vehicle control and test article solutions were administered once per day for two consecutive days by gavage and observed for clinical signs. The animals were observed for mortality and recorded for body weight daily thereafter for 2 days after the last administration day. If there is animal death occurred due to the toxicity of test article, the  $LD_{50/3}$  value with 95% confidence limits and the slope of the dose response curve are calculated for each sex by the method of Bliss (1934) and performed using Linear Regression method.

#### D. Micronucleus Induction Assay

##### 1. Dose levels

The MTD found by the dose-range finding test was set as the top dose for micronucleus assay. Since there was neither mortality nor significant toxicity signs showed in the observation period at volume of 20 ml/kg with origin liquid of MS-20 in both male and female mice, the top dose was set as 20 ml/kg origin liquid (Group 4) and only male mice were used for micronucleus induction assay. Two additional doses were tested, one-half and one-fourth of the top dose (Groups 3 and 2). Vehicle control (Group 1) and positive control (mitomycin C; Group 5) were included in the test simultaneously.



Table 1. Dose Levels of Micronucleus Assay with MicrSoy-20

Group Number	Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	No. of Mice
1	0 (vehicle)	0 (vehicle)	20	5
2	1/4X origin liquid	1/4X origin liquid	20	5
3	1/2X origin liquid	1/2X origin liquid	20	5
4	origin liquid	origin liquid	20	5
5	1.0 (MMC)	0.05	20	5

2. Test/control article preparation

- High dose group (origin liquid): test article from origin liquid was dosed once per day for two consecutive days.
- Middle dose group (1/2X origin liquid): 5.0 ml of high dose group solution was added to the bottle containing 5.0 ml of vehicle to make a 1/2X origin liquid.
- Low dose group (1/4X origin liquid): 3.0 ml of middle dose group solution was added to the bottle containing 3.0 ml of vehicle to make a 1/4X origin liquid.
- Vehicle control group (0 mg/kg): injection water.
- Positive control group (1.0 mg/kg mitomycin C, CAS #50-07-7): 2.0 ml of saline was added to the injection bottle containing 2.0 mg of MMC to make a 1.0 mg/ml solution. 0.5 ml of 1.0 mg/ml solution was added to a tube containing 9.5 ml saline to make a 0.05 mg/ml solution which was then transferred to an injection bottle.

3. Blood sampling and examinations

a. Body weights recording

Individual body weight was recorded on the days of dosing (days 1 and 2, the first dosing day was recorded as day 1), then at days 3 and 4. The body weights are expressed as mean  $\pm$  S.D.

b. Blood sampling

Thirty-six to 48 hours after the last dose administration, five animals per dose and vehicle control groups were blood sampled from the tails. The positive control group animals were sampled once approximately 48 hours after dose administration. The blood samples collected were smeared on acridine orange-coated slides. The slides were wrapped to protect from light and stored at 0-6 °C for at least 4 h before scoring. A minimum of two slides was prepared for each animal.



c. Scoring for micronuclei

Slides were coded by an individual not involved in the scoring process. Using medium magnification, areas of acceptable quality were selected such that the cells were well spread and stained. A minimum of two thousands reticulocytes was scored for the presence of micronuclei for each animal. The data were expressed as numbers of MN per 1000 RETs (MN/1000 RETs) and Mean  $\pm$  S.D. of each group was calculated.

d. Percentages of reticulocytes

The proportion of reticulocytes to total erythrocytes was recorded for each animal as an indicator of bone marrow toxicity with test article. This ratio should not be less than 10% of the control value. Percentages of reticulocytes were measured at 36 to 48 h after the last test article administration using a flow cytometer (Becton Dickinson, FACSort) with the Retic-Fit™ reticulocyte enumeration software. The results were based on analysis of 50,000 erythrocytes.

E. Criteria for a Valid Test

The concurrent control data were compared to the historical control data. The acceptable range is the mean frequency of micronucleated reticulocytes  $\pm$  3 standard deviation in the negative control. The incidence of micronucleated reticulocytes in the positive control group must be significantly increased relative to the concurrent negative control ( $p \leq 0.05$ , *t*-test). A minimum of 3 analyzable doses should be obtained for data analysis.

F. Interpretation of Results

All conclusions were based on sound scientific judgment. However, as a guide to interpret the data, the test article is considered to induce a positive response if a dose-responsive increase in micronucleated reticulocytes is observed and one or more doses are statistically elevated relative to the vehicle control ( $p \leq 0.05$ , *t*-test) at sampling time. If a single treatment group is significantly elevated with no evidence of a dose-response, the assay is considered a suspect or unconfirmed positive and a repeat experiment will be recommended. The test article is judged as negative if no statistically significant increase in micronucleated reticulocytes above the vehicle control values.

G. Data Retention

All raw data, documentation, records, protocols and final reports generated as a result of this study will be inventoried and archived by the Quality Assurance Unit at DCB's archives located in Drug Safety Building. The retaining duration of those records will be in accordance with the relevant regulations.



## RESULTS AND DISCUSSION

### A. Preliminary Dose-range Finding Test

The preliminary test results showed that there was no toxicity at volume of 20 ml/kg with origin liquid of MicrSoy-20 treatment both in male and female mice (up to day 4). The administration of test article did not show adverse effect on body weight gain in animals for the entire post-administration observation period. Therefore, only male mice were used for micronucleus induction assay. The top dose used for micronucleus assay was set as origin liquid with volume of 20 ml/kg.

### B. Concurrent Measurement of Toxicity

#### 1. Body Weights

A summary of group body weights recorded for four study days since first administration of the test article in micronucleus assay is presented in Table 2. However, there was no significant difference in body weight gain of all dosing groups compared to the vehicle control group during the study period.

#### 2. Reticulocytes Ratios

The proportion of reticulocytes (RETs) to total erythrocytes for each individual animal and mean  $\pm$  S.D. for each dosage group are presented in Table 3. Reduction of RETs ratio indicates the effect of the test article on erythropoiesis. The average RETs ratio of vehicle control animals was 4.2%. In all dosing groups at 36 to 48 h after the last administration of MicrSoy-20 did not show reduction of RETs ratios. Positive control at 48 h after dosing caused 38% significant decrease in RETs ratio. Therefore, MicrSoy-20 did not cause bone marrow toxicity in the testing dose range.

### C. Micronucleus Assay

The incidence of micronucleated reticulocytes in peripheral blood of each treatment is presented as MN/1000 RETs for individual animal and mean  $\pm$  S.D. for each group in Table 4. A comparison of concurrent and historical control values of MN/1000 RETs is presented in Table 5. The mean frequencies of micronucleated reticulocytes in vehicle control at 36 to 48 h was 0.5 MN/1000 RETs after the last administration. The positive control, MMC 1.0 mg/kg, gave 28.0 MN/1000 RETs at about 48 h after treatment. Therefore, the frequency of micronucleated reticulocytes in vehicle control was within acceptable range (0 ~ 6.4 MN/1000 RETs). The positive control induced significant increase of frequencies of micronucleus compared to vehicle control. These results met the criteria for a valid test.

Thirty-six to 48 h after the last test article administration, the mean frequencies of micronucleus induction at 1/4X origin liquid, 1/2X origin liquid and origin liquid of MicroSoy-20 gave 1.0, 0.8 and 1.4 MN/1000 RETs, respectively. Therefore, there was no dose-response effect on micronucleus induction of MicroSoy-20, neither significant elevation of micronucleus frequency in any dose group compared to the vehicle control group.

### CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the micronucleus assay in ICR mice indicate that, under the conditions of this study, MicroSoy-20 did not cause a positive response in the test system and was concluded to be negative in mouse peripheral blood micronucleus assay.

### COMMENTS AND/OR PROBLEMS

This study was conducted in compliance with (1) Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR 58), FDA, U.S.A., 1987; (2) Good Laboratory Practice for Nonclinical Laboratory Studies, Department of Health, R.O.C., 3rd ed., 2000, with the exceptions of test article identification and related analyses.

To the best of our knowledge, there were no deviations from the study protocol that would affect the integrity of this study. No problems were encountered that would adversely affect the study results or interpretation.

### REFERENCES

1. The Collaborative Study Group for the Micronucleus Test (CSGMT/JEMS. MMS, The Mammalian Mutagenesis Study Group of the Environmental Mutagen Society of Japan) (1995) Protocol recommended for the short-term mouse peripheral blood micronucleus test. *Mutagenesis*, 10, 153-159.
2. Hayashi, M., Morita, T., Kodama, Y., Sofuni, T. and Ishidate, M. Jr. (1990) The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutation Research*, 245, 245-249.
3. The Collaborative Study Group for the Micronucleus Test (1992) Micronucleus test with mouse peripheral blood erythrocytes by acridine orange supravital staining: The summary report of the 5th collaborative study by CSGMT/JEMS.MMS *Mutation Research*, 278, 83-98.



4. OECD Guideline for The Testing of Chemicals #474 "Mammalian Erythrocyte Micronucleus Test", 1997.
5. Quality Manual, Drug Development Division, Development Center for Biotechnology, 6th edition, 1999.
6. Health Food Safety Evaluation Guideline, Department of Health, Executive Yuan, Republic of China, 1999.



Table 2. Body Weights of Male ICR Mice Treated with MicrSoy-20

Dose Group	Dose (mg/kg)	No. Mice Tested	Body Weight (g)			
			Day 1*	Day 2*	Day 3	Day 4
1	0 (Vehicle)	5	36.36 ± 1.38	35.98 ± 1.73	36.66 ± 1.81	37.09 ± 2.01
2	1/4 X origin liquid	5	36.52 ± 2.45	35.79 ± 2.02	37.41 ± 2.06	37.14 ± 1.83
3	1/2 X origin liquid	5	36.52 ± 1.50	35.90 ± 1.35	37.85 ± 1.92	37.60 ± 2.02
4	origin liquid	5	36.98 ± 1.69	36.52 ± 1.51	37.98 ± 1.73	38.00 ± 1.60
5	Pos. Ctl.	5	35.78 ± 1.05	36.96 ± 1.13	36.82 ± 1.12	36.66 ± 1.05

Data of body weights are presented as group Mean ± S.D.

\*Day 1 and Day 2 were the days of dosing; both data were recorded immediately before test article administration



Table 3. The Percentages of Reticulocytes to Total Erythrocytes in Male ICR Mice Treated with MicrSoy-20

Dose (mg/kg)	Individual Animal Data (% reticulocytes)					Mean $\pm$ S.D. (% reticulocytes)
0 (Vehicle control)	4.2	4.1	4.0	4.5	4.1	4.2 $\pm$ 0.2
1/4 X origin liquid	4.9	4.5	5.2	5.5	5.1	5.0 $\pm$ 0.4
1/2 X origin liquid	4.2	4.1	4.0	4.2	4.2	4.1 $\pm$ 0.1
Origin liquid	4.9	4.8	4.8	7.6	5.1	5.4 $\pm$ 1.2
Positive control	2.2	2.6	1.8	2.4	3.8	2.6 $\pm$ 0.8



Table 4. The Frequencies of Micronucleated Reticulocytes in Peripheral Blood of Male ICR Mice Treated with MicrSoy-20

Dose (mg/kg)	Individual Animal Data					Mean $\pm$ S.D. (MN/1000 RETs)
	(MN/1000 RETs)					
0 (Vehicle control)	0.5	0.5	1.0	0.5	0	0.5 $\pm$ 0.4
1/4 X origin liquid	1.5	1.0	0.5	0.5	1.5	1.0 $\pm$ 0.5
1/2 X origin liquid	0	1.5	2.0	0.5	0	0.8 $\pm$ 0.9
Origin liquid	1.5	0	1.5	2.5	1.5	1.4 $\pm$ 0.9
Positive control	36.0	22.5	33.0	28.5	20.0	28.0 $\pm$ 6.8



Table 5. Concurrent and Historical Control Values of Micronucleated Reticulocytes in Mice

	No. of MN / 1000 RETs	
	Vehicle Control*	1.0 mg/kg Mitomycin C**
Concurrent control	0.5 ± 0.4	28.0 ± 6.8
Historical control		
Mean ± S.D.	2.2 ± 1.4	34.2 ± 9.6
Maximum	6.0	62.0
Minimum	0.0	19.0

\*Vehicle control (water, saline, 2.5% cremophor/H<sub>2</sub>O, 10 % PEG saline, 50 % PEG saline, 50 % PEG/H<sub>2</sub>O, 0.5 % CMC and sugar water); concurrent vehicle control: injection grade water

\*\* Mitomycin C was purchased from Boehringer Mannheim (cat. No. 107409, Germany) administered by i.p.

Data recording period: 4/13/1995 to 10/18/2001

# Appendix A

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- ◆ Test Article Information Sheet



## Test Article Information Sheet

1/1

DV00199-1

DV-QA00033E

Sponsor : MICROBIO Co., Ltd.Address : No.81 Gauyang N. Rd., Lung tan Shiang, Tao Yuan, TaiwanTelephone : 886-3-4710888 Fax : 886-3-4710288Delivery Date : 10 / 05 / 2001 (MM/DD/YY)Category : Health Food Herb Medicine Drugs Cleanser Medical Devices  
Cosmetics Pesticides Others : \_\_\_\_\_1. Sample Name : Product code MicrSoy-20(MS-20)

2. a. Ingredients :

MS-20 is a Chinese medicine. The components are very complicated. Until now, its effective components are still unable to determine.

b. Purity : \_\_\_\_\_

3. Batch / Lot No. : 20010209

4. Physical Appearance :

a. Powder Liquid Others : \_\_\_\_\_b. Odor : No Yes : Prune Juicec. Color : Dark-brown5. How Supplied (Amount / Pack) : 30 ml/Bottle6. Amount Supplied : 2 Bottle



Test Article Information Sheet

DV00199-1

7. Solubility (Approx. \_\_\_ g/L)
H2O Souble, DMSO, Other Solvents

8. Storage

a. Storage Temperature: [X] Room Temperature [ ] Refrigeration [ ] Frozen

b. Other Environment Condition: [ ] Desiccation [X] Protect from Light

[ ] Others: \_\_\_\_\_

c. Expiration Date: 02/ 09/ 2004 (MM / DD / YY)

9. Treatment of Residual Samples

[X] Retrieved by the Sponsor

[ ] Managed by DCB with Extra Fees

[ ] Disposed by DCB with Waste Disposal Method Provided:

10. Handling Precautions and Others

Directions: To drink 1~5c.c. daily by dilution with 100c.c. water before breakfast. It's not suggested to drink water in 10 minutes after MS-20. After 10 minutes later, we suggest you to drink water as usual. Before dilution, the product can be stored at room temperature after opening, but please use the product immediately after dilution.

Undiluted product has a high acidity of pH around 3.8

MS-20 has two packages which are 180ml/Bottle and 30ml/Bottle

Product Chemist :

[Handwritten signature]

[Handwritten signature] (Signature)
(MM / DD YY)

Sponsor Representative :

[Handwritten signature]

[Handwritten signature] (Signature)
(MM / DD YY)

## Appendix B

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◆ Protocol



財團法人生物技術開發中心  
DEVELOPMENT CENTER FOR BIOTECHNOLOGY

SERIAL NO: DV-PR-MN00019E
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# MICRONUCLEUS ASSAY IN MICE PRODUCT CODE MicrSoy-20 (MS-20)

## PROTOCOL

DEVELOPMENT CENTER FOR BIOTECHNOLOGY  
DRUG DEVELOPMENT DIVISION



財團法人生物技術開發中心  
DEVELOPMENT CENTER FOR BIOTECHNOLOGY

SERIAL NO: DV-PR-MN000193  
PROJECT CODE: DV-TA00199  
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### Signature Page

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Shwu-Fei Lcc-Chen Oct. 1 231 2001  
Dr. Shwu-Fei Lcc-Chen, Ph.D.

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**Quality Assurance Officer:**

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Jiun-Min Lai, M.S.

**Facility Manager:**

Jiuan Judy Liu Oct 1 23 12001  
Dr. Jiuan Judy Liu DVM, Ph.D.

**Sponsor's Representative:**

William Lu Oct 1 26/ 2001  
William Lu



## MICRONUCLEUS ASSAY IN MICE – Product Code MicSoy-20 (MS-20)

### I. PURPOSE

The objective of this study is to evaluate the clastogenic potential of the test article as measured by its ability to induce micronucleated reticulocytes in mouse peripheral blood.

### II. TESTING FACILITY

A. Name: Genetic Toxicology Laboratory, Drug Development Division, Development Center for Biotechnology

B. Address: 103, Lane 169, Kang-Ning St., Hsi-Chih City, Taipei County 221, Taiwan, R.O.C.

### III. SPONSOR

A. Name: MICROBIO Co., Ltd.

B. Address: No. 81, Gauyang N. Road, Lung Tan Shiang, Tao Yuan, Taiwan.

C. Representative: Lu, William

### IV. TEST ARTICLE (To be supplied by the sponsor prior to study initiation)

A. Name/Identification: Product code MicSoy-20 (MS-20)

B. Receiving Date: Oct. 05, 2001

C. Batch/Lot Number: 20010209

D. DCB Code: DV00199-1

E. Ingredients: The components are very complicated. Until now, its effective components are still unable to determine.

F. Storage Conditions: Room temperature and protect from light

G. Expiration Date: Feb. 09, 2004

H. Physical Appearance: Dark-brown liquid with prune juice odor

#### Statement:

The test article is a proprietary product of the sponsor, therefore the sponsor will be responsible for the requirements listed under "Test Article" of the GLP regulation.



## V. TEST SCHEDULE

- A. Proposed Dates of Dosing: Oct. 29, 2001 and Oct. 30, 2001
- B. Proposed Date of Peripheral Blood Collection: Nov. 01, 2001
- C. Proposed Blood Slides Observation Period: Nov. 02, 2001 ~ Nov. 09, 2001

## VI. TEST SYSTEM

- A. Species: Mouse
- B. Strain: ICR
- C. Source: National Laboratory Animal Breeding and Research Center, Taipei, Taiwan
- D. Method of Identification: Each animal will be individually identified by ear notch, cage and sex.
- E. Age at Initiation of Study: 8 ~ 9 weeks
- F. Body Weights at Start of Study: 30 ~ 42 g
- G. Justification for Selection: It is one of the rodent species recommended by OECD guideline for peripheral blood micronucleus assay, and is acceptable by the Health authorities worldwide for the assay.
- H. Animal Use Approval Number: # 2000-DV-002-o

## VII. EXPERIMENTAL DESIGN

### A. Selection of Test Article Vehicle

The test article is dark-brown liquid with prune juice odor and has good water solubility. Injection water will be used as the vehicle to prepare the test solution.

### B. Dose Selection

Selection of doses for the micronucleus assay of MicrSoy-20 was based on the toxicity of the test article. In the absence of direct toxicity data, a dose-range finding test was performed to estimate LD<sub>50</sub> and sex-related difference. However, for test article with anticipated low toxicity, only the highest dose at 20 ml/kg with origin liquid from MICROBIO CO., Ltd. was tested. Animals were treated at 20 ml/kg with origin liquid sample of MicrSoy-20 once daily for two consecutive days via gavage. After first administration, animals were observed for clinical signs of toxicity for another three days.



The result of preliminary toxicity study showed that no evidence of toxicity was found during observation period at 20 ml/kg of MicrSoy-20 either in male or in female mice. Therefore, in the micronucleus induction assay only male mice at three dose levels, origin liquid, 1/2 origin liquid and 1/4 origin liquid with volume of 20 ml/kg will be used (Table I). Negative (vehicle) control (Group 1) and positive control (Group 5) will be included in the test simultaneously.

Table I. Dose Levels of Micronucleus Assay with MicrSoy-20

Group Number	Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	No. of Mice
1	0 (vehicle)	0 (vehicle)	20	5
2	1/4 X origin liquid	1/4 X origin liquid	20	5
3	1/2 X origin liquid	1/2 X origin liquid	20	5
4	origin liquid	origin liquid	20	5
5	1.0 (MMC)	0.05	20	5

Test article will be prepared freshly at the days of dosing.

#### C. Route and Frequency of Administration

Animals will be dosed by gavage (P.O.) which is also the route for human consumption of the test article. Animals will be fasted about 4 hours before dosing. The dose volume is 20 ml/kg of body weight for both test article and controls. All mice in the experimental groups will be weighed before dosing. The test article will be dosed once per day for two consecutive days.

Animals may be replaced if death occurred after first dosing. All of the animal death after dosing will be checked by gross necropsy and examined by a veterinarian.

#### D. Controls

##### 1. Negative control

The vehicle, injection water, for the test article will be used as the negative control.



## 2. Positive control

Mitomycin C (CAS # 50-07-7) dissolved in normal saline as 0.05 mg/ml solution, will be administered as the positive control at the dose of 1.0 mg/kg. MMC will be administered once by intraperitoneal (i.p.) injection.

## E. Animal Receipt and Quarantine

Animals will be quarantined for no less than 5 days when they enter the Animal Facility at DCB on 4<sup>th</sup> floor. The animals will be observed each working day for signs of illness, unusual food and water consumption, and other general conditions of poor health determined by the facility veterinarian as necessary. All animals will be judged to be healthy prior to utilization in the study.

## F. Animal Care

Animals are housed in an AAALAC-accredited facility with controlled environment of  $50 \pm 20\%$  relative humidity and  $21 \pm 2$  °C with a 12-hour light/dark cycle. Mice are housed up to five per cage in polycarbonate autoclavable cages. Animals have free access to laboratory autoclavable rodent diet 5010 (PMI<sup>®</sup> Feeds, Inc.) and to drinking water, except feed will be removed for 4 h before each dosing. The animals are acclimated to the animal facility conditions for at least seven days.

## G. Animal Grouping

The animals are randomly assigned to groups of five animals the day before first administration. All animals will be weighed and the animals with body weights beyond the range of mean  $\pm 20\%$  mean will be removed and replaced with other one(s).

## H. Body Weight Measurement

Body weight of each testing animal will be measured at the days of dosing and the following two consecutive days.

## I. Peripheral Blood Sampling

Samples of peripheral blood will be taken once from each of five animals per test dose and vehicle control groups. The blood samples will be collected between 36 hours and 48 hours after the last treatment. The positive control group will be sampled once approximate 48 hours after administration. The blood samples will be collected by the tail trimming and smeared on acridine orange-coated slides. The slides will be wrapped to protect from light and stored at 0-6 °C for at least 4 h



before scoring. A minimum of two slides will be prepared from each animal.

## VIII. OBSERVATION AND EXAMINATION

### A. Scoring for Micronuclei (SOP: DCB-DV-TE00227)

Slides will be blind coded by an individual not involved in the scoring process. Using medium magnification, an area of acceptable quality will be selected such that the cells are well spread and stained. A minimum of 2000 reticulocytes per animal will be scored for the presence of micronuclei.

### B. Ratio of Reticulocyte Analysis (SOP: DCB-DV-TE00228)

The proportion of reticulocytes to total erythrocytes will also be recorded and measured by a flow cytometer. The results are based on analysis at least 50,000 erythrocytes.

### C. Criteria for Determination of a Valid Test

The concurrent control data are compared with the historical control data. The acceptable range is the mean frequency of micronucleated reticulocytes  $\pm 3$  standard deviation in the negative control. The incidence of micronucleated reticulocytes in the positive control group must be significantly increased relative to the concurrent negative control ( $p \leq 0.05$ , *t*-test).

### D. Evaluation of Results

In order to quantify the test article effect on erythropoiesis as an indicator of bone marrow toxicity, the proportion of reticulocytes to total erythrocytes will be presented for each animal and treatment group. This ratio in treated group should be no less than 10% of the negative control.

The incidence of micronucleated reticulocytes per 1000 reticulocytes (MN/1000 RETs) will be presented for each animal and mean  $\pm$  SD for each treatment group. At least 3 analytical dose groups, negative and positive control groups, 5 animals per group are required for data analysis.



## IX. STATISTICAL ANALYSIS

First, the test data will be assessed the dose-response relationship, the trend test. Secondly, the data from each treatment group are compared with the historical negative control data. All conclusions will be based on sound scientific judgment; however, as a guide to interpretation of the data, the test article will be considered to induce a positive response if a dose-responsive increase in micronucleated reticulocytes is observed and one or more doses are statistically elevated relative to the vehicle control ( $p \leq 0.05$ ). If a single treatment group is significantly elevated with no evidence of a dose-responsiveness, the assay will be considered a suspect or unconfirmed positive and a repeat experiment will be recommended. The test article will be judged negative if no statistically significant increase in micronucleated reticulocytes above the vehicle control values is observed at any treatment group.

## X. RECORDS RETENTION

All raw data, documentation, records, protocols and final reports generated as a result of this study will be inventoried and archived by the Quality Assurance Unit at DCB's archives located in Drug Safety Building. Those records will be retained in accordance with the periods regulated in the relevant regulations.

## XI. REGULATORY REQUIREMENTS

This study will be performed in compliance with (1) Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR 58), FDA, U.S.A., 1987; (2) Good Laboratory Practice for Nonclinical Laboratory Studies, Department of Health, R.O.C., 3rd ed., 2000; (3) General Requirements for the Competence of Calibration and Testing Laboratories (ISO/IEC Guide 25), ISO/IEC, 3rd ed., 1990; (4) Specific Criteria for Biological Testing, Chinese National Laboratories Accreditation, R.O.C., 2nd ed., 2000.

## XII. REFERENCES

1. The Collaborative Study Group for the Micronucleus Test (CSGMT/JEMS. MMS, The Mammalian Mutagenesis Study Group of the Environmental Mutagen Society of Japan) (1995). Protocol recommended for the short-term mouse peripheral blood micronucleus test. *Mutagenesis*, 10, 153-159.



2. Hayashi, M., Morita, T., Kodama, Y., Sofuni, T. and Ishidate, M. Jr. (1990). The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutation Research*, 245, 245-249.
3. The Collaborative Study Group for the Micronucleus Test (1992). Micronucleus test with mouse peripheral blood erythrocytes by acridine orange supravital staining: The summary report of the 5th collaborative study by CSGMT/JEMS. *MMS Mutation Research*, 278, 83-98.
4. OECD Guideline for the Testing of Chemicals #474: Mammalian Erythrocyte Micronucleus Test, 1997.
5. Quality Manual, Drug Development Division, Development Center for Biotechnology, 6th ed., 1999.
6. Health Food Safety Evaluation Guideline, Department of Health, Executive Yuan, Republic of China, 1999.

## Appendix C

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- ◆ Animal Use Approval Form

Date Received: Oct. 18, 2001

Date Approved: Oct. 18, 2001

DEVELOPMENT CENTER FOR BIOTECHNOLOGY  
PROTOCOL FOR USE OF LIVE VERTEBRATES  
FOR RESEARCH OR TEACHING

**ANIMAL USE APPROVAL FORM**

A.  Increasing Animal Usage

B.  Changing Test Article

Date Filed: Oct. 18, 2001

Principal Investigator or Study Director: Shwu-Fei Lee-Chen

Original Approved Protocol Number: #2000-DV-002

Original Approved Protocol Title: Micronucleus Assay with Mouse Peripheral  
Blood of DL-028A

Animal Species/Strain: mice-BALB/c

A. Increasing Animal Usage

1. Number/Sex of Animals Already Approved for Project: \_\_\_\_\_
2. Additional Number/Sex of Animals Requested: \_\_\_\_\_
3. Reason (Justification) for Additional Animals (enter text): \_\_\_\_\_
4. New total number of Animals to Complete or Finish Project: \_\_\_\_\_
5. Duration of New Experimental Period: \_\_\_\_\_

B. Changing Test Article

1. New Protocol Number: #2000-DV-002-o
2. New Test Article: DV00199-1
3. Number/Sex of Animals Requested: 40 male mice - ICR
4. New Protocol Title: Micronucleus Assay with Mouse Peripheral Blood of MicrSoy-20
5. New Experimental Period: Oct. 29 ~ Nov. 01, 2001

Other Statement or Amendment:

Reason to Change Animal Strain for Micronucleus Assay  
Due to the supply shortage of BALB/c mice from the National Laboratory Animal Breeding  
and Research Center, Taiwan, R.O.C., ICR mice are used for substitutes. Both BALB/c and ICR  
mice are acceptable strains for micronucleus assay (SOP: DCB-DV-TE00227)

Signature of Responsible PI or Study Director and Date: Shwu-Fei Lee-Chen Oct. 18, 2001

Signature of Chairman of IACUC and Date: C. C. Chang Oct. 18, 2001

## Appendix D

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- ◆ Laboratory Animal Quarantine Report

實驗動物檢疫報告  
Laboratory Animal Quarantine Report

DV-AC00032B

動物來源 (Animal Source): 國立動物中心 (NLABRC)

P.I.: Dr. Shwu-Fei Lee - chen

IACUC Protocol No.: #2000-DV-002-0 Project Code: DV-TA-00199-1 Study No.: MN 00019

接收日期 Received Date	品種/品系 Species/Strain	性別/數量 No./Sex	檢疫期 Quarantine Period*	通過日期 Release Date**
Oct. 3, 2001	Mice / ICR	43 / ♂	Oct. 3, 2001 ~ Oct. 9, 2001	Oct. 9, 2001

備註 (Remarks): 批 201020

\*: 檢疫項目及結果皆存放於動物房檔案室。

The documents and results of the quarantine are kept at the archive of the Laboratory Animal resource Division (LARD).

\*\* : 通過檢疫後，才轉移到飼育室。

The animals are moved to the designated animal rooms after passing the quarantine procedures.

Jay-Te Li Oct. 9, 2001

Attending Veterinarian

Date

## Appendix E

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### ◆ Individual Weight of the Animals at the Start of the Assay

## Micronucleus Assay in Mice — MicrSoy-20

### Individual Weight of the Animals at the Start of the Assay

Project Code: DV-TA00199

Study No: MN00019

Species/ Stain: mice/ ICR

Sex: male

Dose Group	Dose (mg/kg)	No. Mice Tested	Body Weight (g)					
			Individual Animal Data					Mean ± SD
1	0 (Vehicle)	5	35.21	34.90	36.12	37.87	37.70	36.36 ± 1.38
2	1/4 X origin liquid	5	32.92	35.36	38.59	36.96	38.79	36.52 ± 2.45
3	1/2 X origin liquid	5	34.29	36.05	36.50	37.73	38.04	36.52 ± 1.50
4	origin liquid	5	35.15	36.45	35.77	38.57	38.94	36.98 ± 1.69
5	Pos. Ctl.	5	34.44	35.52	36.14	35.51	37.31	35.78 ± 1.05

Data of body weights were recorded immediately before test article administration

## Appendix F

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### ◆ Information of Laboratory Rodent Diet



**Description** Laboratory Autoclavable Rodent Diet 5010 is the companion product of Laboratory Rodent Diet 5001. It has been formulated with extra nutrients to compensate for the nutrient losses that occur during steam sterilization.

The product is coated with a small amount of silicon dioxide in soybean oil to reduce clumping during the autoclaving process.

Refer to the Shelf Life section at the end of this book for product longevity information and storage suggestions.

**Features and Benefits**

- Constant formula helps minimize nutritional variables
- Processed with silicon dioxide to reduce sticking and clumping
- Similar to Laboratory Rodent Chow 5001 in nutrient composition and animal performance

**Product Forms Available**

- Oval pellet, 10 mm x 16 mm x 25 mm length (3/8" x 5/8" x 1" length)
- Meal (ground pellets), special order

**Autoclaving Suggestions**

To autoclave the pellets, place on trays, in small bags, or in larger bags, to a depth of no more than 3 inches.

When steam autoclaved, the pellets swell and exert force on adjacent pellets. Confinement by a bag or container creates additional pressure, which may result in sticking.

**Assay before and after autoclaving:**

Conditions of sterilization must be determined for each autoclaving unit. Microbiological evaluation should be done to insure sterilization is achieved. It is best to assay the diet before and after sterilization to determine nutrient losses desired.

**Guaranteed Analysis**

Crude protein not less than.....	23.0%
Crude fat not less than.....	4.5%
Crude fiber not more than.....	6.0%
Ash not more than.....	8.0%
Added minerals not more than.....	3.0%

**Ingredients**

Ground yellow corn, soybean meal, wheat middlings, fish meal, ground wheat, wheat germ meal, brewers' dried yeast, ground oats, alfalfa meal, calcium carbonate, animal fat preserved with BHA, dried beet pulp, soybean oil, salt, ground soybean hulls, dicalcium phosphate, cyanocobalamin, biotin, DL-methionine, calcium pantothenate, choline chloride, folic acid,

riboflavin, cholecalciferol, vitamin A acetate, dl-alpha tocopheryl acetate, thiamin mononitrate, nicotinic acid, pyridoxine hydrochloride, menadione dimethylpyrimidinol bisulfite (source of vitamin K), silicon dioxide, calcium iodate, manganous oxide, copper sulfate, cobalt carbonate, ferrous carbonate, zinc sulfate, zinc oxide.

**Feeding Directions**

Feed ad libitum to rodents. Plenty of fresh, clean water should be available to the animals at all times.

**Rats-** Adult rats will eat 12 to 15 grams of diet per day. Feeders in rat cages should be designed to hold two to three days' supply of feed at one time.

**Mice-** Adult mice will eat 4 to 5 grams of pelleted ration daily. Some of the larger strains may eat as much as 8 grams per day per animal. Feed should be available on a free choice basis in wire feeders above the floor of the cage.

**Hamsters-** Adults will eat 10 to 14 grams per day.



Chemical  
Composition<sup>1</sup>

<b>Nutrients<sup>2</sup></b>	
<b>Protein %</b> .....	<b>23.5</b>
Arginine %.....	1.40
Cystine %.....	0.34
Glycine %.....	1.20
Histidine %.....	0.58
Isoleucine %.....	1.24
Leucine %.....	1.87
Lysine %.....	1.42
Methionine %.....	0.49
Phenylalanine %.....	1.08
Tyrosine %.....	0.64
Threonine %.....	0.94
Tryptophan %.....	0.29
Valine %.....	1.22
Serine %.....	1.23
Aspartic Acid %.....	2.68
Glutamic Acid %.....	5.02
Alanine %.....	1.49
Proline %.....	1.73
Taurine %.....	0.03
<b>Fat (ether extract) %</b> .....	<b>5.1</b>
<b>Fat (acid hydrolysis) %</b> .....	<b>6.2</b>
Cholesterol, ppm.....	275
Linoleic Acid %.....	1.82
Linolenic Acid %.....	0.12
Arachidonic Acid %.....	<0.01
Omega-3 Fatty Acids %.....	0.42
Total Saturated Fatty Acids %.....	1.40
Total Monounsaturated Fatty Acids %.....	1.52
<b>Fiber (Crude) %</b> .....	<b>3.9</b>
Neutral Detergent Fiber <sup>3</sup> %.....	12.7
Acid Detergent Fiber <sup>4</sup> %.....	4.5
<b>Nitrogen-Free Extract (by difference) %</b> ...	<b>50.3</b>
Starch %.....	36.2
Glucose %.....	0.26
Fructose %.....	0.30
Sucrose %.....	1.02
Lactose %.....	0
<b>Total Digestible Nutrients %</b> .....	<b>76.0</b>
<b>Gross Energy, kcal/gm</b> .....	<b>4.06</b>
<b>Physiological Fuel Value<sup>5</sup>, kcal/gm</b> .....	<b>3.41</b>
<b>Metabolizable Energy, kcal/gm</b> .....	<b>3.17</b>

<b>Minerals</b>	
<b>Ash %</b> .....	<b>7.2</b>
Calcium %.....	1.00
Phosphorus (total) %.....	0.67
Phosphorus (non-phytate) %.....	0.43
Potassium %.....	0.92
Magnesium %.....	0.22
Sulfur %.....	0.24
Sodium %.....	0.28
Chlorine %.....	0.39
Fluorine, ppm.....	35.0
Iron, ppm.....	184.0
Zinc, ppm.....	124.3
Manganese, ppm.....	115.0
Copper, ppm.....	19.6
Cobalt, ppm.....	0.44
Iodine, ppm.....	1.19
Chromium, ppm.....	1.95
Selenium, ppm.....	0.32
<b>Vitamins</b>	
Carotene, ppm.....	4.5
Vitamin K (total), ppm.....	3.4
Menadione (added), ppm.....	2.9
Thiamin, ppm.....	80.7
Riboflavin, ppm.....	8.0
Niacin (available), ppm.....	100.0
Niacin (total), ppm.....	128.1
Pantothenic Acid, ppm.....	25.4
Choline, ppm.....	2200
Folic Acid, ppm.....	6.0
Pyridoxine, ppm.....	16.5
Biotin, ppm.....	0.35
B <sub>12</sub> , mcg/kg.....	33.0
Vitamin A, IU/gm.....	44.1
Vitamin D <sub>3</sub> (added), IU/gm.....	4.4
Vitamin E, IU/kg.....	66.1
Ascorbic Acid, mg/gm.....	—

\* Product Code  
<sup>1</sup> Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly.  
<sup>2</sup> Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purpose of calculations.  
<sup>3</sup> NDF = approximately cellulose, hemicellulose and lignin.  
<sup>4</sup> ADF = approximately cellulose and lignin.  
<sup>5</sup> Physiological Fuel Value (kcal/gm) = Sum of decimal fractions of protein, fat and carbohydrate (Use Nitrogen Free Extract) x 4.9.4 kcal/gm respectively.

## Appendix G

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◆ Temperature Record of Animal Room C420 (Oct. 09 ~ Nov. 01, 2001)

飼育室編號: C-420

### 動物飼育室溫度統計表

記錄期間: 10/9~11/1/2001

日期	溫度 (°C)		
	當日平均值(Mean±SD)	當日最高	當日最低
10/9/2001	20.9 ± 0.2	21.3	20.5
10/10/2001	21.0 ± 0.3	21.4	20.5
10/11/2001	21.0 ± 0.2	21.4	20.6
10/12/2001	21.0 ± 0.2	21.4	20.6
10/13/2001	21.0 ± 0.2	21.4	20.6
10/14/2001	21.0 ± 0.2	21.4	20.6
10/15/2001	21.0 ± 0.2	21.3	20.6
10/16/2001	21.0 ± 0.2	21.3	20.7
10/17/2001	21.0 ± 0.2	21.4	20.7
10/18/2001	21.0 ± 0.2	21.3	20.6
10/19/2001	21.0 ± 0.2	21.4	20.5
10/20/2001	21.0 ± 0.2	21.3	20.6
10/21/2001	21.0 ± 0.2	21.4	20.6
10/22/2001	21.0 ± 0.2	21.3	20.6
10/23/2001	20.9 ± 0.2	21.3	20.5
10/24/2001	20.9 ± 0.2	21.3	20.5
10/25/2001	21.1 ± 0.2	21.5	20.6
10/26/2001	21.1 ± 0.2	21.3	20.7
10/27/2001	20.9 ± 0.7	21.5	18.1
10/28/2001	21.1 ± 0.2	21.4	20.5
10/29/2001	21.0 ± 0.2	21.3	20.6
10/30/2001	20.9 ± 0.3	21.4	20.5
10/31/2001	20.9 ± 0.2	21.3	20.5
11/1/2001	21.0 ± 0.2	21.3	20.7

Prepared by: 劉文彬 11/15/2001

## Appendix H

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- ◆ Humidity Record of Animal Room C420 (Oct. 09 ~ Nov. 01, 2001)

動物飼育室溼度統計表

飼育室編號: C-420

記錄期間: 10/9~11/1/2001

日期	溼度(H%)		
	當日平均值(Mean±SD)	當日最高	當日最低
10/9/2001	54.4 ± 1.9	59.0	50.8
10/10/2001	53.3 ± 2.0	57.1	50.9
10/11/2001	53.8 ± 2.0	57.4	50.8
10/12/2001	53.6 ± 2.0	57.2	50.7
10/13/2001	53.3 ± 2.3	57.5	48.0
10/14/2001	53.1 ± 2.1	58.0	50.6
10/15/2001	53.6 ± 2.1	57.4	50.6
10/16/2001	53.6 ± 1.8	56.8	50.7
10/17/2001	53.0 ± 1.6	57.6	50.8
10/18/2001	53.3 ± 2.1	56.9	50.6
10/19/2001	53.1 ± 2.0	57.7	50.1
10/20/2001	54.3 ± 2.3	59.2	50.4
10/21/2001	55.3 ± 2.5	59.4	51.9
10/22/2001	54.5 ± 2.7	62.3	48.7
10/23/2001	53.6 ± 1.7	56.4	50.8
10/24/2001	54.1 ± 2.5	62.7	50.9
10/25/2001	52.2 ± 4.7	70.1	46.1
10/26/2001	51.7 ± 1.7	56.4	50.1
10/27/2001	52.9 ± 2.4	59.1	50.0
10/28/2001	52.1 ± 1.8	56.2	50.2
10/29/2001	52.3 ± 2.2	57.5	47.3
10/30/2001	53.1 ± 1.9	57.3	49.9
10/31/2001	53.8 ± 3.5	65.5	48.4
11/1/2001	52.9 ± 1.6	55.8	50.5

Prepared by: 劉文彬 11/1/2001