



MICROBIO

Appendix E

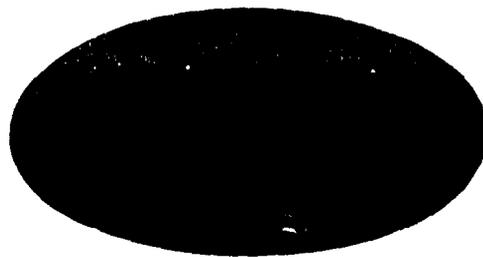


DEPARTMENT OF TOXICOLOGY AND PRECLINICAL SCIENCES
DEVELOPMENT CENTER FOR BIOTECHNOLOGY

SERIAL NO DV-TR-SA00032E
PROJECT CODE: DV-TA00199
PAGE 1 OF 35

**28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS
PRODUCT CODE MICRSOY-20 (MS-20)**

FINAL REPORT



DEPARTMENT OF TOXICOLOGY AND PRECLINICAL SCIENCES
DEVELOPMENT CENTER FOR BIOTECHNOLOGY



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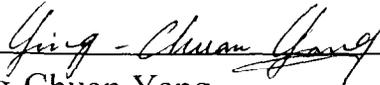
QUALITY ASSURANCE STATEMENT

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 Department of Toxicology and Preclinical Sciences, 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.

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. 24, 2001	Protocol	Oct. 24, 2001	Oct. 24, 2001
Oct. 29, 2001	Body weight recording on males Oral dosing on male rats	Oct. 29, 2001	Oct. 30, 2001
Nov. 05, 2001	Body weight recording on males Test article administration Weekly diet measurement on males Clinical observation	Nov. 05, 2001	Nov. 07, 2001
Nov. 13, 2001	Body weight recording on females Test article administration Weekly diet measurement on females Clinical observation	Nov. 13, 2001	Nov. 13, 2001
Nov. 26, 2001	Necropsy and gross examination Organ collection and weighing Blood collection (On male rats)	Nov. 26, 2001	Dec. 04, 2001
Nov. 27, 2001	Necropsy and gross examination Organ collection and weighing Blood collection (On female rats)	Nov. 30, 2001	Dec. 04, 2001
Mar. 25, 2002	Raw data; study records	Mar. 27, 2002	-----
Apr. 12, 2002	Final report	Apr. 12, 2002	Apr. 12, 2002


Ying-Chuan Yang
Quality Assurance Officer

Apr. 12, 2002
Date



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28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS PRODUCT CODE MICRSOY-20 (MS-20)

ABSTRACT

The purpose of this study was to evaluate the toxicity of "Product Code MicrSoy-20 (MS-20)", and to determine the No Observed Adverse Effect Level (NOAEL), when administered to Sprague-Dawley rats for 28 days via oral gavage. Eighty rats were randomized into four groups, each consisting of ten males and ten females. The dose levels were 0 (vehicle control), 1.5 (low), 5 (medium) and 15 (high) milliliter per kilogram of body weight (ml/kg). Examinations were conducted on the rats with respect to general demeanor, clinical signs, mortality, body weights/total body weight gains, food consumption, ophthalmologic changes, urinalysis, hematology, serum chemistry, organ weights and organ to brain weight ratios, and histopathological evaluation. No mortality was observed in treated and control rats in this study. Intermittent salivation, since study day 10 and thereafter, was observed in some male and female rats at high dose group (15 ml/kg/day), the total incidences for males and females were 9/10 and 10/10, respectively. Slight but statistically significant decreases in food consumption were noted on week 2 and week 3 in male rats and on week 2 and week 4 in female rats at 15 ml/kg/day treatment group ($p < 0.05$). There were no significant differences in mean body weights, mean body weight gains, ophthalmologic changes, urinalysis, hematology, serum chemistry, organ weights and organ to brain weight ratios, as well as gross changes between the vehicle control and Product Code MicrSoy-20 (MS-20) treated groups. There was no treatment related histopathological changes observed in the control and treated animals. In conclusion, the results of this study suggest that Product Code MicrSoy-20 (MS-20) at dose levels limited by dose volume can be administered (15 ml/kg/day) did not induce any adverse effects in SD rats. Therefore, the 28-day NOAEL for rats ingesting of "Product Code MicrSoy-20" is determined to be greater than 15 ml/kg/day. The NOAEL dose (15 ml/kg) established in this study provided a safety margin of 15 over the maximal human recommended daily dose (0.167 ml/kg), basing on a dosage conversion factor of 6 in surface area from a 150-g rat to a 60-kg human. A corresponding safety margin of 90 was provided basing on a body weight conversion. The results generated from this subacute toxicity study can be served as a safety reference for human use.



PURPOSE

Product Code MicrSoy-20 (MS-20) is a Chinese medicine. The purpose of this study was to evaluate the toxicity of MS-20, and to determine the No Observed Adverse Effect Level (NOAEL), when administered to Sprague-Dawley rats via oral gavage for 28 consecutive days. The results of this study will be used to establish safety margin for human daily use. This study was designed in accordance to the "Guidance for Industry Botanical Drug Products" [Center for Drug Evaluation and Research (CDER), August 2000]. The study processes were inspected for compliance with Good Laboratory Practices (GLP). All information generated from this study will be used as a part of an Investigational New Drug (IND) application.

GENERAL INFORMATION

- A. Study Number: SA00032
- B. Name of Sponsor: MICROBIO Co., Ltd.
Address: Address: No. 81, Gauyang N. Rd., Lung Tan Shiang, Tao Yuan, Taiwan, R.O.C.
Representative: William Lu
- C. Testing Facility: General/Reproductive Toxicology Laboratory, Department of Toxicology and Preclinical Sciences, Development Center for Biotechnology (DCB)
Note: According to SOP: DCB-DV-QA00032 issued on Mar. 11, 2002, the original name of 'Drug Development Division' was renamed as 'Department of Toxicology and Preclinical Sciences'.
Address: 103, Lane 169, Kang-Ning St., Hsi-Chih City, Taipei County, Taiwan 221, R.O.C.
- D. Location of Study: This study was conducted in General/Reproductive Toxicology Laboratory, Department of Toxicology and Preclinical Sciences and in animal room C-415.
- E. Personnel:
1. General/Reproductive toxicology unit
 - a. Study director: Jiuan Judy Liu, DVM, PhD
 - b. Associates: Yu-Mai Chen, MS
Chih-Peng Wang, MS
Tan-Fu Yuen, MS
Di-Sheng Wang, MS



Kuo-Tai Yang, MS
Yu-Hui Yang, MS
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Jihn-Shiun Chao, DVM, MS
Jyh-Min Tzeng, DVM, MS
Chien Ming Shih, PhD

c. Veterinary histopathologist: Shin-Yung Hsu, DVM, PhD

2. Collaborated units:

- a. Chief of laboratory animal research division: Chou-Chu Hong, DVM, PhD, DACVM
- b. Attending veterinarian: Tsung-Te Lin, DVM, MS

F. Study Schedule:

- 1. Animal procurement: Oct. 17, 2001
- 2. Quarantine and acclimation: Oct. 17, 2001 to Oct. 28, 2001
- 3. Date of dosing initiation: Oct. 29, 2001 for males and Oct. 30, 2001 for females
- 4. Date of study termination and necropsy: Nov. 26, 2001 for males and Nov. 27, 2001 for females

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 archives room at Drug Safety Building of DCB. All specimens will be stored and archived by General/Reproductive Toxicology Laboratory at DCB. The retention duration of these records and specimens will be in accordance with the relevant regulations.

Statement:

The test results relate only to the items tested; the final report shall not be reproduced except in full, without the written approval of the laboratory.



MATERIALS AND METHODS

A. Test Article and Vehicle Control

1. Test article

- a. Name: Product Code MicSoy-20 (MS-20)
- b. Date of receipt: Oct. 5, 2001 & Oct. 25, 2001
- c. Batch/Lot Number: 20010209
- d. DCB Code: DV00199 & DV00199-a
- e. Ingredients: MS-20 is a Chinese medicine. The components are very complicated. Until now, its effective components are still unable to determine.
- f. Other Characteristics: Dark brown liquid with prune juice odor
- g. Solubility: Water-soluble
- h. Amount Supplied: A total of 24 bottles (180 ml/bottle)
- i. Storage Conditions: Room temperature and protect from light
- j. Expiration Date: Feb. 9, 2004

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 (21 CFR §58.105, FDA).
2. The result and the report are generated by DCB for the test article submitted by the sponsor, and are intended for petition to government agency for product registration.

2. Vehicle control:

- a. Name: Injection grade water
- b. Source and manufacturer: Sin Tong Chemical Industrial Co., Ltd. Taiwan, ROC
- c. Lot number: 4DS1287
- d. Expiration date: June 12, 2002
- e. Storage condition: Room temperature

3. Preparation of test article solutions (SOP: DCB-DV-TE00463): During the 28-day study period, the calculated total volume of test article solution for each day was distributed into sterilized brown bottles at a weekly interval, and the solutions were kept in a dessicator until use. The daily volume of test article was 100 ml for the first week, and 165 ml for the last week.



B. Description of the Test System

1. Species: Rats
2. Strain: Crl:CD(SD)fSPF
3. Source: National Laboratory Animal Breeding & Research Center, National Science Council, Taipei, Taiwan
4. Age at initiation of study: Approximately 6 weeks old, with body weight ranges of 198 to 252 g for males and 157 to 192 g for females.
5. Quarantine and acclimation: After 8 days of quarantine (SOP: DCB-DV-AC00032) in room 431, rats were moved to room 415 for 5 days of acclimation prior to use. All suitable rats were accepted to this study.
6. Method of identification: Rats were identified with a unique number by ear notch (SOP: DCB-DV-TE00446) and cage tags.
7. Method of caging: The rats were housed in a stainless steel wire mesh cage (two rats per cage). Each cage was labeled with the animal number, study number, test article code, dose level, species and sex.
8. Environmental conditions:
 - a. Housing: Each suspended stainless steel cage contains 2 rats. The cages have wire mesh bottoms that allow urine and fecal material to fall through onto the tray containing absorbent wooded chips.
 - (1) Temperature (SOP: DCB-DV-AC00023): maintained at $21 \pm 2^\circ\text{C}$.
 - (2) Humidity (SOP: DCB-DV-AC00023): maintained at $50 \pm 20\%$ relative humidity.
 - (3) Light cycle (SOP: DCB-DV-AC00023): 12 hours light and 12 hours dark, automatic.
 - b. Diet (SOP: DCB-DV-AC00025): Laboratory Autoclavable Rodent Diet 5010 (PMI® Feeds Inc., St. Louis, MO) was supplied *ad libitum* throughout the study period. The dates of manufacturing were Aug. 9, 2001 and Aug. 31, 2001 (See Appendix C).
 - c. Water (SOP: DCB-DV-AC00026): Tap water was supplied *ad libitum* via water bottles attached to the cages.
 - d. Contaminants: Results of the Purina feed and the DCB water supply assays were reviewed and determined that any contaminants covered by those assays were at concentrations which were not affecting the results of the present study (See Appendix C).
9. Laboratory animal quarantine report (See Appendix C).
10. IACUC protocol number: 2001-TP-011-b.



C. Experimental Design

1. Treatment groups: Four groups of rats, each consisting of 10 males and 10 females, were used with a total of 40 males and 40 females. One group served as control and was dosed with injection grade water. The remaining three groups were dosed respectively to low (1.5 ml/kg/day), medium (5 ml/kg/day) and high dose (15 ml/kg/day) of Product Code MicrSoy-20 (MS-20).
2. Randomization: The rats were divided into weight classes with weight variation not exceeding $\pm 20\%$ of the mean body weight. The rats were then randomized and assigned into 4 groups (SOP: DCB-DV-TE00462) using the computerized Laboratory Integration Management System (Computer Service Center, DCB).
3. Basic design

Group no., dose levels, dosing solutions, dosing volumes and animal no. in this subacute oral toxicity study are presented as below:

Group No.	Dose Levels (ml/kg/day)	Dosing Solutions	Dosing volumes (ml/kg/day)	Animal No. M/F
1	0	injection grade water	15	10 M, 10 F
2	1.5	MS-20	1.5	10 M, 10 F
3	5	MS-20	5	10 M, 10 F
4	15	MS-20	15	10 M, 10 F

4. Rationales for dose level selection
 - a. Basing on the letter from the sponsor issued on Dec. 26, 2001, the recommended daily human dose was changed to 1-10 ml; for a 60-kg subject, therefore, the maximal recommended daily dose estimated is 0.167 ml/kg approximately.
 - b. The acute oral toxicity study in SD rats (DV-PR-AC00139E) revealed no observable adverse effects at the dose level up to 20 ml/kg.
 - c. The rats were dosed with MS-20 solution at the dose levels of 1.5, 5 and 15 ml/kg/day in this study, which provided a corresponding safety margin of 15, 30 and 90, respectively, basing on a body weight conversion. A corresponding safety margin of 1.5, 5 and 15, respectively, was also provided basing on a dosage conversion factor of 6 in surface area from a 150-g rat to a 60-kg human.



5. Dosing process: Oral gavage was conducted according to SOP: DCB-DV-TE00447.

D. Experimental Procedures

1. Dosing: Test article solutions at three dose levels (1.5, 5 and 15 ml/kg) and injection grade water (15 ml/kg) were administered to rats (10 males and 10 females per group) once every morning by gavage for 28 days. The dosing volume were calculated on the basis of the most recently recorded individual body weight, and 1~10 ml plastic disposable syringes (needle size: 16 gauge, 80 mm in length) were used. The first dosing day is denoted as SD1 (Study Day 1). The range of first dosed volume for group 1 to group 4 were 3.0~3.7 ml 、 0.3~0.3 ml 、 1.0~1.3 ml and 3.0~3.7 ml for males, and 2.5~2.9 ml 、 0.2~0.3 ml 、 0.8~0.9 ml and 2.5~2.8 ml for females. The range of last dosed volume for group 1 to group 4 were 5.6~6.6 ml 、 0.6~0.7 ml 、 1.8~2.3 ml 、 and 4.9~6.6 ml for males, and 3.6~4.2 ml 、 0.3~0.4 ml 、 1.2~1.4 ml and 3.2~4.4 ml for females.

2. Observation and examination

a. Animal observations (SOP: DCB-DV-TE00469): The rats were observed for clinical signs and mortality after dosing. All rats were observed twice daily at least six-hours apart (before 10:00 AM and after 3:00 PM) on working days and once daily on weekends during the 28-day study. Any clinical signs, moribundity and mortality were recorded and documented.

b. Body weight (SOP: DCB-DV-TE00450): Body weights were recorded on all rats before first dosing at study day 1 (SD1), weekly thereafter for the duration of the study period, prior to the termination of study (SD28) and on the day of necropsy (SD29).

c. Food consumption (SOP: DCB-DV-EQ00074): The food consumption for two rats in each cage was measured at weekly interval throughout the 28-day study period. The average food intake of per kilogram body weight rat per day was calculated.

d. Ophthalmologic examination: Gross examination of the eyes was performed on all 4 groups of animals on the days before the first dosing (SD0) and before the termination of study on SD28.



e. Clinical studies

(1) Urinalysis (SOP: DCB-DV-TE00451): One day before the termination of study on SD28, rats from each treatment group were placed individually in a polycarbonate metabolism cage with free access to water only. Urine specimens were collected for 16 hours. The volume, color, odor and turbidity of the urine samples were recorded. Urinary sediments were examined under light microscopy. The following parameters were analyzed using the Bayer Clinitek 100 Urine Chemistry Analyzer (SOP: DCB-DV-EQ00066).

pH	Glucose
Specific gravity ¹	Ketones
Nitrites	Urobilinogen
Occult blood	Bilirubin
Protein	Leukocytes

¹: Specific gravity was examined by Clinical Refractometer (SOP: DCB-DV-EQ00070).

(2) Hematology: One day before the termination of the study on SD28, all rats were fasted over 16 hours. At necropsy (SD29), the surviving rats were euthanized by i.p. injection with an overdose of sodium pentobarbital solution (SOP: DCB-DV-TE00424). Blood samples were obtained through femoral vein (SOP: DCB-DV-TE00438) and collected in sampling tubes containing EDTA. The following parameters were determined using the Baker System 9118⁺AX Automated Hematology Analyzer (SOP: DCB-DV-EQ00076).

-
- Erythrocytes (Red blood cells) count
 - Hemoglobin
 - Hematocrit
 - Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration (calculated)
 - Leukocytes (White blood cells) count
 - Leukocyte differential¹ count (Lymphocytes, Monocytes, Granulocytes)
 - Platelet count
 - Prothrombin time²
 - Activated partial thromboplastine time²
-

¹: Leukocyte differential count — Microscopic examination of blood smear (SOP: DCB-DV-TE00444) stained with the Hemacolor stain (SOP: DCB-DV-TE00445) were performed with at least one hundred leukocytes (SOP: DCB-DV-TE00453).

²: The blood samples were mixed with CTAD anticoagulant (0.109 M Sodium Citrate, 15 mM Theophylline, 3.7 mM Adenosine, 0.198 mM Dipyridamole) (9:1,v/v), then centrifuged for plasma collection. The items were performed by CA-1000 Sysmex Coagulation Analyzer (SOP: DCB-DV-EQ00071).

(3) Clinical chemistry (SOP: DCB-DV-TE00442): Clinical chemistry was determined on aliquots of the blood samples which were collected in blood sampling tubes without anticoagulant. The collected blood specimens were centrifuged and the sera were aspirated for assays. The determination of clinical chemistry parameters was performed using the Hitachi 7060E Automated Analyzer (SOP: DCB-DV-EQ00075).

Alanine aminotransferase	Lactate dehydrogenase
Aspartate aminotransferase	Creatine phosphate kinase
Alkaline phosphatase	Glucose
γ-glutamyltransferase	Blood urea nitrogen
Total bilirubin	Uric acid
Direct bilirubin	Creatinine
Total protein	Calcium
Albumin	Phosphorus
Albumin/Globulin ratio (calculated)	Sodium
Triglycerides	Potassium
Cholesterol	Chloride

- f. Gross necropsy: Gross necropsies were performed on found dead and all surviving rats at the end of study (SD29). In a randomized order, the surviving rats were euthanized by i.p. injection of an overdose of sodium pentobarbital followed by exsanguination and necropsy. Any gross lesions observed at necropsy were recorded, sampled and fixed in 10% buffered formalin (SOP: DCB-DV-TE00407) for further microscopy evaluations.
- g. Organ weight (SOP: DCB-DV-EQ00074): Organs were taken from all rats surviving to the end of study (SD29) including brain, liver, kidneys, heart, adrenals, thymus, spleen, and testes (male only). The above organs were weighed to the nearest 10 mg, except for adrenals and thymus which were weighed to the nearest 1.0 mg. Organ weight to brain weight ratios were also calculated.



- h. Histopathology: Representative samples of the following tissues collected at necropsy were fixed in 10% buffered formalin or a 3% glutadialdehyde solution. The tissues in control and high dose (15 ml/kg/day) groups and all gross lesions were trimmed, embedded (SOP: DCB-DV-TE00408), sectioned (SOP: DCB-DV-TE00409) and H&E-stained (SOP: DCB-DV-TE00412), followed by microscopic examination. While the tissues at medium and low dose groups were fixed and preserved in 10% buffered formalin.

Adrenals	Ovaries and oviduct* (female only)
Aorta	Pituitary
Bone & bone marrow (femur)	Pancreas
Brain (fore, mid, and hind)	Prostate (male only)
Cecum	Salivary glands
Colon	Sciatic nerve
Corpus and cervix uteri	Seminal vessels (male only)
Duodenum	Skeletal muscle
Epididymis* (male only)	Skin
Esophagus	Spinal cord (cervical, thoracic & lumbar)
Eyes*	Spleen
Harderian glands*	Stomach
Heart	Testes* (male only)
Ileum	Thymus (or thymic region)
Jejunum	Thyroid/parathyroid
Kidneys	Trachea
Lung (with main-stem bronchi)	Urinary bladder
Lymph nodes (mandibular & mesenteric)	Vagina (female only)
Mammary glands (female only)	All gross lesions
	Ears (for identification)

*: indicated that organs will be fixed in 3% glutadialdehyde.

3. Statistical analysis: All measured parameters were calculated as mean \pm standard deviation (SD). Comparisons of all data collected on body weights, food consumption, clinical pathology values and organ weights (absolute and relative to brain weights) were performed using one-way analysis of variance (ANOVA) method, followed by Dunnett's method (SigmaStatTM, V2.03, 1997). Probability of 0.05 or small ($p < 0.05$) was used as the criterion of significance.



F. Clinical Pathology

1. Urinalysis

The urinalysis results in terms of volume, pH and urobilinogen are presented in Table 5. The other urinalysis results are listed in Appendix D: "Individual Animal Data of Urinalysis" and "Individual Animal Data of Urinalysis-Urine Sediment". A slight increase in mean pH value was observed in female rats at 15 ml/kg/day dose group ($p < 0.05$), however, this value was still within the range of normal variation. There were no differences between all treated and control groups in other parameters examined at the termination of the 28-day dosing study.

2. Hematology

The hematology results are presented in Table 6-1 and Table 6-2. Individual animal data are presented in Appendix D. Slight decrease in mean corpuscular hemoglobin concentration (MCHC) and increases in platelets counts and white blood cells were found in male rats at 15 ml/kg/day dose group ($p < 0.05$). However, those values were still within normal range of variation. There were no differences in other observed hematological parameters in male and female rats between the control and all treated groups.

3. Serum chemistry

The serum chemistry results are presented in Table 7-1 and Table 7-2. Individual animal data are presented in Appendix D. Scattered changes but statistical significances were observed in alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine phosphate kinase (CPK), chloride (Cl) in male rats at 15 ml/kg/day dose group ($p < 0.05$). A slight increase in triglycerides was also noted in female rats at 15 ml/kg/day dose group ($p < 0.05$). Those differences were still within normal physiological range and considered not treatment-related. There were no differences in other parameters between the control and the treated animals at the termination of the 28-day dosing study.

G. Gross Necropsy

A summary of the gross findings is presented in Table 8. Several changes/lesions were observed in the treated and control animals, but all were considered to be unrelated to the test article administration. They consisted of: (1) A fat-like mass at the serosal surface of urinary bladder – The change was observed in one male rat of control group, the size of the change was 1.0 cm in diameter. It was confirmed to be an adipose tissue by



RESULTS AND DISCUSSION

A. Mortality

A summary of mortality incidence is presented in Table 1. There was no death observed in treated and control animals throughout the 28-day study period. The mortality for each group per sex was 0/10.

B. Clinical Observations

Table 2 presents a summary of the clinical observations. Salivation, since study day 10 and thereafter, was observed in some male and female treated rats at 15 ml/kg/day dose level. The total incidence was 9/10 for males and 10/10 for females. Hair loss and wound at the left side of head were observed in one male rat at 15 ml/kg/day dose on study day 23, which was resulted by animal fighting. Audible respiration was noted in one female rat at 15 ml/kg/day dose on study day 3 and study day 4, which was suspected to be individual animal response to handling for oral gavage. There were no other clinical signs observed in the animals of the control and other treatment groups during the 28-day study period.

C. Body Weights

The mean body weights and mean body weight gains of the animals in all groups are presented in Table 3. Individual animal data are presented in Appendix D. The mean body weight growth curves are shown in Figure 1. There were no differences in the mean body weights, mean body weight gains at weekly intervals, and the total mean body weight gains between treated and control groups during the study period.

D. Food Consumption

The average daily food consumption, calculated as grams of food/kg of body weight per rat/per day is presented in Table 4. Individual animal data are presented in Appendix D. A slight but statistically significant decrease in food consumption was noted on week 2 and week 3 in male rats and on week 2 and week 4 in female rats at 15 ml/kg/day dose group ($p < 0.05$). There were no other statistical differences found between control and the other treatment groups throughout the 28-day study period.

E. Ophthalmologic Examination

A summary of ophthalmologic examination is presented in Table 1. There was no observable abnormality in the rats of control and the treatment groups before the first dosing (SD0) and one day before the termination of the study (SD28).



histopathological evaluation. (2) Two whitish spots at the surface of spleen – The changes were observed in one male rat of medium dose group. The whitish spots measured 0.1-0.2 cm in diameter. Histopathologically, a small piece of tissue detached from the spleen, but there was no inflammatory or hemorrhagic response at and around the focus. It was confirmed to be the result of abrasion. (3) Hemorrhagic spot at the anterior portion of the thymus – The change was noted in one male rat of medium dose group. The change showed evidence of hemorrhage, but there was no inflammatory response at and around the focus. It was considered to be the result of circulatory disturbance due to the pentobarbital sodium administration. (4) Bilateral dilatation and accumulation of fluid in the uterine horns – The change was observed in 2 females, one for each of control group and low dose group. It was confirmed to be a normal physiological response of uterine horns during diestrus phase.

H. Organ Weights and Organ to Brain Weight Ratios

A summary of the organ weights and organ to brain weight ratios of the animals in all groups is presented in Table 9-1 and Table 9-2. Individual animal data are presented in Appendix D. There were no statistical significant differences in organ weights and the organ to brain weight ratios between the control and the treated groups.

I. Histopathology

A summary of microscopic findings is listed in Table 10. Correlation of Gross and Microscopic Findings is listed in Table 11. Individual animal data of histopathology evaluation are presented in Appendix D. Several microscopic changes were observed in animals of the test article-treated and control groups, but all those were considered to be spontaneous changes/lesions, and were not related to the test article administration. They consisted of: (1) Mineralization of renal tubules – The incidence of the change in female rats of control and high dose groups were 3/10 and 5/10, respectively; the change was also seen in one male rat of control group. Mineralization of renal tubules was mostly observed at the junction between the cortex and medulla. (2) Chronic progressive nephropathy – This change was noted in 2 male rats of control group, they were all in minimal changes. (3) Infiltration of lymphocytic cells in prostate – The change was noted in 2 male rats of high dose group. The major change was infiltration of lymphocytic cells in the interstitial tissues. The cause of this change was unknown. In summary, the test article (product code MicrSoy-20) when administered orally to rats at the dose level of 15 ml/kg/day for 28 days did not cause any pathological changes/lesions in the tissues/organs. Gross observations noted at necropsy were correlated to microscopic findings as appropriate listed in Table 11.

CONCLUSION

The results of this 28-day oral toxicity study of Product Code MicrSoy-20 (MS-20) in rats indicated that no mortality was observed at low (1.5 ml/kg/day), medium (5 ml/kg/day), high doses treated and control rats. Salivation, since study day 10 and thereafter, was observed in some male and female rats at high dose group (15 ml/kg/day), the total incidences for male and females were 9/10 and 10/10, respectively. A slight but statistically significant decrease in food consumption was noted on week 2 and week 3 in male rats and on week 2 and week 4 in female rats at 15 ml/kg/day dose group ($p < 0.05$). There were no significant differences in mean body weights, mean body weight gains, ophthalmologic changes, urinalysis, hematology, serum chemistry, organ weights and organ to brain weight ratios, as well as gross changes between the vehicle control and Product Code MicrSoy-20 (MS-20) treated groups. There was no treatment related histopathological changes observed in the control and treated animals. In conclusion, the results of this study suggest that Product Code MicrSoy-20 (MS-20) at dose levels limited by dose volume can be administered (15 ml/kg/day) did not induce any adverse effects in SD rats. Therefore, the 28-day NOAEL for rats ingesting of "Product Code MicrSoy-20" is determined to be greater than 15 ml/kg/day. The NOAEL dose (15 ml/kg) established in this study provided a safety margin of 15 over the maximal human recommended daily dose (0.167 ml/kg), basing on a dosage conversion factor of 6 in surface area from a 150-g rat to a 60-kg human. A corresponding safety margin of 90 was provided basing on a body weight conversion. The results generated from this subacute toxicity study can be served as a safety reference for human use.

COMMENTS AND/OR PROBLEMS

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 during the study that would adversely affect the study results or interpretation.



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3. Guidance for Industry Botanical Drug Products, Center for Drug Evaluation and Research (CDER), August 2000.
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Table 1. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Mortality and Ophthalmologic Abnormality Examination (N/N)

Dose (ml/kg/day)	0 (Control)		1.5		5		15	
Sex	M	F	M	F	M	F	M	F
Number of animals per group	10	10	10	10	10	10	10	10
Mortality	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Ophthalmologic abnormality								
Before initiation (SD0)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Before necropsy (SD28)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

N/N: Number of rats with death or abnormality/Number of rats examined

Control: injection grade water (15 ml/kg/day)

M: Male

F: Female

Table 2. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Clinical Signs and Total Incidence (N/N)

Dose (ml/kg/day)	0 (Control)		1.5		5		15	
Number of animals per group	10		10		10		10	
<u>Males</u>								
Salivation	0/10		0/10		0/10		9/10	
Hair loss	0/10		0/10		0/10		1/10	
Wound	0/10		0/10		0/10		1/10	
<u>Females</u>								
Audible respiration	0/10		0/10		0/10		1/10	
Salivation	0/10		0/10		0/10		10/10	

N/N: Number of rats with clinical signs/Number of rats examined

Control: injection grade water (15 ml/kg/day)



Table 3. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Body Weights and Body Weight Gains (N=10)

Sex	Dose (ml/kg/day)	Body Weights During the Study Period (g, Mean ±SD)				
		SD1	SD8	SD15	SD22	SD28
Male	0 (Control)	226.1±17.0	293.9±21.1	355.3±21.6	405.6±20.4	443.3±21.6
	1.5	221.6±12.6	297.4±22.8	360.1±25.6	411.6±29.1	449.2±31.4
	5	225.7±15.0	297.6±20.0	356.5±23.4	402.0±28.5	436.3±33.1
	15	226.0±17.4	295.9±18.4	351.8±27.8	397.1±34.3	435.8±36.5
Female	0 (Control)	179.3±7.3	218.3±12.5	240.1±11.9	259.1±15.5	279.2±15.3
	1.5	173.9±13.2	214.9±10.5	235.2±13.4	254.8±14.3	267.5±14.8
	5	175.4±8.0	216.3±17.1	240.3±17.6	257.1±13.8	268.9±14.1
	15	178.7±6.5	215.0±17.9	232.4±25.1	250.0±23.5	262.0±27.5

Sex	Dose (ml/kg/day)	Body Weight Gains During the Study Period (g, Mean ±SD)			
		Week 1	Week 2	Week 3	Total Gains at Final
Male	0 (Control)	67.8±5.8	129.2±7.8	179.5±10.7	217.2±13.2
	1.5	75.8±13.6	138.5±18.3	190.0±22.5	227.6±25.6
	5	71.9±7.1	130.8±10.7	176.3±16.2	210.6±21.9
	15	69.9±5.5	125.8±19.2	171.1±26.7	209.8±31.2
Female	0 (Control)	39.0±12.0	60.8±10.1	79.8±12.9	99.9±14.8
	1.5	41.0±10.3	61.3±12.4	80.9±11.6	93.6±14.0
	5	40.9±16.4	64.9±15.5	81.7±11.5	93.5±12.7
	15	36.3±15.4	53.7±22.8	71.3±21.3	83.3±26.0

Control : Injection grade water (15 ml/kg/day)



Table 4. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Average Daily Food Consumption (Mean ±SD, N=5)

Sex	Dose (ml/kg/day)	Average Daily Food Consumption (g/kg/day)			
		During 28-day study period			
		week 1	week 2	week 3	week 4
Male	0 (Control)	158.6 ± 8.4	132.6 ± 5.2	110.6 ± 6.8	96.6 ± 5.3
	1.5	169.2 ± 11.0	132.6 ± 5.2	110.0 ± 4.9	96.0 ± 3.6
	5	162.6 ± 5.0	128.2 ± 5.5	105.4 ± 4.0	92.4 ± 3.4
	15	153.2 ± 10.4	120.8 ± 3.8*	100.4 ± 4.2*	90.0 ± 3.1
Female	0 (Control)	154.8 ± 10.6	124.2 ± 4.6	115.8 ± 6.5	107.6 ± 6.8
	1.5	158.8 ± 18.0	122.8 ± 4.4	115.2 ± 6.2	104.4 ± 2.4
	5	155.2 ± 18.4	124.6 ± 10.4	109.6 ± 9.4	100.4 ± 4.4
	15	141.0 ± 9.8	109.8 ± 11.6*	103.4 ± 10.2	92.6 ± 8.2*

Control : Injection grade water (15 ml/kg/day)

* : p < 0.05



Table 5. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Urinalyses (Mean ± SD, N=10)

Sex	Parameter	Dose (ml/kg/day)			
		0 (Control)	1.5	5	15
Male	Volume (mL)	28.4 ± 5.9	24.4 ± 6.0	29.8 ± 8.7	31.2 ± 6.1
	Specific gravity	1.020 ± 0.006	1.022 ± 0.005	1.018 ± 0.005	1.017 ± 0.003
	pH	7.05 ± 0.16	6.85 ± 0.47	6.95 ± 0.28	7.25 ± 0.54
	Urobilinogen (EU/dl)	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
Female	Volume (mL)	14.7 ± 6.3	14.7 ± 3.7	14.3 ± 4.3	18.7 ± 5.4
	Specific gravity	1.025 ± 0.005 ^a	1.024 ± 0.004	1.024 ± 0.006	1.020 ± 0.006
	pH	6.60 ± 0.46	6.80 ± 0.26	6.90 ± 0.32	7.30 ± 0.26*
	Urobilinogen (EU/dl)	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00

Control : Injection grade water (15 ml/kg/day)

^a : n=9 , one datum was out of the detection limit and was not included.

* : p < 0.05



Table 6-1. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Male Rats — Hematology Parameters (Mean ± SD, N=10)

Parameter	Dose (ml/kg/day)			
	0 (Control)	1.5	5	15
Red blood cells (RBCs; $\times 10^6/\mu\text{l}$)	7.48 ± 0.43	7.52 ± 0.24	7.58 ± 0.40	7.88 ± 0.28
Hemoglobin (Hb; g/dl)	15.03 ± 0.49	14.83 ± 0.40	14.92 ± 0.67	15.18 ± 0.49
Hematocrit (%)	45.47 ± 1.50	44.91 ± 1.23	45.84 ± 1.62	46.94 ± 1.56
Mean corpuscular volume (μm^3)	60.89 ± 2.02	59.78 ± 1.91	60.53 ± 1.98	59.57 ± 0.54
Mean corpuscular Hb (pg)	20.13 ± 0.88	19.74 ± 0.64	19.71 ± 0.93	19.27 ± 0.43
Mean corpuscular Hb concentration (%)	33.06 ± 0.51	33.03 ± 0.45	32.54 ± 0.67	32.33 ± 0.68*
Platelets count ($\times 10^3/\mu\text{l}$)	914.3 ± 54.0	904.1 ± 49.7	919.4 ± 50.7	1039.5 ± 112.2*
White blood cells (WBCs; $\times 10^3/\mu\text{l}$)	6.91 ± 1.32	7.18 ± 1.71	7.02 ± 1.15	9.30 ± 1.88*
Bands (%)	0	0	0	0.1 ± 0.3
Lymphocytes (%)	88.2 ± 4.7	88.6 ± 6.2	88.9 ± 4.6	88.2 ± 5.5
Monocytes (%)	3.2 ± 2.1	2.5 ± 1.6	2.6 ± 2.3	2.5 ± 1.3
Neutrophils (%)	8.2 ± 3.9	8.7 ± 5.5	8.4 ± 3.2	8.6 ± 5.1
Eosinophils (%)	0.4 ± 0.5	0.2 ± 0.4	0.1 ± 0.3	0.6 ± 0.7
Basophils (%)	0	0	0	0
Granulocytes (%)	8.6 ± 3.7	8.9 ± 5.4	8.5 ± 3.1	9.3 ± 5.4
Prothrombin time (sec)	15.8 ± 1.5	16.1 ± 1.4	16.5 ± 1.3	15.8 ± 1.3
Activated partial thromboplastine time (sec)	20.2 ± 2.9	21.1 ± 2.5	22.8 ± 2.5	22.3 ± 2.1

Control : Injection grade water (15 ml/kg/day)

* : $p < 0.05$



Table 6-2. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Female Rats — Hematology Parameters (Mean ± SD, N=10)

Parameter	Dose (ml/kg/day)			
	0 (Control)	1.5 ^a	5	15
Red blood cells (RBCs; x10 ⁶ /μl)	7.24 ± 0.25	7.38 ± 0.33	7.32 ± 0.28	7.39 ± 0.38
Hemoglobin (Hb; g/dl)	14.55 ± 0.32	14.76 ± 0.52	14.57 ± 0.48	14.53 ± 0.62
Hematocrit (%)	43.24 ± 1.05	43.57 ± 1.72	43.63 ± 1.59	43.47 ± 2.00
Mean corpuscular volume (μm ³)	59.74 ± 1.25	59.01 ± 1.08	59.59 ± 1.05	58.91 ± 1.85
Mean corpuscular Hb (pg)	20.10 ± 0.57	19.99 ± 0.52	19.89 ± 0.53	19.70 ± 0.79
Mean corpuscular Hb concentration (%)	33.66 ± 0.50	33.88 ± 0.70	33.40 ± 0.51	33.44 ± 0.59
Platelets count (x10 ³ /μl)	906.8 ± 114.1	930.7 ± 135.7	941.4 ± 110.1	1014.7 ± 65.1
White blood cells (WBCs; x10 ³ /μl)	3.77 ± 0.72	4.28 ± 0.59	3.75 ± 0.81	4.10 ± 0.99
Bands (%)	0.1 ± 0.3	0	0	0
Lymphocytes (%)	88.4 ± 7.0	89.4 ± 5.6	90.2 ± 4.4	89.8 ± 6.3
Monocytes (%)	2.0 ± 1.6	2.0 ± 1.6	1.5 ± 1.4	2.1 ± 1.9
Neutrophils (%)	8.9 ± 5.4	8.0 ± 5.5	7.6 ± 3.4	7.0 ± 4.7
Eosinophils (%)	0.6 ± 0.8	0.6 ± 0.8	0.7 ± 0.8	1.1 ± 1.0
Basophils (%)	0	0	0	0
Granulocytes (%)	9.6 ± 5.8	8.6 ± 5.9	8.3 ± 3.9	8.1 ± 4.9
Prothrombin time (sec)	15.0 ± 1.0	15.1 ± 0.8	14.9 ± 0.8 ^a	15.5 ± 0.9
Activated partial thromboplastine time (sec)	16.1 ± 1.7	16.3 ± 2.1	17.5 ± 2.0 ^a	17.4 ± 0.9

Control: Injection grade water (15 ml/kg/day)

^a: n=9, one sample was not calculated due to blood coagulation.

Table 7-1. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Male Rats — Serum Chemistry Parameters (Mean ± SD, N=10)

Parameter	Dose (ml/kg/day)			
	0 (Control)	1.5	5	15
Alanine aminotransferase (U/l)	29.9 ± 4.6	30.0 ± 3.4	29.1 ± 3.0	25.3 ± 4.4*
Aspartate aminotransferase (U/l)	170.6 ± 25.6	151.8 ± 35.5	134.8 ± 22.7*	132.9 ± 40.5*
Alkaline phosphatase (U/l)	171.8 ± 39.7	178.8 ± 20.5	166.3 ± 29.6	151.9 ± 38.6
Total protein (g/dl)	4.74 ± 0.16	4.83 ± 0.20	4.83 ± 0.16	4.96 ± 0.26
Albumin (g/dl)	3.10 ± 0.08	3.14 ± 0.08	3.13 ± 0.11	3.20 ± 0.14
Albumin/Globulin ratio	1.90 ± 0.16	1.86 ± 0.13	1.85 ± 0.11	1.82 ± 0.08
γ-glutamyltransferase (U/l)	0.60 ± 0.38 ^a	0.60 ± 0.30 ^b	0.60 ± 0.24	0.68 ± 0.41 ^a
Direct bilirubin (mg/dl)	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Total bilirubin (mg/dl)	0.02 ± 0.01 ^b	0.03 ± 0.02 ^a	0.02 ± 0.01	0.02 ± 0.02 ^a
Triglycerides (mg/dl)	56.6 ± 22.7	54.9 ± 19.4	53.8 ± 27.9	57.0 ± 21.4
Cholesterol (mg/dl)	59.7 ± 6.7	61.8 ± 7.7	64.5 ± 7.3	62.3 ± 6.2
Lactate dehydrogenase (U/l)	1916.3 ± 455.0	1596.4 ± 661.4	1292.3 ± 446.6	1250.7 ± 677.8*
Creatine phosphate kinase (U/l)	1381.5 ± 466.2	1103.8 ± 445.7	855.5 ± 289.2*	849.6 ± 443.4*
Glucose (mg/dl)	112.5 ± 32.6	97.0 ± 23.1	102.0 ± 25.2	89.7 ± 14.2
Blood urea nitrogen (mg/dl)	18.02 ± 3.12	17.15 ± 2.34	17.04 ± 2.59	16.34 ± 2.01
Uric acid (mg/dl)	1.07 ± 0.21	0.96 ± 0.12	0.96 ± 0.07	1.01 ± 0.15
Creatinine (mg/dl)	0.70 ± 0.07	0.73 ± 0.07	0.69 ± 0.07	0.70 ± 0.09
Calcium (mg/dl)	10.27 ± 0.36	10.18 ± 0.17	10.25 ± 0.17	10.36 ± 0.34
Phosphorus (mg/dl)	8.64 ± 0.59	8.85 ± 0.56	8.84 ± 0.60	9.06 ± 0.57
Sodium (mEq/l)	146.2 ± 2.0	146.3 ± 0.8	147.2 ± 1.3	147.1 ± 1.3
Potassium (mEq/l)	4.18 ± 0.19	4.11 ± 0.34	4.01 ± 0.34	4.32 ± 0.39
Chloride (mEq/l)	100.9 ± 2.8	101.6 ± 1.3	103.2 ± 2.2*	103.6 ± 1.6*

Control: Injection grade water (15 ml/kg/day)

^a: n=9, one datum was out of the detection limit and was not calculated.

^b: n=8, two data were out of the detection limit and were not calculated.

*: p < 0.05



Table 7-2. 28-Day Subacute Oral Toxicity Study of Product Code MicSoy-20 (MS-20) in Female Rats — Serum Chemistry Parameters (Mean ± SD, N=10)

Parameter	Dose (ml/kg/day)			
	0 (Control)	1.5	5	15
Alanine aminotransferase (U/l)	25.0±3.9	24.5±4.2	25.0±5.4	23.0±4.5
Aspartate aminotransferase (U/l)	142.3±35.6	119.8±24.7	136.4±37.5	121.2±27.2
Alkaline phosphatase (U/l)	99.7±21.8	98.5±26.1	95.7±26.9	94.5±22.3
Total protein (g/dl)	5.11±0.19	5.28±0.36	5.24±0.21	5.12±0.37
Albumin (g/dl)	3.38±0.14	3.46±0.23	3.43±0.08	3.36±0.20
Albumin/Globulin ratio	1.93±0.13	1.88±0.08	1.90±0.18	1.93±0.21
γ-glutamyltransferase (U/l)	0.65±0.35	0.57±0.29	0.65±0.30	0.59±0.35 ^a
Direct bilirubin (mg/dl)	0.01±0.00	0.01±0.01 ^b	0.00±0.01 ^a	0.00±0.01
Total bilirubin (mg/dl)	0.02±0.01	0.01±0.02 ^a	0.02±0.02 ^d	0.01±0.01 ^c
Triglycerides (mg/dl)	11.2±4.4	17.5±7.4	13.0±3.4	18.1±7.6*
Cholesterol (mg/dl)	79.1±8.8	69.6±12.4	80.4±11.5	85.5±19.1
Lactate dehydrogenase (U/l)	1583.2±595.1	1314.5±444.7	1526.1±654.6	1321.7±398.9
Creatine phosphate kinase (U/l)	1088.2±366.1	887.3±370.2	973.9±486.0	812.6±263.9
Glucose (mg/dl)	83.6±19.1	101.0±23.8	93.0±8.5	88.6±15.7
Blood urea nitrogen (mg/dl)	23.05±4.27	22.66±4.02	23.51±2.62	23.80±3.90
Uric acid (mg/dl)	1.16±0.32	1.00±0.17	1.02±0.28	0.98±0.21
Creatinine (mg/dl)	0.80±0.07	0.79±0.07	0.82±0.06	0.79±0.09
Calcium (mg/dl)	10.05±0.29	10.28±0.29	10.22±0.30	10.30±0.40
Phosphorus (mg/dl)	7.11±0.56	7.02±0.85	7.40±1.08	7.25±0.61
Sodium (mEq/l)	146.1±1.6	146.3±1.3	147.1±1.7	147.3±1.2
Potassium (mEq/l)	3.77±0.31	3.80±0.37	3.76±0.29	4.04±0.40
Chloride (mEq/l)	106.1±1.8	105.6±1.0	106.7±1.8	107.5±2.1

Control: Injection grade water (15 ml/kg/day)

^a: n=9, one datum was out of the detection limit and was not calculated.

^b: n=8, two data were out of the detection limit and were not calculated.

^c: n=7, three data were out of the detection limit and were not calculated.

^d: n=6, four data were out of the detection limit and were not calculated.

*: p < 0.05



Table 8. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Gross Necropsy Findings (N/N)

Dose level (ml/kg/day)	0	1.5	5	15
Volume administered (ml/kg/day)	15	1.5	5	15
Number of animals examined per group	10	10	10	10
<u>Males</u>				
<u>Urinary bladder</u>				
Fat-like mass, serosal surface	1/10	0/10	0/10	0/10
<u>Spleen</u>				
White spots	0/10	0/10	1/10	0/10
<u>Thymus</u>				
Hemorrhage, anterior portion	0/10	0/10	1/10	0/10
<u>Females</u>				
<u>Uterine horn</u>				
Dilatation and accumulation of fluids, bilateral	1/10	1/10	0/10	0/10

N/N: Number of rats with gross changes/Number of rats examined



Table 9-1. 28-Day Subacute Oral Toxicity Study of Product Code MicSoy-20 (MS-20) in Male Rats — Organ Weights and Organ to Brain Weight Ratios (Mean ± SD, N=10)

Dose (ml/kg/day)	Brain		Liver		Kidneys	
	Organ wt.(g)	Ratio	Organ wt.(g)	Ratio	Organ wt.(g)	Ratio
0 (對照溶液)	2.01 ± 0.08	7.24 ± 0.32	14.56 ± 0.41	7.24 ± 0.32	3.28 ± 0.28	1.63 ± 0.12
1.5	2.01 ± 0.11	7.12 ± 0.80	14.28 ± 1.61	7.12 ± 0.80	3.23 ± 0.32	1.61 ± 0.17
5	2.02 ± 0.07	6.92 ± 0.57	13.96 ± 1.24	6.92 ± 0.57	3.29 ± 0.34	1.63 ± 0.14
15	2.05 ± 0.06	6.93 ± 1.02	14.15 ± 1.82	6.93 ± 1.02	3.29 ± 0.26	1.61 ± 0.15

Dose (ml/kg/day)	Spleen		Heart	
	Organ wt.(g)	Ratio	Organ wt.(g)	Ratio
0 (對照溶液)	0.93 ± 0.08	0.47 ± 0.06	1.37 ± 0.13	0.68 ± 0.08
1.5	0.98 ± 0.12	0.49 ± 0.06	1.37 ± 0.13	0.68 ± 0.06
5	0.93 ± 0.14	0.46 ± 0.06	1.32 ± 0.12	0.66 ± 0.05
15	0.89 ± 0.09	0.44 ± 0.05	1.34 ± 0.12	0.66 ± 0.07

Dose (ml/kg/day)	Adrenals		Thymus	
	Organ wt.(g)	Ratio ^a (%)	Organ wt.(g)	Ratio
0 (對照溶液)	0.062 ± 0.011	3.09 ± 0.59	0.679 ± 0.087	0.34 ± 0.05
1.5	0.067 ± 0.011	3.34 ± 0.61	0.625 ± 0.118	0.31 ± 0.06
5	0.067 ± 0.013	3.30 ± 0.66	0.602 ± 0.136	0.30 ± 0.06
15	0.061 ± 0.009	2.99 ± 0.37	0.671 ± 0.103	0.33 ± 0.05

Dose (ml/kg/day)	Testes	
	Organ wt.(g)	Ratio
0 (Control)	3.53 ± 0.19	1.76 ± 0.11
1.5	3.71 ± 0.20	1.85 ± 0.12
5	3.75 ± 0.30	1.85 ± 0.11
15	3.75 ± 0.20	1.83 ± 0.10

Control: Injection grade water (15 ml/kg/day)

Ratio: organ weight/brain weight

^a: (organ weight/brain weight) x 100



Table 9-2. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Female Rats — Organ Weights and Organ to Brain Weight Ratios (Mean \pm SD, N=10)

Dose (ml/kg/day)	Brain		Liver		Kidneys	
	Organ wt.(g)	Ratio	Organ wt.(g)	Ratio	Organ wt.(g)	Ratio
0 (Control)	1.85 \pm 0.07	4.73 \pm 0.35	8.76 \pm 0.63	4.73 \pm 0.35	1.98 \pm 0.17	1.07 \pm 0.09
1.5	1.89 \pm 0.07	4.65 \pm 0.47	8.75 \pm 0.79	4.65 \pm 0.47	2.03 \pm 0.23	1.08 \pm 0.12
5	1.87 \pm 0.05	4.75 \pm 0.39	8.85 \pm 0.67	4.75 \pm 0.39	1.93 \pm 0.19	1.03 \pm 0.10
15	1.83 \pm 0.06	4.88 \pm 1.01	8.90 \pm 1.66	4.88 \pm 1.01	1.92 \pm 0.27	1.05 \pm 0.18

Dose (ml/kg/day)	Spleen		Heart	
	Organ wt.(g)	Ratio	Organ wt.(g)	Ratio
0 (Control)	0.63 \pm 0.12	0.34 \pm 0.06	0.96 \pm 0.07	0.52 \pm 0.04
1.5	0.61 \pm 0.08	0.32 \pm 0.04	0.90 \pm 0.04	0.48 \pm 0.03
5	0.55 \pm 0.11	0.30 \pm 0.06	0.95 \pm 0.11	0.51 \pm 0.06
15	0.57 \pm 0.11	0.31 \pm 0.06	0.88 \pm 0.11	0.48 \pm 0.07

Dose (ml/kg/day)	Adrenals		Thymus	
	Organ wt.(g)	Ratio ^a (%)	Organ wt.(g)	Ratio
0 (Control)	0.075 \pm 0.007	4.07 \pm 0.43	0.522 \pm 0.082	0.28 \pm 0.04
1.5	0.075 \pm 0.013	4.00 \pm 0.67	0.504 \pm 0.075	0.27 \pm 0.05
5	0.075 \pm 0.013	3.99 \pm 0.66	0.487 \pm 0.067	0.26 \pm 0.04
15	0.070 \pm 0.018	3.84 \pm 0.97	0.470 \pm 0.110	0.26 \pm 0.06

Control: Injection grade water (15 ml/kg/day)

Ratio: organ weight/brain weight

^a: (organ weight/brain weight) x 100



Table 10. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Incidence and Severity of Histopathology Lesions (N/N)

Sex	Males		Females	
	0 ¹	15	0 ¹	15
Dose level (ml/kg/day)				
Volume administered (ml/kg/day)	15	15	15	15
Number of animals examined per group	10	10	10	10
Kidney				
Mineralization, renal tubules				
Incidence	1/10	0/10	3/10	5/10
Degree of severity ^a				
Minimal	1/10	0/10	3/10	3/10
Slight	0/10	0/10	0/10	2/10
Nephropathy, progressive, chronic				
Incidence	2/10	0/10	0/10	0/10
Degree of severity ^a				
Minimal	2/10	0/10	0/10	0/10
Prostate				
Infiltration, lymphocytic cells				
Incidence	0/10	2/10		
Degree of severity ^a				
Minimal	0/10	2/10		

N/N: Number of rats with microscopic lesions/Number of rats examined

¹: injection grade water

^a: Degrees of lesions were graded from one to five depending on severity (EPL)



Table 11. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Correlation of Gross and Microscopic Findings

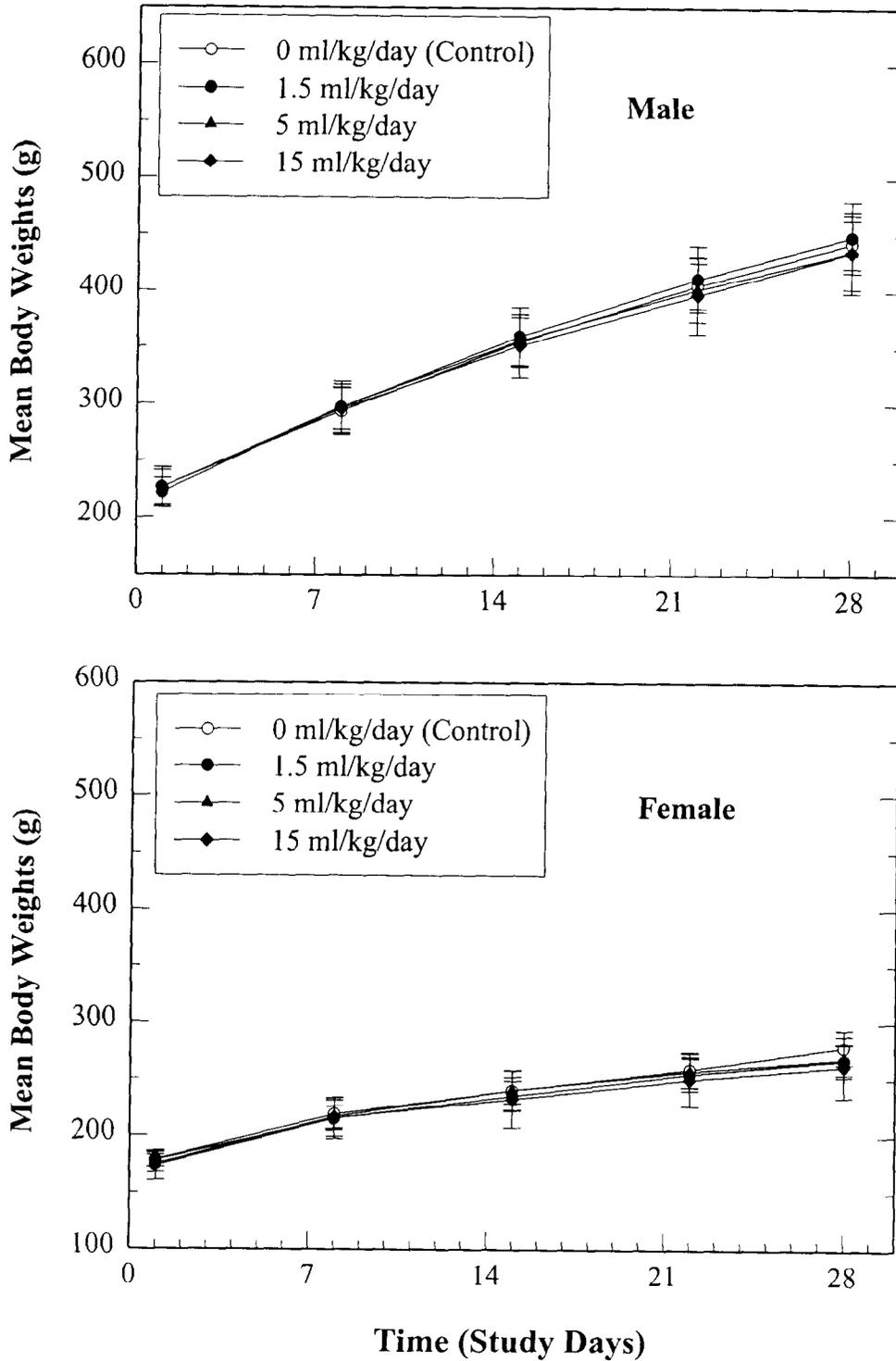
Dose level (ml/kg/day)	Animal ID ¹	Topography /Site	Gross Observation	Microscopic Findings
0 (Control)	1010261041	Urinary bladder	Fat-like mass	Adipose tissue
	1010260015	Uterine horn, bilateral	Dilatation and accumulation of fluid	Minimal dilatation, physiological change, proestrus phase
1.5	1010260025	Uterine horn, bilateral	Dilatation and accumulation of fluid	Slight dilatation, physiological change, proestrus phase
5	1010261020	Spleen	White-spot	Detachment of surface tissue, others were nothing remarkable
	1010261031	Thymus	Hemorrhagic spot	Hemorrhagic spot

Control: injection grade water (15 ml/kg/day)

¹: Animal ID (batch no. + sex no. + rat no.) contained 10 digits, the first 6 digits indicated the serial batch of the rats, the next digit indicated sex (1 is male and 0 is female), the remaining 3 digits indicated the rat number of that batch.



Fig.1. 28-Day Subacute Oral Toxicity Study of Product Code MicrSoy-20 (MS-20) in Rats — Mean Body Weight Growth Curves



Control: Injection grade water (15 ml/kg/day)

Appendix A (附件一)

Test Article Information Sheet

試驗物質資料表

Correspondence Letters from the Sponsor for Correction of Test Article Information

委託單位提供之試驗物質資料更正函

Final Report Amendment

試驗報告變更說明書

Test Article Information Sheet

試驗物質資料表



DEVELOPMENT CENTER FOR BIOTECHNOLOGY

1/1

Test Article Information Sheet

DV00199

DV-QA00033E

Sponsor : MICROBIO Co., Ltd.

Address : No.81 Gauyang N. Rd., Lung tan Shiang, Tao Yuan, Taiwan

Telephone : 886-3-4710888 Fax : 886-3-4710288

Delivery 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 Juice

c. Color : Dark-brown

5. How Supplied (Amount / Pack) : 180 ml/Bottle

6. Amount Supplied : 22 Bottle



Test Article Information Sheet

DV00199

2/2

7. Solubility (Approx. ___ g/L)
H2O Soluble, 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 : Jason Iku 10/5/2004 (Signature)
(MM / DD/YY)

Sponsor Representative : William Lu 10/5/2004 (Signature)
(MM / DD/YY)



DEVELOPMENT CENTER FOR BIOTECHNOLOGY

Test Article Information Sheet

DV00199-a

1/1

DV-QA00033E

Sponsor : MICROBIO Co., Ltd.

Address : No.81 Gauyang N. Rd., Lung tan Shiang, Tao Yuan, Taiwan

Telephone : 886-3-4710888 Fax : 886-3-4710288

Delivery Date : 10 / 25 / 2001 (MM/DD/YY)

Category : Health Food Herb Medicine Drugs Cleanser Medical Devices
Cosmetics Pesticides Others : _____

1. Sample Name : Product code MicSoy-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 Juice

c. Color : Dark-brown

5. How Supplied (Amount / Pack) : 180 ml/Bottle

6. Amount Supplied : 2 Bottle



Test Article Information Sheet

DV00199-a

7. Solubility (Approx. ___ g/L)
H2O Soluble, 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: Jason Hsu 10/25/2003 (Signature) (MM / DD / YY)

Sponsor Representative: William Lu 10/25/2003 (Signature) (MM / DD / YY)

- 
- Correspondence Letters from The Sponsor for Correction of Test Article Information**
委託單位提供之試驗物質資料更正函

中天生物科技股份有限公司 函

公司地址：桃園縣龍潭鄉高楊北路81號
承辦人：胡·智·傑
電話：(03) 47110888#1106
傳真：(03) 47110288

受文者：財團法人生物技術開發中心

速 別：一般

發文日期：中華民國九十年十二月二十六日

發文字號：90中(總)字第053號函

主旨：委託 貴中心藥品安全性試驗，修正所載人體建議劑量。

說明：一、修正貴單位計畫編碼 DVI-PR-SN00032 物質資料表中所載試驗藥品『代號：MiciSoy-20(MS-20)』人體建

議使用劑量，由成人每天1.5毫升修正至成人每天1.0毫升。

二、請勿於實驗報告中標示安全指數。

三、敬請 貴單位配合修正相關報告。

正本：財團法人生物技術開發中心
副本：

中天生物科技股份有限公司



中天生物科技股份有限公司 函

受文者：財團法人生物技術開發中心

速 別：速件

密等及解度條件：

發文日期：中華民國九十一年四月十五日

發文字號：二中(總)字第026號函

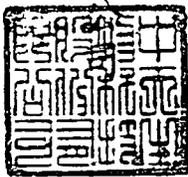
附件：

主旨：藥品安全性試驗報告請載明安全指數換算方式

說明：一、貴單位計畫編號 DV-PR-SA00032 之藥品安全性試驗報告，請載明安全指數換算方式，包括體重及體表面積等之換算方式。

二、敬請 貴單位配合修正相關報告。

中天生物科技股份有限公司



公司地址：桃園縣龍潭鄉高楊北路81號
承辦人：胡智傑
電話：(03) 4710888#106
傳真：(03) 4710288

中天生物科技股份有限公司 總機電話：(03) 4710888

Final Report Amendment
試驗報告變更說明書

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Final Report Amendment

DV-QA00032F

Final Report Serial No.: DV-TR-SA00032E

Amendment No.: 1

Project Code: DV-TA00199

Study Title: 28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS PRODUCT
CODE MICRSOY-20 (MS-20)

Date Issued: Sep. 10, 2002

Changed from:

1. Abstract Line 5th: "...millimeter per kilogram of body"
2. Abstract Line 20th: "...at dose levels up to 15 ml/kg/day did not induce...."
3. Abstract Line 22nd: "... is determined to be 15 ml/kg/day...."
4. Conclusion Line 13th: "... at dose levels up to 15 ml/kg/day did not induce...."
5. Conclusion Line 15th: "determined to be 15 ml/kg/day."

Changed to:

1. Abstract Line 5th: "...milliliter per kilogram of body"
2. Abstract Line 20th: "...at dose levels limited by dose volume can be administered (15 ml/kg/day) did not induce...."
3. Abstract Line 22nd: "... is determined to be greater than 15 ml/kg/day. ..."
4. Conclusion Line 13th: "... at dose levels limited by dose volume can be administered (15 ml/kg/day) did not induce...."
5. Conclusion Line 15th: "determined to be greater than 15 ml/kg/day."

Reasons for Change:

1. Typing error
2. To increase the clarity of the sentences to fit the requests from the sponsor.
3. To increase the clarity of the sentences to fit the requests from the sponsor.

Study Director: Jiuan Judy Liu Sept 17, 2002 (Signed and dated)

QA Officer: Ying-Chuan Yang Sept. 17, 2002 (Signed and dated)

Director: C. C. Chang Sept. 17, 2002 (Signed and dated)

Appendix B (附件二)

Protocol (DV-PR-SA00032E)

試驗計畫書

Study Schedule

試驗進度表

Protocol (DV-PR-SA00032E)

試驗計畫書



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

SERIAL NO: DV-PR-SA00032E
PROJECT CODE: DV-TA00199
PAGE 1 OF 11

28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS
PRODUCT CODE MICRSOY-20 (MS-20)

PROTOCOL

DEVELOPMENT CENTER FOR BIOTECHNOLOGY
DRUG DEVELOPMENT DIVISION

製藥工業發展計畫
PHARMACEUTICAL R&D LABORATORY PROGRAM



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

SERIAL NO: DV-PR-8A00032
PROJECT CODE: DV-TA0019
PAGE 2 OF 11

Signature Page

Study Director:

Jiuan Judy Liu Oct. 124 / 2001
Jiuan Judy Liu, PhD, DVM

Investigators:

- Yu-Mai Chen, MS Tan-Fu Yuen, MS Ming-Fen Yang, DVM, MS
- Yu-Hui Yang, MS Kuo-Tai Yang, MS Mei-Lun Wang, DVM, MS
- Di-Sheng Wang, MS Jyh-Min Tzeng, DVM, MS Jih-Shiun Chao, DVM, MS
- Chih-Peng Wang, MS Chien Ming Shih, PhD Hung-Chun Chu
- Shin-Yung Hsu, DVM, PhD (Veterinary Histopathologist)

Quality Assurance Officer:

Ying-Chuan Yang Oct. 124 / 2001
Ying-Chuan Yang, MS

Facility Manager:

Jiuan Judy Liu Oct. 124 / 2001
Dr. Jiuan Judy Liu, PhD, DVM

Sponsor's Representative:

William Lu Oct. 26 / 2001
William Lu

製藥工業發展計畫
PHARMACEUTICAL R&D LABORATORY PROGRAM

28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS
PRODUCT CODE MICRSOY-20 (MS-20)

I. PURPOSE

Product Code MicrSoy-20 (MS-20) is a Chinese medicine. The purpose of this study is to evaluate the toxicity of MS-20, and to determine the "No Observed Adverse Effect Level" (NOAEL), when administered to Sprague-Dawley rats daily for 28 days via gavage. The results of this study will be used to establish safety margin for human daily use. This study will be designed in accordance to the "Guidance for Industry Botanical Drug Products" [Center for Drug Evaluation and Research (CDER), August 2000]. The study processes will be inspected for compliance with Good Laboratory Practices (GLP). All information generated from this study will be used as a part of an Investigational New Drug (IND) application.

II. TESTING FACILITY

- A. Name: General/Reproductive Toxicology Laboratory, Drug Development Division, Development Center for Biotechnology
- B. Address: 103, Lane 169, Kang-Ning St., Hsi-Chih City, Taipei County, Taiwan, R.O.C.

III. SPONSOR

- A. Name: MICROBIO Co., Ltd.
- B. Address: No. 81, Gauyang N. Rd., Lung Tan Shiang, Tao Yuan, Taiwan, R.O.C.
- C. Representative: William Lu

IV. TEST ARTICLE AND VEHICLE CONTROL

- A. Name: Product Code MicrSoy-20 (MS-20)
- B. Received Date: Oct. 5, 2001 & Oct. 25, 2001
- C. Batch/Lot Number: 20010209
- D. DCB Code: DV00199 & DV00199-a
- E. Ingredients: MS-20 is a Chinese medicine. The components are very complicated. Until now, its effective components are still unable to determine.

- F. Other Characteristics: Dark brown liquid with prune juice odor
- G. Solubility: Water-soluble
- H. Amount Supplied: A total of 24 bottles (180 ml/bottle)
- I. Storage Conditions: Room temperature and protect from light
- J. Expiration Date: Feb. 9, 2004
- K. Vehicle Control: Injection grade water

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 (21CFR §58.105, FDA).

V. STUDY SCHEDULE

- A. Proposed Study Initiation Date for First Dosing:
 - Male — Oct. 29, 2001
 - Female — Oct. 30, 2001
- B. Proposed Study Completion Date for Necropsy:
 - Male — Nov. 26, 2001
 - Female — Nov. 27, 2001

VI. TEST SYSTEM

- A. Species: Rats
- B. Strain: CrI:CD(SD)fSPF
- C. Source: National Laboratory Animal Breeding & Research Center, National Science Council, Taipei, Taiwan
- D. Age at Initiation of Study: Approximately 6 weeks old
- E. Method of Identification: Rats will be identified by earmotch (SOP: DCB-DV-TE00446) and cage tags.
- F. Number on Study: Ten males and 10 females per control and dose group; 40 males and 40 females in total.
- G. Justification for Selection: The Sprague-Dawley (SD) rat is chosen as rodent species since it is widely accepted by Health Authorities as an appropriate experimental model, with documented susceptibility to a wide range of toxic substances.
- H. IACUC Approved Protocol Number: 2001-TP-011-b

VII. EXPERIMENTAL DESIGN AND METHODS

A. Test animals

1. Quarantine and acclimatization

Animals will be housed in an AAALAC accredited animal facility with a controlled environment of 21 ± 2 °C and $50 \pm 20\%$ relative humidity, in a 12-hr/12-hr light/dark cycle with light on at 6:00 AM and off at 6:00 PM (SOP: DCB-DV-AC00023). Rats will be quarantined (SOP: DCB-DV-AC00032) and acclimated for at least 7 days prior to use. The unsuitable animals will be excluded from this study. The rats will be housed two per cage in stainless steel wire mesh cages.

2. Diet and water supply

Laboratory Autoclavable Rodent Diet 5010 (PMI® Feeds Inc., St. Louis, MO) and tap water will be supplied *ad lib* throughout the study (SOPs: DCB-DV-AC00025, DCB-DV-AC00026).

3. Randomization (SOP: DCB-DV-TE00462)

Computerized LIMS system (Computer Service Center, DCB) will be used for randomization and dose groups assignment. The rats will be divided into weight classes, then randomly assigned into four groups (10 males and 10 females per group). The weight variation of the animals to be used should not exceed $\pm 20\%$ of the mean body weight.

B. Basic design

1. Study design

Group no., dose levels, dosing solutions, dosing volumes and animal no. in this subacute oral toxicity study are presented as below:

Group No.	Dose Levels (ml/kg/day)	Dosing Solutions	Dosing volumes (ml/kg/day)	Animal No. M/F
1	0	injection grade water	15	10 M, 10 F
2	1.5	MS-20	1.5	10 M, 10 F
3	5	MS-20	5	10 M, 10 F
4	15	MS-20	15	10 M, 10 F

2. Rationales for dose level selection:

- a. The recommended daily human dose is 1-5 ml; for a 60-kg subject, therefore, the maximal recommended daily dose estimated is 0.083 ml/kg approximately.
- b. The acute oral toxicity study in SD rats (DV-PR-AC00139E) revealed no observable adverse effects at the dose level up to 20 ml/kg.
- c. The rats will be dosed with MS-20 solution at the dose levels of 1.5, 5 and 15 ml/kg/day in this study, which will provide 18, 60 and 180 folds of safety margin over the anticipated human recommended dose. A corresponding safety margin of 2.9, 9.6 and 28.8, respectively, will also be provided basing on a surface area conversion.

3. Test article preparation (SOP: DCB-DV-TE00463)

During the 28-day study period, the calculated total volume of test article solution for each day will be distributed into sterilized reagent bottles at a weekly interval, and the solutions will be kept in room temperature before use.

4. Test article administration (SOP: DCB-DV-TE00447)

The test article at dose levels of 1.5, 5 and 15 ml/kg/day and the injection grade water (15 ml/kg/day) will be administered to rats (10 males and 10 females per group) once every morning by gavage for 28 days. The dosing volume will be calculated on the basis of the most recently recorded individual body weight, and 1~10 ml plastic disposable syringe (needle size: 16 or 18 gauge, 80 mm in length) will be used. The first dosing day is denoted as SD1 (Study Day 1).

Animals can be replaced if death occurred after first dosing and is confirmed by gross necropsy as gavage accident. No replacement will be made if the death is observed after second dosing.

5. Reason for selection of route

The oral route is the route to be used in human.

VIII. OBSERVATION AND EXAMINATION

A. Observations of animals (SOP: DCB-DV-TE00469)

The rats will be observed for mortality and clinical signs after dosing. All animals will be observed twice daily (before 10:00 AM and after 3:00 PM, at least

six hours apart) on working days and once daily on weekends for 28 days. Any mortality, moribundity and clinical signs will be recorded and documented.

B. Body weight (SOP: DCB-DV-TE00450)

Body weights will be recorded on all animals before first dosing at study day 1 (SD1), weekly thereafter for the duration of the study period, prior to the termination of study (SD28) and on the day of necropsies (SD29).

C. Food consumption (SOP: DCB-DV-EQ00074)

The food consumption for each cage (2 rats per cage) will be measured weekly throughout the 28-day study period.

D. Ophthalmologic examination

Gross examination of the eyes will be performed on all animals at the days before first dosing (SD0) and before the end of study (SD28).

E. Clinical pathology

1. Urinalysis (SOP: DCB-DV-TE00451)

The day before termination (SD28), rats from each group will be placed individually in polycarbonate metabolism cages with free access to water only, to collect urine specimens over 16-hour. The volume, color and appearance of the urine specimen will be examined and the following parameters will be analyzed using Bayer Clinitek 100 Urine Chemistry Analyzer (SOP: DCB-DV-EQ00066). Urinary sediments will be examined under light microscopy.

pH	Glucose
Specific gravity ¹	Ketones
Nitrites	Urobilinogen
Occult blood	Bilirubin
Protein	Leukocytes

¹ Specific gravity will be examined by Clinical Refractometer (SOP: DCB-DV-EQ00070).

2. Hematology

The day before termination (SD28), all rats will be fasted over 16 hours. At the day of necropsy (SD29), The surviving rats will be anesthetized by i.p. injection of pentobarbital sodium solution (SOP: DCB-DV-TE00424). Blood

samples will be obtained through femoral vein (SOP: DCB-DV-TE00438) and collected in tubes containing EDTA. The following items will be determined using Baker System 9118⁺AX Automated Hematology Analyzer (SOP: DCB-DV-EQ00076).

Erythrocytes (Red blood cells) count
Hemoglobin
Hematocrit
Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration (calculated)
Leukocytes (White blood cells) count
Leukocyte differential ¹ count (Lymphocytes, Monocytes, Granulocytes)
Platelet count
Prothrombin time ²
Activated partial thromboplastine time ²

¹: Leukocyte differential count — Microscopic examination of blood smear (SOP: DCB-DV-TE00444) stained with the Hemacolor stain (SOP: DCB-DV-TE00445) will be performed with at least one hundred leukocytes (SOP: DCB-DV-TE00453).

²: The blood samples will be mixed with CTAD anticoagulant (0.109 M Sodium Citrate, 15 mM Theophylline, 3.7 mM Adenosine, 0.198 mM Dipyridamole) (9:1,v/v), then centrifuged for plasma collection. The items will be performed by CA-1000 Sysmex Coagulation Analyzer (SOP: DCB-DV-EQ00071).

3. Clinical chemistry (SOP: DCB-DV-TE00442)

Aliquots of the blood samples will be collected in tubes without anticoagulant for clinical chemistry determinations. The collected blood specimens will be centrifuged and the serum will be used for assays. The clinical chemistry determinations will be performed on Hitachi 7060 Automated Analyzer (SOP: DCB-DV-EQ00075).

Alanine aminotransferase	Lactate dehydrogenase
Aspartate aminotransferase	Creatine phosphate kinase
γ-glutamyltransferase	Glucose
Direct bilirubin	Blood urea nitrogen
Total bilirubin	Uric acid
Alkaline phosphatase	Creatinine
Total protein	Calcium



Albumin	Phosphorus
Albumin/Globulin ratio (calculated)	Sodium
Triglycerides	Potassium
Cholesterol	Chloride

F. Gross necropsy

Gross necropsies will be performed on found dead, moribund sacrificed animals and all surviving animals at the end of study (SD29). The surviving rats will be anesthetized prior to blood sampling, exsanguinated and necropsied in a randomized order. Gross lesions observed at necropsy will be recorded and sampled. The gross lesions and all tissues will be fixed in 10 % neutral buffered formalin (SOP: DCB-DV-TE00407) for further microscopic evaluations.

G. Organ weight (SOP: DCB-DV-EQ00074)

Organs will be taken from all rats surviving to the end of study (SD29) including liver, thymus, kidneys, heart, spleen, adrenals, testes (male only) and brain. Organs will be weighed to the nearest 10 mg, except for adrenals and thymus which will be weighed to the nearest 1.0 mg. Organ: brain weight ratios will also be calculated.

H. Histopathology

Representative samples of the following tissues will be collected and fixed in 10 % neutral buffered formalin or 3% glutadialdehyde at necropsy (SD29). The tissues (as listed below) in control, high dose (15 ml/kg/day) groups, found dead/moribund sacrificed rats at the study period, and gross lesions will be trimmed, embedded, sectioned and H&E-stained (SOP: DCB-DV-TE00470), followed by microscopic examination. A complete histopathologic evaluation will be performed on control and high dose groups of rats, plus all rats inclusive of early deaths and those sacrificed as moribund. Treatment-related lesions (target organs), if any, will be identified, and these organs, plus gross lesions, will be further examined in lower dose groups until a no-observed treatment effect level is determined. The rest tissues will be fixed and preserved in 10% neutral buffered formalin.

Adrenals	Ovaries and oviduct* (female only)
Aorta	Pituitary
Bone & bone marrow (femur)	Pancreas
Brain (fore, mid, and hind)	Prostate (male only)



Cecum	Salivary glands
Colon	Sciatic nerve
Corpus and cervix uteri	Seminal vessels (male only)
Duodenum	Skeletal muscle
Epididymis* (male only)	Skin
Esophagus	Spinal cord (cervical, thoracic & lumbar)
Eyes*	Spleen
Harderian glands*	Stomach
Heart	Testes* (male only)
Ileum	Thymus (or thymic region)
Jejunum	Thyroid/parathyroid
Kidneys	Trachea
Liver	Urinary bladder
Lung (with main-stem bronchi)	Vagina (female only)
Lymph nodes (mandibular & mesenteric)	All gross lesions
<u>Mammary glands (female only)</u>	<u>Ears (for identification)</u>

*: indicated that organs will be fixed in 3% glutaraldehyde.

IX. STATISTICAL ANALYSIS

Results will be expressed as mean \pm standard deviation. Comparisons of all data collected at each interval on body weights, food consumption, clinical pathology data and organ weights (absolute and relative to brain weights) will be performed using one-way analysis of variance (ANOVA) method, followed by Dunnett's method (SigmaStat™, V2.03, 1997). The 0.05 level of probability is used as the criterion of significance.

X. RECORDS RETENTION

All raw data, documentation, records, protocols and final reports generated as a result of this study will be inventoried and stored by the Quality Assurance Unit at DCB's archives. All applicable specimens will be stored and archived by General/Reproductive Toxicology Laboratory at DCB. The retaining duration of those records and specimens will be in accordance with 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., 2000; (3) General Requirements for the Competence of Calibration and Testing Laboratories (ISO/IEC Guide 25), ISO/IEC, third edition, 1990; (4) Specific Criteria for Biological Testing, Chinese National Laboratories Accreditation, R.O.C., second edition, 2000.

XII. REFERENCES

1. Derelanko MJ, Hollinger MA. 1995. CRC Handbook of Toxicology. CRC press, USA.
2. OECD Guideline for the Testing of Chemicals # 407. 1981. Repeated Dose Oral Toxicity - Rodent: 28-day or 14-day Study.
3. Guidance for Industry Botanical Drug Products, Center for Drug Evaluation and Research (CDER), August 2000.
4. Guideline for the Nonclinical Pharmacology/Toxicology Studies for Medicinal Products Application, DOH, ROC, 3rd ed., 2000.

Study Schedule
試驗進度表

試驗進度表
Study Schedule

DV-QA00045A

Laboratory: General/Reproductive Toxicology Test Article No.: DV00199

Study No.: SA00032 (E) Project Code: DV-TA00199

Study Director: Juan Judy Liu Oct 24 2001 QA Officer: Ying-Chuan Yang Oct 24 2001

DATE	WK	PROGRESS		QAU INSP.	REMARK
		Male	Female		
Oct. 26, 2001	Fri	Pre-study clinical observation Body weight recording Animal randomization and grouping	Pre-study clinical observation Body weight recording Animal randomization and grouping		
Oct. 27, 2001	Sat	Pre-study clinical observation	Pre-study clinical observation		
Oct. 28, 2001	Sun	Pre-study clinical observation Ophthalmologic examination	Pre-study clinical observation		
Oct. 29, 2001	Mon	Day 1 (SD1) Body weight recording Test article administration via gavage Clinical observation	Pre-study clinical observation Ophthalmologic examination	<u>Ying-Chuan Yang</u> Oct. 29, 2001	
Oct. 30, 2001	Tue	Test article administration via gavage Clinical observation	Day 1 (SD1) Body weight recording Test article administration via gavage Clinical observation		
Oct. 31, 2001	Wed	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 1, 2001	Thu	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 2, 2001	Fri	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 3, 2001	Sat	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 4, 2001	Sun	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 5, 2001	Mon	Day 8 (SD8) Body weight recording Weekly diet measurement Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation	<u>Ying-Chuan Yang</u> Nov. 5, 2001	

DATE	WK	PROGRESS		QAU INSP.	REMARK
		Male	Female		
Nov. 6, 2001	Tue	Test article administration via gavage Clinical observation	Day 8 (SD8) Body weight recording Weekly diet measurement Test article administration via gavage Clinical observation		
Nov. 7, 2001	Wed	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 8, 2001	Thu	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 9, 2001	Fri	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 10, 2001	Sat	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 11, 2001	Sun	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 12, 2001	Mon	Day 15 (SD15) Body weight recording Weekly diet measurement Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 13, 2001	Tue	Test article administration via gavage Clinical observation	Day 15 (SD15) Body weight recording Weekly diet measurement Test article administration via gavage Clinical observation	<i>Ying-Chuan Yang</i> Nov. 13, 2001	
Nov. 14, 2001	Wed	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 15, 2001	Thu	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 16, 2001	Fri	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 17, 2001	Sat	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 18, 2001	Sun	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		

DATE	WK	PROGRESS		QAU INSP.	REMARK
		Male	Female		
Nov. 19, 2001	Mon	Day 22 (SD22) Body weight recording Weekly diet measurement Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 20, 2001	Tue	Test article administration via gavage Clinical observation	Day 22 (SD22) Body weight recording Weekly diet measurement Test article administration via gavage Clinical observation		
Nov. 21, 2001	Wed	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 22, 2001	Thu	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 23, 2001	Fri	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 24, 2001	Sat	Test article administration via gavage Clinical observation	Test article administration via gavage Clinical observation		
Nov. 25, 2001	Sun	Day 28 (SD28) Test article administration via gavage Clinical observation Body weight recording Weekly diet measurement Ophthalmologic examination Place individual rat into the metabolic cage with only water access by 5:00 pm	Test article administration via gavage Clinical observation		
Nov. 26, 2001	Mon	Study termination (SD29) Urine collection Body weight recording Necropsy/ Gross examination Blood collection Organ collection and weighing	Day 28 (SD28) Test article administration via gavage Clinical observation Body weight recording Weekly diet measurement Ophthalmologic examination Place individual rat into the metabolic cage with only water access by 5:00 pm	<i>Yip Chuan Hung</i> Nov. 26, 2001	
Nov. 27, 2001	Tue		Study termination (SD29) Urine collection Body weight recording Necropsy/ Gross examination Blood collection Organ collection and weighing	<i>Yip Chuan Hung</i> Nov. 27, 2001	

Appendix C (附件三)

- Records of Animal Room Temperature during Study Period
動物飼育室溫度紀錄統計表
- Records of Animal Room Relative Humidity during Study Period
動物飼育室相對溼度紀錄統計表
- Record of Animal Diet
動物飼料紀錄
- Analysis Records of Animal Drinking Water
水質分析報表
- Analysis Records of Animal Diet
動物飼料分析報表
- Laboratory Animal Quarantine Report
實驗動物檢疫報告

- Records of Animal Room Temperature during Study Period
動物飼育室溫度紀錄統計表

動物飼育室溫度紀錄統計表
Records of Animal Room Temperature during Study Period

飼育室編號
Animal Room No.: C-415

試驗期間
Study Period: 10/29/2001~11/27/2001

日期 Date	溫度 (Temperature ; °C)		
	平均值 ± 標準差 Mean ± SD	當日最高 Maximum	當日最低 Minimum
10/29/2001	21.0 ± 0.1	21.2	20.8
10/30/2001	21.0 ± 0.2	21.2	20.8
10/31/2001	21.0 ± 0.2	21.2	20.8
11/1/2001	21.0 ± 0.1	21.2	20.8
11/2/2001	21.0 ± 0.1	21.2	20.7
11/3/2001	21.0 ± 0.1	21.2	20.8
11/4/2001	21.0 ± 0.1	21.2	20.8
11/5/2001	21.0 ± 0.2	21.4	20.8
11/6/2001	21.0 ± 0.1	21.2	20.7
11/7/2001	21.0 ± 0.2	21.2	20.8
11/8/2001	21.0 ± 0.1	21.3	20.8
11/9/2001	21.0 ± 0.1	21.2	20.7
11/10/2001	22.7 ± 1.8	25.2*	20.8
11/11/2001	21.0 ± 0.1	21.2	20.8
11/12/2001	21.0 ± 0.1	21.2	20.8
11/13/2001	21.0 ± 0.1	21.2	20.8
11/14/2001	21.0 ± 0.1	21.2	20.7
11/15/2001	21.0 ± 0.1	21.2	20.8
11/16/2001	21.0 ± 0.1	21.2	20.8
11/17/2001	21.0 ± 0.1	21.2	20.8
11/18/2001	21.0 ± 0.1	21.2	20.8
11/19/2001	21.0 ± 0.1	21.2	20.8
11/20/2001	20.9 ± 0.1	21.3	20.8
11/21/2001	21.0 ± 0.2	21.2	20.8
11/22/2001	21.0 ± 0.2	21.4	20.8
11/23/2001	21.0 ± 0.1	21.2	20.8
11/24/2001	21.0 ± 0.1	21.2	20.8
11/25/2001	21.0 ± 0.1	21.2	20.7
11/26/2001	21.0 ± 0.2	21.2	20.8
11/27/2001	21.0 ± 0.2	21.2	20.7

* : The temperature of the animal room on Nov. 10, 2001 was out of the normal range, due to shut-down of the A/C system temporarily for cleaning the cooling tower. This higher temperature only lasted for few hours and did not affect the normal growth of the animals.

* : 關閉空調系統以清洗冷卻水塔，導致11/10/2001動物房之溫度偏高，但並不影響動物成長。

Records of Animal Room Relative Humidity during Study Period
動物飼育室相對溼度紀錄統計表

動物飼育室相對溼度紀錄統計表
Records of Animal Room Relative Humidity during Study Period

飼育室編號
Animal Room No.: C-415

試驗期間
Study Period: 10/29/2001~11/27/2001

日期 Date	相對溼度 (Relative Humidity ; %)		
	平均值 ± 標準差 Mean ± SD	當日最高 Maximum	當日最低 Minimum
10/29/2001	54.3 ± 2.3	58.3	50.5
10/30/2001	54.6 ± 2.9	59.5	50.4
10/31/2001	54.9 ± 3.7	61.8	49.5
11/1/2001	55.0 ± 2.9	60.8	50.4
11/2/2001	54.8 ± 3.5	61.2	48.1
11/3/2001	55.2 ± 3.4	60.7	50.1
11/4/2001	55.0 ± 3.1	61.5	50.5
11/5/2001	56.0 ± 2.8	60.5	50.4
11/6/2001	53.4 ± 3.6	59.6	47.1
11/7/2001	54.1 ± 3.2	59.6	45.1
11/8/2001	53.5 ± 2.4	59.3	50.4
11/9/2001	54.5 ± 3.0	59.3	50.5
11/10/2001	58.5 ± 6.3	79.1*	49.3
11/11/2001	53.6 ± 2.4	58.9	49.6
11/12/2001	53.1 ± 3.7	58.1	47.8
11/13/2001	52.8 ± 2.8	58.7	48.3
11/14/2001	49.3 ± 4.3	55.1	37.2
11/15/2001	52.5 ± 3.1	58.1	48.2
11/16/2001	52.3 ± 3.4	59.1	44.9
11/17/2001	48.9 ± 4.2	57.8	43.2
11/18/2001	47.3 ± 3.6	54.9	42.4
11/19/2001	50.6 ± 3.2	56.2	45.1
11/20/2001	53.9 ± 3.7	60.8	46.6
11/21/2001	51.8 ± 3.8	58.7	46.3
11/22/2001	54.7 ± 4.2	61.3	48.7
11/23/2001	55.6 ± 3.1	60.9	50.6
11/24/2001	54.7 ± 3.1	60.0	51.1
11/25/2001	56.0 ± 3.7	63.9	50.3
11/26/2001	54.7 ± 3.3	59.2	49.8
11/27/2001	52.5 ± 3.6	59.2	45.1

* : The relative humidity of the animal room on Nov. 10, 2001 was out of the normal range, due to shut-down of the A/C system temporarily for cleaning the cooling tower. This higher humidity only lasted for few hours and did not affect the normal growth of the animals.

* : 關閉空調系統以清洗冷卻水塔，導致11/10/2001動物房之相對溼度偏高，但並不影響動物成長。

Record of Animal Diet
動物飼料紀錄

1.]
2.]
3.]

Record of Animal Diet

動物飼料紀錄

1. Experiment Date : From Oct. 29, 2001 to Nov. 27, 2001.
2. Room Number : Room 415.
3. Animal Feed Production Company : PMI. Feeds , Inc.
 - a. Product Name : Laboratory Autoclavble Rodent Diet 5010
 - b. Production Date : Aug. 9, 2001 and Aug. 31, 2001
 - c. Bag Serials Number :
Aug. 9, 2001: 0391 0393 0397 0399 0445.

Aug. 31, 2001: 0651 0658 0661 0665 0672 0674.

Prepared by: W.B.Liu Apr. 09, 2002 *W.B. Liu Apr. 9, 2002*

Analysis Records of Animal Drinking Water
水質分析報表

各淨水場清水平均水質

資料來源：臺北自來水事業處水質中心
 ※註：ND表示低於偵測極限

時間：90年1至11月

偵測極限：氨氮：0.03mg/L；亞硝酸氮：0.0009mg/L；氟鹽：0.01mg/L；鐵：0.02mg/L；錳0.008mg/L；鉛：0.00088mg/L；三鹵甲烷包括(三氯甲烷=0.00012mg/L；一溴二氯甲烷=0.00007mg/L；二溴一氯甲烷=0.00008mg/L；三溴甲烷=0.00005mg/L)

檢驗項目	水質標準	單位\場別	直潭淨水場	長興淨水場	公館淨水場	雙溪淨水場			陽明淨水場			
						雙溪水源	士林水源	三角埔	頂北投取水口	大屯水源	中山樓水源	秀山路水源
水溫	---	°c	22.0	21.4	21.6	21.7	19.7	23.7	21.6	19.1	20.6	20.9
濁度	<2	NTU	0.19	0.20	0.24	0.13	0.61	0.17	0.34	0.30	0.22	0.21
色度	<5	UNIT	1	1	1	1	1	1	1	---	1	1
總鹼度	---	mg/L	20	21	19	25	64	57	31	---	32	65
pH值	6.0-8.5	---	7.0	7.0	7.0	7.1	6.7	6.9	7.5	7.5	7.4	7.2
氯鹽	<250	mg/L	6.15	6.31	6.23	10.65	14.32	31.07	15.08	---	20.34	13.98
硫酸鹽	<250	mg/L	8.40	9.24	8.87	16.48	17.05	51.75	29.54	---	30.74	18.32
氨氮	<0.1	mg/L	ND	ND	ND	ND						
硝酸亞氮	<0.1	mg/L	ND	---	ND	ND						
硝酸氮	<10	mg/L	0.58	0.60	0.58	0.72	3.43	1.14	0.90	---	0.57	3.96
溶解固體量	<600	mg/L	52	58	59	89	159	243	164	144	146	159
氟鹽	<0.8	mg/L	0.07	0.07	0.07	0.07	0.07	0.11	0.08	---	0.07	0.07
游離氯	0.2-1.0	mg/L	0.59	0.56	0.56	0.57	0.50	0.40	0.56	0.62	0.54	0.5
總硬度	<400	mg/L	27.6	29.4	29.5	43.7	81.8	125.1	96.3	---	71.1	82.7
鈣	---	mg/L	6.7	7.4	7.9	10.8	20.4	34.4	26.5	---	20.4	21.2
鎂	---	mg/L	2.7	2.8	2.4	5.0	7.7	9.8	7.6	---	4.9	7.4
鐵	<0.3	mg/L	0.08	ND	ND	ND	ND	ND	ND	---	0.02	ND
錳	<0.05	mg/L	ND	---	ND	ND						
總菌落數	<100	CFU/mL	<1	7	<1	<1	<1	<1	7	<1	21	9
大腸桿菌群	<6	CFU/100mL	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
總有機碳	---	mg/L	0.37	0.35	0.38	0.55	0.33	0.26	0.51	0.32	0.38	0.33
三鹵甲烷	<0.1	mg/L	0.0041	0.0055	0.0052	0.0137	ND	0.0002	0.0048	---	0.0021	0.0001
鉛	<0.05	mg/L	ND	---	ND	ND						
鋁	---	mg/L	0.342	0.07	0.049	0.063	0.085	0.021	0.132	---	0.033	0.022
砷	<0.01	mg/L	ND	ND	ND	ND	ND	ND	0.0032	---	ND	ND
汞	<0.002	mg/L	ND	0.0002	ND	ND	ND	ND	ND	---	ND	ND
鎘	<0.005	mg/L	ND	---	ND	ND						
鉻	<0.05	mg/L	0.0011	0.0005	ND	0.0029	0.0005	0.0009	0.0011	---	ND	0.0035
銀	<0.05	mg/L	ND	---	ND	ND						
銅	<1	mg/L	0.0015	0.0079	0.0033	0.001	0.0013	0.0031	0.001	---	ND	0.0005
鋅	<5	mg/L	0.0049	0.0156	0.0058	0.0141	0.0035	0.0017	0.0025	---	ND	ND
水質合格否(Y/N)	---	---	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

說明：本中心之動物飲水係由長興淨水場供應，此飲用水經動物房檢測並未發現大腸桿菌及生菌數，符合動物飲用水標準。

Analysis Records of Animal Diet
動物飼料分析報表

HMCP
25.11.98

Lab Diet

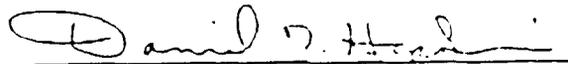
The Guarantee of Consistency

PMI Feeds, Inc. guarantees that Lab Diets are the same diets and formulation that you may currently be using in your study. In fact, in order for you to maintain total dietary control of your experiment for now and in the future, Lab Diets, the Richmond standard, are the only diets you should use.

We, the undersign, guarantee that Lab Diets conform in every manner to your current Laboratory feed.



Damon C. Shelton, Ph.D.
Lab Animal Diet Consultant



Daniel T. Hopkins, Ph.D.
Director, Technical Services



William C. Sadler, Ph.D.
General Manager,
Specialty Businesses



Laboratory Autoclavable Rodent Diet 5010

5010

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 6 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.



Laboratory Autoclavable Rodent Diet

5010

Nutrients²

Protein %.....	23.5
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
Selfine %.....	1.23
Aspartic Acid %.....	2.68
Glutamic Acid %.....	5.02
Alanine %.....	1.49
Proline %.....	1.73
Taurine %.....	0.03
Fat (ether extract) %.....	5.1
Fat (acid hydrolysis) %.....	6.2
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
Fiber (Crude) %.....	3.9
Neutral Detergent Fiber ³ %.....	12.7
Acid Detergent Fiber ⁴ %.....	4.5
Nitrogen-Free Extract (by difference) % ...	50.3
Starch %.....	36.2
Glucose %.....	0.26
Fructose %.....	0.30
Sucrose %.....	1.02
Lactose %.....	0
Total Digestible Nutrients %.....	76.0
Gross Energy, kcal/gm.....	4.06
Physiological Fuel Value ⁵, kcal/gm.....	3.41
Metabolizable Energy, kcal/gm.....	3.17

Minerals

Ash %.....	7.2
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

Vitamins

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 ₁₂ , mcg/kg.....	33.0
Vitamin A, IU/gm.....	44.1
Vitamin D ₃ (added), IU/gm.....	4.4
Vitamin E, IU/kg.....	66.1
Ascorbic Acid, mg/gm.....	—

* Product Code

¹ Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly.

² Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purpose of calculations.

³ NDF = approximately cellulose, hemicellulose and lignin.

⁴ ADF = approximately cellulose and lignin.

⁵ 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.

Laboratory Animal Quarantine Report
實驗動物檢疫報告

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. Juan Judy Lin

IACUC Protocol No.: 2001-JP-011-b Project Code: DV-TA001PP Study No.: SA00032

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. 19, 2001	Rat / SD	50/♂	Oct. 19, 2001 ~ Oct. 23, 2001	Oct. 23, 2001
"	"	50/♀	"	"

備註 (Remarks): 加子 10/026

*: 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.

Judy Lin

Attending Veterinarian

Oct. 23, 2001

Date

Appendix D (附件四)

- Individual Animal Data on Body Weights and Body Weight Gains
個別試驗動物體重及體重變化數據
- Individual Animal Data on Food Consumption
個別試驗動物飼料消耗量數據
- Individual Animal Data on Organ Weights and Organ to Brain Weight Ratios
個別試驗動物臟器重量、臟器與腦重量比數據
- Individual Animal Data on Urinalysis
個別試驗動物尿液學數據
- Individual Animal Data on Urinalysis — Urine Sediment
個別試驗動物尿液學數據 — 尿渣檢查
- Individual Animal Data on Hematology
個別試驗動物血液學數據
- Individual Animal Data on Hematology — Coagulation Analysis
個別試驗動物血液學數據 — 血液凝固分析
- Individual Animal Data on Hematology — White Blood Cells Differential Count
個別試驗動物血液學數據 — 白血球分類計數
- Individual Animal Data on Serum Chemistry
個別試驗動物血清生化學數據
- Quality Control Values of Clinical Parameters
臨床病理品管數據
- Individual Animal Data on Histopathology Evaluation
個別試驗動物組織病理鏡檢紀錄

28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS - PRODUCT CODE MICRSOY-20(MS-20)

大鼠28天口服重覆劑量(亞急性)毒性測試 - 代號 MICRSOY-20 (MS-20)

Individual Animal Data of Organ Weights and Organ/Brain Weight Ratios

個別試驗動物臟器重量、臟器與腦重量比數據

Project Code: DV-TA00199

Study No.: SA00032

Day on Test: SD 29

Sex: Male

Group	Animal I.D.	Dose (ml/kg/day)	Brain		Liver		Spleen		Kidneys		Adrenal Gls.		Thymus		Heart		Testis	
			weight (g)	ratio ^a	weight (g)	ratio ^b (%)	weight (g)	ratio ^a	weight (g)	ratio ^a	weight (g)	ratio ^a						
I	1010261005	0	2.11	14.59	6.91	0.87	0.41	3.47	1.64	0.066	3.13	0.591	0.28	1.26	0.60	3.41	1.62	
	1010261023	0	1.99	13.96	7.02	1.01	0.51	3.32	1.67	0.070	3.52	0.675	0.34	1.37	0.69	3.70	1.86	
	1010261017	0	2.05	14.12	6.89	0.93	0.45	3.19	1.56	0.061	2.98	0.558	0.27	1.63	0.80	3.44	1.68	
	1010261029	0	1.92	14.42	7.51	1.06	0.55	3.06	1.59	0.080	4.17	0.819	0.43	1.39	0.72	3.43	1.79	
	1010261022	0	2.00	15.17	7.59	0.92	0.46	3.70	1.85	0.051	2.55	0.699	0.35	1.49	0.75	3.72	1.86	
	1010261034	0	2.06	14.70	7.14	0.86	0.42	3.03	1.47	0.045	2.18	0.672	0.33	1.31	0.64	3.47	1.68	
	1010261027	0	2.06	15.25	7.40	0.90	0.44	3.57	1.73	0.073	3.54	0.598	0.29	1.32	0.64	3.50	1.70	
	1010261035	0	1.85	14.49	7.83	1.05	0.57	3.02	1.63	0.060	3.24	0.749	0.40	1.42	0.77	3.50	1.89	
	1010261015	0	2.08	14.53	6.99	0.85	0.41	3.56	1.71	0.066	3.17	0.636	0.31	1.31	0.63	3.88	1.87	
	1010261041	0	2.02	14.32	7.09	0.87	0.43	2.90	1.44	0.049	2.43	0.793	0.39	1.15	0.57	3.24	1.60	
	N		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
	Mean		2.01	14.56	7.24	0.93	0.47	3.28	1.63	0.062	3.09	0.679	0.34	1.37	0.68	3.53	1.76	
	SD		0.08	0.41	0.32	0.08	0.06	0.28	0.12	0.011	0.59	0.087	0.05	0.13	0.08	0.19	0.11	
	SEM		0.02	0.13	0.10	0.03	0.02	0.09	0.04	0.004	0.19	0.028	0.02	0.04	0.02	0.06	0.04	

^a:Ratio=Organ weight/Brain weight

^b:Ratio=(Organ weight/Brain weight) x 100

Prepared by: Chik Peng Wang Mar, 26, 2002

Checked by: Jui-Min Chen Mar 26, 2002

28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS - PRODUCT CODE MICRSOY-20(MS-20)

大鼠28天口服重覆劑量(亞急性)毒性測試 - 代號 MICRSOY-20 (MS-20)

Individual Animal Data of Organ Weights and Organ/Brain Weight Ratios

個別試驗動物臟器重量、臟器與腦重量比數據

Project Code: DV-TA00199

Study No.: SA00032

Day on Test: SD 29

Sex: Male

Group	Animal I.D.	Dose (ml/kg/day)	Brain		Liver		Spleen		Kidneys		Adrenal Gls.		Thymus		Heart		Testis	
			weight (g)	ratio ^a	weight (g)	ratio ^b (%)	weight (g)	ratio ^a	weight (g)	ratio ^a	weight (g)	ratio ^a						
2	1010261024	1.5	2.10	13.71	6.53	1.06	0.50	3.20	1.52	0.069	3.29	0.787	0.37	1.42	0.68	3.76	1.79	
	1010261044	1.5	1.90	12.72	6.69	0.99	0.52	2.83	1.49	0.052	2.74	0.635	0.33	1.13	0.59	3.33	1.75	
	1010261003	1.5	2.09	14.35	6.87	0.88	0.42	3.22	1.54	0.061	2.92	0.479	0.23	1.33	0.64	3.76	1.80	
	1010261028	1.5	1.85	13.20	7.14	0.83	0.45	3.08	1.66	0.076	4.11	0.561	0.30	1.29	0.70	3.44	1.86	
	1010261016	1.5	1.96	14.98	7.64	1.02	0.52	3.13	1.60	0.071	3.62	0.590	0.30	1.41	0.72	3.66	1.87	
	1010261037	1.5	2.03	14.49	7.14	1.00	0.49	3.24	1.60	0.056	2.76	0.635	0.31	1.25	0.62	3.71	1.83	
	1010261010	1.5	1.86	13.79	7.41	0.92	0.49	3.29	1.77	0.075	4.03	0.614	0.33	1.35	0.73	3.96	2.13	
	1010261046	1.5	2.18	11.90	5.46	0.82	0.38	2.91	1.33	0.080	3.67	0.529	0.24	1.45	0.67	3.68	1.69	
	1010261047	1.5	2.03	16.48	8.12	1.23	0.61	3.99	1.97	0.079	3.89	0.863	0.43	1.61	0.79	3.93	1.94	
	1010261049	1.5	2.10	17.15	8.17	1.02	0.49	3.42	1.63	0.050	2.38	0.552	0.26	1.46	0.70	3.90	1.86	
	N		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
	Mean		2.01	14.28	7.12	0.98	0.49	3.23	1.61	0.067	3.34	0.625	0.31	1.37	0.68	3.71	1.85	
	SD		0.11	1.61	0.80	0.12	0.06	0.32	0.17	0.011	0.61	0.118	0.06	0.13	0.06	0.20	0.12	
	SEM		0.04	0.51	0.25	0.04	0.02	0.10	0.05	0.004	0.19	0.037	0.02	0.04	0.02	0.06	0.04	

^a:Ratio=Organ weight/Brain weight

^b:Ratio=(Organ weight/Brain weight) x 100

Prepared by: Chia-Ping Chang Mar. 26, 2002

Checked by: Yu-Hsin Chen Mar. 26, 2002

28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS - PRODUCT CODE MICRSOY-20(MS-20)

大鼠28天口服重覆劑量(亞急性)毒性測試 - 代號 MICRSOY-20 (MS-20)

Individual Animal Data of Organ Weights and Organ/Brain Weight Ratios

個別試驗動物臟器重量、臟器與腦重量比數據

Project Code: DV-TA00199

Study No.: SA00032

Day on Test SD 29

Sex: Male

Group	Animal I.D.	Dose (ml/kg/day)	Brain		Liver		Spleen		Kidneys		Adrenal Gls.		Thymus		Heart		Testis	
			weight (g)	ratio ^a	weight (g)	ratio ^b (%)	weight (g)	ratio ^a	weight (g)	ratio ^a	weight (g)	ratio ^a						
3	1010261020	5	2.13	12.33	5.79	1.03	0.48	3.18	1.49	0.051	2.39	0.731	0.343	1.29	0.61	3.91	1.84	
	1010261030	5	2.01	13.10	6.52	0.80	0.40	2.69	1.34	0.044	2.19	0.534	0.266	1.16	0.58	3.54	1.76	
	1010261001	5	1.97	13.53	6.87	0.83	0.42	3.25	1.65	0.070	3.55	0.468	0.238	1.35	0.69	3.37	1.71	
	1010261042	5	1.97	14.70	7.46	0.78	0.40	3.14	1.59	0.060	3.05	0.489	0.248	1.27	0.64	3.41	1.73	
	1010261002	5	2.06	14.18	6.88	0.79	0.38	3.53	1.71	0.068	3.30	0.668	0.324	1.20	0.58	3.97	1.93	
	1010261013	5	2.09	15.40	7.37	1.11	0.53	3.78	1.81	0.065	3.11	0.583	0.279	1.40	0.67	3.87	1.85	
	1010261026	5	2.08	16.17	7.77	1.04	0.50	3.71	1.78	0.093	4.47	0.875	0.421	1.57	0.75	4.02	1.93	
	1010261031	5	1.95	14.08	7.22	0.87	0.45	3.15	1.62	0.072	3.69	0.486	0.249	1.33	0.68	3.95	2.03	
	1010261011	5	1.89	12.30	6.51	0.91	0.48	2.97	1.57	0.067	3.54	0.490	0.259	1.26	0.67	3.32	1.76	
	1010261019	5	2.05	13.85	6.76	1.16	0.57	3.51	1.71	0.076	3.71	0.700	0.341	1.41	0.69	4.11	2.00	
	N			10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean			2.02	13.96	6.92	0.93	0.46	3.29	1.63	0.067	3.30	0.602	0.30	1.32	0.66	3.75	1.85
	SD			0.07	1.24	0.57	0.14	0.06	0.34	0.14	0.013	0.66	0.136	0.06	0.12	0.05	0.30	0.11
SEM			0.02	0.39	0.18	0.04	0.02	0.11	0.04	0.004	0.21	0.043	0.02	0.04	0.02	0.10	0.04	

^a:Ratio=Organ weight/Brain weight

^b:Ratio=(Organ weight/Brain weight) x 100

Prepared by: Ch. L. Peng Chang Mar. 26, 2002

Checked by: Mr. Min Chen Mar 26, 2002

28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS - PRODUCT CODE MICRSOY-20(MS-20)

大鼠28天口服重覆劑量(亞急性)毒性測試 - 代號 MICRSOY-20 (MS-20)

Individual Animal Data of Organ Weights and Organ/Brain Weight Ratios

個別試驗動物臟器重量、臟器與腦重量比數據

Project Code: DV-TA00199

Day on Test: SD 29

Study No.: SA00032

Sex: Male

Group	Animal I.D.	Dose (ml/kg/day)	Brain		Liver		Spleen		Kidneys		Adrenal Gls.		Thymus		Heart		Testis	
			weight (g)	ratio ^a	weight (g)	ratio ^b (%)	weight (g)	ratio ^a	weight (g)	ratio ^a	weight (g)	ratio ^a						
4	1010261004	15	2.15	12.69	5.90	0.82	0.38	3.09	1.44	0.069	3.21	0.670	0.312	1.21	0.56	3.49	1.62	
	1010261050	15	2.11	14.34	6.80	0.98	0.46	3.53	1.67	0.070	3.32	0.659	0.312	1.37	0.65	3.97	1.88	
	1010261045	15	2.02	15.42	7.63	1.01	0.50	3.54	1.75	0.061	3.02	0.821	0.406	1.47	0.73	3.81	1.89	
	1010261048	15	2.09	10.75	5.14	0.75	0.36	2.69	1.29	0.066	3.16	0.487	0.233	1.22	0.58	3.92	1.88	
	1010261038	15	1.98	13.14	6.64	0.80	0.40	3.33	1.68	0.049	2.47	0.532	0.269	1.28	0.65	3.64	1.84	
	1010261043	15	2.09	13.13	6.28	1.02	0.49	3.12	1.49	0.054	2.58	0.614	0.294	1.44	0.69	3.89	1.86	
	1010261007	15	1.96	16.33	8.33	0.89	0.45	3.44	1.76	0.047	2.40	0.753	0.384	1.51	0.77	3.61	1.84	
	1010261021	15	2.05	16.37	7.99	0.89	0.43	3.37	1.64	0.067	3.27	0.758	0.370	1.31	0.64	4.05	1.98	
	1010261012	15	1.99	15.74	7.91	0.85	0.43	3.34	1.68	0.061	3.07	0.704	0.354	1.40	0.70	3.58	1.80	
	1010261040	15	2.04	13.61	6.67	0.91	0.45	3.48	1.71	0.070	3.43	0.709	0.348	1.18	0.58	3.54	1.74	
	N		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
	Mean		2.05	14.15	6.93	0.89	0.44	3.29	1.61	0.061	2.99	0.671	0.33	1.34	0.66	3.75	1.83	
	SD		0.06	1.82	1.02	0.09	0.05	0.26	0.15	0.009	0.37	0.103	0.05	0.12	0.07	0.20	0.10	
	SEM		0.02	0.58	0.32	0.03	0.01	0.08	0.05	0.003	0.12	0.033	0.02	0.04	0.02	0.06	0.03	

^a:Ratio=Organ weight/Brain weight

^b:Ratio=(Organ weight/Brain weight) x 100

Prepared by: Car Peng Wang Mar. 26, 2002

Checked by: Yu-Mao Chen Mar. 26, 2002

D-20

28-DAY SUBACUTE ORAL TOXICITY STUDY IN RATS - PRODUCT CODE MICRSOY-20 (MS-20)

大鼠28天口服重覆劑量(亞急性)毒性測試 - 代號 MICRSOY-20 (MS-20)

Individual Animal Data of Organ Weights and Organ/Brain Weight Ratios

個別試驗動物臟器重量、臟器與腦重量比數據

Project Code DV-TA00199

Study No: SA00032

Day on Test SD 29

Sex: Female

Group	Animal I.D.	Dose (ml/kg/day)	Brain		Liver		Spleen		Kidneys		Adrenal Gls.		Thymus		Heart	
			weight (g)	ratio ^a	weight (g)	ratio ^b (%)	weight (g)	ratio ^a	weight (g)	ratio ^b						
I	1010260035	0	1.86	9.04	4.86	0.61	0.33	2.24	1.20	0.069	3.71	0.446	0.24	1.06	0.57	
	1010260046	0	1.77	8.74	4.94	0.42	0.24	1.81	1.02	0.078	4.41	0.387	0.22	0.88	0.50	
	1010260004	0	1.84	8.63	4.69	0.59	0.32	1.96	1.07	0.089	4.84	0.562	0.31	0.84	0.46	
	1010260034	0	1.90	9.02	4.75	0.64	0.34	2.08	1.09	0.067	3.53	0.579	0.30	0.90	0.47	
	1010260038	0	1.81	9.36	5.17	0.86	0.48	2.12	1.17	0.084	4.64	0.649	0.36	1.02	0.56	
	1010260042	0	1.85	9.69	5.24	0.73	0.39	2.05	1.11	0.075	4.05	0.525	0.28	0.98	0.53	
	1010260015	0	1.73	7.86	4.54	0.58	0.34	1.80	1.04	0.071	4.10	0.522	0.30	1.00	0.58	
	1010260031	0	1.96	8.88	4.53	0.69	0.35	1.91	0.97	0.075	3.83	0.462	0.24	0.98	0.50	
	1010260013	0	1.94	8.74	4.51	0.60	0.31	2.13	1.10	0.074	3.81	0.616	0.32	1.03	0.53	
	1010260045	0	1.87	7.61	4.07	0.53	0.28	1.70	0.91	0.071	3.80	0.469	0.25	0.94	0.50	
	N		10	10	10	10	10	10	10	10	10	10	10	10	10	
	Mean		1.85	8.76	4.73	0.63	0.34	1.98	1.07	0.075	4.07	0.522	0.28	0.96	0.52	
	SD		0.07	0.63	0.35	0.12	0.06	0.17	0.09	0.007	0.43	0.082	0.04	0.07	0.04	
	SEM		0.02	0.20	0.11	0.04	0.02	0.05	0.03	0.002	0.14	0.026	0.01	0.02	0.01	

^a: Ratio = Organ weight/Brain weight

^b: Ratio = (Organ weight/Brain weight) x 100

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