final report

ICR mice gavage administered sbk002 and potassium dichromate raw material

Single-dose toxicity studies

Test Facility

|  |  |
| --- | --- |
| name: | Suzhou Huace Biological Technology Co., Ltd. |
| Address: | Jiangsu Province, Kunshan City, New & Hi-tech Industrial Development Zone, Yuanfeng Road, No. 166 |
| Telephone: | 0512-36801688 |

Sponsor

|  |  |
| --- | --- |
| name: | Chengdu Shibeikang Bio-pharmaceutical Technology Co., Ltd. |
| Address: | No. 17, West Core Avenue, Chengdu Hi-tech Industrial Development Zone |
| Telephone: | 028-62532315 |

study duration: 2018-12-10 ~ 2019- 01-21

# signature page

Gao Momo, M.M. Date

study director

He Yan Date

QA

Statement of GLP Compliance

GLP regulations:

This assay, completed at Suzhou Huace Biotechnology Co., Ltd. (test facility), adhered to the study protocol and the test facility's standard operating procedures (SOPs). The assay complied with the following Good Laboratory Practices (GLPs) for non-clinical studies.

The original National Food and Drug Administration (CFDA) 'Good Laboratory Practices (GLPs) for Non-clinical Studies of Pharmaceuticals' (Order No. 34), effective on September 1, 2017.

FDA Good Laboratory Practice for Nonclinical Laboratory Studies（21 CFR 58）.

animal welfare:

In this assay, all parts comply with the following regulations and guidelines related to animal management and welfare:

1) The guidelines listed in the book 'Guide for the Care and Use of Laboratory Animals' from AAALAC, compiled by the National Research Council's Institute for Laboratory Animal Research, revised in 2011;

2) Ministry of Science and Technology of the People's Republic of China, 'Regulations on Laboratory Animal Management', amended in 2013.

No known events that could affect data integrity occurred during the study process.

Gao Xiaosister, M.M. Date

study director

# 

# QA statement

Study director: Gao Shaosha

Test article code: sbk002

Study Name: ICR mouse gavage administering sbk002 and sulfuric acid ethylation precursor single dose toxicity study

Study number: A2018030-T001-01

According to the General Administration of Food and Drug Administration's Good Laboratory Practices for Non-clinical Pharmaceutical Research (Order No. 34) effective from September 1, 2017, economic cooperation and development group OECD Principles of Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17, US Food and Drug Administration Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR Part 58), examination of study protocols and standard operating procedures (SOPs), and listing examination reports with the date reported to the study director and test facility management. Additionally, this statement confirms that the final report accurately reflects the assay's raw data.

| Inspection Contents | Inspection Date | Report Date (SD/Test Facility Management) |
| --- | --- | --- |
| study protocol | 2018-12-05 | 2018-12-05 |
| Study protocol amendments (No.01) | 2018-12-12 | 2018-12-12 |
| dose formulations preparation | 2018-12-14 | 2018-12-14 |
| Sampling of dose formulations | 2018-12-14 | 2018-12-14 |
| dose formulation analysis | 2018-12-14 | 2018-12-14 |
| Dose | 2018-12-14 | 2018-12-14 |
| Study Protocol Amendments (No.02) | 2018-12-25 | 2018-12-25 |
| anatomy | 2018-12-28 | 2018-12-28 |
| organ weighing | 2018-12-28 | 2018-12-28 |
| original data | 2019-01-17~2019-01-18 | 2019-01-18 |
| final report | 2019-01-18 | 2019-01-18 |

In addition, the quality assurance department will follow SOPs to implement quarterly process inspections and annual organizational inspections.

QA: He Yan

Signature： Date ：

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Summary

Objective: Observe for 14 days after a single oral gavage of sbk002 and chloroform (clo) raw material to ICR mice, to investigate the nature, extent, and reversibility of potential toxic reactions caused by the test article, preliminarily determining the target organ or target tissue toxicity, providing a reference for future clinical study designs and safe drug administration.

Method: The assay consists of 3 groups, namely the vehicle control group, sbk002 dose group, and clo dose group, with 20 mice in each group, half male and half female. The sbk002 dose group and clo dose group each received a single oral gavage dose of 5000 mg/kg of sbk002 and clo, respectively; observation continued for 14 days post-dosing. The vehicle control group received an equivalent volume of vehicle. The dosing day is defined as dosing phase day 1 (D1).

Symptoms observed and testing indicators include: (1) General condition: observation of general performance, behavioral status, eyes, oral cavity, muzzle, ears, hair and skin, feces, urine, genitalia, death and other toxic symptoms; (2) Body weight; (3) Food consumption; (4) Gross necropsy, organ weighing, and histopathological examination: gross observation of any remarkable findings in organs, weighing and recording the absolute weight of tissues/organs, calculating organ-to-body and organ-to-brain ratios, and performing histopathological examination on tissues/organs with abnormal findings from the gross necropsy observations.

result:

1. General status observation

Under the assay conditions, ICR mice were separately given a single gavage dose of sbk002 and clo, with dosages of 5000 mg/kg, followed by continuous observation for 14 days after dosing. The laboratory animals in the vehicle control group and sbk002 dose group survived until the scheduled necropsy, and no treatment-related abnormalities were observed. On D1 after dosing, male animals in the clo dose group exhibited different extents of lying down, decreased activity, balance disorders, accelerated/depressed respiration, lethargy, convulsions, unstable gait, and other symptoms. Except for female animal 3F009, other female animals showed different extents of salivation, lying down, decreased activity, accelerated respiration, lethargy, convulsions, unstable gait, balance disorders, muscle spasms, and arched back symptoms. On D2, female animals 3F001 and 3F008 in the clo dose group still exhibited symptoms such as low body temperature, lying down, decreased activity, balance disorders, and trembling. Between D1 and D3, a total of 19 out of 20 animals (10 males and 9 females) in the clo dose group died.

1. body weight

During the assay period, the body weights of laboratory animals in the vehicle control group and the sbk002 dosage group were within normal fluctuation ranges before and after dosing, with no significant abnormalities related to the test article treatment.

1. food consumption

During the assay period, no significant abnormalities were observed in the food consumption of the vehicle control group and sbk002 dose group animals before and after dosing related to the test article. However, in the clo dose group, food consumption decreased significantly in surviving animals on D2 post-dosing.

(2) Gross necropsy, organ weighing and histopathological examination

Under the conditions of this assay, at D15, compared with the solvent control group, the brain weight of female animals in the sbk002 dosage group decreased, and the weight and organ-to-body ratio of the uterus (including the cervix) decreased, while the lung and main bronchus organ-to-brain ratio of male animals increased. Although the above test results showed some statistically significant differences, they fluctuated within the normal range. Furthermore, no obvious pathological changes were observed during the general necropsy, so these changes are considered to have no toxicological significance. Additionally, there were no statistically significant differences (P>0.05) in the remaining organ weights, organ-to-body ratio, and organ-to-brain ratio calculations between the laboratory animals in the solvent control group and the sbk002 dosage group. No remarkable findings related to the test article treatment were observed. No significant pathological findings were observed in the general necropsy of animals in the sbk002 dosage group. In the clo dosage group, the death animals showed symptoms of lung congestion, stomach necrosis, and pigment deposition in the stomach. The direct cause of death of the animals could not be determined. The deaths of 3F003 and 3M007 could possibly be related to gastrointestinal lesions, while pathological changes in the lungs could be related to post-mortem organ congestion.

Conclusion: Based on the above, under the conditions of this assay, a single oral gavage administration of sbk002 at a maximum tolerated dose (MTD) of ≥5000 mg/kg in ICR mice showed a median lethal dose (LD50) <5000 mg/kg.

# General Information

* 1. Study name and number

Study Name: Single dose toxicity studies of sbk002 and chloroform raw material administered via gavage to ICR mice;

study number: A2018030-T001-01.

* 1. Study Objective

ICR mice were given a single oral gavage of sbk002 and phosphoric acid quinine cholecalciferol raw material drug, observed for 14 days, observation of test article may cause toxic reactions in terms of nature, extent and reversibility, initially determining the toxicity target organ or target tissue, providing references for subsequent clinical trial design and safe drug use.

* 1. Test Facility

name: Suzhou Huace Biological Technology Co., Ltd.;

Address: Jiangsu Province, Kunshan City, New & Hi-tech Industrial Development Zone, Yuanfeng Road 166;

Postal Code: 215300;

Contact person: Xia Yuye;

telephone: 0512-36801688;

Fax: 0512-36802288;

Email: xiayuye@cti-cert.com.

* 1. Sponsor

Name: Chengdu Shibeikang Biotechnology Co., Ltd.;

Address: 17 Xixin Avenue, Chengdu New & Hi-tech Industrial Development Zone;

Postal code: 611731;

contact: Mu Yan;

Telephone: 028-62532315;

email: 779061281@qq.com.

* 1. study personnel composition
     1. study director

name: Gao Yayun;

Address: Jiangsu Province, Kunshan City, New & Hi-tech Industrial Development Zone, Yuanfeng Road 166;

telephone: 0512-36801688;

Fax: 0512-36802288;

email: gaotingting@cti-cert.com.

* + 1. Assay mainly composed of personnel

Assay operations: Min Shen, Jialing Xu, Chang He, Jiameng Cheng;

Veterinarian: Wang Haiyang, Xu Rui, Wu Ziqiang, Xu Yongqiang, Shen Shuangya;

Animal Housing Management: Li Yongchao;

Test Article Management: Chen Xiaoyan, Li Xuan;

Dose formulations preparation: Shi Jingjing, Liu Lulu;

Dose Formulations Analyzed: Li Peng, Lu Mingmin, Zhao Yan;

Necropsy personnel: Chen Yong, Li Jialin, Li Zhengdao, Yang Gang, Lu Wanhao, Jiang Fazhi, Chen Mingming, Lu Yufeng, Zhang Hailiang, Liu Xing;

Gross observation: Jinglong, Tian Tian, Li Rongxia;

Histopathological Examination: Li Yanchuan;

Data collation and statistical analysis: Gao Xiaoxia, Shen Xiaoxia.

* 1. Quality Assurance Personnel

name: He Yan；

telephone: 0512-36801688;

email: cti-btc-qa.list@cti-cert.com.

* 1. Regulations and Technical Guidelines Followed

This assay will comply with the regulations and technical guidelines, including but not limited to:

'Good Laboratory Practices (GLPs) for Non-clinical Studies of Pharmaceuticals' (CFDA, September 2017);

Good Laboratory Practice for Nonclinical Laboratory Studies （21 CFR 58, FDA） ；

OECD Principles of Good Laboratory Practice [ENV/MC/CHEM (98)17, OECD]；

《Pharmaceuticals Registration Management Measures》 (Former CFDA, October 2007);

Guideline for Single Dose Toxicity Study of Drugs (former CFDA, May 2014);

Q&A on Non-clinical Safety Evaluation test article testing Requirements (Former CFDA, May 2014);

ICH M3 （R2）: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. June，2009；

The implementation of this study, except as specifically noted in the protocol, complies with the standard operating procedures of this facility (Standard Operating Procedures, SOPs).

* 1. Quality Assurance

Quality Assurance Department in accordance with 'Non-clinical Study Good Laboratory Practices (GLPs)' (September 2017), U.S. Food and Drug Administration (FDA) (21 CFR 58, Good Laboratory Practice For Nonclinical Laboratory Studies), Organization for Economic Cooperation and Development (OECD) [OECD Principles of Good Laboratory Practice, ENV/MC/CHEM (98)17] GLP regulations, and the institution's SOPs for strict supervision, inspection, and review of study protocols, protocol amendments, the study process, raw data, and final reports to ensure the reliability of the study process and results.

* 1. assay time schedule

Assay Start Date: 2018-12-12;

Dosing date: 2018-12-14;

Necropsy date: 2018-12-28;

Assay end date: 2018-12-28.

# Study Materials

1. 1. Test article

2.1.1. General Information

Name/Code: sbk002;

Facility Code: W2018027;

Characterization: White crystalline powder;

Specification: 20 g/bag;

content: 99.9%;

Batch number: 180803;

Expiry date: 2020-08-23 (tentative);

Storage condition: 15 ℃~ 25 ℃, airtight, protected from light, dry;

Manufacturer: Chengdu Shipribec Bio-Pharmaceutical Technology Co., Ltd.;

Supplier: Chengdu Sibicon Biomedical Technology Co., Ltd.

2.1.2. Preparation of test article

preparation method: according to the most recent body weight of ICR mice and the dosage to calculate the amount of test article needed. Based on the amount of test article and preparation concentration, calculate the prepared volume [volume = (amount of test article × content) / concentration]. At room temperature and protected from light, accurately weigh a certain amount of test article into a mortar, add an appropriate amount of vehicle, grind fully, then completely transfer to a container of a certain volume, and use the vehicle to make up to the required concentration of test article dose formulations.

Example of preparation method: For preparing 100mL of dose formulation:

| group | Dosage  **（mg/kg）** | Concentration  **（mg/mL）** | Preparation method |
| --- | --- | --- | --- |
| sbk002  dose group | 5000 | 250 | Accurately weigh 25050 mg of sbk002, add an appropriate amount of 0.5 % CMC-Na solution and grind thoroughly, completely transfer to the calibrated beaker (100 mL), add 0.5 % CMC-Na solution to volume to the mark, obtaining 100 mL dose formulations of 250 mg/mL sbk002 dose group. |

Preparation condition: room temperature, protected from excessive light;

Identification method: Dose formulations of the sbk002 dosage group were labeled with red labels, including study number, name, concentration, number, preparation date, preparer, storage conditions, expiry date, and finished product number.

Storage conditions and expiry after preparation: Room temperature; protect from light, use within 4 hours.

2.1.3. Retention of samples and remaining dose formulations/test article disposal

Remaining dose formulations disposal: return to test article management department, and handle according to the requirements for medication/chemical waste disposal;

test article retention samples: perform test article retention samples according to company related SOP;

Retained test articles disposal: This batch of test article retention samples shall be documented in accordance with the related records of the Test Article Management Department. After the end of the project, archiving will be performed according to SOP regulations and stored in the sample archive room of the institution's archive management department;

Remaining test article handling: to be returned to the sponsor after all studies of this project end.

* 1. positive reference item
     1. General Information

Name/Code: Sodium bromate/clo;

Characterization: white or nearly white powder;

Specification: 30 g/bottle;

Purity: 98.8%;

Batch number: D163703;

Expiry date: 2019-11-25;

Storage condition: 15 ℃~ 25 ℃, airtight, protected from light, dry;

Manufacturer: Chengdu Best Reagents Co., Ltd.;

Supplier: Chengdu Sibicon Biomedical Technology Co., Ltd.

* + 1. Positive reference item preparation

Preparation method: Based on the most recent body weight of ICR mice and dose calculation, determine the amount of the test article (reference item). Based on the quantity of the test article and the required concentration, calculate the preparation volume [Volume = (Test article amount × content) / concentration]. Under room temperature and light-avoiding conditions, accurately measure a specific amount of the reference item into a mortar, add an appropriate amount of vehicle for thorough grinding, then transfer completely into a container of a specific volume, adjust with vehicle to the desired concentration, and obtain the necessary concentration dose formulations for the control group.

Preparation method example: Using preparation of 100 mL dose formulation as an example:

| group | Dosage  **（mg/kg）** | Concentration  **（mg/mL）** | Preparation method |
| --- | --- | --- | --- |
| clo  dose group | 5000 | 250 | Accurately weigh 25,304 mg of clo, fully grind it with 0.5% CMC-Na solution, completely transfer it to a labeled beaker (100 mL), dilute to the mark with 0.5% CMC-Na solution to make a dose formulation of 250 mg/mL clo dose group up to 100 mL. |

Preparation condition: room temperature, protected from excessive light;

Identification method: The prepared clodose group dose formulations are marked with yellow labels, indicating the study number, name, concentration, quantity, preparation date, preparer, storage conditions, expiration date, and product number.

Storage conditions and expiry date after preparation: room temperature, light-resistant, use within 4 hours.

* + 1. Retention of samples and disposal of remaining positive control items dose formulations/positive control items

Positive control item retention samples: Returned to the Test Article Management Department and disposed of according to medication/chemical waste requirements;

Handling of remaining dose formulations: Performing test article retention samples according to the company's related SOP;

retention samples positive reference item disposal: this batch of test article retention samples follow the records of the Test Article Management Department, after project completion perform archiving as per SOP regulations, store at this institution's archives management department retention samples archive room;

Remaining positive reference item disposal: Return to the sponsor after all studies of this project are completed.

* 1. Vehicle
     1. General Information

name: carboxymethylcellulose sodium (CMC-Na)；

Characterization: White to grayish powder or crystal;

Specification: 500g ;

Batch Number: F1719015;

Expiry date: Three years after opening (2021-10-22);

Storage condition: room temperature;

Manufacturer: Shanghai Alading Biochemical Technology Co., Ltd.

* + 1. Vehicle preparation

Preparation method: Accurately measure a specific amount of CMC-Na powder, using a volumetric cylinder to measure a specific volume of pure water and transfer it to a beaker. Evenly disperse the CMC-Na powder into the beaker, add a stirring bar, and use a magnetic stirrer to stir until completely dissolved, thus obtaining 0.5% CMC-Na solution;

preparation method example: preparation of 100 mL 0.5 % CMC-Na as an example:

| group | Concentration | Preparation method |
| --- | --- | --- |
| Vehicle control group | 0.5 % | Accurately weigh 0.5 g of CMC-Na powder, transfer 100 mL of pure water in the measuring cylinder to the beaker, uniformly disperse the CMC-Na powder in the beaker, add the stirring rod, and use the magnetic stirrer to stir until completely dissolved, resulting in 100 mL of a 0.5% CMC-Na solution. Dispense the required volume of 0.5% CMC-Na solution to prepare dose formulations for the vehicle control group. |

Preparation condition: room temperature;

Identification method: Formulations for the control group were labeled with white labels and include the study number, name, concentration, quantity, preparation date, preparer, storage conditions, expiry date, and product number;

Storage conditions and expiry after preparation: Store at room temperature and use within 7 days.

* + 1. disposal of remaining vehicle control group dose formulations

After administration, the remaining solvent control group dose formulations: return to test article management, and handle according to the requirements for medication/chemical waste disposal.

* 1. dose formulation analysis

The content and stability materials of the test product are provided by the sponsor. The analysis method refers to “sbk002 and sodium bicarbonate raw material drug dose formulation analysis methodology validation test (special topic number: A2018030-FA01)”. The stability analysis of the test product is conducted within this special topic, hence, this experiment will not be independently performing stability determination.

Analyze the content of dose formulations administered on the day of dosing. Collect 2 samples each from the top, middle, and bottom layers of the dose formulations to be tested (only the middle layer for the vehicle control group). Use one sample for analysis and keep the other at room temperature and protected from light for backup. Vehicle control group samples are kept at room temperature for backup. The analyzed sample is labeled with the dose formulation finished product number, position abbreviation + two-digit serial number (e.g., 181214-G1-M01). Prior to sample collection for dose formulation analysis, use a magnetic stirrer to stir at a certain speed for at least 15 minutes (set the speed so that an obvious vortex is formed without causing splashing or a large amount of bubbles). Sample volume, handling, and testing methods are described in “ICR mouse gavage with sbk002 and dichlorvos vehicle single-dose toxicity study dose formulation analysis method (method number: W2018027-FA02)".

Result acceptance criteria: For the sbk002 dose group and the clo dose group, the accuracy of the dose formulations (ratio of detected concentration to labeled concentration) is between 85 % ~ 115 %, and the relative standard deviation (RSD) of the measured concentrations of the top, middle, and bottom layers of the dose formulations is ≤10 %. For the vehicle control group, the dose formulations have no interference peaks or the peak area of interfering peaks is ≤10 % of the peak area at the lower limit of quantification at the retention time of sbk002 and clopidogrel bisulfate raw material.

The disposal of remaining sample: Remaining samples post-analysis are returned to the Test Article Management Department and disposed of according to medication/chemical waste requirements.

* 1. operation/Safety measures

Research institution operations follow the Occupational Health Safety and Protection Manual. When performing assay operations, wear appropriate personal protective equipment (PPE).

* 1. Dose formulations transfer within the test facility

Test article dose formulations under room temperature and light-shielded conditions are transferred from the Test Article Management to the test site. Vehicle control group dose formulations under room temperature conditions are also transferred from the Test Article Management to the test site. Test article dose formulations retrieved from the Test Article Management are stored under room temperature and light-shielded conditions when not in use. Similarly, vehicle control group dose formulations not in use are stored under room temperature conditions.

* 1. Other major reagents

|  |  |  |
| --- | --- | --- |
| name | source | Grade |
| pentobarbital sodium | Shanghai Junhao Biotechnology Co., Ltd. | NA |

* 1. Major Instruments and Equipment

| Equipment Name | Manufacturer | Model |
| --- | --- | --- |
| pure water apparatus | Millipore | ELIX® Advantage 5 |
| Microtome | Leica | Leica RM2235 |
| Sealing Machine | Leica | Leica CV5030 |
| Stainer | Leica | Leica ST5020 |
| Tissue Embedding Machine | Leica | Leica EG1150C+ EG1150H |
| Fully automatic dehydration machine | Leica | Leica ASP300S |

# test system

* 1. Product/Strain/Grade

Species: ICR mouse;

Grade: SPF grade.

* 1. Sex and Number

Purchased animal number and sex: 66, half male and half female;

number and sex of animals used: 60, female and male each half;

Disposal of remaining animals: The remaining laboratory animals were transferred to the Toxicology Operations Department on the 5th day after dosing.

* 1. source

Supplier: Zhejiang Weitonglihua Laboratory Animal Technique Co., Ltd.

Production license No: SCXK (Zhe) 2018-0001;

Animal quality certificate number: No 1812050025.

* 1. Body Weight and Age

Body weight: At purchase, the body weight of females was 22.2 ~ 26.5 g, and males were 22.0 ~ 27.5 g. At group assignment, the body weight of females was 23.5 ~ 27.4 g, and males were 27.0 ~ 32.0 g. During group assignment, individual body weights were within ± 20% of the mean body weight for the same sex group.

age: At purchase approximately 5 ~ 7 weeks, at group assignment approximately 6 ~ 8 weeks.

* 1. Animal Identification

According to the test facility's SOPs, cage cards are made, with each animal identified through tail labeling and cage cards for animal identification.

* 1. Selection and Justification of Laboratory Animals and Number

laboratory animals selection reasons: refer to 'medication single dose toxicity research technical guidance principles' (former CFDA, May 2014), chemical drug single dose toxicity studies should adopt at least two types of mammals for experimentation, typically choosing one rodent animal and one non-rodent animal. Preliminary research indicates that ICR mice are the sensitive animals for this test article, hence, ICR mice are used as the test animals for this assay, and their genetic, biological background (including anatomical, physiological, clinical pathology, and other data ranges) are well understood.

Justification for the selection of the number of laboratory animals: Under the premise of meeting the research objectives, scientific standards, and regulatory requirements, use the least possible number of animals. This assay is designed according to the MTD method, observing the tolerance situation after a single administration of sbk002, while also setting up a clo administration group with an animal number of 20 per group. To prevent the situation where the purchased laboratory animals cannot meet the experimental needs due to unknown reasons, an additional 3 animals per gender were purchased.

# Animal Housing and Management

* 1. animal management and use

Suzhou Huace Biotechnique Co., Ltd. is an institution accredited by the 'International Council for Laboratory Animal Evaluation and Accreditation (AAALAC International)', and its use of laboratory animals has been approved by the Science & Technology Department of Jiangsu Province.

The assay is not a simple repetition of any previous assays, and there are no alternative assays that can resolve it. Furthermore, literature searches did not reveal any methods that could replace the procedures mentioned in this assay with less pain and tension. During the study process, no conditions were found that could cause pain, tension, or diseases affecting the animal experiment results.

The contents and procedures related to animal experiments involved in this assay comply with the relevant laws and regulations on the use and management of laboratory animals and the relevant provisions of the Institutional Animal Care and Use Committee (IACUC) of this institution. The number of animals, study design, and disposal of animals were approved by the IACUC of this institution (approval number: IACUC-A2018030-T001-01), and strictly followed the contents approved by the IACUC.

* 1. Animal Receipt and Acclimation

Laboratory animals receive 6 days of acclimation after reception.

* 1. Animal Housing

laboratory animals use license number: SYXK (Su) 2018-0051;

Laboratory animal use license No.: Suzhou Huace Biology Technology Co., Ltd., Building 1, 2nd Floor, within the barrier system;

cage type: polysulfone mouse cage, size (L×W×H): 26 cm×16 cm×13 cm;

housing density: ≤4 animals per cage.

* 1. Housing Environment

Housing Environment Conditions Standards: National Standards of the People's Republic of China GB14925-2010；

Housing environment control system: MSEA-MVE 6.0 Jiangsen animal housing environment monitoring system;

temperature: 21.1 ~ 23.4 ℃ (daily temperature variation 0.6 ~ 2.2 ℃);

Relative humidity: 44.6 % ~ 64.4 %;

Lighting: Artificial lighting; 12-hour light/dark cycles;

ventilation rate: at least 15 air changes per hour.

* 1. Environmental Enrichment

Provide toys for laboratory animals as environmental enrichment measures.

* 1. feed

Type: SPF rat and mouse maintenance feed;

Feed Batch Number: 18103213, 18113213;

Manufacturer: Beijing Sino-Australian Cooperation Feed Co., Ltd.;

Production license No: SCXK(Beijing)2014-0010;

Feeding method: ad libitum (except when the assay has special requirements);

Feed testing: The supplier provides a quality certificate for the feed, certificate numbers: 1112621800002026, 1112621800002136;

testing result:

Nutritional composition: Nutritional composition inspection report of feed batches 18103213 and 18113213 provided by SONY Testing Group Co., Ltd. (Report numbers: GMAXKCAK40861508, GMAJKSIK27539508), including indicators for moisture, crude protein, crude fat, crude fiber, crude ash, calcium, and phosphorus. Test results conform to GB14924.3-2010;

chemical pollutants: including aflatoxin, lead, cadmium, mercury, dioxins, ochratoxin A, aflatoxin B1. The test results meet the standards of GB/T 14924.2-2001;

Microbiological indicators: a microbiological testing report of feed batches 18103213 and 18113213 provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. and Charles River Laboratories Inc. (Report IDs: BJVRL-FR-20181018C, GMABENWK32548508) includes total bacterial count, coliforms, mold and yeast counts, and Salmonella. The test results conform to GB/T 14924.2-2001.

* 1. bedding

bedding type: corn cob;

bedding batch number: 20180712;

Manufacturer: Jiangsu Hengrui Medicine Co., Ltd.

Storage and use: low temperature, dry, hygienic; Spread sterilized bedding on the bottom of the mouse cage, spread evenly across the whole bottom;

Bedding testing: The supplier provides a quality assurance certificate for the feed, certificate number: No. 1201812080010; with testing reports provided by Juniperus Test Group Co., Ltd. for batch number 20180712 on bedding chemical pollutants (report number: BMA5HG9J16849708A). The testing standards include water absorbency (48-hour water absorption), moisture, total ash, lead, cadmium, arsenic, hexavalent chromium, mercury, and aflatoxin B1. The testing results meet the requirements.

* 1. Drinking Water

Type: Reverse osmosis water (testing report as domestic drinking water);

water supply method: Drinking Water bottles provided, ad libitum (collection urine period remove the water bottles);

Testing of water quality routine indicators: with testing reports provided by Shanghai Huace Pinhui Testing Technology Co., Ltd. (Report numbers: A20180219731101, A20180219731102b). The testing items include: pH value, color, turbidity, odor and taste, visible matter to the naked eye, total bacterial colonies, total coliform bacteria, Escherichia coli, thermotolerant coliform bacteria, oxygen demand, total dissolved solids, total hardness, anionic synthetic detergents, total alpha radioactivity, total beta radioactivity, hexavalent chromium, cyanide compounds, fluoride compounds, chloride compounds, bromide compounds, phosphate compounds, acetate compounds, formate compounds, tetrahydrofuran, zinc, lead, selenium, mercury, aluminum, iron, cadmium, copper, nickel, and various fermentation substances. The testing results meet the requirements.

* 1. Animal Selection

Selection of healthy (non-pregnant, nulliparous) ICR mice as test animals. All ICR mice passed the examination during the acclimatization period, selecting 60 of them for the study.

# study design

Group design: Vehicle control group, sbk002 dose group, and clo dose group;

animal number: total 60, each group 20;

sex ratio: female and male each half;

Group assignment method: Using Pristima system for sex-random group assignment;

Detailed group assignment information can be found in the table below:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| group | Test substance | Dosage  **（mg/kg）** | Dose Concentration  **（mg/mL）** | Animal number | |
| Female | male |
| Vehicle control group | 0.5 % CMC-Na | / | / | 1F001 ~ 1F010 | 1M001 ~ 1M010 |
| sbk002 dose group | sbk002 | 5000 | 250 | 2F001 ~ 2F010 | 2M001 ~ 2M010 |
| clodose group | clo | 5000 | 250 | 3F001 ~ 3F010 | 3M001 ~ 3M010 |

Note: The first digit of the animal number represents the group (1, 2, 3 respectively represent vehicle control group, sbk002 dose group, and clo dose group). The second letter represents the sex (F for female, M for male), and the last 3 digits represent the animal sequence number. “/” represents Not Applicable.

* 1. Dosing Information

Dosage: sbk002 dose group and clo dose group, dosage is 5000 mg/kg;

dose volume: dose volume is 20 mL/kg;

Dose Concentration: sbk002 dose group and clo dose group dose concentration are both 250 mg/mL;

Route of administration: oral gavage, pre-dose with magnetic stirrer stirring at a certain speed for 15 min or more (speed set to form a clear depression but without foaming or large bubbles).

selection reason: consistent with clinical medication route;

Frequency of administration and period: Administer once within 24 hours, followed by continuous observation for 14 days post-dosing, with fasting the night before dosing;

Pretest phase day 1 defined as P1, dosing day defined as dosing phase day 1 (D1).

* 1. dose design justification

According to the Technical Guiding Principle for Single Dose Toxicity Study of Medicinal Products (Original CFDA, May 2014) and ICH M3 recommendations, the dosage for single dose toxicity studies should range from a dose with no observed toxic reaction to a dose presenting severe toxic reactions, or up to the Maximum Tolerated Dose (MTD). According to the data provided by the Sponsor: the single dose toxicity study dosage for the same class of drugs in mice is 5000 mg/kg, with no animal deaths. Considering the feasible concentration for preparing the test product, this study will employ sbk002 dose group with supposed oral gavage, with a dose volume of 20 mL/kg and a dosage of 5000 mg/kg. The clo dose group will be administered an equivalent dose of positive reference item sodium bicarbonate (clo). Additionally, a vehicle control group will be set, administered with an equivalent dose volume of the vehicle. This will provide reference for subsequent clinical study design and for safe drug usage.

# observation and examination

* 1. General status observation

Observation time: Continuously observe for 4 h after each dose; during the assay period, observe twice daily (once in the morning and once in the afternoon). If animals exhibit clear signs of toxicity, increase the observation frequency and record (observe once on the day of necropsy).

Observation items: including but not limited to general conditions, behavioral status, eyes, oral cavity, muzzle, ears, hair and skin, feces, urine, genitalia, and other toxicity symptoms;

Animals to be observed: all surviving laboratory animals.

* 1. body weight

Measurement time: assay P1, D1, D2, D7, D14, and D15 each measured once (P1 signal weight for group assignment; D1 is pre- dose body weight, used for calculating the dose volume; D15 body weight is used only for calculating organ-to-body ratio and for calculating anesthesia dose, not included in the statistical analysis of body weight indicators; D1 and D15 body weight is not included in the analysis of body weight indicators, and the measurement frequency can be appropriately adjusted according to the toxic responses of the animals);

Animals to be measured: all surviving laboratory animals.

* 1. food consumption

Measurement time: P1 ~ P2, D1 ~ D2, D6 ~ D7, D13 ~ D14, each measured once (Pristima data report displays the leftover measurement dates, i.e., P2, D2, D7, D14);

Animals to be measured: All surviving laboratory animals;

Method for measurement: Day 1 determination of the feed amount given to each cage; Day 2 determination of the remaining feed amount at approximately the same time. The difference between the two is the total feed intake for each cage over 24 hours. This is then divided by the number of animals per cage to calculate the average intake per animal (measurement frequency can be adjusted according to the characteristics of the test article).

# Gross necropsy, organ weighing, and histopathological examination

* 1. Necropsy Time

D15, animals to be necropsied fasted overnight.

* 1. anatomy animal

All surviving laboratory animals.

* 1. anesthesia and euthanasia method

Before anesthesia, fast the mice for at least 8 hours, anesthetize with intraperitoneal injection of pentobarbital sodium at a dose of 80 mg/kg, concentration of 10 mg/mL, and injection volume of 8 mL/kg (adjustments can be made according to the animal's anesthesia status), after anesthesia, perform euthanasia by exsanguination via the abdominal aorta.

* 1. gross necropsy observation

First, perform general examinations, examining the animal's external appearance, including body shape, nutritional status, hair and skin, external genitalia, and various cavities; open the abdominal cavity, pelvic cavity, and thoracic cavity and examine internal organs, observing the positioning, color, size, hardness, and presence of hemorrhage or adhesion of each organ; and record the necropsy findings.

* 1. organ weight

weigh and record the absolute weight of tissues/organs (tissues/organs that need to be weighed are detailed in 7.6), and calculate organ-to-body ratio and organ-to-brain ratio

organ-to-body ratio = organ weight (g) / body weight (g)× 100 %；

Organ-to-brain ratio = organ weight (g) / brain weight (g) × 100%.

* 1. Tissue fixation, pathology examination

Only for gross necropsy observation of any organs/tissues with abnormalities in volume, color, or consistency, perform trimming and fixation, followed by routine histology processes such as paraffin embedding, sectioning, slide preparation, and HE staining, and conduct histopathological examination. If storage is required, preserve bilateral eyeballs and optic nerves in 2.5% glutaraldehyde solution, testes, and epididymides in modified Davidson’s fixative, and other tissues/organs in 10% neutral buffered formalin.

Tissues/organs that require extraction, weighing, fixation, and histopathological examination are listed in the following table:

| Tissues name | weigh | fixative | Retention | histopathological examination | |
| --- | --- | --- | --- | --- | --- |
| Aorta |  | F | # | # | |
| adrenal glands | √ | F | # | # | |
| Bone and bone marrow (sternum) |  | F | # | # | |
| Bone (Femur, including knee joint) |  | F | # | # | |
| brain (cerebrum, cerebellum, brain stem) | √ | F | # | # | |
| testes | √ | MD | # | # | |
| epididymis | √ | MD | # | # | |
| eye and optic nerve\* |  | 2.5%G | # | # | |
| esophagus |  | F | # | # | |
| harderian glands |  | F | # | # | |
| heart | √ | F | # | # | |
| kidney | √ | F | # | # | |
| large intestine (colon) |  | F | # | # | |
| large intestine (cecum) |  | F | # | # | |
| large intestine (rectum) |  | F | # | # | |
| Liver with gallbladder | √ | F | # | # | |
| lungs and main bronchi | √ | F | # | # | |
| lymph node (Mesentery) |  | F | # | # | |
| pancreas |  | F | # | # | |
| prostate gland | √ | F | # | # | |
| pituitary |  | F | # | # | |
| Sciatic nerve |  | F | # | # | |
| Skeletal muscle (biceps femoris) |  | F | # | # | |
| spleen | √ | F | # | # | |
| Stomach |  | F | # | # | |
| small intestine (duodenum) |  | F | # | # | |
| small intestine (jejunum) |  | F | # | # | |
| small intestine (ileum) |  | F | # | # | |
| Seminal vesicles |  | F | # | # | |
| Skin and mammary gland (inguinal) |  | F | # | # | |
| Salivary gland (submandibular gland, sublingual gland) |  | F | # | # | |
| Spinal cord (neck, thoracic, lumbar) |  | F | # | | # |
| thymus (or thymus region) | √ | F | # | | # |
| thyroid gland containing parathyroid gland\* |  | F | # | | # |
| Trachea |  | F | # | | # |
| urinary bladder |  | F | # | | # |
| Ovary | √ | F | # | | # |
| fallopian tube |  | F | # | | # |
| uterus (including cervix) | √ | F | # | | # |
| vagina |  | F | # | | # |
| dosing site draining lymph node (mandibular lymph node) |  | F | # | | # |
| Gross lesion |  | F | √ | | √ |

Note: F with 10% neutral buffered formalin fixative; MD indicates modified Davidson's fixative; 2.5% G with 2.5% glutaraldehyde solution; \* at least microscopic examination unilateral; # for gross necropsy, observe for abnormal volume, color, or consistency.

* 1. Handling of deceased animals

clo dose group animals 3M001, 3M002, 3M003, 3M004, 3M008, 3M009, 3F004, 3F005, 3F010 were found dead on D1; 3M005, 3M006, 3M007, 3M010, 3F002, 3F003, 3F006, 3F007 were found dead on D2; 3F001, 3F008 were found dead on D3.

# data acquisition and analysis

All raw data within the facility will be collected manually according to the study protocol and the SOP of Suzhou Huachao Bio-Technology Co., Ltd., or collected using a data acquisition system. Manually collected data can be transcribed into Excel tables for analysis and reporting.

The collection system for collecting and reporting electronic data is as follows:

| System | Version | Purpose |
| --- | --- | --- |
| Johnson Control | MSEA-MVE 6.0 | Environmental control and testing of the animal housing |
| Pristima | 7.0.0 | assay data collection and/or statistical analysis |

# statistical analysis

Quantitative parameters are expressed by mean ± standard deviation. Data from groups with fewer than 3 samples are not included in statistical comparison.

All quantitative parameters are collected and statistically analyzed using Pristima 7.0.0 data acquisition system; use SPSS for statistical analysis when necessary.

If the number of groups to be statistically compared is < 3, the statistical analysis method is as follows: first use the LEVENE method to perform the test for homogeneity of variances. When the variances are homogeneous (P > 0.05), use the t-test for statistical analysis; when the variances are not homogeneous (P ≤ 0.05 ), use the approximate t-test (t-test) for statistical analysis. When the results of the t-test or the approximate t-test show no statistically significant differences (P > 0.05 ), the statistical analysis ends. All tests are bilateral tests with α = 0.05.

If the number of groups to be statistically compared is ≥3, the statistical analysis method is as follows: first perform LEVENE's test for homogeneity of variances. When the variances are homogeneous (P≥0.05), use a one-way ANOVA for statistical testing. When the variances are not homogeneous (P<0.05), use a Kruskal-Wallis H test for statistical analysis. When a one-way ANOVA indicates significant overall differences (P≤0.05), use Dunnett's LSD test to compare inter-group differences. When a one-way ANOVA shows no significant overall differences (P>0.05), the statistical analysis ends. When a Kruskal-Wallis H test shows significant overall differences (P≤0.05), use a Mann-Whitney U test to compare inter-group differences. When a Kruskal-Wallis H test shows no significant overall differences (P>0.05), the statistical analysis ends. Inter-group comparisons are conducted between dose groups and the vehicle control group. Dunnett's LSD test (P<0.05) and Mann-Whitney U test (P≤0.05) indicate statistical significance.

general state observation result listing analyzed.

Pathological data described in detail.

This study used various computational models (regression models) and computer programs for data analysis. Due to different methods of performing rounding or truncation by different models on the data, some values in the tables (such as averages, standard deviations, or individual data values) may show slight discrepancies compared to data in other tables, standalone calculations, or statistical analysis data. These discrepancies do not affect the integrity of the data or the interpretation of the results.

# Test results and discussion

* 1. assay result
     1. dose formulation analysis results

The assay for dose formulations underwent content analysis, with detailed analysis results provided in Appendix 1. Results showed: After testing, system suitability, standard curve linear range, and quality control all met the analysis method requirements. The accuracy of the sbk002 dose group (the ratio of testing concentration to the standard concentration of dose formulations) ranged from 102.47% to 104.60%. The relative standard deviation (RSD) of testing concentrations in the upper, middle, and lower layers of sbk002 dose group was 1.04%. The accuracy for the clo dose group (the ratio of testing concentration to the standard concentration of dose formulations) ranged from 99.61% to 102.44%. The RSD of testing concentrations in the upper, middle, and lower layers of the clo dose group was 1.41%. These analysis results indicate that the dose formulations preparation met the requirements.

* + 1. General state observation

General state observation results are summarized in Appendix 2, Tables 1-1, 1-2, and individual data are shown in Appendix 3, Tables 7-1 ~ Tables 7-4. The results show that under the test conditions, ICR mice were administered with sbk002 and clo by single gavage at a dosage of 5000 mg/kg each, and observed continuously for 14 days after administration. In the solvent control group, one male animal showed scabbing of the tail from D11 to D15, considered as an accidental injury due to animal activity. Apart from this, all animals in the solvent control group and the sbk002 dose group survived until the scheduled necropsy, with no abnormalities observed in general state observation related to the test article administration.

After dosing with the clodose group on D1, male animals exhibited varying degrees of prostration, decreased activity, imbalance, rapid/shallow breathing, lethargy, twitching, and unstable gait. Female animals, except for 3F009, exhibited varying degrees of salivation, prostration, decreased activity, rapid/shallow breathing, lethargy, twitching, and unstable gait, as well as imbalance, muscle tremors, and hunchback symptoms. On D2, females 3F001 and 3F008 continued to exhibit symptoms of body chills, prostration, decreased activity, imbalance, and tremors. During D1 to D3, a total of 19 out of 20 animals (10 males and 9 females) died: 3M001, 3M002, 3M003, 3M004, 3M008, 3M009, 3F004, 3F005, 3F010 were found dead on D1; 3M005, 3M006, 3M007, 3M010, 3F002, 3F003, 3F006, 3F007 were found dead on D2; and 3F001, 3F008 were found dead on D3.

* + 1. body weight

The results of body weight measurements are summarized in Appendix 2; individual data can be found in Appendix 3, Tables 8-1 and 8-2. Results indicate that the body weights of laboratory animals in the vehicle control group and the sbk002 dosage group were within normal fluctuation ranges before and after dosing, with no significant abnormalities related to the test article treatment.

* + 1. food consumption

Summary of food consumption determination results is in Appendix Two Table 3, individual data is in Appendix Three Table 9-1, 9-2. Results show: Compared to the vehicle control group, on D14 after dosing, food consumption in the male animals of the sbk002 dose group decreased (P<0.01), but was still within the normal fluctuation range. No test article treatment-related remarkable findings were observed in food consumption of laboratory animals in the vehicle control group and sbk002 dose group before and after dosing.

* + 1. Gross necropsy, organ weighing, and histopathological examination

Details of gross necropsy and histopathological examination results are provided in Appendix Four.

10.1.5.1. Death or moribund condition

clo dose group animals 3M001, 3M002, 3M003, 3M004, 3M008, 3M009, 3F004, 3F005, 3F010 were found dead on D1; 3M005, 3M006, 3M007, 3M010, 3F002, 3F003, 3F006, 3F007 were found dead on D2; 3F001, 3F008 were found dead on D3.

10.1.5.2. Organ Weighing

Animal organ absolute weight determination results are summarized in Appendix 2, tables 4-1 and 4-2, with individual data in Appendix 3, tables 10-1 and 10-2. Organ-to-body ratio calculation results are given in Appendix 2, tables 5-1 and 5-2, with individual data in Appendix 3, tables 11-1 and 11-2. Organ-to-brain ratio calculation results are in Appendix 2, tables 6-1 and 6-2, with individual data in Appendix 3, tables 12-1 and 12-2. Results show that on D15, compared with the vehicle control group, the sbk002 dose group female animals showed a significant reduction in brain weight (P<0.05), uterus weight (including cervical uteri weight), and organ-to-body ratio (P<0.05), while male animals showed a significant increase in lung and main bronchi organ-to-brain ratio (P<0.05). Despite these statistical differences, they are within normal fluctuation ranges. Besides these findings, no statistically significant differences (P>0.05) were observed for the organ weight, organ-to-body ratio, and organ-to-brain ratio of other laboratory animals compared to the vehicle control group, and there were no remarkable findings related to the test article treatment.

10.1.5.3. Gross necropsy observation

Non-scheduled necropsy: gross necropsy observations revealed autolysis in 3F001, 3F002, 3F004, 3F006, 3F007, 3F008, 3F010, 3M001, 3M002, 3M003, 3M004, 3M005, 3M006, 3M009, 3M008, and 3M010; gastric expansion in 3F003 and 3M007; the gastric wall of 3F003 was transparent, with its contents being yellowish-orange pulp; focal dark red lung areas in 3M007; and yellowish change in the left lobe of the liver in 3F005.

scheduled necropsy: gross necropsy observation did not reveal abnormalities.

10.1.5.4. Histopathological examination

clo dose group animal 3F003 showed severe necrosis in the stomach with moderate pigment deposition; animal 3M007 showed moderate necrosis in the stomach with minimal pigment deposition, and mild congestion in the lungs. These results are consistent with gross necropsy observations. No significant pathological changes were observed in the liver of 3F005. The direct cause of death of the animals could not be determined. The deaths of 3F003 and 3M007 may be related to digestive system toxicity, and the pathological changes in the lungs may be related to post-mortem organ congestion.

* 1. Discussion

Under the conditions of this assay, ICR mice were gavaged with sbk002 and clo at a dosage of 5000 mg/kg in a single administration, followed by continuous observation for 14 days. The laboratory animals in the solvent control group and the sbk002 dosage group survived until the scheduled necropsy, and no remarkable findings related to the test article treatment were observed in general state observation, body weight, or food consumption. In the clo dosage group, 19/20 animals (10 males and 9 females) died during the D1 to D3 period. After administration on D1, the laboratory animals (except for 3F009) exhibited various degrees of salivation, lying down, decreased activity, accelerated respiration, weakness, convulsions, and unsteady gait, along with symptoms such as ataxia, muscle spasms, and hunched posture. By D2, female animals 3F001 and 3F008 in the clo dosage group still exhibited symptoms of low body temperature, lying down, decreased activity, ataxia, and muscle spasms.

Under assay conditions, at D15, compared to the vehicle control group, female animals in the sbk002 dose group exhibited a decrease in brain weight, uterus (including cervix) weight, and organ-to-body ratio, while male animals exhibited an increase in lung and main bronchi organ-to-brain ratio. Although these testing results showed statistical differences, they were within normal fluctuation ranges, and no obvious pathological changes were observed through gross observation, therefore these findings are considered to be of no toxicological significance. Additionally, the differences in organ weight, organ-to-body ratio, and organ-to-brain ratio of the laboratory animals compared to the vehicle control group were statistically insignificant (P>0.05), and no remarkable findings related to the test article administration were observed. No obvious pathological changes were observed in the sbk002 dose group through gross necropsy. In the clo dose group, the deceased animals showed signs of lung congestion, stomach necrosis, and pigment deposition in the stomach upon gross lesion histopathological examination. The direct cause of death for these animals could not be determined; the death of 3F003 and 3M007 could be related to toxicity in the digestive system, and the pathological changes in the lungs may be related to post-mortem organ congestion.

1. Conclusion

In summary, under the conditions of this experiment, the maximum tolerated dose (MTD) of sbk002 administered via single oral gavage to ICR mice is ≥5000 mg/kg, while the median lethal dose (LD50) of clo under the same dose is <5000 mg/kg.

1. Study protocol amendments and deviations

study protocol and amendments detailed in Appendix 5;

During this assay, two protocol amendments were made: 1. Changes in the preparation method of the test article, vehicle control group sampling method, dose formulation sampling time, and the stirring time with a magnetic stirrer before dosing. 2. Changes in the statistical analysis method.

No deviations occurred in this assay.

1. Storage of Relevant Data
   1. Archiving Time

Within 2 weeks of the subject's conclusion, the person in charge of the subject will transfer the experimental materials to the archives management department for filing. Paper materials will be stored for at least five years after the pharmaceutical product is marketed, while electronic documents will be stored permanently. Wet specimens and other biological specimens will be stored for a limited time, ensuring they do not affect their quality.

* 1. Storage site and Storage condition

Storage location: Suzhou Huace Biomedical Technology Co., Ltd. Archives Management Department;

Storage condition: routine;

Contact person: Lu Li;

Contact telephone: 0512-36801688.

1. Mainly reference documents

[1] Bo Li, Boyun Yuan, Mingyang Liao. Pharmacotoxicology. People's Health Publishing House, 2015: 523-525.

[2] Yuan Bojun, Liao Mingyang, Li Bo. Laboratory Methods and Techniques in Toxicology. Chemical Industry Press, 2007: 200-210.

[3] Redbook 2000. Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients. FDA. 2007.

1. Appendixes

15.1. Appendix One: Dose Formulations Analysis Report

15.2. Appendix II: assay result statistical summary

15.3. Appendix Three: Assay result individual data

15.4. Appendix IV: Pathology examination report

15.5. Appendix Five: study protocol and amendments

15.6. Appendix VI: temperature and humidity report

* 1. Appendix One: Dose Formulation Analysis Report

Study Name: ICR mouse gavage administration of sbk002 and phosphorous acid cyanohydroxyguanidine primary intermediates

Single-dose toxicity studies

Study number: A2018030-T001-01

* 1. Appendix Two: Experiment Results Statistical Summary

Study Name: ICR mouse gavage with sbk002 and sodium bisulfite paraben raw material drug

Single-dose toxicity studies

Study number: A2018030-T001-01

* 1. Appendix III: Assay result individual data

Study Name: ICR mouse gavage administration of sbk002 and phosphorous acid cyanohydroxyguanidine primary intermediates

Single-dose toxicity studies

Study number: A2018030-T001-01

* 1. Appendix IV: Pathology examination report

Study Name: ICR mouse gavage administration of sbk002 and phosphorous acid cyanohydroxyguanidine primary intermediates

Single-dose toxicity studies

Study number: A2018030-T001-01

* 1. Appendix V: study protocol and amendments

Study Name: ICR mouse gavage administration of sbk002 and phosphorous acid cyanohydroxyguanidine primary intermediates

Single-dose toxicity studies

Study number: A2018030-T001-01

* 1. Appendix Six: Temperature and Humidity Report

Study Name: ICR mouse gavage administration of sbk002 and phosphorous acid cyanohydroxyguanidine primary intermediates

Single-dose toxicity studies

Study number: A2018030-T001-01