FINAL REPORT

[redacted]

Assessment of Acute Dermal Toxicity with [redacted] in the Rat

[redacted]

TEST FACILITY:
Charles River Laboratories Den Bosch BV
Hambakenwetering 7
5231 DD ‘s-Hertogenbosch
The Netherlands

[redacted]

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[redacted]

LIST OF APPENDICES

QUALITY ASSURANCE STATEMENT

Study title: Assessment of Acute Dermal Toxicity with [redacted] in the Rat.

This report was inspected by the Test Facility Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s). The reported method and procedures were found to describe those used and the report reflects the raw data. The Test Facility inspection program was conducted in accordance with Standard Operating Procedure. During the on-site process inspections, procedures applicable to this type of study were inspected.

The dates of Quality Assurance inspections are given below.

[redacted]

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date to TFM and SD*
Study	Study Plan	13-Nov-2017	13-Nov-2017	13-Nov-2017
	Study Plan Amendment 01	11-Dec-2017	11-Dec-2017	11-Dec-2017
	Report	05-Feb-2018	05-Feb-2018	05-Feb-2018
Process	**Necropsy**	12-Sep-2017	26-Sep-2017	29-Sep-2017
	Observations/Measurements			
	Specimen Handling			
	Animal Facilities	02-Oct-2017	13-Oct-2017	18-Oct-2017
	Test Item Handling			
	Exposure			
	Observations/Measurements			
	Specimen Handling			
	Test Item Formulation	07-Nov-2017	16-Nov-2017	16-Nov-2017
	Test Item Handling			
	Test Item Receipt	07-Nov-2017	14-Nov-2017	16-Nov-2017
	Test Item Handling			

*TFM=Test Facility Management SD = Study Director

The review of the final report was completed on the date of signing this QA statement.

Britta van Vessem, MSC
Quality Assurance Auditor

BvVessem Date: 20 Feb 2018

COMPLIANCE STATEMENT AND REPORT APPROVAL

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

Exceptions from the above regulations are listed below.

- Analyses conducted to support the information cited in the Certificate of Analysis or equivalent document for the test item were not conducted in compliance with the GLP or Good Manufacturing Practice (GMP) regulations. The characterization of the test item was conducted in an ISO 9001 environment.

This study was conducted in accordance with the procedures described herein. There were no deviations from the study plan and standard operating procedures. The report represents an accurate and complete record of the results obtained. There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

Date: 21 Feb 2018

P.H.T. van Sas, MSc.

Study Director

[redacted]

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director	P.H.T. van Sas, MSc.
Test Facility Management	H.H. Emmen, MSc.

1.2. Sponsor

Sponsor Representative [redacted]

2. SUMMARY

The objective of this study was to determine the potential toxicity of [redacted], when given by a single dermal dose.

The study was carried out based on the guidelines described in:

- OECD No. 402 (1987) "Acute Dermal Toxicity"
- EC No 440/2008, part B: "Acute Toxicity (Dermal)"
- EPA, OPPTS 870.1200 (1998), "Acute Dermal Toxicity"
- JMAFF Guidelines (2000), including the most recent revisions.

[redacted] was administered to five Wistar rats of each sex by a single dermal application at 6420 mg active ingredient/kg body weight for 24 hours. Animals were subjected to daily observations and weekly determination of body weight. Macroscopic examination was performed after terminal sacrifice (Day 15).

No mortality occurred.

Chromodacryorrhoea of the snout was noted for two males and one female on Days 1 and/or 2. General erythema of the left flank, neck or treated skin and brown or yellow discoloration of the treated skin were seen for the animals during the observation period, these local effects were considered not to have affected the conclusion of the study.

The changes noted in body weight gain in males and females were within the range expected for rats used in this type of study and were therefore considered not indicative of toxicity.

No abnormalities were found at macroscopic post mortem examination of the animals.

The dermal LD50 value of [redacted] in Wistar rats was established to exceed 6420 mg active ingredient/kg body weight.

Based on these results, [redacted] does not have to be classified and has no obligatory labelling requirement for acute dermal toxicity according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments) and Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments).

3. INTRODUCTION

The objective of this study was to determine the potential toxicity of [redacted], when given by a single dermal dose. This study is intended to provide information on the potential health hazards of [redacted] and data produced can be used for classification/labelling of the test item. This study should provide a rational basis for risk assessment in man.

The design of this study is in compliance with the following study guidelines:

- OECD Guideline 402. *Acute Dermal Toxicity*, 1987.
- EPA Health Effects Test Guideline OPPTS 870.1200. *Acute Dermal Toxicity*, August 1998.
- EC No 440/2008 Part B. *Acute Toxicity (Dermal)*, May 2008.
- Appendix to Director General Notification, No. 12-Nousan-8147. Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan (JMAFF), November 2000, including the most recent revisions.

The Study Director signed the study plan on 13 Nov 2017, and dosing was initiated on 28 Nov 2017. The in-life phase of the study was completed on 12 Dec 2017. The experimental start date was 27 Nov 2017, and the experimental completion date was 12 Dec 2017. The study plan and amendment are presented in Appendix 3.

4. MATERIALS AND METHODS

4.1. Test item

4.1.1. Test Item

[redacted]

Appearance:	Brown viscous liquid

[redacted]

Purity/Composition:	44.4%
Test item storage:	At room temperature
Stable under storage conditions until:	09 August 2019 (expiry date)

Additional information

[redacted]

Purity/Composition correction factor:	Yes, correction factor is 2.25 based on active ingredient
Specific gravity / density:	0.935 g/cm^3 (15.6°C)

4.2. Test Item Characterization

The Sponsor provided to the Test Facility documentation of the identity, purity, composition, and stability for the test item(s). A Certificate of Analysis or equivalent document was provided to the Test Facility and is presented in Appendix 2.

4.3. Reserve Samples

For each batch (lot) of test item, a reserve sample (about 0.5 gram) was collected and maintained under the appropriate storage conditions by the Test Facility and destroyed after the expiration date.

4.4. Test and Reference Item Inventory and Disposition

Records of the receipt, distribution, and storage of test item(s) were maintained. With the exception of reserve samples, all unused Sponsor-supplied test item will be discarded or returned to the Sponsor after completion of the scheduled program of work. Records of the decisions made will be kept at the Test Facility.

4.5. Preparation of Test Item

The Test Item, [redacted], was administered as received.

Adjustment was made for specific gravity of the test item. A factor of 2.89 was used to correct for the purity/composition of the test item. After dosing the correction factor was changed to 2.25 based on the purity after new analysis by the Sponsor. Therefore, the actual dose was calculated at 6420 mg active ingredient/kg.

Any residual volumes were discarded.

4.6. Sample Collection and Analysis

The test item was used as received from the Sponsor; therefore, samples for dose formulation analysis were not collected by the Test Facility.

4.7. Test System

Species:	Rat
Strain:	Crl: WI(Han)
Condition:	Outbred, SPF-Quality
Source:	Charles River Deutschland, Sulzfeld, Germany
Number of Animals:	5 males and 5 females (females were nulliparous and non-pregnant).
Age at the Initiation of Dosing:	Young adult animals (approximately 10 weeks old) were selected.
Weight at the Initiation of Dosing:	Males: 274 to 287 g. Females: 182 to 191 g.

4.7.1. Justification for Test System and Number of Animals

The Wistar Han rat was chosen as the animal model for this study as recognized by international guidelines as a recommended test system. The test method and number of animals were based on the test guidelines.

This type of study plan was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee of Charles River Den Bosch as required by the Dutch Act on Animal Experimentation (February 1997).

4.7.2. Animal Identification

At study assignment, each animal was identified using a tail mark with indelible ink.

4.7.3. Environmental Acclimation

The animals were allowed to acclimate to the Test Facility toxicology accommodation for at least 5 days before the commencement of dosing.

4.7.4. Selection, Assignment, Replacement, and Disposition of Animals

Animals were assigned to the study at the discretion of the coordinating biotechnician according to body weights, with all animals within ± 20% of the sex mean. Animals in poor health or at extremes of body weight range were not assigned to the study.

Before the initiation of dosing, a health inspection was performed and any assigned animal considered unsuitable for use in the study were replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

The disposition of all animals was documented in the study records.

4.7.5. Husbandry

4.7.5.1. Housing

On arrival, animals were group housed (up to 5 animals of the same sex together) in polycarbonate cages (Makrolon MIV type; height 18 cm.) and following assignment to the study, animals were individually housed in polycarbonate cages (Makrolon MIII type; height 18 cm.) containing sterilized sawdust as bedding material (Lignocel S 8-15, JRS - J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) equipped with water bottles. The room(s) in which the animals were kept were documented in the study records.

Animals were separated during designated procedures/activities. Each cage was clearly labeled.

4.7.5.2. Environmental Conditions

Target temperatures of 18 to 24°C with a relative target humidity of 40 to 70% were maintained. The actual daily mean temperature during the study period was 20 to 21°C with an actual daily mean relative humidity of 43 to 51%. A 12-hour light/12-hour dark cycle was maintained. Ten or greater air changes per hour with 100% fresh air (no air recirculation) were maintained in the animal rooms.

4.7.5.3. Food

Pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) was provided ad libitum throughout the study, except during designated procedures.

The feed was analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis were provided by the supplier and are on file at the Test Facility.

It is considered that there were no known contaminants in the feed that would interfere with the objectives of the study.

4.7.5.4. Water

Municipal tap-water was freely available to each animal via water bottles.

Periodic analysis of the water was performed, and results of these analyses are on file at the Test Facility.

It is considered that there were no known contaminants in the water that would interfere with the objectives of the study.

4.7.5.5. Animal Enrichment

For psychological/environmental enrichment, animals were provided with paper (Enviro-dri, Wm. Lillico & Son (Wonham Mill Ltd), Surrey, United Kingdom), except when interrupted by study procedures/activities.

4.7.5.6. Veterinary Care

Veterinary care was available throughout the course of the study; however, no examinations or treatments were required.

4.8. Experimental Design

4.8.1. Administration of Test item

A single dose of test item was administered to the appropriate animals by dermal application on Day 1. One day before dosing, an area of approximately 5x7 cm on the back of the animals was clipped. The test item was applied in an area of approximately 10% of the total body surface, i.e. approximately 25 cm^2 for males and 18 cm^2 for females. The test item was held in contact with the skin with a dressing, consisting of a surgical gauze patch (Surgy 1D), successively covered with aluminum foil and Coban elastic bandage. A piece of Micropore tape was additionally used for fixation of the bandages in females only. The application period was 24 hours, after which the dressing was removed and the skin cleaned of residual test item using water.

The dose level was 6420 mg active ingredient/kg body weight.

The dose volume for each animal was based on the body weight measurement prior to dosing. Dose volume (mL/kg body weight) was calculated as follows:
Dose level (g/kg) / spec.gravity or density (g/mL) * purity correction factor.

The dosing formulations were stirred continuously during dose administration.

4.8.2. Justification of Route and Dose Levels

The dermal route was selected as it is a possible route of human exposure during manufacture, handling or use of the test item. The dose level was based on the OECD test guidelines.

4.9. In-life Procedures, Observations, and Measurements

4.9.1. Mortality/Moribundity Checks

Throughout the study, animals were observed for general health/mortality and moribundity twice daily, in the morning and at the end of the working day. Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings.

4.9.2. Clinical Observations

4.9.2.1. Postdose Observations

Postdose observations were performed at periodic intervals on the day of dosing (at least three times) and once daily thereafter. The observation period was 14 days.

All the animals were examined for reaction to dosing. The onset, intensity and duration of these signs was recorded (if appropriate), particular attention being paid to the animals during and for the first hour after dosing.

4.9.3. Body Weights

Animals were weighed individually on Day 1 (predose), 8 and 15.

4.10. Terminal Procedures

All moribund animals and animals surviving to the end of the observation period were sacrificed by oxygen/carbon dioxide procedure. All animals assigned to the study were subjected to necropsy and descriptions of all internal macroscopic abnormalities were recorded.

5. ANALYSIS

All results presented in the tables of the report are calculated using values as per the raw data rounding procedure and may not be exactly reproduced from the individual data presented.

The results can be evaluated according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (including all amendments) and the Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of items and mixtures (including all amendments).

6. COMPUTERIZED SYSTEMS

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

Text Table 1
Critical Computerized Systems

System name	Version No.	Description of Data Collected and/or Analyzed
REES Centron	SQL 2.0	Temperature, relative humidity and/or atmospheric pressure monitoring Animal and Laboratory facilities
ToxData	8.0	In-life phase (mortality; clinical signs; body weights) data collection

7. RETENTION OF RECORDS

All study-specific raw data, documentation, study plan and final report from this study were archived at the Test Facility by no later than the date of final report issue. At least five years after issue of the final report, the Sponsor will be contacted.

Electronic data generated by the Test Facility were archived as noted above.

8. RESULTS

For detailed results see Appendix 1.

8.1. Mortality

No mortality occurred.

8.2. Clinical Observations

Chromodacryorrhoea of the snout was noted for two males and one female on Days 1 and/or 2.

General erythema of the left flank, neck or treated skin and brown or yellow discoloration of the treated skin were seen for the animals during the observation period, these local effects were considered not to have affected the conclusion of the study.

8.3. Body Weights

The changes noted in body weight gain in males and females were within the range expected for rats used in this type of study and were therefore considered not indicative of toxicity.

8.4. Macroscopic Findings

No abnormalities were found at macroscopic post mortem examination of the animals.

9. CONCLUSION

The dermal LD50 value of [redacted] in Wistar rats was established to exceed 6420 mg active ingredient/kg body weight.

Based on these results, [redacted] does not have to be classified and has no obligatory labelling requirement for acute dermal toxicity according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2015) (including all amendments) and Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments).

[redacted]

Appendix 1
Tables

[redacted]

TABLE 1 MORTALITY DATA

TEST DAY HOURS AFTER TREATMENT	1 0	1 2	1 4	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MALES 6420 MG A.I./KG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 6420 MG A.I./KG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2 CLINICAL SIGNS

TEST DAY HOURS AFTER TREATMENT	MAX GRADE	1 0	1 2	1 4	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MALES 6420 MG A.I./KG																		
ANIMAL 1																		
Various																		
Brown (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 2																		
Skin / fur																		
General erythema (Flank left)	(4)	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Various																		
Brown (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 3																		
Secretion / excretion																		
Chromodacryorrhoea (Snout)	(3)	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Various																		
Brown (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 4																		
Secretion / excretion																		
Chromodacryorrhoea (Snout)	(3)	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Various																		
Brown (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 5																		
Various																		
Brown (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
FEMALES 6420 MG A.I./KG																		
ANIMAL 6																		
Various																		
Yellow (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 7																		
Skin / fur																		
General erythema (Treated skin)	(4)	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Various																		
Yellow (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 8																		
Skin / fur																		
General erythema (Neck)	(4)	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Secretion / excretion																		
Chromodacryorrhoea (Snout)	(3)	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Various																		
Yellow (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 9																		
Various																		
Yellow (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-
ANIMAL 10																		
Various																		
Yellow (Treated skin)	(1)	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-

- = Sign not observed

[redacted]

TABLE 3 BODY WEIGHTS (GRAM)

SEX/DOSE LEVEL	ANIMAL	DAY 1	DAY 8	DAY 15
MALES 6420 MG A.I./KG				
	1	284	303	339
	2	287	319	361
	3	274	306	355
	4	279	303	327
	5	283	313	354
	MEAN	281	309	347
	ST.DEV.	5	7	14
	N	5	5	5
FEMALES 6420 MG A.I./KG				
	6	191	192	201
	7	186	190	200
	8	188	186	195
	9	182	194	201
	10	187	192	202
	MEAN	187	191	200
	ST.DEV.	3	3	3
	N	5	5	5

TABLE 4 MACROSCOPIC FINDINGS

ANIMAL ORGAN	FINDING	DAY OF DEATH
MALES 6420 MG A.I./KG		
1	No findings noted	Scheduled necropsy Day 15 after treatment
2	No findings noted	Scheduled necropsy Day 15 after treatment
3	No findings noted	Scheduled necropsy Day 15 after treatment
4	No findings noted	Scheduled necropsy Day 15 after treatment
5	No findings noted	Scheduled necropsy Day 15 after treatment
FEMALES 6420 MG A.I./KG		
6	No findings noted	Scheduled necropsy Day 15 after treatment
7	No findings noted	Scheduled necropsy Day 15 after treatment
8	No findings noted	Scheduled necropsy Day 15 after treatment
9	No findings noted	Scheduled necropsy Day 15 after treatment
10	No findings noted	Scheduled necropsy Day 15 after treatment

[redacted]

Appendix 2
Test Item Characterization

[redacted]

[redacted]

[redacted]

Appendix 3
Study Plan and Amendment

FINAL STUDY PLAN

[redacted]

Assessment of Acute Dermal Toxicity with [redacted] in the Rat

[redacted]

TEST FACILITY:
Charles River Laboratories Den Bosch B.V.
Hambakenwetering 7
5231 DD ‘s-Hertogenbosch
The Netherlands

TABLE OF CONTENTS

1. OBJECTIVE(S)

The objective of this study is to determine the potential toxicity of [redacted], when given by a single dermal dose. This study is intended to provide information on the potential health hazards of [redacted] and data produced can be used for classification/labelling of the test item. This study should provide a rational basis for risk assessment in man.

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report.

Experimental Start Date:	20 Nov 2017 (Week 47) (First date of study-specific data collection; weighing of test item)
Experimental Completion Date:	21 Jan 2018 (Week 03) (Last date data are collected from the study; necropsy)
Initiation of Dosing	27 Nov 2017 (Week 48)
Completion of In-life	14 Jan 2018 (Week 02) (Last date of necropsy)
Unaudited Draft Report:	28 Jan 2018 (Week 04)

3. GUIDELINES FOR STUDY DESIGN

The design of this study was based on the study objective(s), the overall product development strategy for the test item and in compliance with the following study design guidelines:

- OECD Guideline 402. *Acute Dermal Toxicity*, 1987.
- EPA Health Effects Test Guideline OPPTS 870.1200. *Acute Dermal Toxicity*, August 1998.
- EC No 440/2008 Part B. *Acute Toxicity (Dermal)*, May 2008.
- Appendix to Director General Notification, No. 12-Nousan-8147. Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan (JMAFF), November 2000.

4. REGULATORY COMPLIANCE

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

5. QUALITY ASSURANCE

5.1. Test Facility

The Test Facility Quality Assurance Unit (QAU) will monitor the study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with Good Laboratory Practice regulations. The QAU will review the study plan, conduct study and/or process inspections at intervals adequate to assure the integrity of the study, and audit the Final Report to assure that it accurately describes the methods and standard operating procedures and that the reported results accurately reflect the raw data of the study.

The Test Facility QAU contact is indicated below:

C.J. Mitchell, BSc.
Address as cited for Test Facility
Tel: +31 73 640 6700
E-mail: Christine.Mitchell@crl.com

6. SPONSOR

Sponsor Representative / Sponsor Study Monitor

[redacted]

7. RESPONSIBLE PERSONNEL

Study Director

P.H.T. van Sas, MSc.
address as cited for Test Facility
Tel: +31 73 640 6700
E-mail: Pieter.vanSas@crl.com

Management Contact

H.H. Emmen, MSc.
address as cited for Test Facility
Tel: +31 73 640 6700
E-mail: Harry.Emmen@crl.com

8. TEST ITEM

8.1. Test Item

[redacted]

Appearance:	Brown viscous liquid

[redacted]

Purity/Composition:	34.6%
Test item storage:	At room temperature
Stable under storage conditions until:	09 August 2019 (expiry date)

Additional information

[redacted]

Purity/Composition correction factor:	Yes, correction factor is 2.89 based on active ingredient
Specific gravity / density:	0.935 g/cm^3 (15.6°C)

8.2. Test Item Characterization

The Sponsor will provide to the Test Facility documentation of the identity, purity, composition, and stability for the test item. If available, a Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report. The Sponsor will also provide information concerning the regulatory standard that was followed for these evaluations.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test item, and this information is available to the appropriate regulatory agencies should it be requested.

8.3. Analysis of Test Item

The stability of the bulk test item will not be determined during the course of this study. Information to support the stability of each lot of the bulk test item will be provided by the Sponsor.

8.4. Reserve Samples

For each batch (lot) of test item, if practically possible a reserve sample will be collected and maintained under the appropriate storage conditions by the Test Facility and destroyed after the expiration date.

8.5. Test Item and Vehicle Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test item will be maintained.

9. SAFETY

The following safety instructions apply to this study:

Standard safety precautions specified in Charles River Den Bosch procedures.

10. DOSE FORMULATION AND ANALYSIS

10.1. Preparation of Test Item

The test item, [redacted] will be administered as received.

Adjustment will be made for specific gravity of the test item. A factor of 2.89 will be used to correct for the purity/composition of the test item.

Any residual volumes will be discarded unless otherwise requested by the Study Director.

10.2. Sample Collection and Analysis

The test item will be used as received from the Sponsor; therefore, samples for dose formulation analysis will not be collected by the Test Facility.

11. TEST SYSTEM

Species:	Rat
Strain:	Crl: WI(Han)
Condition:	Outbred, SPF-Quality
Source:	Based on availability, one of the following sources will be used and specified in the report: • Charles River France, L'Arbresle, France • Charles River Deutschland, Sulzfeld, Germany
Number of Animals:	5 males and 5 females per dose group (females will be nulliparous and non-pregnant).
Target Age at the Initiation of Dosing:	Between 10 and 12 weeks old. Animals to be used within the study will be of approximately the same age.
Target Weight at the Initiation of Dosing:	230 to 380 g (males) and 130 to 280 (females).

The actual age and weight of animals dosed will be listed in the Final Report.

11.1. Justification of Test System and Number of Animals

The Wistar Han rat was chosen as the animal model for this study as recognized by international guidelines as a recommended test system (e.g. OECD, FDA, MHW). The test method and number of animals are based on the test guidelines.

11.2. Animal Identification

At study assignment, each animal will be identified using a tail mark with indelible ink.

11.3. Environmental Acclimation

The animals will be allowed to acclimate to the Test Facility toxicology accommodation for at least 5 days before the commencement of dosing.

11.4. Selection, Assignment, Replacement, and Disposition of Animals

Animals will be assigned to the study at the discretion of the coordinating biotechnician according to body weights, with all animals within ± 20% of the sex mean. Animals in poor health or at extremes of body weight range will not be assigned to the study.

Before the initiation of dosing, a health inspection will be performed and any assigned animals considered unsuitable for use in the study will be replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

The disposition of all animals will be documented in the study records.

12. HUSBANDRY

12.1. Housing

On arrival, animals will be group housed (up to 5 animals of the same sex together) in polycarbonate cages (Makrolon MIV type; height 18 cm.) and following assignment to the study, animals will be individually housed in polycarbonate cages (Makrolon MIII type; height 18 cm.) containing sterilized sawdust as bedding material (Lignocel S 8-15, JRS - J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) equipped with water bottles. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room(s) in which the animals will be kept will be documented in the study records.

Animals will be separated during designated procedures/activities. Each cage will be clearly labeled.

12.2. Environmental Conditions

The target conditions for animal room environment will be as follows:

Temperature:	18 to 24°C
Humidity:	40 to 70%
Light Cycle:	12-hours light and 12-hours dark (except during designated procedures)
Ventilation:	At least 10 air changes per hour

12.3. Food

Pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) will be provided ad libitum throughout the study, except during designated procedures.

The feed is analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and are on file at the Test Facility.

It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

12.4. Water

Municipal tap-water will be freely available to each animal via water bottles.

Periodic analysis of the water is performed, and results of these analyses are on file at the Test Facility.

It is considered that there are no known contaminants in the water that would interfere with the objectives of the study.

12.5. Animal Enrichment

For psychological/environmental enrichment, animals will be provided with paper (Enviro-dri, Wm. Lillico & Son (Wonham Mill Ltd), Surrey, United Kingdom), except when interrupted by study procedures/activities.

12.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

13. EXPERIMENTAL DESIGN

13.1. Administration of Test item

A single dose of test item will be administered to the appropriate animals by dermal application on Day 1. One day before dosing, an area of approximately 5x7 cm on the back of the animals will be clipped. The test item will be applied in an area of approximately 10% of the total body surface, i.e. approximately 25 cm^2 for males and 18 cm^2 for females. The test item will be held in contact with the skin with a dressing, consisting of a surgical gauze patch (Surgy 1D), successively covered with aluminum foil and Coban elastic bandage. A piece of Micropore tape will additionally be used for fixation of the bandages in females only. The application period will be 24 hours, after which the dressing will be removed and the skin cleaned of residual test item using water or an appropriate vehicle.

The dose level will be 5000 mg/kg body weight.

The dose volume for each animal will be based on the body weight measurement prior to dosing. Dose volume (mL/kg body weight) will be calculated as follows:
Dose level (g/kg) / spec.gravity or density (g/mL) * purity correction factor.

The dosing formulations will be stirred continuously during dose administration.

13.2. Justification of Route and Dose Levels

The dermal route is selected as it is a possible route of human exposure during manufacture, handling or use of the test item. The dose level is based on the OECD test guidelines.

14. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS

14.1. Mortality/Moribundity Checks

Frequency:	Twice daily throughout the study.
Procedure:	Animals will be observed for general health/mortality and moribundity. Animals will not be removed from cage during observation, unless necessary for identification or confirmation of possible findings.

14.2. Clinical Observations

14.2.1. Postdose Observations

Frequency:	At periodic intervals on the day of dosing (at least three times) and at least once daily thereafter. The observation period will be 14 days.
Procedure:	All the animals will be examined for reaction to dosing. The onset, intensity and duration of these signs will be recorded (if appropriate), particular attention being paid to the animals during and for the first hour after dosing.

14.3. Body Weights

Frequency:	On Days 1 (predose), 8 and 15.
Procedure:	Animals will be individually weighed. Terminal body weights will also be collected from animals if found dead or euthanized moribund after Day 1.

15. TERMINAL PROCEDURES

All moribund animals and animals surviving to the end of the observation period will be sacrificed by oxygen/carbon dioxide procedure. All animals assigned to the study are subjected to necropsy and descriptions of all internal macroscopic abnormalities will be recorded.

16. ANALYSIS

A dermal LD50 value will be derived.

The results can be evaluated according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (including all amendments) and the Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of items and mixtures (including all amendments).

17. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by study plan, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by study plan and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

Critical Computerized Systems

System Name	Description of Data Collected and/or Analyzed
REES Centron	Temperature and Humidity (Animal and Laboratory facilities) Data Collection
TOXDATA	In-life phase (mortality; clinical signs; body weights) data collection

18. AMENDMENTS AND DEVIATIONS

Changes to the approved study plan shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary study plan changes in advance with the Sponsor.

All study plan and SOP deviations will be documented in the study records. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

19. RETENTION OF RECORDS

All study-specific raw data, electronic data, documentation, study plan and final reports will be archived by no later than the date of Final Report issue. All materials generated by Charles River from this study will be transferred to a Charles River archive. At least five years after issue of the Final Report, the Sponsor will be contacted.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Study plan, study plan amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system, health, and husbandry
- Test item receipt, identification, preparation
- In-life measurements and observations
- Gross observations and related data

20. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft Report. The Final Report will be provided in Adobe Acrobat PDF format (hyperlinked and searchable) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Facility's handwritten signatures will be retained. The Sponsor will receive a paper copy of the report.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Test Facility unless other arrangements are made by the Sponsor.

21. ANIMAL WELFARE

This type of study plan was reviewed and agreed by the Laboratory Animal Welfare Officer and the Ethical Committee (DEC 14-19) as required by the Dutch Act on Animal Experimentation (February 1997).

Animals showing pain, distress or discomfort, which is considered not transient in nature or is likely to become more severe, will be sacrificed for humane reasons based on OECD guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (ENV/JM/MONO/ 2000/7).

By approving this study plan, the Sponsor affirms that this study is required by a relevant government regulatory agency and that it does not unnecessarily duplicate any previous experiments.

TEST FACILITY APPROVAL

The signature below acknowledges Test Facility Management's responsibility to the study as defined by the relevant GLP regulations.

Date: 15-Mar-2017

K. Scase, MSc.
Section Head General Toxicology

The signature below indicates that the Study Director approves the study plan.

Date: 13-Nov-2017

P.H.T. van Sas, MSc.
Study Director

SPONSOR APPROVAL

The signature of the Sponsor Representative below indicates approval of this study plan.

[redacted]

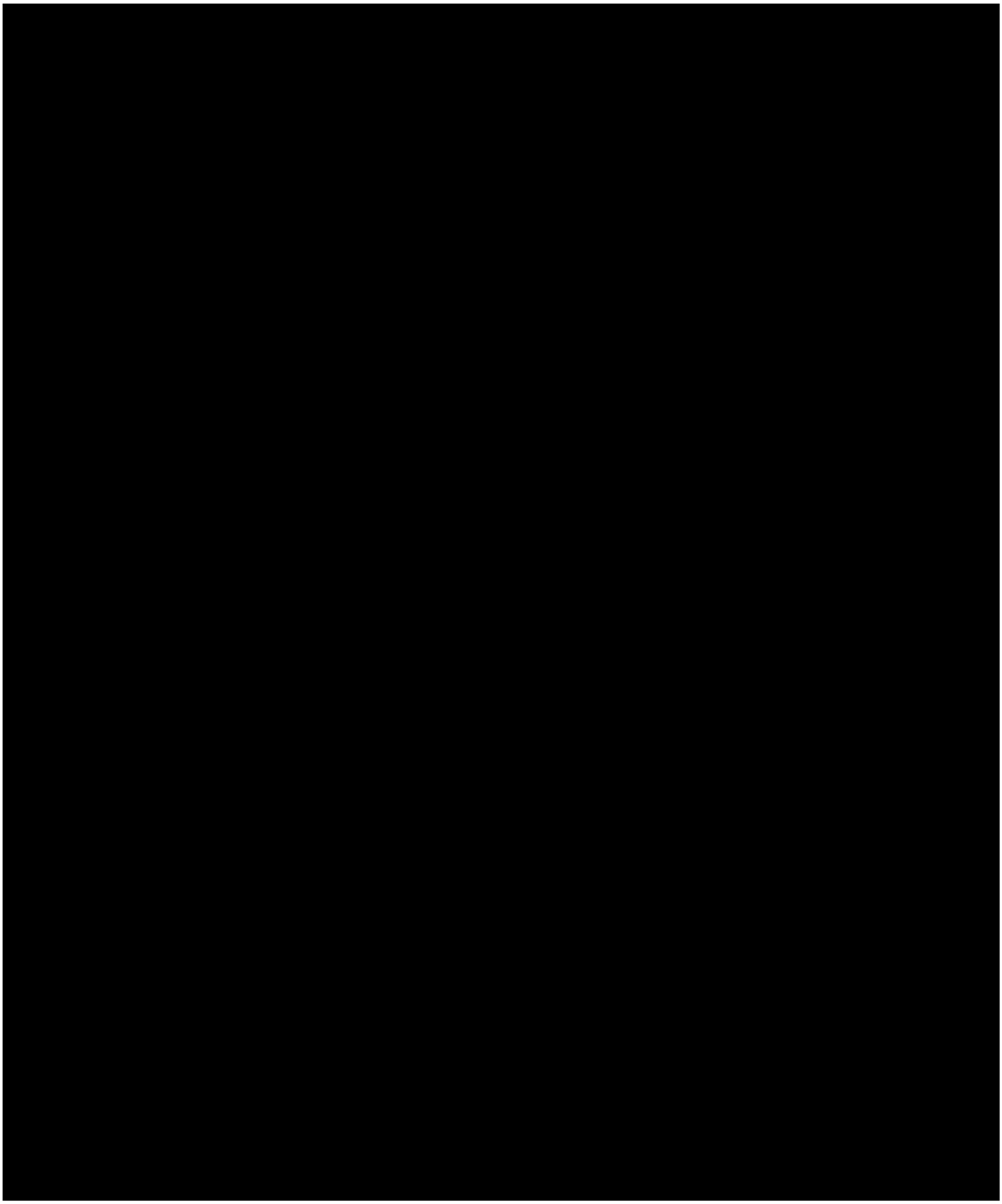
[redacted]

ATTACHMENT A

Distribution List

Electronic copies will be supplied unless otherwise specified below.

Version	**Recipient**	
Original	Study Director	
1 Copy	Sponsor Representative / Study Monitor	
1 Copy	QAU / Management	
1 Copy	Necropsy	Baartman, M;
1 Copy	Necropsy	van der Steen, A;
1 Copy	Formulations	Tsfher;
1 Paper Copy	Cordinating Biotechnician	van Voorden, K;



charles river

STUDY PLAN AMENDMENT NUMBER: 1

Study Title	Assessment of Acute Dermal Toxicity with [redacted] in the Rat
Sponsor	[redacted]
Study Monitor	
Test Facility Test Item No.	
Test Facility Study No	

AMENDMENT DESCRIPTION

1. Page 5, **8.1. Test Item:**

Purity/Composition:	44.4%
Purity/Composition correction factor:	Yes, correction factor is 2.25 based on active ingredient

REASONS FOR AMENDMENT

1. The data has been changed according to information of the sponsor, dated 30 November 2017. This has no effect on other data.

APPROVAL

Study director

Petervansas ..... 07 Dec 2017 .....

P.H.T. van Sas, MSc. Date

[redacted]