Determination of in vivo acute eye irritation/corrosion potential of manganese chelate of lysine.

Aleksandra Pawlik, Panos Voudouris and Theo Verkleij

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Determination of *in vivo* acute eye irritation/corrosion potential of manganese chelate of lysine.

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Institute: Wageningen Food & Biobased Research

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Experiments were carried out by Charles River Laboratories Den Bosch BV

Wageningen Food & Biobased Research
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Summary

A new product is developed by Metex Noovistago (Metex) and is aimed for animal nutrition. The new product is a manganese chelate of lysine. The evaluation of the eye irritation/corrosion potential, relevant to workers safety aspects for the handling of this product, is an important aspect for Metex. To evaluate the eye irritation/corrosion potential, the test item was assessed in the conjunctival sac of the rabbit's eye. The study described in this report conforms to the most recent eye irritation/corrosion tests guidelines [1-4].

A single dose of 76.2 mg of manganese chelate of lysine was installed into an eye of a sentinel rabbit and the observations were made for 21 days following the exposure. The installation of the test item resulted in severe toxicological effects on the cornea and conjunctivae, with no evidence of ocular corrosion. Since the observed irritation did not resolve completely in the sentinel animal within the observation period of 21 days, the test item was considered to have irreversible toxicological effects on the eye and furthermore no other animals could be exposed via the conjunctival sac.

Based on these results and according to the guideline/regulations [5-6], the manganese chelate of lysine should be:

- classified as Category 1 for having irreversible effects on the eyes.
- labeled as H318: Causes serious eye damage.

1 Introduction

A new product is developed by Metex Noovistago (Metex) with the aim to be used for animal nutrition. The new product is a manganese chelate of lysine. In this report, the above-described product will be referred as “manganese chelate of lysine” or as “test item”.

The objective of this study was to assess the possible irritation or corrosion potential of the manganese chelate of lysine when administered as a single dose into the conjunctival sac of the rabbit’s eye. This study provides information that can be used for classification and labelling of the test item.

The design of this study is based on the following study guidelines:

- OECD Guideline 405. Acute Eye Irritation/Corrosion, 2021 [1]
- EPA Health Effects Test Guideline OPPTS 870.2400. Acute Eye Irritation, August 1998 [2]
- EC No 440/2008 Part B. Acute Toxicity, Eye Irritation/Corrosion, May 2008 [3]
- 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 [4]

In short, the test item is installed, as one dose only, into the conjunctival sac of the rabbit’s eye and observations are made approximately 1, 24, 48 and 72 hours and 7, 14 and 21 days after the exposure to the test item.

The experimental part was outsourced to a third party – Charles River Laboratories Den Bosch BV - while results and conclusions were assembled by WFBR in the present report.

2 Methods

2.1 Test system

The test system consisted of a rabbit, New Zealand White strain, SPF-Quality, sourced from Charles River France, L'Arbresle, France. Exposure group consisted of 1 animal, appr. 12-13 weeks old, male.

The New Zealand White rabbit was chosen as the animal model for this study being recognized by international guidelines as a recommended test system (e.g., OECD, FDA, MHLW). The test method and number of animals were based on the test guidelines. The ocular route was selected because the test item may accidentally come into contact with the human eyes during manufacture, handling and/or use. This study was designed such that it did not require an unnecessary number of animals to accomplish its objectives. The study plan was reviewed and agreed by the Animal Welfare Body of Charles River Laboratories Den Bosch B.V.

Animal husbandry details including housing, environmental conditions, food, water, animal enrichment and veterinary care were closely monitored and are described in the report of Charles River Laboratories Den Bosch BV (Annex 1).

2.2 Test item

The test item, manganese chelate of lysine, was delivered by Metex to Wageningen Food & Biobased Research (WFBR). WFBR stored the test item for 3 months at ambient conditions. 200g of the test item was shipped to Charles River Laboratories Den Bosch BV, the third party, which carried out the evaluation of the acute eye irritation/corrosion.

The experimental study began on 20th June 2023 and was completed on 17th July 2023.

2.3 Experimental design and administration procedure

The study was performed following a stepwise exposure scenario and was started on a single (sentinel) rabbit.

As a part of the pre-emptive pain management strategy, one hour prior to the instillation of the test item, buprenorphine (Buprenodale®) 0.01 mg/kg was administered by subcutaneous injection, in order to provide a therapeutic level of systemic analgesia. Five minutes prior to the instillation of the test item, two drops of the topical anaesthetic 0.5% proparacaine hydrochloric ophthalmic solution (Tetracaine eye drops®) were applied to both rabbit's eyes.

The animal was treated by instillation of 76.2 mg of the test item (a volume of approximately 0.1 mL, as described by the guidelines), in the conjunctival sac of one of the eyes after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test item. The other eye remained untreated and served as the reference (i.e., negative) control.

Immediately after the 1-hour observation, the treated eye was rinsed with approximately 50 mL tepid tap water, using a velocity of flow which did not affect the eye, to remove any visible residual test item. For reference control the other eye was also rinsed.

Additional injections of buprenorphine 0.01 mg/kg were administered immediately after the 1-hour observation and at the end of the first day, to reduce pain and distress. In order to provide a continued level of systemic analgesia, buprenorphine 0.01 mg/kg and meloxicam (Metacam®, Boehringer Vetmed GmbH, Ingelheim/Rhein, Germany) 0.5 mg/kg were administered by subcutaneous injection. Additional injections of buprenorphine 0.01 mg/kg and meloxicam 0.5 mg/kg were administered immediately after the 48-hour observation to reduce pain and distress, following the internal Standard Procedure for pain assessment.

Immediately after the 24-hour observation, a solution of 2% fluorescein (Minims® fluoresceinesodium, 20 mg/ml solution) was introduced into both eyes of the animal to quantitatively determine corneal epithelial damage. This procedure was repeated to assess recovery. Any bright green stained area, indicating epithelial damage, was estimated as a percentage of the total corneal area.

2.4 In-life procedures, observations and measurements

Throughout the study, the animal was observed for general health/mortality and moribundity twice daily, in the morning and at the end of the working day.

Observations for toxicity were performed once daily throughout the study.

The animal was weighed on day 1 (pre-dose) and on the day of the final observation.

The eyes of the animal were examined approximately 1, 24, 48 and 72 hours and 7, 14 and 21 days after instillation of the test item. The irritation scores and a description of all other (local) effects were recorded.

The eye irritation was assessed according to the numerical scoring system, where at each observation point the highest given scores were recorded.

In cases where the standard lighting was considered inadequate for observing minor effects, eye examinations were performed using an ophthalmic examination lamp.

After the final observation, the animal was euthanized according to laboratories Standard Operating Procedures.

A detailed description on the test system preparation, handling and exposure details along with the specifics on data collection and calculations can be found on the report of Charles River Laboratories Den Bosch BV provided in Annex 1.

3 Results

3.1 Irritation and corrosion

Instillation of 76.2 mg of the manganese chelate of lysine (a volume of approximately 0.1 mL) into one eye of the sentinel animal resulted in effects on the cornea and conjunctivae.

The corneal injury consisted of opacity and epithelial damage. The corneal injury resolved within 7 days.

The irritation of the conjunctivae consisted of redness, chemosis and discharge, which did not resolve completely within 21 days. In addition, reduced flexibility of the eye lid was noted and was resolved within 7 days.

There was no evidence of ocular corrosion.

Since the observed conjunctival irritation did not resolve completely in the sentinel animal within the observation period of 21 days, the test item was considered to have irreversible toxicological effects on the eye and no other animals could be exposed *via* the conjunctival sac.

3.2 Coloration/Remnants

Remnants of the test item were present in the eye and on the outside of the eyelids between 1 hour and 7 days after the installation. This is due to innate protective physiological mechanisms of the eye against entry of foreign compounds.

3.3 Toxicity/Mortality

No signs of systemic toxicity were observed in the animal during the test period and no mortality occurred.

For detailed information on data collection, calculations and recordings see Annex 1, which refers to the report of Charles River Laboratories Den Bosch BV.

4 Conclusions

The objective of this acute eye irritation study was to assess the eye irritation or corrosion potential of the manganese chelate of lysine in humans, following a single exposure in rabbit, as a model animal. The test item of a defined concentration was placed in the conjunctival sac of a sentinel, New Zealand White rabbit and the animal was retained for a 21-day post exposure observation period. Gathered data provided information on the potential health hazards of the manganese chelate of lysine and can be used for classification/labelling of the test item, thus a rational basis for risk assessment in human.

The study results showed severe eye effects in the sentinel animal. For animal welfare reasons, the other animals were therefore not exposed to the test item. Since the observed irritation did not resolve completely within the 21-day observation period in the sentinel animal, the test item was considered to have irreversible toxicological effects on the eye.

Based on these results and according to the guideline/regulations [5-6], the manganese chelate of lysine:

- should be classified as Category 1 for having irreversible effects on the eyes.
- should be labeled as H318: Causes serious eye damage.

Literature

1. OECD Guideline 405. Acute Eye Irritation/Corrosion, 2021.
2. EC No 440/2008 Part B. Acute Toxicity, Eye Irritation/Corrosion, May 2008.
3. EPA Health Effects Test Guideline OPPTS 870.2400. Acute Eye Irritation, August 1998.
4. 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.
5. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), United Nations, New York and Geneva (2021) (including all amendments).
6. Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments).

Annex 1 Charles River report

FINAL REPORT

Test Facility Study No. 20418373

Acute Eye Irritation/Corrosion Study with Chélate-Manganese in the Rabbit

GLP

SPONSOR:
Wageningen Food & Biobased Research
P.O.Box 17,
6700 AA Wageningen
The Netherlands

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

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QUALITY ASSURANCE STATEMENT

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.

Test Facility Study No. 20418373

Type of Inspections	**Phase/Process**	**Start Inspection date**	**End Inspection date**	**Reporting date to TFM and SD***
Study				
	Final Study Plan	16-Jun-2023	16-Jun-2023	19-Jun-2023
	Report	22-Aug-2023	23-Aug-2023	25-Aug-2023
	Final Report	02-Nov-2023	02-Nov-2023	02-Nov-2023
Process				
	Animal Facilities	05-Apr-2023	28-Apr-2023	28-Apr-2023
		05-May-2023	30-May-2023	30-May-2023
		09-Jun-2023	30-Jun-2023	30-Jun-2023
	Test Item Handling			
	Exposure			
	Observations/Measurements			
	Specimen Handling			
	Test Item Formulation	06-Jun-2023	08-Jun-2023	08-Jun-2023
		16-Jun-2023	23-Jun-2023	23-Jun-2023
		05-Jul-2023	07-Jul-2023	07-Jul-2023
	Test Item Handling			
	Test Item Receipt	17-Mar-2023	27-Mar-2023	27-Mar-2023
		12-Jun-2023	21-Jun-2023	21-Jun-2023
	Test Item Handling			

*TFM=Test Facility Management SD = Study Director

All electronic signatures appear at the end of this Report upon finalization.

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 (FDA and EPA), Japan (MHLW, MAFF and METI) and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions from the above regulations are listed below.

- No Certificate of Analysis or analytical report was available. However, sufficient information was available regarding the batch number, expiry date, composition, physical description and the test material was stored under the conditions described. Based on the available information it was considered that the study integrity was not affected by this.

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.

All electronic signatures appear at the end of the document upon finalization.

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Role/Phase	Quality Assurance Unit	Name	Contact Information
Study Director	Charles River	Sandra van de Wiel, PhD	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: Sandra.vandeWiel@crl.com
Test Facility Management	Charles River	Harry Emmen, MSc	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: Harry.Emmen@crl.com
Test Facility QAU	Charles River	Lead QA	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: QADenBosch@crl.com

2. SUMMARY

The objective of this acute eye irritation study was to assess the possible irritation or corrosion potential when a single dose of Chélate-Manganese was placed in the conjunctival sac of the rabbit eye.

The study was carried out in compliance with the guidelines described in:

- OECD No.405 (2021) "Acute Eye Irritation / Corrosion".
- EC No 440/2008, part B: "Acute Toxicity: Eye Irritation/Corrosion".
- EPA, OPPTS 870.2400 (1998), "Acute Eye Irritation".
- JMAFF Guidelines (2000), including the most recent revisions.

A single sample of 76.2 mg of Chélate-Manganese (a volume of approximately 0.1 mL) was instilled into one eye of one rabbit. Observations were made 1, 24, 48 and 72 hours and 7, 14 and 21 days after instillation.

Instillation of the test material resulted in effects on the cornea and conjunctivae. The corneal injury consisted of opacity and epithelial damage. The corneal injury resolved within 7 days. The irritation of the conjunctivae consisted of redness, chemosis and discharge, which did not resolve completely within 21 days. In addition, reduced flexibility of the eye lid was noted and was resolved within 7 days. There was no evidence of ocular corrosion.

Exposure of the sentinel animal to the test material resulted in severe eye effects. For animal welfare reasons, the other animals could therefore not be exposed via the conjunctival sac. Since the observed irritation did not resolve completely within 21 days in the sentinel animal, the test material was considered to have irreversible toxicological effects on the eye.

- According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2021) (including all amendments), Chélate-Manganese should be classified as: having irreversible effects on the eyes (Category 1).
- According to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments), Chélate-Manganese should be classified as Irreversible effects on the eye (Category 1) and labeled as H318: Causes serious eye damage.

3. INTRODUCTION

The objective of this acute eye irritation study was to assess the possible irritation or corrosion potential when a single dose of Chélate-Manganese was placed in the conjunctival sac of the rabbit eye. This study is intended to provide information on the potential health hazards of Chélate-Manganese and data produced can be used for classification/labelling of the test material. 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 405. *Acute Eye Irritation/Corrosion*, 2021.
- EPA Health Effects Test Guideline OPPTS 870.2400. *Acute Eye Irritation*, August 1998.
- EC No 440/2008 Part B. *Acute Toxicity, Eye Irritation/Corrosion*, 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 plan is presented in Appendix 2.

Study Initiation Date:	14 Jun 2023
Initiation of Dosing:	20 Jun 2023
Completion of In-life:	17 Jul 2023
Experimental Start Date:	20 Jun 2023
Experimental Completion Date:	17 Jul 2023

4. MATERIALS AND METHODS

4.1. Test Material

Identification:	Chélate-Manganese
Batch (Lot) Number:	Chélate-Manganese
Expiry date:	01 June 2025
Physical Description:	Brown powder (determined by Charles River Den Bosch)
Purity/Composition:	Manganese: 19%, lysine: 23%, Sulfates: 39%
Storage Conditions:	At room temperature

Additional information

Quality system or regulatory standard for CoA or equivalent document:	Not applicable
Test Facility test material number:	500476/A
Purity/Composition correction factor:	No correction factor required
Test material handling:	No specific handling conditions required

4.2. Test Material Characterization

The Sponsor provided to the Test Facility documentation of the identity, purity, composition, and stability for the test material. The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test material, and this information is available to the appropriate regulatory agencies should it be requested.

4.3. Reserve Samples

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

4.4. Test and Reference Material Inventory and Disposition

Records of the receipt, distribution, and storage of test material were maintained. With the exception of reserve samples, all unused Sponsor-supplied test material 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 Material

The powdery test material was instilled as delivered by the Sponsor. No correction was made for the purity/composition of the test material, since the guidelines require a fixed amount to be instilled.

4.6. Sample Collection and Analysis

The test material 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:	Rabbit
Strain:	New Zealand White
Condition:	SPF-Quality
Source:	Charles River France, L'Arbresle, France
Number of Animals:	1 Male.
Age at the Initiation of Dosing:	Young adult animal (approximately 12-13 weeks old) was selected.
Weight at the Initiation of Dosing:	2691 g.

4.7.1. Justification for Test System and Number of Animals

The New Zealand White rabbit was chosen as the animal model for this study as recognized by international guidelines as a recommended test system (e.g. OECD, FDA, MHLW). The test method and number of animals were based on the test guidelines.

The study plan was reviewed and agreed by the Animal Welfare Body of Charles River Laboratories Den Bosch B.V. within the framework of Appendix 1 of project license AVD23600202216274 approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation (December 2014).

4.7.2. Animal Identification

At study assignment, each animal was identified using an ear mark with indelible ink.

4.7.3. Environmental Acclimation

The animal was 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, with all animals within ± 20% of the sex mean body weights. 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 was replaced by alternate animal 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 and following assignment to the study, the animal was housed individually in labeled cages with perforated floors (Ebeco, Germany, dimensions 67 x 62 x 55 cm) equipped with water bottles. The rooms in which the animal was kept were documented in the study records. The cage was clearly labeled.

4.7.5.2. Environmental Conditions

Target temperatures of 17 to 21°C with a relative target humidity of 40 to 70% were maintained. The actual daily mean temperature during the study period was 19 to 20°C with an actual daily mean relative humidity of 51 to 82%. The values that were outside the targeted mean humidity range occurred for several days and were without a noticeable effect on the clinical condition of the animal or on the outcome of the study. 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 diet for rabbits (KLIBA NAFAG Rabbit Diet 3409 maintenance and breeding, from Granovit AG, Kaiseraugst, Switzerland) was provided once daily throughout the study. In addition, hay (TecniLab-BMI BV, Someren, The Netherlands) was available during the study period.

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, the animal was provided with a shelter (Ebeco, Germany, dimensions 40 x 32 x 23 cm) and wooden sticks (Swedish aspen wood, Bioservices, Schaijk, The Netherlands) 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

The study was performed in a stepwise manner and was started by treatment of a single rabbit (sentinel).

4.9. Pre - Emptive Pain Management

One hour prior to instillation of the test material, buprenorphine (Buprenodale®) 0.01 mg/kg was administered by subcutaneous injection in order to provide a therapeutic level of systemic analgesia.

Five minutes prior to instillation of the test material, two drops of the topical anaesthetic 0.5% tetracaine hydrochloric ophthalmic solution (Tetracaine eye drops®) were applied to both eyes.

4.10. Administration of Test material

The animal was treated by instillation of 76.2 mg of the test material (a volume of approximately 0.1 mL), in the conjunctival sac of one of the eyes after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test material. The other eye remained untreated and served as the reference control.

Immediately after the 1-hour observation, the treated eye was rinsed with approximately 50 mL tepid tap water, using a velocity of flow which did not affect the eye, to remove any visible residual test material. For reference control the other eye was also rinsed. Additional injections of buprenorphine 0.01 mg/kg were administered immediately after the 1-hour observation and at the end of the first day to reduce pain and distress.

Immediately after the 24-hour observation, a solution of 2% fluorescein (Minims® fluoresceinesodium, 20 mg/ml solution) was instilled into both eyes of each animal to quantitatively determine corneal epithelial damage. This procedure was repeated to assess recovery. Any bright green stained area, indicating epithelial damage, was estimated as a percentage of the total corneal area.

In order to provide a continued level of systemic analgesia, buprenorphine 0.01 mg/kg and meloxicam (Metacam®, Boehringer Vetmed GmbH, Ingelheim/Rhein, Germany) 0.5 mg/kg were administered by subcutaneous injection.

Additional injections of buprenorphine 0.01 mg/kg and meloxicam 0.5 mg/kg were administered immediately after the 48-hour observation to reduce pain and distress.

Additional injections of buprenorphine 0.03 mg/kg were supplied to reduce pain and distress.

4.11. Justification of Route and Dose Level

The ocular route was selected because the test material may accidentally come into contact with the eyes during manufacture, handling and/or use. The dose level was based on the test guidelines.

4.12. In - Life Procedures, Observations, and Measurements

4.12.1. Mortality/Moribundity Checks

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

4.12.2. Toxicity

Observations for toxicity were performed once daily throughout the study.

4.12.3. Body Weights

The animal was weighed individually on Day 1 (pre-dose) and on the day of the final observation.

4.12.4. Irritation

The eyes of the animal were examined approximately 1, 24, 48 and 72 hours and 7, 14 and 21 days after instillation of the test material. The irritation scores and a description of all other (local) effects were recorded.

The irritation was assessed according to the following numerical scoring system. At each observation, the highest scores given were recorded:

CORNEAL IRRITATION

Opacity: degree of density (area most dense taken for reading)
No ulceration or opacity (may include slight dulling of normal luster) 0
Scattered or diffuse areas of opacity, details of iris clearly visible 1
Easily discernible translucent area, details of iris slightly obscured 2
Nacreous area, no details of iris visible, size of pupil barely discernible 3
Opaque cornea, iris not discernible through the opacity 4

Area of cornea involved:
No ulceration or opacity 0
One quarter or less but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

IRIS

Normal 0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection, any of these or combination thereof, iris still reacting to light (sluggish reaction is positive) 1
No reaction to light, hemorrhage, gross destruction (any or all of these) 2

CONJUNCTIVAL IRRITATION

Redness (refers to palpebrae and sclera, excluding cornea and iris):
Blood vessels normal 0
Some blood vessels definitely hyperaemic (injected).....1
Diffuse, crimson color, individual vessels not easily discernible 2
Diffuse beefy red 3

Chemosis (refers to lids and/or nictitating membranes):
No swelling …… 0
Any swelling above normal (includes nictitating membranes) 1
Obvious swelling with partial eversion of lids 2
Swelling with lids about half closed 3
Swelling with lids more than half closed.....4

Discharge:
No discharge (may include small amounts observed in inner canthus of normal animals) 0
Any amount different from normal and/or lacrimation 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs (considerable area around the eye) 3

Where standard lighting was considered inadequate for observing minor effects, eye examinations were performed using an ophthalmic examination lamp.

4.13. Terminal Procedures

After the final observation, the animal was euthanized according to laboratories Standard Operating Procedures.

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 were evaluated according to:

- Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2021) (including all amendments).
- 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

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
Computerized Systems

System Name	Description of Data Collected and/or Analyzed
M-Files®	Reporting and collection of 21 CFR Part 11 compliant signature
REES Centron	Temperature, relative humidity and/or atmospheric pressure monitoring Animal and Laboratory facilities

7. RETENTION AND DISPOSITION OF RECORDS

All study-specific raw data, documentation, study plan and final report from this study were archived at the Test Facility at finalization of the report. At least two years after issue of the final report, the Sponsor will be contacted.

Electronic data generated by the Test Facility were archived as noted above, except that files stored on M-Files® (Study Plan, documentation and reporting files) were archived at the Charles River Laboratories facility location in Wilmington, Massachusetts, USA.

8. RESULTS

For detailed results see Appendix 1.

8.1. Irritation and Corrosion

Instillation of 76.2 mg of Chélate-Manganese (a volume of approximately 0.1 mL) into one eye of one rabbit resulted in effects on the cornea and conjunctivae.

The corneal injury consisted of opacity and epithelial damage. The corneal injury resolved within 7 days.

The irritation of the conjunctivae consisted of redness, chemosis and discharge, which did not resolve completely within 21 days. In addition, reduced flexibility of the eye lid was noted and was resolved within 7 days.

There was no evidence of ocular corrosion.

8.2. Coloration / Remnants

Remnants of the test material were present in the eye and on the outside of the eyelids between 1 hour and 7 days after instillation. This is considered to be due to innate protective physiological mechanisms of the eye against entry of foreign compounds.

8.3. Toxicity / Mortality

No signs of systemic toxicity were observed in the animal during the test period and no mortality occurred.

9. CONCLUSION

Exposure of the sentinel animal to the test material resulted in severe eye effects. For animal welfare reasons, the other animals could therefore not be exposed via the conjunctival sac. Since the observed irritation did not resolve completely within 21 days in the sentinel animal, the test material was considered to have irreversible toxicological effects on the eye.

- According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2021) (including all amendments), Chélate-Manganese should be classified as: having irreversible effects on the eyes (Category 1).
- According to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments), Chélate-Manganese should be classified as Irreversible effects on the eye (Category 1) and labeled as H318: Causes serious eye damage.

Appendix 1 Tables

Table 1 Individual Eye Irritation Scores

		Cornea			*Iris*	*Conjunctivae*			
Animal	**Time After Dosing**	**Opacity**	**Area**	**Fluor Area (%)** [2]		**Redness**	**Chemosis**	**Discharge**	**Comments**
115 [1]	1 hour	3	2	n.a.	0	2	3	2	b,c,f
	24 hours	2	2	50	0	2	4	3	b,c,f
	48 hours	2	2	n.a.	0	2	3	2	b,f
	72 hours	1	1	10	0	2	2	1	b,f
	7 days	0	0	0	0	2	1	0	-
	14 days	0	0	n.a.	0	1	0	0	-
	21 days	0	0	n.a.	0	1	0	0	-

[1] Sentinel
[2] Green staining after fluorescein treatment (% of total corneal area) indicating corneal epithelial damage

n.a. Not applicable
Comments:
- No comments
b Remnants of the test material in the eye.
c Remnants of the test material on the outside of the eyelids.
f Reduced elasticity of the eyelids.

Corneal Irritation
Opacity
0 = No ulceration or opacity (may include slight dulling of normal lustre)
1 = Scattered or diffuse areas of opacity, details of iris clearly visible
2 = Easily discernible translucent area, details of iris slightly obscured
3 = Nacreous area, no details of iris visible, size of pupil barely discernible
Area
0 = No ulceration or opacity
1 = One quarter or less but not zero
2 = Greater than one quarter, but less than half

Iris
0 = Normal

Conjunctival Irritation
Redness
0 = Blood vessels normal
1 = Some blood vessels definitely hyperemic (injected)
2 = Diffuse, crimson color, individual vessels not easily discernible
Chemosis
0 = No swelling
1 = Any swelling above normal (includes nictitating membranes)
2 = Obvious swelling with partial eversion of lids
3 = Swelling with lids about half closed
4 = Swelling with lids more than half closed
Discharge
0 = No discharge (may include small amounts observed in inner canthus of normal animals)
1 = Any amount different from normal and/or lacrimation
2 = Discharge with moistening of the lids and hairs just adjacent to lids
3 = Discharge with moistening of the lids and hairs (considerable area around the eye)

Table 2 Mean Value Eye Irritation Scores

Animal	Mean 24, 48 and 72 hours			
	Corneal Opacity	Iris	Conjunctivae Redness	Conjunctivae Chemosis
115	1.66	0	2	3

Table 3 Animal Specifications

Animal	Sex	Age at Start (weeks)	Body weights (grams)	
			Prior to Application	at Termination
115	♂	12-13	2691	3125

Appendix 2 Study Plan

FINAL STUDY PLAN

Test Facility Study No. 20418373

Acute Eye Irritation/Corrosion Study with Chélate-Manganese in the Rabbit

GLP

SPONSOR:
Wageningen Food & Biobased Research
P.O.Box 17,
6700 AA Wageningen
The Netherlands

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 acute eye irritation study is to assess the possible irritation or corrosion potential when a single dose of Chélate-Manganese was placed in the conjunctival sac of the rabbit eye. This study is intended to provide information on the potential health hazards of Chélate-Manganese and data produced can be used for classification/labelling of the test material. This study should provide a rational basis for risk assessment in man.

2. PROPOSED STUDY SCHEDULE

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

Experimental Starting Date:	14 Jun 2023 (Week 24) (First date of study-specific data collection)
Initiation of Dosing:	25 Jun 2023 (Week 25)
Completion of In-life:	06 Aug 2023 (Week 31) (Last date of necropsy)
Experimental Completion Date:	13 Aug 2023 (Week 32) (Last date on which data are collected)
Unaudited Draft Report:	20 Aug 2023 (Week 33)

3. SPONSOR

Role	Name	Contact Information
Sponsor Representative Name	Theo Verkleij	Address as cited for Sponsor Tel: +31 317 481 096 E-mail: theo.verkleij@wur.nl
Study Monitor Name	Panagiotis Voudouris	Address as cited for Sponsor Tel: + 31 317 48 6758 E-mail: panagiotis.voudouris@wur.nl

4. RESPONSIBLE PERSONNEL

Role/Phase	Quality Assurance Unit	Name	Contact Information
Study Director	Charles River	Sandra van de Wiel, PhD	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: Sandra.vandeWiel@crl.com
Test Facility Management	Charles River	Harry Emmen, MSc	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: Harry.Emmen@crl.com
Test Facility QAU	Charles River	Lead QA	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: QADenBosch@crl.com

5. TEST MATERIALS

5.1. Test Material Characterization

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity, composition, and stability for the test material. A Certificate of Analysis or equivalent documentation may be provided for inclusion in the Final Report.

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

5.2. Test Material Identification

5.2.1. Test Material

Identification:	Chélate-Manganese
Batch (Lot) Number:	Chélate-Manganese
Expiry date:	01 June 2025
Physical Description:	Brown powder (determined by Charles River Den Bosch)
Purity/Composition:	Manganese: 19%, lysine: 23%, Sulfates: 39%
Storage Conditions:	At room temperature
Additional information	
Test Facility test material number:	500476/A
Purity/Composition correction factor:	No correction factor required
Test material handling:	No specific handling conditions required

5.3. Reserve Samples

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

5.4. Test Material Inventory and Disposition

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

5.5. Safety

The following safety instruction(s) apply to this study:

- Standard safety precautions specified in Charles River Den Bosch procedures

6. DOSE FORMULATION AND ANALYSIS

6.1. Preparation of Formulations

Chélate-Manganese will be kept at room temperature and dosed undiluted.

If necessary, Chélate-Manganese will be ground to a fine powder prior to administration.

No correction will be made for the purity/composition of the test material, since the guidelines mention a fixed amount that has to be dosed.

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

6.2. Sample Collection and Analysis

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

7. TEST SYSTEM

Species:	Rabbit
Strain:	New Zealand White
Condition:	SPF-Quality
Source:	Charles River France, L'Arbresle, France
Number of Animals:	Three animals of one sex.
Target Age at the Initiation of Dosing:	Between 12 and 24 weeks old. Animals to be used within the study will be of approximately the same age.
Target Weight at the Initiation of Dosing:	At least 1.5 kg.

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

7.1. Animal Identification

Method:	Each animal will be identified using a ear mark with indelible ink. Further identification marks may be applicable, to be documented in the study file.

7.2. 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.

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

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

Replacement:	Before the initiation of dosing, 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.
Disposition:	The disposition of all animals will be documented in the Study Files.

8. HUSBANDRY

8.1. Housing

Caging:	Individually in labeled cages with perforated floors (Ebeco, Germany, dimensions 67 x 62 x 55 cm) 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.
Cage Identification:	Cage cards indicating at least Test Facility Study No. and animal number.

8.2. Animal Enrichment

For psychological/environmental enrichment, animals will be provided with shelters (Ebeco, Germany, dimensions 40 x 32 x 23 cm) and wooden sticks (Swedish aspen wood, Bioservices, Schaijk, The Netherlands) except when interrupted by study procedures/activities.

8.3. Environmental Conditions

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

Temperature:	17 to 21°C
Humidity:	40 to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)
Ventilation:	Ten or more air changes per hour

8.4. Food

Diet:	Global Diet 2030 from Envigo Teklad®, Mucedola, Milanese, Italy or KLIBA NAFAG Rabbit Diet 3409 maintenance and breeding, from Granovit AG, Kaiseraugst, Switzerland, to be specified in the report
Type:	Pellets (alternate diet may be provided on individual animal basis as warranted as approved by the Study Director). In addition, hay will be available during the study period.
Frequency:	*Ad libitum* except during designated procedures.

Analysis:	Results of analysis for nutritional components and environmental contaminants 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.

In addition, washed fresh fruits and/or vegetables may be provided twice weekly.

8.5. Water

Type:	Municipal tap water.
Frequency/Ration:	Freely available to each animal via water bottles.
Analysis:	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 could interfere with the outcome of the study.

8.6. 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. In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or attending veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

9. EXPERIMENTAL DESIGN

The study will be performed in a stepwise manner and will start with the dosing of one animal (sentinel). The criteria to treat additional animals are described in Charles River Den Bosch Standard Operating Procedure (SOP) DIE J/125. If no severe effects on the eyes are observed in the sentinel, the other animal(s) will be treated in a similar manner. If results suggest severe eye irritation or a corrosive effect, further testing in the other animal(s) will not be done.

9.1. Pre-emptive Pain Management

One hour prior to instillation of the test material, buprenorphine (Buprenodale®) 0.01 mg/kg will be administered by subcutaneous injection in order to provide a therapeutic level of systemic analgesia.

Five minutes prior to instillation of the test material, two drops of the topical anaesthetic 0.5% proparacaine hydrochloric ophthalmic solution (Alcaine eye drops®) or 0.5% tetracaine hydrochloric ophthalmic solution (to be specified in the report) will be applied to both eyes.

9.2. Administration of Test Material

Dose Route:	Conjunctival sac of one of the eyes
Frequency:	Once
Method:	Instillation of 0.1 mL of the test material (the weight will be recorded, a maximum of approximately 100 mg will be instilled). The eye lids will then be gently held together for approximately one second to prevent loss of test material. The non-treated eye will serve as the control.

9.3. Removal of Test Material

Immediately after completion of the 1 and 24 hour examination, the test material dosed eye of each animal may be rinsed with tap water to remove residual test material visually present. For reference control, the other eye will also be rinsed.

9.4. Pain management

Following the 24-hours examination of the eyes, buprenorphine 0.01 mg/kg and meloxicam 0.5 mg/kg (Metacam®) will be administered by subcutaneous injection, in order to provide a continued level of systemic analgesia.

Additional injections may be supplied during the observation period if it is expected that these may reduce possible pain and distress. Details will be specified in the study files and report.

10. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS

The in-life procedures, observations, and measurements listed below will be performed for all animals.

In-life Assessments

Parameter	Frequency (minimum required)	Comments
Mortality	At least twice daily during the study	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Toxicity	At least once daily	If toxicity is observed, all clinical signs will be recorded until they have disappeared.
Individual Body Weights	Day 1 (pre-dose), at final observation, at death	No body weights will be determined for animals found dead on Day 1.
Corneal Damage	Immediately after the 24 hour examination and in case of findings	Both eyes will be further examined with the aid of fluorescein stain (Minims® fluoresceinesodium, 20 mg/ml solution). Any bright green stained area, indicating epithelial damage, will be estimated as a percentage of the total corneal area. If epithelial damage is observed this procedure will be repeated at later observation times, to assess recovery.

Parameter	Frequency (minimum required)	Comments
Irritation	Approximately 1, 24, 48 and 72 hours after instillation and at least for the duration of the irritation (maximum 21 days)	At least for the duration of the irritation (including local effects and staining of the eye; criteria described in SOP DIE J/125), further observations will be made 7, 14 and 21 (maximum) days after instillation. The irritation scores and a description of all other (local) effects will be recorded according to the numerical scoring system shown below. Where standard lighting is considered inadequate for observing minor effects, eye examinations will be performed using an ophthalmic examination lamp.

CORNEAL IRRITATION

Opacity: degree of density (area most dense taken for reading)

No ulceration or opacity (may include slight dulling of normal luster) 0
Scattered or diffuse areas of opacity, details of iris clearly visible 1
Easily discernible translucent area, details of iris slightly obscured 2
Nacreous area, no details of iris visible, size of pupil barely discernible 3
Opaque cornea, iris not discernible through the opacity 4

Area of cornea involved:
No ulceration or opacity 0
One quarter or less but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

IRIS
Normal 0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia, or injection, any of these or combination thereof, iris still reacting to light (sluggish reaction is positive) 1
No reaction to light, hemorrhage, gross destruction (any or all of these) 2

CONJUNCTIVAL IRRITATION

Redness (refers to palpebrae and sclera, excluding cornea and iris):
Blood vessels normal 0
Some blood vessels definitely hyperemic (injected) 1
Diffuse, crimson color, individual vessels not easily discernible 2
Diffuse beefy red 3

Chemosis (refers to lids and/or nictitating membranes):
No swelling 0
Any swelling above normal (includes nictitating membranes) 1
Obvious swelling with partial eversion of lids 2
Swelling with lids about half closed 3
Swelling with lids more than half closed 4

Discharge:
No discharge (may include small amounts observed in inner canthus of normal animals) 0
Any amount different from normal and/or lacrimation 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs (considerable area around the eye) 3

11. TERMINAL PROCEDURES

11.1. Unscheduled Euthanasia

Animals found dead or animals sacrificed for humane reasons will be subjected to necropsy. Descriptions of all internal macroscopic abnormalities will be recorded. No necropsy will be performed on animals sacrificed for severe effects on the eyes.

11.2. Scheduled Euthanasia, Tissue Collection and Processing

After final observation and weighing, animals will be euthanized according to laboratories Standard Operating Procedures after approval of the Study Director in the study files or withdrawn from the study. This will be specified in the raw data and report.

12. ANALYSIS

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).

13. COMPUTERIZED SYSTEMS

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

Computerized Systems

System Name	Description of Data Collected and/or Analyzed
M-Files®	Reporting and collection of 21 CFR Part 11 compliant signature
REES Centron	Temperature and Humidity (Animal and Laboratory facilities) Data Collection

14. 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 (FDA and EPA), Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

15. QUALITY ASSURANCE

15.1. 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 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.

16. 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. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

17. RETENTION AND DISPOSITION OF RECORDS, SAMPLES, AND SPECIMENS

All applicable study-specific raw data, electronic data, documentation, Study Plan, retained samples and specimens, and Final Reports will be archived at finalization of the report. All materials generated by Charles River from this study will be transferred to a Charles River archive. At least 2 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 receipt, health, and husbandry
- Test material receipt, identification and preparation
- In-life measurements and observations

18. 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). 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.

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.

19. JUSTIFICATIONS AND GUIDELINES

19.1. Justification of Test System and Number of Animals

The New Zealand White rabbit was chosen as the animal model for this study as recognized by international guidelines as a recommended test system (e.g. OECD, FDA, MHLW). The test method and number of animals are based on the test guidelines.

19.2. Justification of Route and Dose Levels

Dose route and dose concentrations are in compliance with the study design guidelines.

19.3. Guidelines for Study

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

- OECD Guideline 405. Acute Eye Irritation/Corrosion, 2021.
- EPA Health Effects Test Guideline OPPTS 870.2400. Acute Eye Irritation, August 1998.
- EC No 440/2008 Part B. Acute Toxicity, Eye Irritation/Corrosion, 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.

20. ANIMAL WELFARE

This study plan was reviewed and agreed by the Animal Welfare Body of Charles River Laboratories Den Bosch B.V. within the framework of Appendix 1 of project license AVD23600202216274 approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation (December 2014).

In the interest of animal welfare and to minimize any testing likely to produce severe responses in animals, a weight of evidence analysis will be performed, prior to start of this *in vivo* eye irritation study in rabbit. As recommended in the test guidelines, all available information will be evaluated (e.g. existing human and animal data, literature, material data supplied by the Sponsor, analysis of structure activity relationships (SAR), physicochemical properties and reactivity (pH, buffering capacity) and *in vitro*, *ex vivo* tests) to determine the need for *in vivo* testing. Results from the *in vitro* eye irritation studies showed that no severe irritation is to be expected. It was concluded that there was need to perform this *in vivo* study in order to fulfill the regulatory requirements for this endpoint.

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.

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	Qaumailboxher;
1 Copy	Coordinating Biotechnician	Eyndhoven, Richard; HER-DL-DIE-DB-rabbits@crl.com;

SPONSOR APPROVAL

The Study Plan was approved by the Sponsor by e-mail on the date designated below. The correspondence giving approval will be archived, as appropriate with other Sponsor communications.

13 Jun 2023

Date of Sponsor Approval

TEST FACILITY APPROVAL

All electronic signatures appear at the end of the document upon finalization.

SIGNATURE(S) FOR DOCUMENT: 20418373 - WUR OECD 405 Study Plan

TFM Approval-GLP:	I approve the Study Director identified in this document and management's responsibility to the study as defined by the relevant GLP.
Name:	**Lourens, Nicky**
Lourens, Nicky	14-Jun-2023 13:51:21 (UTC+00:00)
Electronically Signed in M-Files	Timestamp

Study Director Approval:	I approve this document.
Name:	**van de Wiel, Sandra**
van de Wiel, Sandra	14-Jun-2023 13:56:56 (UTC+00:00)
Electronically Signed in M-Files	Timestamp

SIGNATURE(S) FOR DOCUMENT: 20418373 General Toxicology Final Report WUR OECD 405

QA Approval:	I approve the Quality Assurance Statement for this report.
Name:	**van Dooren, Maaike**
van Dooren, Maaike	02-Nov-2023 15:31:22 (UTC+00:00)
Electronically Signed in M-Files	Timestamp

Study Director Approval:	I approve this Report.
Name:	**van de Wiel, Sandra**
van de Wiel, Sandra	02-Nov-2023 15:40:19 (UTC+00:00)
Electronically Signed in M-Files	Timestamp

To explore the potential of nature to improve the quality of life

Wageningen Food & Biobased Research
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The Netherlands
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Report 2508

Confidential

The mission of Wageningen University and Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.