

FINAL REPORT

Study Title: Acute Eye Irritation/Corrosion Study in Rats with Tembotrione 35g/L +Atrazine 200g/L OD

Study No.: S213460951

Study Director: Sun Lei

Sponsor

EASTCHEM Co., Ltd.

Floor 26, Haoyuan Mansion, NO.266, Middle Tongjiang Road, Changzhou,
Jiangsu, China

Test Facility

Liaoning Qianyi Testing and Evaluation Technology Development Co., Ltd.
No. 136 Xianghuai Road, Economic-Technological Development Area of
Benxi, Liaoning Province, China.

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SIGNATURE PAGE

Study Director

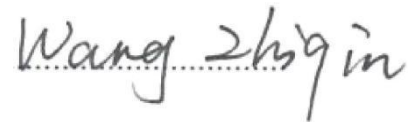
Sun Lei



Date: 2021.9.20

Test Facility Management

Wang Zhiqin



Date: 2021.9.20

GLP COMPLIANCE STATEMENT

STUDY TITLE Acute Eye Irritation/Corrosion Study in Rats with Tembotrione 35g/L
 +Atrazine 200g/L OD

STUDY NO. S213460951

SPONSOR EASTCHEM Co., Ltd.

This study was conducted under my guidance in accordance with the NY/T718-2003 Good Laboratory Practice for Toxicological Safety Evaluation of Pesticides issued by the Ministry of Agriculture on December 1, 2003 and implemented on March 1, 2004.

This study was conducted in accordance with the GB15670-1995 Toxicological Test Method for Pesticide Registration approved by the State Bureau of Technical Supervision on August 17, 1995 and implemented on January 1, 1996. The report faithfully and accurately reflects the results of the test.

Study Director

Sun Lei



Date: 2021.9.20

QUALITY ASSURANCE STATEMENT

STUDY TITLE Acute Eye Irritation/Corrosion Study in Rats with Tembotrione 35g/L
 +Atrazine 200g/L OD

STUDY NO. S213460951

SPONSOR EASTCHEM Co., Ltd.

The Quality Assurance Unit (QAU) inspected/reviewed this study in accordance with the following GLP (Good Laboratory Practice) regulations:

Good Laboratory Practice for Toxicological Safety Evaluation of Pesticides (NY/T 718-2003), Ministry of Agriculture of the People's Republic of China (MOA).

QAU reviewed the report, which stated the test materials and methods, and the results truly reflected the original data.

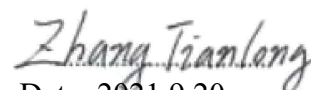
Item of Audit	Date of Inspection	Date of Reporting
Draft Study Plan	2021-03-16	2021-03-16
Supplementary Study Plan	2021-06-26	2021-06-26
Administration*	2021-08-09	2021-08-09
Observation of eye irritation response*	2021-08-10	2021-08-10
Weigh*	2021-08-29	2021-08-29
Raw data and Final Study Report	2021-09-19	2021-09-19

Note: * Indicates the procedure - based check item

All the inspection results are accurate reflections of the performances of the study phases. The test report represents accurate descriptions of the test method, test procedure and observation results. The results audited are accurate reflections of the raw data.

Study Director

Zhang Tianlong



Date: 2021.9.20

SUMMARY

Introduction

The purpose of this experiment is to study whether the test products provided by the client can irritate or corrode the eye of mammals, so as to provide a basis for safety protection in production and use.

Method

Four healthy adult New Zealand white rabbits were selected for the experiment. 24 h before the experiment, no abnormality was found in both eyes. When administering the drug, gently pull down the right lower eyelid, drop 0.1 mL of the test product into the conjunctival sac, and immediately gently close the eyelid for about 1min. The left eye was left for self control. The eyes of the subjects were examined by slit-lamp at 1h, 24h, 48h and 72h after administration, and the eyes of all animals were further examined by sodium fluorescein at 24h after observation and recording. The degree of eye injury was scored after each examination. According to the highest total score and the mean of total score at each observation time point, combined with other observed eye injuries and recoverability, the degree of eye irritation was evaluated according to the grading standard of eye irritation.

Results

1h after administration: the highest score index of eye irritation (I.A.O.I.) was 2, and the mean index of eye irritation (M.I.O.L.) was 0.50.

24h after administration: the highest score index of eye irritation (L.A.O.I.) was 0, and the average index of eye irritation (M.I.O.I.) was 0.

48h after administration: the highest score index of eye irritation (L.A.O.I.) was 0, and the average index of eye irritation (M.I.O.I.) was 0.

72h after administration: the highest score index of eye irritation (I.A.O.I.) was 0, and the mean index of eye irritation (M.I.O.I.) was 0.

Observation after drug administration in animals showed that:

1 hour after administration, conjunctival vascularization occurred in 1 animal.

24h after administration, the animals returned to normal at all time points, and no irritant symptoms were found on the control side.

No abnormal clinical symptoms other than ocular irritation were found after administration.

Conclusion

Under the experimental conditions:

Among all the observation time points of the subjects provided by the client, the highest score index of eye irritation of New Zealand White rabbit was 2, and the average index of eye irritation after 48h was 0. According to the grading standard of eye irritation of pesticide rabbits, the grading of eye irritation intensity of test subject Tembotrione 35g/L +Atrazine 200g/L OD on New Zealand White rabbit was determined to be: no irritation.

1 GENERAL

1.1 Study title and study No.

Study Title: Acute Eye Irritation/Corrosion Study in Rats with Tembotrione 35g/L +Atrazine 200g/L OD

Study No.: S213460951

1.2 Objective

The purpose of this experiment is to study whether the test products provided by the client can irritate or corrode the eye of mammals, so as to provide a basis for safety protection in production and use.

1.3 Specifications and guidance documents to follow

1.3.1 GLP

This study was conducted under my guidance in accordance with the NY/T718-2003 Good Laboratory Practice for Toxicological Safety Evaluation of Pesticides issued by the Ministry of Agriculture on December 1, 2003 and implemented on March 1, 2004.

1.3.2 Test instruction

This study was conducted in accordance with the GB15670-1995 Toxicological Test Method for Pesticide Registration approved by the State Bureau of Technical Supervision on August 17, 1995 and implemented on January 1, 1996. The report faithfully and accurately reflects the results of the test.

1.4 Animal welfare

Animal use complied with national animal welfare laws and regulations "Instructive Notions with Respect to Caring for Laboratory Animals" (2006, PRC Ministry of Science). The animal care and use activities required for conduct of this study are reviewed and approved by the test facility Animal Care and Use Committee (IACUC).

1.5 Test facility

Name: Liaoning Qianyi Testing and Evaluation Technology Development Co., Ltd.

Address: No.136 Xianghuai Road, Economic-Technological Development Area of Benxi, Liaoning Province, China.

Test Facility Management: Wang Zhiqin

Tel: 024-47505200

1.6 Sponsor

Sponsor: EASTCHEM Co., Ltd.

Address: Floor 26, Haoyuan Mansion, NO.266, Middle Tongjiang Road, Changzhou, Jiangsu, China

Tel: 86-519-68786677

1.7 Key personnel

Experiment Operators: Tang Yue, Zhang Jingru Wang Xiulan,

Test item Administrators: Tang Fengyang

Archivist: Lu Sibao

Veterinarian: Jiao Shouming

1.8 Quality assurance

Address: No.136 Xianghuai Road, Economic-Technological Development Area of Benxi, Liaoning Province, China.

Tel: 86-24-47505280

The test plan, test implementation, raw data and final report are all supervised and reviewed by the Quality Assurance Department (QAU) of the institution. Conduct periodic inspections of certain operations or processes in the conduct of the study in accordance with the requirements of the relevant SOPs of the Institution. The quality Assurance Department will issue a quality assurance statement in the final report.

1.9 Test protocol changes and deviations

Before the start of the test, the test plan has been approved by the person in charge of the subject and the person in charge of the institution. At the same time, the test protocol has been reviewed by the QA staff in accordance with GLP requirements.

1.10 Proposed schedule

Study Initiation date: 2021-06-29

Experimental starting date: 2021-07-04

Experimental completion date: 2021-07-13

Final report date: 2021-09-20

2 Materials

2.1 Test item

2.1.1 Test item information

Name: Tembotrione 35g/L +Atrazine 200g/L OD

Batch No.: 20201220

Content: Tembotrione 35g/L , Atrazine 200g/L

Type of formulation: Oil-based suspension concentrates

Physical condition: Off-white liquid

Expiry date: 2022-12-20

Storage condition: Room temperature (10-30°C)

Stability: Stable under storage conditions

Safety precautions: Wear overalls, gloves and masks

Supplied by: EASTCHEM Co., Ltd.

2.1.2 Preparation of test item

The test product is administered directly without preparation.

2.1.3 Test product analysis

There is no need for sample analysis in this experiment.

2.1.4 Sample retention for test products

Sample retention: 10.00g

Responsible department for sample retention: Sample Department

2.1.5 Surplus test item disposal

The residual test item after study indicated by sponsor, any residual test item was disposed totally.

2.2 Test system

2.2.1 Test animal information

Species and Strain: New Zealand white rabbits

Grade: Conventional animals

Total number and sex: 4 (half male and half female, female is not pregnant and does not give birth)

Age: 189-219days

Body weight: 2.460-2.704kg

Source: Liaoning Changsheng Biological Technology Co., Ltd.

Treatment of animals: No group remaining animals.

2.2.2 Reasons for the selection of test system

The test guidelines recommend the use of white rabbits, and the domestic and foreign research background data of this line of animals are abundant, so healthy adult New Zealand white rabbits were selected for the study of acute eye irritation test.

2.2.3 Selection of experimental animals

Healthy adult New Zealand white rabbits were selected, two eyes of the experimental animals were examined by slit-lamp within 24h before the experiment, and all eyes were further examined by using fluorescein sodium. No abnormal reaction was found, which could be used in the experiment.

2.2.4 Identification and marking of animals

For temporary numbering during quarantine and acclimation, use an oil marker to mark the animal's left ear, and for formal testing to mark the animal's right ear. Each cage is hung with a cage card. The cage card information includes the topic number, animal number, dosage, topic leader and other information. The color of the cage card for the drug administration group is white.

2.2.5 Quarantine and adaptive feeding

All animals underwent quarantine and adaptive feeding for 9 days, and qualified animals were used for testing.

2.2.6 Cage

The animals were kept in single cages in stainless steel cages of 42 cm×50 cm×35 cm.

2.2.7 Environmental conditions

Rom No.: A1058

Photoperiod: 12h light and 12h dark

Air change times: ≥ 8 times /h

Temperature: 23.7-24.2°C

Humidity: 58.3-68.3% RH

Maximum daily temperature difference range: $<4^{\circ}\text{C}$

2.2.8 Diet

Type of feed: Rat full nutritive solid feed

Feed lot number: 20210511

Manufacturer: Liaoning Changsheng Biological Technology Co., Ltd

Storage conditions: Store in special feed room, keep low temperature, dry, hygienic

Feeding method: Full nutritive solid feed about 75 g/ day ration supply.

Feed testing: Each batch of feed shall have the quality certificate provided by the manufacturer, and the regular nutrient composition index, chemical pollutant index and microbial index shall be tested once a year by a qualified third-party professional testing unit.

Feed test results: All tested items were qualified, and the test results were stored at our laboratory.

2.2.9 Drinking

Type of drinking water: reverse osmosis water

Water supply method: uninterrupted water supply free intake

Water quality test: Test once every quarter

Water quality test results: All the tested items were qualified, and the test results were stored at our laboratory.

2.3 Main test instrument and reagent

2.3.1 Main Instrument

Name	Type	Manufacturer
Electronic scales	TC10K	Changshu Shuangjie test Instrument factory
Hand-held slit lamp microscope	YZ-3	Suzhou Liuliu Vision Technology Co., LTD

2.3.2 Main reagent

Name	Specification	Batch No.	Manufacturer
2% Sodium fluorescein Solution	10mL/ bottle	20210709-1	TC: Sinopharm Chemical Reagent Co., LTD
		20210711-1	Solution: Suzhou Xishan Zhongke Drug R&D Co., Ltd
Sodium chloride injection (0.9%)	500mL/ bottle	B161219022	Shandong Kelun Pharmaceutical Co. LTD

3 Experiment design

3.1 Group and dose

Four healthy New Zealand white rabbits (half male and half female) were selected. The information was shown in the table below:

Group	Test product	Dose (ml/ Test eye)	Sex	Amount	Animals No.
1	Tembotrione	35g/L	♂	2	1101-1102
	+Atrazine 200g/L OD	0.1	♀	2	2103-2104

3.2 Reasons for dose design

According to the requirements of eye irritation test in GB 15670-1995 Toxicological test Method for Pesticide Registration.

3.3 Information about administration

3.3.1 Information about administration

Administration route: After intraocular administration, the lower eyelid of the right eye was gently pulled down, 0.1mL of the test product was dropped into the conjunctival sac, and the eyelid was immediately gently closed for about 1 min. The left eye was the control, and the eye was not washed within 24 h after administration.

Frequency and duration of administration: Single dose.

Dose: 0.1ml/each eye.

3.3.2 Reasons for the selection of route, frequency and duration of administration

According to the requirements of eye irritation test in GB 15670-1995 Toxicological test Method for Pesticide Registration.

3.4 Detection frequency and methods of various indicators

3.4.1 Clinical signs

During the observation period, clinical observation was conducted on the tested animals once a day, mainly to observe other clinical toxic reactions and death or near-death situations except eye reactions.

3.4.2 Body weights

Individual body weights were measured before dosing and at the end of the test.

3.4.3 Eye reaction

The eyes were examined at 1h, 24h, 48h and 72h after administration, and scored according to the scoring criteria for the degree of eye injury. Eyes of all animals were further examined with sodium fluorescein at 24h post-administration after observation and recording. The experiment was finished 72h after administration.

Grading scale

Ocular lesions		Score
Cornea		
A	Opacity-degree area most dense taken for reading)	
	No opacity	0
	Scattered or diffuse areas of opacity, details of iris clearly visible	1
	Easily discernible translucent areas, details of iris slightly obscured	2
	Opalescent area, no details of iris visible, size of pupil barely discernil	3
	Opaque cornea, iris not discernible through the opacity.	4
B	Area of corner involved (with fluorescein staining)	
	One quarter (or less), but not zero ($<1/4$)	1
	Greater than one quarter, but less than half ($1/4-1/2$)	2
	Greater than half, but less than three quarters ($1/2-3/4$)	3
	Greater than three quarters, up to whole area ($3/4-1$)	4
Total Maximum ($A \times B \times 5$) =80		
Iris		
	Normal	0
	Folds above normal, congestion, swelling, moderate circumcorneal hyperemia or (any of these or combination thereof), iris still reaction to light	1
	No reaction to light, hemorrhage, gross destruction (any of all of these)	2
Total Maximum ($A \times 5$) =10		
Conjunctivae		
A	Redness (refer to palpebral and bulbar conjunctivae)	
	Blood vessels normal	0

	Definitely hyperemic blood vessels scarlet	1
	Crimson color, individual vessels not easily discernible	2
	Diffuse beefy red	3
B	Chemosis: lids and/or nictitating membrane	
	No swelling	0
	Any swelling above normal (includes nictitating membrane)	1
	Obvious swelling with partial eversion of lids	2
	Swelling with lids about half closed	3
	Swelling with lids more than half closed	4
C	Secretion	
	No secretion	0
	Small amounts secretion	1
	Secretion with moistening of the lids and hairs just adjacent to lids	2
	Secretion with moistening of the lids and hairs, and considerable area around the eye	3
Total Maximum (A+B+C) ×2=20		
The total irritation score for the eye is the sum of scores obtained for the cornea, iris and conjunctivae with the possible maximum total score for the eye being equal to 110.		

4 Data processing

Data was processed using Microsoft Excel 2013 software. The data is summarized in tabular form. The scores of the cornea, iris, and conjunctival stimuli for each animal are added together to give the total score of an animal's eye stimuli response. The sum of the stimulus response scores for each animal divided by the number of animals is the mean of the total number of eye irritants for the pesticide tested. At the same time, the degree of eye irritation was evaluated according to the grading criteria of eye irritation based on other eye injuries and recoverability observed.

Classification of Test Substance

Descriptive Rating	Index average of ocular irritation (I.A.O.I)	Mean index of ocular irritation (M.I.O.I)
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Non-irritating	0-5	0 48 hours later
Slightly Irritating	5-15	< 5 48 hours later
Slightly to Moderately Irritating	15-30	< 5 4 days later
Moderately Irritating	30-60	< 20 7 days later
Moderately to Severely Irritating	60-80	< 40 7 days later
Severely Irritating	80-110	

5 Archiving

After filing, the following information shall be kept in the Archives Department of the institution for a period of at least 5 years from the date of registration:

Test scheme;

All raw data;

Electronic file;

Final report, etc.

In addition, the sample can be stored for 5 years after archiving or until the test product fails.

The data saved shall be returned to the principal within the specified time limit. If the Principal needs to extend the retention time, the Principal and the Agency shall decide the specific matters through consultation.

6 Changes and deviations

No changes related to the test scheme have occurred in this study, and the deviations are as follows:

Scheme stipulation	Actual situation	Evaluation
The age range of experimental animals was 6-7 months	The age range of experimental animals was 189-219days	No effect

7 Result

7.1 Clinical observation

No abnormal clinical symptoms other than ocular irritation were found after administration.

7.2 Body weights

No significant changes in body weight and body weight were observed after administration

Data on animal body weight and body weight change are shown in Table 2 in Schedule I.

7.3 Eye irritation response observation and scoring

1h after administration: the highest score index of eye irritation (I.A.O.I.) was 2, and the mean index of eye irritation (M.I.O.I.) was 0.50.

24h after administration: the highest score index of eye irritation (L.A.O.I.) was 0, and the average index of eye irritation (M.I.O.I.) was 0.

48h after administration: the highest score index of eye irritation (L.A.O.I.) was 0, and the average index of eye irritation (M.I.O.I.) was 0.

72h after administration: the highest score index of eye irritation (I.A.O.I.) was 0, and the mean index of eye irritation (M.I.O.I.) was 0.

Observation after drug administration in animals showed that:

1 hour after administration, conjunctival vascularization occurred in 1 animal.

24h after administration, the animals returned to normal at all time points, and no irritant symptoms were found on the control side.

No abnormal clinical symptoms other than ocular irritation were found after administration.

The eye irritation response scores at each time point and the mean scores of all animals are shown in Table 1 of Attached Table I.

8 Conclusion

Under the experimental conditions:

Among all the observation time points of the subjects provided by the client, the highest score index of eye irritation of New Zealand White rabbit was 2, and the average index of eye irritation after 48h was 0. According to the grading standard of eye irritation of pesticide

rabbits, the grading of eye irritation intensity of test subject Tembotrione 35g/L +Atrazine 200g/L OD on New Zealand White rabbit was determined to be: no irritation.

9 References

- 1) GB 15670-1995 Toxicological test methods for pesticide registration State Bureau of Technical Supervision. Approved on August 17, 1995.1.1 implemented.
- 2) NY/T 718-2003 Good Laboratory Practice for Toxicological Safety Evaluation of pesticides, Ministry of Agriculture of the People's Republic of China. Issued on December 1, 2003, implemented on March 1, 2004.

10 Appendix

Table 1 Individual eye reaction scores

Observation time	Irritation Effects	Animal ID and irritation score								I.A.O.I	M.I.O.I	Irritation intensity classification
		1101		1102		2103		2104				
		Control	Administration	Control	Administration	Control	Administration	Control	Administration			
1h	Cornea	0	0	0	0	0	0	0	0	2	0.5	No-Irritation
	Iris	0	0	0	0	0	0	0	0			
	Conj.	0	0	0	0	0	0	2	0			
	Total score	0	0	0	0	0	0	2	0			
24h	Cornea	0	0	0	0	0	0	0	0	0	0	
	Iris	0	0	0	0	0	0	0	0			
	Conj.	0	0	0	0	0	0	0	0			
	Total score	0	0	0	0	0	0	0	0			

48h	Cornea	0	0	0	0	0	0	0	0		
	Iris	0	0	0	0	0	0	0	0	0	0
	Conj.	0	0	0	0	0	0	0	0		
	Total score	0	0	0	0	0	0	0	0		
72h	Cornea	0	0	0	0	0	0	0	0		
	Iris	0	0	0	0	0	0	0	0	0	0
	Conj.	0	0	0	0	0	0	0	0		
	Total score	0	0	0	0	0	0	0	0		

Table 2 Animal body weight individual value

Group	Sex	Animal ID	Body weight (kg)		Body weight change (kg)
			Before administration	Off-test	Off-test--- Before administration
1	♂	1101	2.666	2.745	0.079
		1102	2.460	2.434	-0.026
	♀	2103	2.704	2.780	0.076

2104	2.468	2.519	0.051
Mean	2.575	2.620	0.045

11 Qualification certificate



China National Accreditation Service for Conformity Assessment

GLP Compliance Statement

(Registration No. CNAS GLP0011)

**Liaoning Qianyi Testing and Evaluation Technology
Development Co., Ltd.**

No.136, Xianghuai Road, Economic-Technological Development Area of
Benxi, Liaoning, China

*is inspected to Principles of Good Laboratory Practice (GLP)
published by Organisation for Economic Co-operation and
Development (OECD) for the study expertise listed in the appendix to
this statement, which bears the same registration number as above.
The appendix forms an integral part of this statement.*

Effective Date: 2019-09-29

Expiry Date: 2022-09-28

Date of Initial Accreditation: 2016-09-30

Signed on behalf of China National Accreditation Service for Conformity Assessment



China National Accreditation Service for Conformity Assessment (CNAS) is authorized by Certification and Accreditation Administration of the People's Republic of China (CNCA) to operate the national accreditation schemes for conformity assessment. CNAS is a member of the International Laboratory Accreditation Cooperation (ILAC) and the Asia Pacific Accreditation Cooperation (APAC).
The validity of the certificate can be checked on CNAS website at <http://www.cnas.org.cn/english/findanaccreditedbody/index.shtml>.