

FINAL REPORT

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

Study No.: S213460941

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 Dermal Irritation/Corrosion Study in Rats with Tembotrione
35g/L +Atrazine 200g/L OD

STUDY NO. S213460941

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 Dermal Irritation/Corrosion Study in Rats with Tembotrione
35g/L +Atrazine 200g/L OD

STUDY NO. S213460941

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	2021-03-13	2021-03-13
Supplementary Study Plan	2021-06-14	2021-06-14
Administration*	2021-07-25	2021-07-25
Observation of skin irritation response*	2021-07-25	2021-07-25
Weigh*	2021-07-28	2021-07-28
Raw data and Final Study Report	2021-08-29	2021-08-29

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

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 skin 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. The coat on both sides of the back of the experimental animal was carefully removed close to the skin within 24 hours before administration, and the range of each piece was about 6cm². Take 0.5mL of the test product and apply it to the right hairless skin of the animal, then cover it with two layers of gauze and one layer of cellophane, and then fix it with non-irritant tape and binding to ensure close contact between the test product and the skin, and prevent falling off and animal licking of the test product. The left side of the skin was the self control, except that the test product was not given, other operations were consistent with the administration site. At the end of administration, wash off any remaining samples with water. The skin reaction at the administration site was observed at 1h, 24h, 48h and 72h after washing and scored. The mean of the total skin response integral of the total number of animals at each observation time point was calculated, and a comprehensive evaluation was carried out according to the grading standard of skin irritation intensity, combined with other skin injuries and reversibility observed.

Results

After removing the test product for 1 hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

After removing the test product for 24hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

After removing the test product for 48hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

After removing the test product for 72hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

Conclusion

Under the experimental conditions:

The mean value of the maximum total score for skin irritation of New Zealand white rabbits provided by the client was 0.

According to the grading standard of acute dermal irritation/corrosion test of pesticides, Tembotrione 35g/L +Atrazine 200g/L OD was no-irritation.

1 GENERAL

1.1 Study title and study No.

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

Study No.: S213460941

1.2 Objective

The purpose of this experiment is to study whether the test products provided by the client can irritate or corrode the skin 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: Zhou Lili

1.8 Quality assurance

QA Manager: Zhang Tianlong

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: 2021-06-19

Experimental starting date: 2021-06-23

Experimental completion date: 2021-07-06

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: 190-220days

Body weight: 2.343-2.997kg

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 skin irritation test.

2.2.3 Selection of experimental animals

Healthy New Zealand white rabbits with intact skin were selected for 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 10 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.: A1059A

Photoperiod: 12h light and 12h dark

Air change times: ≥ 8 times /h

Temperature: 22.4-24.5°C

Humidity: 54.4-67.8% 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

2.3.1 Main Instrument

Name	Type	Manufacturer
Electronic scales	TC10K	Changshu Shuangjie test Instrument factory

3 Experiment design

3.1 Group and dose

Animals were not grouped in this study. Dosage is shown in the table below:

Group	Test product	Dose (ml/skin)	Test	Sex	Amount	Animals No.
1	Tembotrione	35g/L	0.5	♂	2	1101-1102
	+Atrazine 200g/L OD			♀	2	2103-2104

3.2 Reasons for dose design

According to the requirements of skin 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: The coat on both sides of the back of the experimental animal was

carefully removed within 24 h before the drug was administered through the skin, with each piece measuring about 6cm². Take 0.5mL of the test product and apply it to the right hairless skin of the animal, then cover it with two layers of gauze and a layer of cellophane, and then fix it with non-irritant tape and binding to ensure close contact between the test product and the skin, to prevent falling off and animal licking of the test product. 4h after administration, scrub the remaining test product with clean water cotton ball. The left side of the skin was the self-control, except that the test product was not given, other operations were consistent with the administration site.

Frequency and duration of administration: The application was continued for 4 h after single administration.

Dose: 0.5ml/each animal.

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

According to the requirements of skin 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 carried out on the tested animals once a day, mainly to observe other clinical toxic reactions and death or dying situations except skin reactions.

3.4.2 Body weights

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

3.4.3 Skin reaction

After 4h of application, wash the residue with water to avoid skin damage. The skin reaction of each animal was observed at 1h, 24h, 48h and 72h after the test product was removed, and the skin reaction score was performed according to the skin irritation response scoring standard. The experiment was finished after 72h observation.

Reactions	Score
A Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2

Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation	4
B Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
The highest score	A+ B

4 Data processing

Data was processed using Microsoft Excel 2013 software. The data is summarized in tabular form, showing the animal's weight, animal deaths and mortality during the test, and recording the animal's toxic symptoms. LC₅₀ of the test product was obtained according to the toxic reaction and death of the animals, and toxicity classification was performed according to the classification standard of acute inhalation toxicity of pesticides.

Toxicity grading standards for pesticide products

Toxicity grading	LC ₅₀ (mg/m ³)
No-Irritation	0-0.4
Mild Irritation	0.5-1.9
Moderate Irritation	2.0-5.9
Strong Irritation	6.0-8.0

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 190-220days	No effect

7 Result

7.1 Clinical observation

No obvious abnormal symptoms were observed during the observation period.

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 Skin response observation and scoring

After removing the test product for 1 hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

After removing the test product for 24hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

After removing the test product for 48hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

After removing the test product for 72hour:

No obvious abnormal symptoms were observed in the skin of the administration area, the mean of the total integral is: 0.

The mortality and clinical observation results of the animals were shown in Table 1 of Schedule I.

No skin irritation symptoms were found on the negative control side at all time points.

The skin 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:

The mean value of the maximum total score for skin irritation of New Zealand white rabbits provided by the client was 0.

According to the grading standard of acute dermal irritation/corrosion test of pesticides, Tembotrione 35g/L +Atrazine 200g/L OD was 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 skin reaction scores

Observation time	Skin reaction	Animal ID and irritation score								Integral mean value	Irritation intensity classification		
		1101		1102		2103		2104					
		Control	Administration	Control	Administration	Control	Administration	Control	Administration				
1h	Erythema	0	0	0	0	0	0	0	0	0	No-Irritation		
	Oedema	0	0	0	0	0	0	0	0				
	Total score	0	0	0	0	0	0	0	0				
24h	Erythema	0	0	0	0	0	0	0	0	0		No-Irritation	
	Oedema	0	0	0	0	0	0	0	0				
	Total score	0	0	0	0	0	0	0	0				
48h	Erythema	0	0	0	0	0	0	0	0	0			No-Irritation
	Oedema	0	0	0	0	0	0	0	0				

	Total score	0	0	0	0	0	0	0	0	
	Erythema	0	0	0	0	0	0	0	0	
72h	Oedema	0	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	2.570	2.574	0.004	
		1102	2.343	2.276	-0.067	
	♀	2103	2.997	2.629	-0.368	
		2104	2.530	2.482	-0.048	
	Mean		2.610	2.490	-0.120	

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>.