

FINAL REPORT

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

Study No.: S213460921

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, Ecohome Technological Development Area of Benxi,
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SIGNATURE PAGE

Study Director

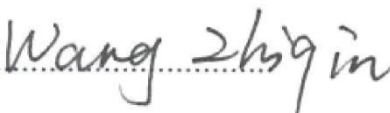
Sun Lei



Date: 2021.8.11

Test Facility Management

Wang Zhiqin



Date: 2021.8.11

GLP COMPLIANCE STATEMENT

STUDY TITLE Acute Dermal Toxicity Study in Rats with Tembotrione 35g/L
 +Atrazine 200g/L OD

STUDY NO. S213460921

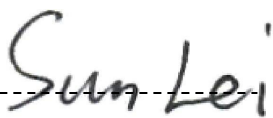
SPONSOR EASTCHEM Co., Ltd.

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: 2021.8.11

QUALITY ASSURANCE STATEMENT

STUDY TITLE: Acute Dermal Toxicity Study in Rats with Tembotrione 35g/L +Atrazine
200g/L OD

STUDY NO.: S213460921

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.

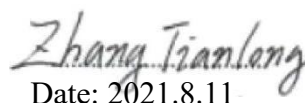
Item of Audit	Date of Inspection	Date of Reporting
Draft Study Plan	2021-03-13	2021-03-13
Supplementary Study Plan	2021-06-14	2021-06-14
Weigh*	2021-07-21	2021-07-21
Administration*	2021-07-22	2021-07-22
Critical Phase Inspection*	2021-07-22	2021-07-22
Raw data and Final Study Report	2021-08-03	2021-08-03

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: 2021.8.11

SUMMARY

Introduction

The purpose of acute percutaneous toxicity test is to study the acute percutaneous toxicity information of the test product provided by the client, so as to obtain the half lethal dose of the test product to the experimental animals after direct contact with the skin, so as to provide the basis for classification and formulation of safety protection measures.

Method

In this study, the limit test method was used, and the drug was administered at the dosage of 2000 mg/kg body weight. A group of 10 rats (half male and half female) were set up. The back hair of the rats was removed within 24 hours before the drug administration, with an area of about 5×5cm². The animal was fixed before administration, and the test product was evenly pasted on the hairless skin, covered with 1 layer of cellophane and 4 layers of gauze, then fixed with medical tape, and finally fixed again with non-irritant transparent tape to ensure close contact between the test product and the skin, to prevent shedding and animal licking of the test product. After 4h of administration, the residual samples were removed and observed immediately, then once a day for 14 days. The animals were weighed once before administration (day 0), on day 8, and on day 15. The animals that died during the study period were examined by gross pathology. LD50 was obtained and classified according to the toxic reaction and death of the animals.

Results

Death of male and female rats: No animal deaths were observed during the test.

Clinical symptoms of animals after administration: No obvious abnormal clinical symptoms were found in animals after administration.

Animal weight and weight changes: The animal weight showed an upward trend, and no abnormal weight changes were found.

Necropsy and gross pathology were not performed because no animal died during the observation period.

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Conclusion

Under the experimental conditions, the acute percutaneous median lethal dose (LD_{50}) of male and female SD rats was $>2000\text{mg/kg}$ body weight. According to the grading standard of acute transdermal toxicity test of pesticides, the acute dermal toxicity of Tembotrione 35g/L +Atrazine 200g/L OD was low toxicity to both male and female SD rats.

1 GENERAL

1.1 Study title and study No.

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

Study No.: S213460921

1.2 Objective

The purpose of acute dermal toxicity test is to study the acute dermal toxicity information of the test product provided by the client, so as to obtain the half lethal dose of the test product to the experimental animals after direct contact with the skin, so as to provide the basis for classification and formulation of safety protection measures.

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: Liu Yizu, Xu Xiuyan, Zhang Yanling

Test item Administers: Tang Fengyang

Histopathology Examination: Wang Yan

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

Reasons should be given for the change of the test plan, and the change should be signed and dated by the person in charge of the subject, the person in charge of quality assurance and the

person in charge of the institution.

The subject leader shall describe and explain the deviation from the test protocol in order, confirm and date it. The material shall be kept together with the original data.

1.10 Proposed schedule

Study Initiation date: 2021-06-19

Experimental starting date: 2021-07-10

Experimental completion date: 2021-07-27

Final report date: 2021-08-11

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 was administered in TC 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: Sprague Dawley Rats

Grade: SPF

Total number and sex: 10(half male and half female)

Age: 7-8 weeks

Body weight: Male, 273.5-300.0g; Female, 203.8-233.0g

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 rats, and this line of animals at home and abroad have abundant research background data, so the SD rats were selected for the study of acute oral toxicity test in rats.

2.2.3 Selection of experimental animals

The mean body weight difference of the same sex animals between groups should be less than 5% in the early adult SD rats with clear strain and genetic background.

2.2.4 Identification and marking of animals

During quarantine and adaptive breeding, animals are provisionally numbered with numbers written directly on their tails. Animal numbers are marked at different locations with animal coloring pens during formal testing. During the test, each animal has a unique animal number. At the same time, the animal cage card is hung on the corresponding animal cage. The cage card contains the special number, animal number and other information. The color of cage card is blue.

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

Animals are raised in plastic rat boxes (size L42cm×W27cm×H20cm). Female and male animals were kept in cages, and the feeding density of each cage was 5 animals per cage during quarantine adaptation period and drug administration period.

2.2.7 Environmental conditions

Rom No.: A1043

Photo period: 12h light and 12h dark

Air change times: ≥ 15 times /h

Temperature: 24.0-26.0°C

Humidity: 44.6-50.0% 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: 20210601

Manufacturer: Beijing Keaoxieli Feed Co., Ltd.

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

Feeding method: continuous supply, unlimited free intake

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	T1000	Changshu Shuangjie test Instrument factory

3 Experiment design

3.1 Group and dose

10 healthy adult SD rats, half male and half female, were selected. The specific group and dose information were as follows:

Group	Test product	Dose (mg/kg)	Sex	Amount	Animals No.
1	Tembotrione 35g/L	2000	♂	5	1101-1105
	+Atrazine 200g/L OD		♀	5	2106-2110

3.2 Reasons for dose design

The dosage was designed according to GB 15670-1995 Toxicological test Method for pesticide Registration through pre-test and literature review.

3.3 Information about administration

3.3.1 Information about administration

Administration route: The back hair of rats was removed within 24 h before administration, and the area was about $5 \times 5 \text{ cm}^2$. When administering the drug, the animal is fixed first, and the test product is evenly coated on the hairless skin, then covered with 1 layer of cellophane and 4 layers of gauze, and then fixed with medical tape, and finally fixed again with non-irritant transparent tape to ensure the close contact between the test product and the skin, to prevent falling off and animals licking the test product.

Frequency and duration of administration: Single dose, the single dose was applied

continuously for 4 h.

Dose: Dose (mL)= animal weight × dose/density

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

According to the requirements of 《GB 15670-1995 Toxicological Test Method for pesticide Registration》.

3.4 Detection frequency and methods of various indicators

3.4.1 Clinical signs

After 4h of administration, the residual samples were removed and observed immediately and recorded, then observed once a day for 14 days. Comprehensively observe and record changes in animal fur, eyes and mucous membranes, respiratory system, circulatory system, nervous system, especially changes in limb movement and behavior.

3.4.2 Body weights

Animals were weighed once before grouping, before administration (day 1), on day 8, and on day 15.

3.4.3 Histopathology

Gross pathology was observed only in dead or dying animals caused by drug administration. CO₂ inhalation anesthesia was used in euthanasia.

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. According to the toxic reaction and death of the animals, LD₅₀ of the test product was obtained and toxicity classification was performed.

Toxicity grading standards for pesticide products

Toxicity grading	LD ₅₀ (mg/kg)
Highly toxic	<20

High toxicity	20-200
Moderate toxicity	200-2000
Low toxicity	2000-5000
Slight toxicity	>5000

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
Group weight range: 200-250g	The males weighed more than 250g when grouped	No effect

7 Result

7.1 Clinical observation

Death of male and female rats: No animal deaths were observed during the test.

Clinical symptoms of animals after administration: No obvious abnormal clinical symptoms were found in animals after administration.

The mortality and clinical observation results of the animals were shown in Table 1 and Table 2 of Schedule I.

7.2 Body weights

Animal weight and weight changes: The animal weight showed an upward trend, and no abnormal weight changes were found.

The data of animal body weight and body weight change are shown in Table 3 in Schedule I.

7.3 Histopathology

Necropsy and gross pathology were not performed because no animal died during the observation period.

8 Conclusion

Under the experimental conditions, the acute percutaneous median lethal dose (LD₅₀) of male and female SD rats was >2000mg/kg body weight.

According to the grading standard of acute transdermal toxicity test of pesticides:

The acute dermal toxicity of Tembotrione 35g/L +Atrazine 200g/L OD was low toxicity to both male and female SD rats.

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 Table of mortality distribution

Dose (mg/kg)	Sex	No. of animals	Time of death distribution														Mortality (%)
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
2000	♂	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	♀	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

Note: "-" indicates that no animals have died

Table 2 Clinical Observation

Dose(mg/kg)	Animals No.	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
2000	1101	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1102	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1103	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1104	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1105	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2106	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2107	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2108	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2109	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2110	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: - indicates normal

Table 3 Animal body weight and weight gain

Dose(mg/kg)	Sex	Animal No.	Body weight (g)			Weight gain (g)
			D0	D8	D15	D15-D0
2000	♂	1101	273.5	342.8	389.0	115.5
		1102	300.0	386.2	428.2	128.2
		1103	295.8	369.5	412.4	116.6
		1104	299.8	373.8	417.8	118.0
		1105	291.3	360.8	396.2	104.9
		Mean	292.1	366.6	408.7	116.6
		SD	11.0	16.2	16.0	8.3
	♀	2106	229.1	253.4	272.4	43.3
		2107	233.0	289.2	334.5	101.5
		2108	227.6	256.5	295.4	67.8
		2109	230.9	257.9	287.4	56.5
		2110	203.8	226.0	245.7	43.7
		Mean	224.9	256.6	287.4	62.6
		SD	12.0	22.4	32.0	24.0

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



Handwritten signature

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>.