FINAL REPORT

Study Title: Skin Sensitization Study in Rats with Tembotrione 35g/L +Atrazine 200g/L OD

Study No.: P2034609061

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 Evaluaiton Technology Development Co., Ltd.

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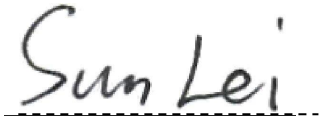
CONTENTS

SIGNATURE PAGE 3
GLP COMPLIANCE STATEMENT 4
QUALITY ASSURANCE STATEMENT 5
SUMMARY 6
1 GENERAL 8
1.1 Study title and study No. 8
1.2 Objective 8
1.3 Specifications and guidance documents to follow 8
1.4 Animal welfare 8
1.5 Test facility 8
1.6 Sponsor 9
1.7 Key personnel 9
1.8 Quality assurance 9
1.9 Test protocol changes and deviations 9
1.10 Proposed schedule 9
2 Materials 10
2.1 Test item 10
2.2 Information of positive control substance 10
2.3 Test system 11
2.4 Main test instrument 13
3 Experiment design 13
3.1 Group and dose 13
3.2 Check the validity and sensitivity of test methods and techniques 13
3.3 Reasons for dose design 14
3.4 Information about administration 14
3.5 Detection frequency and methods of various indicators 14
4 Data processing 15
5 Archiving 16
6 Changes and deviations 16
7 Result 17
7.1 Sensitization ratio 17
7.2 Clinical observation 17
7.3 Body weights 17
7.4 Histopathology 17
8 Conclusion 17
9 References 18
10 Appendix 18
10.1 Appendix I: Summary of Test results 18
10.2 Appendix II: Historical Data of positive control group 18
11 Qualification certificate 23

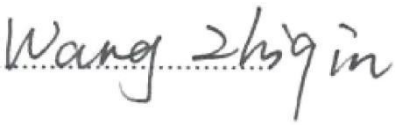
SIGNATURE PAGE

Study Director Sun Lei



Date: 2021.9.20

Test Facility Management Wang Zhiqin



Date: 2021.9.20

GLP COMPLIANCE STATEMENT

STUDY TITLE	Acute Skin Sensitizaiton Study in Rats with Tembotrione 35g/L +Atrazine 200g/L OD
STUDY NO.	S213460961
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

Sun Lei

Date: 2021.9.20

QUALITY ASSURANCE STATEMENT

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

STUDY NO. S213460961

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	2021-04-15	2021-04-15
Supplementary Study Plan	2021-06-26	2021-06-26
Animal grouping and identification*	2021-08-09	2021-08-09
Administration*	2021-08-10	2021-08-10
Observation of skin sensitization response*	2021-08-17	2021-08-17
Weigh*	2021-08-29	2021-08-29
Raw date 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

Zhang Tianlong

Date: 2021.9.20

SUMMARY

Introduction

The purpose of this test is to investigate the likelihood of immune-transmitted skin reactions in the animal after repeated exposure to the test product provided by the client, so as to determine the skin allergy (sensitization) of the test product.

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

Animal skin reaction 24h after exposure in test group and negative control group: no abnormal reaction was observed.

The sensitization rate of test group and negative control group 24h after exposure was 0%.

Animal skin reaction 48h after exposure in test group and negative control group: no abnormal reaction was observed.

The sensitization rate of test group and negative control group 48h after exposure was 0%.

Observation results of clinical symptoms except skin: no abnormality was found.

Animal body weight and its change: showed an upward trend.

Conclusion

Under the experimental conditions:

The sensitization rate of Tembotrione 35g/L +Atrazine 200g/L OD provided by EASTCHEM Co., Ltd. to Hartley guinea pigs was 0%.

According to the grading standard of skin sensitization test of pesticides, Tembotrione 35g/L +Atrazine 200g/L OD was non-sensitizing to guinea pigs.

1 GENERAL

1.1 Study title and study No.

Study Title: Skin Sensitization Study in Rats with Tembotrione 35g/L +Atrazine 200g/L OD

Study No.: P213460961

1.2 Objective

The purpose of this test is to investigate the likelihood of immune-transmitted skin reactions in the animal after repeated exposure to the test product provided by the client, so as to determine the skin allergy (sensitization) of the test product.

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: Sun Xiaoguang, Zhang Jingru, Yang Haixia, Liang Jiayu

Test item Administers: Tang Fengyang

Archivist: Lu Sibo

Veterinarian: Jiao Shouming

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: 2021-06-29

Experimental starting date: 2021-06-30

Experimental completion date: 2021-08-13

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℃)

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 residualtest item was disposed totally.

2.2 Information of positive control substance

Reference product name:	2, 4-dinitrochlorobenzene
Active ingredient:	2, 4-dinitrochlorobenzene, 99.5%
Batch number:	20190108
Valid date:	2021-06-04
Character:	Yellow rhombic yellow crystal

CAS No.:	97-00-7
Specification:	100 g/ bottle
Safety protection:	Wear a mask and gloves during operation
Stability:	Stable under storage conditions
Storage conditions:	Room temperature 10-30℃, away from light, dry
Manufacturer:	Chengdu Gracia Chemical Technology Co., LTD

2.3 Test system

2.3.1 Test animal information

Species and Strain: Hartley Guinea Pigs

Grade: Conventional animals

Total number and sex: 20 (half male and half female, female is not pregnant and does not give birth)

Age: 59-73days

Body weight: Male: 273.2-328.5g; Females: 268.6-345.8g

Source: Liaoning Changsheng Biological Technology Co., Ltd.

Treatment of animals: No group remaining animals.

2.3.2 Reasons for the selection of test system

According to the recommendation of the guidelines, guinea pigs are the experimental animals given priority in sensitization experiments, and there are abundant domestic and foreign research background data on this kind of animals used in sensitization experiments. Therefore, healthy adult Hartley guinea pigs were selected for the study in this experiment.

2.3.3 Selection of experimental animals

Healthy guinea pigs with intact skin were selected for the experiment.

2.3.4 Identification and marking of animals

Temporary numbering marks shall be used for animals during quarantine and adaptive breeding. During the experiment, the body surface staining method was used, and the same color was used to mark the different positions. During the experiment, 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 topic number, animal number, group and gender

information. The cage cards of the test group were red, and the cage cards of the negative control group were white.

2.3.5 Quarantine and adaptive feeding

All animals underwent quarantine and adaptive feeding for 17 days, and qualified animals were used for testing.

2.3.6 Cage

Animals were kept in plastic cages measuring 93 cm×61 cm×24 cm. Quarantine adaptation period was divided into gender groups with appropriate density, and the experiment period was based on 5 animals per cage.

2.3.7 Environmental conditions

Rom No.: A1051

Photoperiod: 12h light and 12h dark

Air change times: ≥8times/h

Temperature: 24.2-26.2℃

Humidity: 60.0-71.8% RH

Maximum daily temperature difference range: <4℃

2.3.8 Diet

Type of feed: Rat full nutritive solid feed

Feed lot number: 20210512

Manufacturer: S 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 40 g/ day ration supply. The full nutritive solid feed was fed in the box on the cage, and the vegetables were fed in the cage.

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.

2.3.9 Drinking

Type of cushion: Corn cob

Pad batch number: 20210619/20210717

Storage conditions and use: low temperature, dry, sanitary, direct use

Pad replacement frequency: once a week

Gasket testing: Each batch of gasket shall have the quality certificate provided by the manufacturer, and the third-party qualified professional testing unit shall test the conventional chemical pollutant index and microbial index of the gasket once a year.

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

2.4 Main test instrument

Name	Type	Manufacturer
Electronic scales	T1000	Changshu Shuangjie test Instrument factory

3 Experiment design

3.1 Group and dose

Twenty healthy adult guinea pigs, half male and half female, were selected and divided into groups according to body weight:

Group	Sensitizing contact concentration	Excited contact concentration	Sex	Amount	Animals ID
Test group	100% (v/v)	100% (v/v)	♂	5	1101-1105
	100% (v/v)	100% (v/v)	♀	5	2106-2110
Negative control group	-	100% (v/v)	♂	5	1211-1215
	-	100% (v/v)	♀	5	2116-2220

Note: (1) "-" means no administration. (2) The test substance was given to the negative control group when exposure was triggered

3.2 Check the validity and sensitivity of test methods and techniques

For the validity and sensitivity of guinea pig locally-closed skin test methods and techniques in our laboratory, positive control 2, 4-dinitrochlorobenzene was used. The positive control group was shared with P1715201D061, and the results showed that the test method and technique were effective in our laboratory. The data of the positive group are shown in Table 3~4 in Appendix II.

3.3 Reasons for dose design

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

3.4 Information about administration

3.4.1 Information about administration

Administration route: Local closed skin administration.

Frequency and duration of administration: Sensitizing exposure was given once on day 0, 7 and 14 for 6 h; Stimulated exposure was performed 14 days after the last sensitization and lasted for 6 h.

Dose: 0.2ml/each animal.

3.4.2 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.

3.4.3 Sensitizing exposure

In the test group, the hair removal area on the left side of the back of the animals was about 3cm×3cm 24 h before the experiment. On day 0, 0.2mL of the test product was placed on 2cm×2cm filter paper and affixed to the hair removal area. It was first covered with two layers of gauze, then with a layer of oil paper, and finally closed and fixed with non-stimulation adhesive cloth for 6 h. Then, the application was removed and the residual test product was removed by scrubbing with clean water cotton balls. Repeat on days 7 and 14 in the same way.

Negative control group, no drug administration, treatment method is the same as above.

3.4.4 Initiate exposure

In the test group, on day 28, 14 days after the last sensitization, 0.2mL of the test product was applied to the hair removal area of about 3cm×3cm on the right back of the guinea pig according to the method described in 3.4.3 (hair removal was performed on the animal the day before the induction). After 6h, the application was removed and the remaining test product was scrubbed with cotton balls of clean water.

The negative control group was treated in the same way as the test group.

3.5 Detection frequency and methods of various indicators

3.5.1 Clinical signs

Clinical observation was carried out on the animals once a day throughout the trial period,

mainly to observe the clinical reactions of the animals and the situation of death or dying.

3.5.2 Body weights

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

3.5.3 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.

Skin Reactions	Score
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
Edema Formation	
No edema	0
Mild edema (barely visible)	1
Moderate edema (skin raised and defined)	2
Severe edema (skin swelling about 1mm or more)	3
The highest score	7

3.5.4 Necropsy and Histopathology

Autopsy and gross pathology should be performed only for animals that have died during the trial period and for dying animals euthanized. The inhalation of excess CO_2 is used in euthanasia.

4 Data processing

Data were analyzed in Microsoft Excel 2013 and summarized in table form. The sensitization rate of animals was calculated by dividing the number of animals with erythema or edema (no

matter the degree or severity) in the corresponding experimental group by the total number of animals in the group. The sensitization rate intensity classification standard in "GB 15670-1995 Toxicological Test Method for Pesticide Registration" was used to evaluate and grade.

Sensitization Ratio Scale

Sensitization Ratio (%)	Grade	Class
0-8	I	Non-sensitizing or extremely slight sensitizing
9-28	II	Slight sensitizing
29-64	III	Moderately sensitizing
65-80	IV	Severely sensitizing
81-100	V	Extremely severely sensitizing

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 7-8weeks	The age range of experimental animals was 59-73days	No effect
Humidity range: 40-70%RH	Humidity range: 60.0-71.8%RH	No effect

7 Result

7.1 Sensitization ratio

Animal skin reaction 24h after exposure in test group and negative control group: no abnormal reaction was observed.

The sensitization rate of test group and negative control group 24h after exposure was 0%.

Animal skin reaction 48h after exposure in test group and negative control group: no abnormal reaction was observed.

The sensitization rate of test group and negative control group 48h after exposure was 0%.

Observation results of clinical symptoms except skin: no abnormality was found.

7.2 Clinical observation

No abnormal clinical toxic reactions were observed in any animal during the whole trial period, and all animals survived.

7.3 Body weights

The animal body weight and its weight change showed an upward trend after administration.

Animal weight and weight change data are shown in Table 2 in Appendix 1.

7.4 Histopathology

No animal died or died during the test period, and further anatomical and gross pathological examination was not required.

8 Conclusion

Under the experimental conditions:

The sensitization rate of Tembotrione 35g/L +Atrazine 200g/L OD provided by EASTCHEM

Co., Ltd. to Hartley guinea pigs was 0%.

According to the grading standard of skin sensitization test of pesticides, Tembotrione 35g/L +Atrazine 200g/L OD was non-sensitizing to guinea pigs.

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

10.1 Appendix I: Summary of Test results

10.2 Appendix II: Historical Data of positive control group

Table 1 Sensitization ratio

Group	No.	Sensitizing contact	Excited contact	Observation time (h)	Erythema					Edema				Number of Animals with Skin Reaction	Sensitization ratio	Sensitization Class
		Dose	Dose		0	1	2	3	4	0	1	2	3			
Test group	10	0.2 ml/each animal	0.2 ml/each animal	24	10/10	0/10	0/10	0/10	0/10	10/10	0/10	0/10	0/10	0	0	Slight sensitizing
				48	10/10	0/10	0/10	0/10	0/10	10/10	0/10	0/10	0/10			
Negative control group	10	-	0.2 ml/each annimal	24	10/10	0/10	0/10	0/10	0/10	10/10	0/10	0/10	0/10	0	0	Slight sensitizing
		-		48	10/10	0/10	0/10	0/10	0/10	10/10	0/10	0/10	0/10			

Note: "-" means no administration.

Table 2 Body weight

Group	Sex	Animals ID	Body weight (g)		Body weight change (g)
			Pre-administration	Off-test	Off-test-- Pre-administration
Test group	♂	1101	328.5	485.7	157.2
		1102	319.1	291.2	172.1
		1103	312.8	403.4	90.6
		1104	307.4	485.6	178.2
		1105	302.9	471.6	168.7

		Mean	314.1	467.5	153.4
		SD	10.1	36.6	35.9
	♀	2106	345.8	432.2	86.4
		2107	307.8	396.7	88.9
		2108	306.6	451.8	145.2
		2109	297.2	467.4	170.2
		2110	288.9	391.5	102.6
		Mean	309.3	427.9	118.7
		SD	21.8	33.3	37.2
Negative control group	♂	1211	325.3	406.8	81.5
		1212	319.6	493.5	173.9
		1213	309.7	477.1	167.4
		1214	307.9	399.9	90.2
		1215	273.2	485.7	212.5
		Mean	307.1	452.6	145.5
		SD	20.3	45.4	56.4
	♀	2216	341.9	493.6	151.7
		2217	308.6	471.2	162.6
		2218	301.4	463.5	162.1
		2219	299.1	418.9	119.8
		2220	268.6	400.3	131.7

Mean	303.9	449.5	145.6
SD	26.2	38.6	19.1

Table 3 Results table of historical data sensitization rate in positive control group

Group	No.	Sensitizing contact	Excited contact	Observation time (h)	Erythema					Edema				Number of Animals with Skin Reaction	Sensitization ratio	Sensitization Class
		Dose	Dose		0	1	2	3	4	0	1	2	3			
Positive control group	10	0.2 ml/each animal	0.2 ml/each annimal	24	2/10	5/10	1/10	0/10	2/10	7/10	3/10	0/10	0/10	8	80%	Severely sensitizing
				48	5/10	3/10	0/10	2/10	0/10	10/10	0/10	0/10	0/10	5	50%	Moderately sensitizing

Table 4 Historical data animal weight table for positive control group

Group	Sex	Animals ID	Body weight (g)		Body weight change (g)
			Pre-administration	Off-test	Off-test-- Pre-administration
Positive control group	♂	1321	245.8	309.9	64.1
		1322	230.2	314.1	83.9
		1323	227.6	308.8	81.2
		1324	207.9	352.4	144.5
		1325	200.5	289.3	88.8
		Mean	222.4	314.9	92.5

	SD	18.2	23.0	30.5
	2326	263.1	335.8	72.7
	2327	250.5	318.2	67.7
	2328	241.3	358.9	117.6
♀	2329	222.6	327.1	104.5
	2330	213.4	309.8	96.4
	Mean	238.2	330.0	91.8
	SD	20.2	18.9	21.2

11 Qualification certificate

CNAS

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.