

STUDY TITLE

Mutagenicity Study (Ames Test) of Tembotrione 35g/L +Atrazine 200g/L OD

SPONSOR

EASTCHEM CO., LTD.

Study No.: S201401064

STUDY DIRECTOR

Xue Liao

DATE

October 30, 2014

PERFORMING LABORATORY

Safety Evaluation Center of Shenyang Research Institute of Chemical Industry Ltd.

600 Shenliao Road, Tiexi District, Shenyang, Liaoning Province.

People's Republic of China (Post code: 110141)

GLP COMPLIANCE STATEMENT

This study was performed in compliance with OECD Guidelines for testing of chemicals, No.471: “Bacterial Reverse Mutation Test”, adopted July 21, 1997 and following OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97) 186/Final].

The stability of the test item dilutions under the test conditions is unknown; therefore it is excluded from this statement.

This report is a complete, true and accurate representation of the study and its results and Study Director accepts the responsibility for validity of the data.



October 30, 2014

Xue Liao
Study Director



October 30, 2014

Wang Jie

Facility Manager

QUALITY ASSURANCE STATEMENT

This study was inspected in compliance with OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

The general facilities and activities are inspected periodically and the results reported to the responsible personnel and the management.

Study procedures with the exception of the solubility and precipitation check were periodically inspected. The study plan and report were audited by the Quality Assurance. The following inspections were performed:

Items of inspection	Dates of inspection	Dates of report
Protocol	June 09, 2014	June 09, 2014
First Administration	August 18, 2014	August 18, 2014
Study report	October 30, 2014	October 30, 2014



October 30, 2014

Bai Xigeng
QAU Manager

1. Abstract

Ames test was performed using the plate penetrating method. Ames test for Tembotrione 35g/L +Atrazine 200g/L OD, was evaluated when treated to the test bacteriums of TA97, TA98, TA100 and TA102 at a dose of level of 40, 200, 1000 and 5000µg per plate including inactive and active system.

The revertant colonies on each test plate were observed and counted. The revertant colonies on doses plates (the mean of six plates) showed negative results whether or not the active system was added.

Based on the results and according to the mutagenicity toxicity criteria, the test article, Tembotrione 35g/L +Atrazine 200g/L OD, under the test experiment condition of this laboratory, is not a mutagen to tester strains.

2. Test title

The Mutagenicity Study (Ames test) of Tembotrione 35g/L +Atrazine 200g/L OD

3. Purpose of Project

This study was performed in compliance with OECD Guidelines for testing of chemicals, No.471: "Bacterial Reverse Mutation Test", adopted July 21, 1997, and under the GLP conditions in the center, to determine the mutagenicity of Tembotrione 35g/L +Atrazine 200g/L OD with the revertant theory of Salmonella, therefore, to evaluate the safety of product and provide the relevant data for registration purpose.

4. Project Number

S201401064

5. Tested by

Safety Evaluation Center of Shenyang Research Institute of Chemical Industry Ltd.

Add: 600 Shenliao Road, Tiexi District, Shenyang, Liaoning Province.

People's Republic of China

(Post code: 110141)

6. Sponsor

Name: EASTCHEM CO., LTD

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

7. Lab director and participants

The Center Director: Wang Jie

The Project Director: Xue Liao

Participants: Yu Gang, Ma Ting

8. Test schedule

Study initiation	August 18, 2014
Test initiation	August 18, 2014
Test completion	September 17, 2014
Study completion	October 30, 2014

9. Quality assurance

Bai Xigeng

10. Materials and method

10.1 Sample

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

Purity: 235g/L

Supplier: EASTCHEM CO., LTD

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

Storage: to be stored in the Center's Sample Room

10.2 Bacterium

10.2.1 Bacterium and its strain

Salmonella typhimurium, TA97, TA98, TA100, TA102.

10.2.2 Genotype confirmation of the tester strains

The following items were performed with routine procedures: the histidine requirement, the Rfa mutation, the ampicillin resistance, the tetracycline resistance, the *uvrB* deletion, and the spontaneous reversion, to confirm the genotypes of the tester strains. Spontaneous reversion was also measured with routine procedures. Before the test, an overnight culture was performed at 37°C to increase the final cell concentration up to 1×10^8 cells/ml.

10.2.3 Bacterium provided

By the Center's reproductive toxic dept.

10.3 Culture Components

Culture components were prepared based on the Ames test, 4 totally.

10.4 S-9m System

S-9 and S-9m are prepared, used and stored based on Ames test.

S-9: A mixture of liver pulp S-9 from male Sprague-Dawley rats, NADPH induced by polychlorinated.

S-9m: on the bases of S-9 system, prepared freshly.

Polychlorinate 1254: (Aroclor 1254)

10.5 Dose designs

Weigh sample on electronic balance, and prepare sample solution with Water. The doses were 40, 200, 1000 and 5000µg per plate.

10.6 Test Procedures

The standard Ames method is introduced into this test. Plate incorporation is adopted for all the tests which were divided into:

10.6.1 Inactive plate penetrating system:

Add 0.1ml sample and 0.1ml bacterial culture into the overlay agar of plate at 45°C, mix well. Triplicates are used for 4 dosages.

Use 0.1ml water per plate and Dexon 50µg per plate as blank experiments. Turn over the plates at 37°C for 48 hrs after the solidification of the upper agar. The colonies of revertant per plate were counted directly.

10.6.2 Active plate penetrating system:

Add 0.33ml S-9m, 0.1ml sample and 0.1ml bacterial culture into the upper soft agar of plate at 45°C in turn, mix well. Triplicates are used for 4 dosages.

Use 0.1ml water per plate and 2-AF 20µg per plate as blank experiments.

Turn over the plates at 37°C for 48 hrs after solidification of the upper agar. The colonies of revertant per plate were counted directly.

10.7 Method

Count the revertant colonies to get the average value of the three counting. If the average exceeds water blank twofold, it is considered positive and draw the response-effect curve.

10.8 Treatment

The positive measurement should be treated according to the regulation, incinerate the waste plate, and cleaning tube after 20 min boiling.

11. Results and Conclusion

11.1 Results:

See attached table. There was no positive result found in all the 4 tester strains under the test condition.

11.2 Conclusion

Based on the results and according to the mutagenicity toxicity criteria in the Toxicological Test Methods of Pesticide for Registration of P. R. China, the test article, Tembotrione 35g/L +Atrazine 200g/L OD, under the test experiment condition of this laboratory, is not a mutagen to tester strains.

12. Files

After the report is sent to the consigner, the following documents will be filed in the Center's Information Dept for at least 10 years.

Test design, initial data and the final report.

Some relevant record for sample

Some relevant record for bacterium

Others which required under GLP conditions

The result of Ames test for Tembotrione 35g/L +Atrazine 200g/L OD

Project: S201401064

concentration of Sample (µg/plate)	Plate No.	No. of mutant recovery /plate							
		TA97		TA98		TA100		TA102	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
5000	1	114	108	44	38	168	181	196	212
	2	108	107	30	40	164	195	173	205
	3	113	125	42	33	153	176	178	204
	Mean	112	113	39	37	162	184	182	207
	SD	3.2	10.1	7.6	3.6	7.8	9.8	12.1	4.4
1000	1	111	108	48	42	180	191	187	201
	2	112	107	38	29	158	204	201	198
	3	110	132	47	36	156	189	200	194
	Mean	111	116	44	36	165	195	196	198
	SD	1.0	14.2	5.5	6.5	13.3	8.1	7.8	3.5
200	1	118	130	38	35	174	198	185	211
	2	102	120	50	40	170	188	199	207
	3	102	127	33	38	163	175	188	203
	Mean	107	126	40	38	169	187	191	207
	SD	9.2	5.1	8.7	2.5	5.6	11.5	7.4	4.0
40	1	117	130	38	35	174	198	185	210
	2	102	120	51	40	169	187	199	207
	3	102	126	33	39	163	175	189	203
	Mean	107	125	41	38	169	187	191	207
	SD	8.7	5.0	9.3	2.6	5.5	11.5	7.2	3.5
Water 0.1ml/Plate	1	103	134	35	35	169	181	191	196
	2	109	113	34	37	168	199	197	208
	3	111	111	38	33	180	181	171	205
	Mean	108	119	36	35	172	187	186	203
	SD	4.2	12.7	2.1	2.0	6.7	10.4	13.6	6.2
Dexon 50µg/Plate	1	4091	--	3012	--	1737	--	2628	--
	2	4278	--	3216	--	1597	--	2691	--
	3	4104	--	3155	--	1401	--	2540	--
	Mean	4158	--	3128	--	1578	--	2620	--
	SD	104.4	--	104.7	--	168.8	--	75.8	--
2-AF 20µg/Plate	1	--	1269	--	3012	--	2272	--	--
	2	--	1206	--	3015	--	2605	--	--
	3	--	1309	--	3187	--	2286	--	--
	Mean	--	1261	--	3071	--	2388	--	--
	SD	--	51.9	--	100.2	--	188.3	--	--

Appendix GLP Compliance



ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 28 February – 7 March 2014 at

Safety Evaluation Center
Shenyang Research Institute of Chemical Industry
600 Shenliao Road
Shenyang, 11041 P.R. China

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Toxicity, Mutagenicity and Environmental toxicity studies, Studies on behaviour in water, soil and air and bioaccumulation, and Analytical and clinical chemistry studies.

Den Haag, 30 April 2014



Dr R. Jaspers
Coordinating Specialist Senior Inspector

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