TECAM
LABORATÓRIOS

CONFIDENTIAL

Final Report RL3393/2007 – 2.0AM-B
December 13th 2007

Study Number 3393/2007 – 2.0AM
Bacterial reverse mutation test (Ames Test) for
GLIFOSATO TÉCNICO HELM

Reference Methodology
OECD Guideline for Testing of Chemicals
Bacterial Reverse Mutation Test 471 (1997)

Sponsor
HELM DO BRASIL MERCANTIL LTDA
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Performing Laboratory
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STUDY COMPLIANCE STATEMENT

The present study was conducted according to the OECD Principles on Good Laboratory Practice (OECD Environment Health and Safety Publications, as revised in 1997) and "Norma NIT-DICLA-028 (INMETRO, Sep/03, Rev.01)" under the supervision of the Study Director.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

[redacted]

12/13/07

Study Director
R. Fábia, 59 – [redacted]
São Paulo, SP.

KEY PERSONNEL

Name	Responsibility
[redacted]	Study Director Test Unit Manager Technical Staff

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QUALITY ASSURANCE STATEMENT

The study plan, final report and original data from this study have been reviewed for adherence to the principles of the Good Laboratory Practice by the Quality Assurance Unit. The final report was considered to be a correct and faithful record of the raw data. Proceedings of the present study were inspected by process-based inspection and the compliance with the Good Laboratory Practice was confirmed.
Dates of inspections and the dates on which the findings were reported to the Study Director and Test Facility Management are given below.

Phase of Study	Inspection	Reporting to Study Director and to Management
Experimental	Nov/27/2007	Nov/28/2007
Study plan	Oct/29/2007	Oct/29/2007
Raw data	Dec/06/2007	Dec/06/2007
Final report	Dec/06/2007	Dec/06/2007
	Dec/13/2007	Dec/13/2007

[redacted]

Dec/13/2007

Quality Assurance Unit

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RESUMO

O Teste de Ames (mutação gênica reversa) foi realizado com a substância-teste **GLIFOSATO TÉCNICO HELM** com o objetivo de avaliar o potencial mutagênico do agente para as cepas TA98; TA100; TA102; TA1535; TA1537 de *Salmonella typhimurium* na ausência e presença de um sistema de ativação metabólica (fração microssomal de fígado de ratos induzido com Aroclor 1254). As concentrações 648; 1080; 1800; 3000; 5000 µg/placa foram utilizadas para o teste definitivo. As razões de mutagenicidade (RM) obtidas após 72 horas de incubação das cepas de *Salmonella typhimurium* foram inferiores a 2. A análise estatística não apresentou resultados significantes tanto na presença quanto ausência de ativação metabólica (pANOVA>0,05). O resultado obtido foi considerado negativo para as cepas TA98; TA100; TA102; TA1535; TA1537 de *Salmonella typhimurium*, uma vez que a substância-teste **GLIFOSATO TÉCNICO HELM** representada pela amostra de lote nº 2007091801, nas condições descritas não apresentou efeito mutagênico.

ABSTRACT

The reverse mutation assay (Ames Test) was carried out with the test substance **GLIFOSATO TÉCNICO HELM** in order to study the possible mutagenic effect of that substance on the strains TA98; TA100; TA102; TA1535; TA1537 of *Salmonella typhimurium* in systems with and without metabolic activation (microsomal fraction of rat liver induced with Aroclor 1254). The definitive test was performed at the following concentrations: 648; 1080; 1800; 3000; 5000 µg/plate. Mutation rates after 72 hours of incubation of *Salmonella typhimurium* strains were lower than 2. Statistical analysis presented no significant results (pANOVA> 0.05). Under the conditions of this study, **GLIFOSATO TÉCNICO HELM** (batch nº 2007091801) has presented no mutagenic effect on *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535; TA1537 both with and without metabolic activation.

INTRODUCTION

The reverse mutation assay (Ames Test) measures the possible mutagenic effect of a test substance on strains of *Salmonella typhimurium* in systems with and without metabolic activation. A set of histidine-requiring strains is used to detect chemicals which cause base changes or frameshifts mutations in the genome of the organism. Bacteria are exposed to the test substance with and without a metabolic activation system and plated onto minimal medium. After incubation, revertant colonies are counted and compared to the number of spontaneous revertant colonies in a solvent control culture.

MATERIALS AND METHODS

The present test was conducted according to methodology described by the "OECD Guideline for Testing of Chemicals" (Bacterial Reverse Mutation Test. 471, 1997).

1. Test substance information

Identification: GLIFOSATO TÉCNICO HELM.

Protocol: 3393/2007 – 2.0.

Received on: Oct/29/2007.

Manufactured on: Sep/17/2007.

Expiry date: Sep/17/2009.

Batch N°: 2007091801.

Common name of a.i.: Glyphosate.

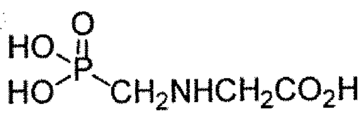
Declared concentration of a.i.: 950 g/kg.

Analysed concentration of a.i.[1]: 980.1 g/kg (Appendix 1).

Class: Herbicide.

IUPAC name: *N*-(phosphonomethyl)glycine.

Chemical and structural formula: $C_3H_8NO_5P$



CAS RN: [107-83-6].

Homogeneity: Homogeneous (visual inspection).

Stability (a.i.): Stable (CIPAC MT 46, 54° C, 14 days).

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Formulation: Technical grade.

Physical state: Solid.

[1]: Analysis performed at Tecam Tecnologia Ambiental.

2. Study dates

Initial date: Oct/29/2007.

Experimental phase (range finding): Nov/06/2007 to Nov/09/2007.

Experimental phase (definitive test): Nov/30/2007 to Dec/03/2007.

3. Test system

Standard strains: Five strains of *Salmonella typhimurium* acquired from Moltox Inc. (Annapolis, MD, USA) were used:

STRAIN	PLASMID	MUTATION	SR
TA98	pKM101	frameshift mutation	17-75
TA100	pKM101	base-pair substitutions	60-220
TA102	pKM101; pAQ1	base-pair substitutions	240-320
TA1535	-	base-pair substitutions	5-50
TA1537	-	frameshift mutation	3-25

SR: spontaneous revertants

Test concentrations: 648; 1080; 1800; 3000; 5000 µg/plate.

4. Methodology

For the direct plate incorporation test without metabolic activation 0.1 mL of test substance and 0.1 mL of a fresh bacterial culture grown overnight were added to 3.0 mL of top agar. For tests with metabolic activation 0.5 mL of S9 (microsomal fraction of rat liver induced with Aroclor 1254) prepared by Moltox (Annapolis, MD) was added to the top agar after the addition of test substance and bacteria. The concentration of protein in S9 fraction employed in this assay was 34.9 mg/mL. Contents of each tube were mixed and poured over the surface of a selective agar plate. At the end of the incubation period, revertant colonies per plate were counted.

5. Genotypes of tester strains

Previously to the test, the requirement of histidine for growth was demonstrated for each strain, as well as other phenotypic characteristics using methods such as crystal violet sensitivity and resistance to ampicillin and tetracyclin. Spontaneous reversion rate was checked with values reported in the literature.

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6. Controls

Concurrent positive and negative controls were included in each assay. Negative controls consisting of vehicle (DMSO, 100 µL/plate) without the test substance were included in each assay. Positive controls should ensure both strain responsiveness and efficacy of the metabolic activation system. The following chemicals were employed as positive controls:

ASSAY	STRAINS	COMPOUND
S9 –	TA98	2-Nitrofluorene
S9 –	TA100; TA1535	Sodium azide
S9 –	TA1537	ICR 191 - Acridine
S9 –	TA102	Mitomycin C
S9 +	TA98; TA100; TA102; TA1535; TA1537	2-aminoanthracene

S9 = metabolic activation.

7. Test concentrations

A range finding assay using TA100 was performed to select concentrations for the definitive test at the following concentrations: 8; 40; 200; 1000 and 5000 µg/plate. No concentration was found to be toxic for bacteria growing. Therefore the definitive test with strains TA98; TA100; TA102; TA1535 and TA1537 was performed at the following concentrations: 648; 1080; 1800; 3000; 5000 µg/plate with and without metabolic activation. All test solutions for both range finding and definitive tests were prepared from stock solutions. All plating was done in triplicate, except for the positive controls, which were done in two replicates.

8. Expression of the results

Results were presented as number of revertant colonies per plate and concentration and by the mutation rate, which corresponds to the rate between number of revertants induced by test substance and number of revertants observed in the negative control. The number of revertants observed in the negative control of each strain is compared to the expected range reported in the literature (Maron & Ames, 1983) and established in the laboratory by historical control values.

9. Analysis of the results

A test substance is considered to be active in the test system if the mutation rates after 72 hours of incubation of strains exposed to the test chemical are higher than 2 for strains TA98, TA100 and TA102 or higher than 3 for strains TA1535 and TA1537. To confirm the positive result the analysis of variance of the data set should indicate significant results (pANOVA<0.05) and a clear dose-related increase in the number of revertants should be observed. The analysis of variance indicate the probability of the number of revertants observed in the different concentrations be increased (mutagenicity) or decreased (toxicity).

10. Evaluation of the assay

The acceptance criteria of the assay are: a) presence of background lawn in the test plates; b) spontaneous revertant colonies of the negative control are in the range reported in the literature (Maron & Ames, 1983) and established in the laboratory by historical control values; c) positive controls show mutagenic activity in all tested strains.

RESULTS

Results of range finding test (TA100) with **GLIFOSATO TÉCNICO HELM** and mutation rates (MR) after 72 hours are presented in Table 1. Results of definitive test after 72 hours of incubation of *Salmonella* strains exposed to the test substance **GLIFOSATO TÉCNICO HELM** are presented in Table 2 to 11. Negative and positive controls showed appropriate results with all tester strains in the range finding and definitive tests so that the assays were considered valid.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment at any concentration level, neither in the presence nor absence of metabolic activation. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological significance. Mutation rates after 72 hours of incubation were lower than 2 and the analysis of variance of the data set indicated no significant difference (pANOVA>0.05) among the number of revertants, except for strains TA1537 with metabolic activation and TA102 without metabolic activation. In these assays toxic effects lead to the reduction in the number of revertants at the highest tested concentrations indicating a significant difference (pANOVA<0.05) among the number of revertants. No concentration-effect relationship was observed (increase of the number of revertants with the increase of tested concentrations).

CONCLUSION

Under the conditions of this study, **GLIFOSATO TÉCNICO HELM** (batch n° 2007091801) did not induce gene mutations by base pair changes or frameshifts in the genome of *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535 and TA1537 at the employed range of concentrations both with and without metabolic activation.

REFERENCES

MARON, D.M. & AMES, B.N. Revised methods for the *Salmonella* mutagenicity test. **Mutation Research, 113**: 173-215, 1983.

OECD **Guideline for testing of chemicals**. Bacterial Reverse Mutation Test. 471, 11p., 1997.

Table 1 - Results of **GLIFOSATO TÉCNICO HELM** tested with *Salmonella typhimurium* TA100 (range finding test).

STRAIN	TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE	MUTATION FREQUENCY
TA100	0[a]	195 ± 9	-
	8	156 ± 6	0.80
	40	152 ± 11	0.78
	200	178 ± 14	0.91
	1000	166 ± 8	0.85
	5000	146 ± 14	0.75
	CP[b]	3066 ± 139	15.75

0[a] = negative control: DMSO (100 µL/plate).

CP[b] = positive control: SA 5 µg/plate (without metabolic activation).

Table 2 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA98 (definitive test; without metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	36	30	36	34 ± 3	-
648	31	30	27	29 ± 2	0.86
1080	29	30	37	32 ± 4	0.94
1800	31	36	33	33 ± 3	0.98
3000	38	30	26	31 ± 6	0.92
5000	36	33	33	34 ± 2	1.00
CP[b]	1120	1032	-	1076 ± 62	31.65

Analysis of variance ⇒ pANOVA = 0.613

Table 3 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA98 (definitive test; with metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	30	35	32	32 ± 3	-
648	31	32	28	30 ± 2	0.94
1080	39	32	31	34 ± 4	1.05
1800	30	43	37	37 ± 7	1.13
3000	37	35	34	35 ± 2	1.09
5000	31	35	41	36 ± 5	1.10
CP[b]	3052	3528	-	3290 ± 337	101.75

Analysis of variance ⇒ pANOVA = 0.455

MR = Mutation rate.
0[a] = negative control: DMSO (100 µL/plate).
CP[b] = positive controls: 2-Nitrofluorene 0.5 µg/plate (without metabolic activation).
2AA 2.5 µg/plate (with metabolic activation).

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Table 4 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA100 (definitive test; without metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	204	208	200	204 ± 4	-
648	200	212	216	209 ± 8	1.03
1080	208	200	200	203 ± 5	0.99
1800	204	204	200	203 ± 2	0.99
3000	208	216	200	208 ± 8	1.02
5000	200	216	212	209 ± 8	1.03
CP[b]	2996	2856	-	2926 ± 99	14.34

Analysis of variance ⇒ pANOVA = 0.589

Table 5 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA100 (definitive test; with metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	204	204	200	203 ± 2	-
648	200	208	204	204 ± 4	1.01
1080	200	204	208	204 ± 4	1.01
1800	212	200	216	209 ± 8	1.03
3000	212	208	220	213 ± 6	1.05
5000	200	204	220	208 ± 11	1.03
CP[b]	2772	2884	-	2828 ± 79	13.95

Analysis of variance ⇒ pANOVA = 0.378

MR = Mutation rate.

0[a] = negative control: DMSO (100 µL/plate).

CP[b] = positive controls: SA 5 µg/plate (without metabolic activation).
2AA 2.5 µg/plate (with metabolic activation).

Table 6 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA102 (definitive test; without metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	316	312	308	312 ± 4	-
648	304	312	316	311 ± 6	1.00
1080	316	308	304	309 ± 6	0.99
1800	316	308	300	308 ± 8	0.99
3000	312	308	304	308 ± 4	0.99
5000	292	276	292	287 ± 9	0.92
CP[b]	3360	2912	-	3136 ± 317	10.05

Analysis of variance ⇒ pANOVA = 0.004

Table 7 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA102 (definitive test; with metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	312	308	300	307 ± 6	-
648	316	312	308	312 ± 4	1.02
1080	304	312	304	307 ± 5	1.00
1800	300	304	312	305 ± 6	1.00
3000	308	300	304	304 ± 4	0.99
5000	308	304	300	304 ± 4	0.99
CP[b]	2044	1736	-	1890 ± 218	6.16

Analysis of variance ⇒ pANOVA = 0.408

MR = Mutation rate.

0[a] = negative control: DMSO (100 µL/plate).

CP[b] = positive controls: Mitomycin C 0.5 µg/plate (without metabolic activation).
2AA 2.5 µg/plate (with metabolic activation).

Table 8 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA1535 (definitive test; without metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	35	34	29	33 ± 3	-
648	34	31	30	32 ± 2	0.97
1080	35	32	33	33 ± 2	1.02
1800	28	29	30	29 ± 1	0.89
3000	34	32	37	34 ± 3	1.05
5000	34	37	27	33 ± 5	1.00
CP[b]	2492	2660	-	2576 ± 119	78.86

Analysis of variance ⇒ pANOVA = 0.370

Table 9 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA1535 (definitive test; with metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	28	32	29	30 ± 2	-
648	29	31	24	28 ± 4	0.94
1080	31	28	35	31 ± 4	1.06
1800	28	26	30	28 ± 2	0.94
3000	31	35	32	33 ± 2	1.10
5000	32	29	23	28 ± 5	0.94
CP[b]	292	252	-	272 ± 28	9.17

Analysis of variance ⇒ pANOVA = 0.355

MR = Mutation rate.

0[a] = negative control: DMSO (100 µL/plate).

CP[b] = positive controls: SA 5.0 µg/plate (without metabolic activation).
2AA 2.5 µg/plate (with metabolic activation).

Table 10 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA1537 (definitive test; without metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	11	11	13	12 ± 1	-
648	12	9	9	10 ± 2	0.86
1080	7	12	10	10 ± 3	0.83
1800	7	11	10	9 ± 2	0.80
3000	11	10	17	13 ± 4	1.09
5000	8	7	5	7 ± 2	0.57
CP[b]	3416	3586	-	3501 ± 120	300.09

Analysis of variance ⇒ pANOVA = 0.094

Table 11 - Results of **GLIFOSATO TÉCNICO HELM** *Salmonella typhimurium* strain TA1537 (definitive test; with metabolic activation).

TEST SUBSTANCE (µg/plate)	REVERTANTS PER PLATE			MEAN AND SD	MR
0[a]	11	7	9	9 ± 2	-
648	16	12	13	14 ± 2	1.52
1080	13	19	10	14 ± 5	1.56
1800	7	9	7	8 ± 1	0.85
3000	8	9	5	7 ± 2	0.81
5000	13	6	8	9 ± 4	1.00
CP[b]	264	252	-	258 ± 8	28.67

Analysis of variance ⇒ pANOVA = 0.042

MR = Mutation rate.
0[a] = negative control: DMSO (100 µL/plate).
CP[b] = positive controls: ICR 191–Acridine 10 µg/plate (without metabolic activation).
2AA 2.5 µg/plate (with metabolic activation).

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APPENDIX 1

REPORT OF ANALYSIS PROTOCOL Nº.: 3393/2007 – 1.0

Sponsor: HELM DO BRASIL MERCANTIL LTDA
Adress: Rua Alexandre Dumas, 2220 – 4º Andar – 04717-004, São Paulo - SP

1. TEST SUBSTANCE INFORMATION

Identification: GLIFOSATO TECNICO HELM
Protocol: 3393/2007 – 1.0
Batch Nº: 2007091801
Manufactured on: 09/17/2007.
Expiry date: 09/17/2009.
Common name of a.i.: Glyphosate.
IUPAC name: *N*-(phosphonomethyl)glycine

2. EXPERIMENTAL

Equipment: High Liquid Performance Chromatograph 1200 Series (HPLC) AGILENT TECHNOLOGIES – TECAM 83.0.EQ

3. DATES

Initial date: 12/07/2007.
Final date: 12/12/2007.

4. RESULTS

Protocol Nº.	Result
3393/2007 – 1.0	980.1 g/kg

5. METODOLOGY

Analytical method: TECAM: POP Nº022/07 Rev. 01 – Teor de Glifosato

6. SIGNATURES

[redacted] 12/13/07 (mm dd yy)
Study Director

[redacted] 12/13/07 (mm dd yy)
Quality Assurance

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TECAM LABORATORIOS

APPENDIX 2

The Swiss GLP Monitoring Authorities

Swiss Federal Office of Public Health

Swiss Agency for the Environment, Forests and Landscape

Swissmedic Swiss Agency for Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of August 29 - 31, 2005

the following Test Facility of

TECAM
Tecnologia Ambiental Ltda
05051-030 São Paulo
Brazil

was inspected by the Federal Office of Public Health with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Test Facility	**Areas of expertise**
TECAM - Tecnologia Ambiental Ltda.	- Environmental toxicity studies on aquatic & terrestrial organisms - Physical-chemical testing - Mutagenicity studies

This inspection has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted May 18th, 2005 [RS 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 28th, 1997 by decision of the OECD Council [C(97)186/Final].

Swiss Federal Office of Public Health
Consumer Protection Directorate
Notification Authority
The Head

Berne, December 2005

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