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Study title

The Salmonella typhimurium reverse mutation by GLIFOS

Data Requirements
Instituto Brasileiro do Meio Ambiente
e Recursos Naturais Renováveis - IBAMA
Portaria Normativa n° 139, of December 21th, 1994

Study Completed on

December 23, 1996

Performing Laboratory

BioAgri - Biotecnologia Agrícola Ltda.
Rod. SP 127 Km 24+62m
13412 - 000
Piracicaba - São Paulo - Brasil

Study Sponsor

CHEMINOVA AGRO S.A.
P.O. Box 9
DK - 7620
Lemvig - Denmark

BioAgri Report #

G.1.1 - 050/96

AA041269

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STUDY COMPLIANCE STATEMENT

We the undersigned, declare that this study was performed under our supervision, according to the procedures herein described. This report represents an accurate and true recording of the results obtained and is scientifically valid.

An exact copy of raw data was provided to CHEMINOVA AGRO S.A. with the final report. All original raw data were retained at BioAgri - Biotecnologia Agrícola Ltda..

[redacted]
Study Director

12/23/96
mm/dd/yy

[redacted]
Director - BioAgri

12/23/96
mm/dd/yy

Sponsor

mm/dd/yy

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Quality Assurance Documentation

This study have been reviewed by the Quality Assurance Unit of BioAgri. It has been found to accurately describe and/or identify the methods, practices and procedures employed the course of the study. Observations and results presents in this final report form a true and accurate representation of the raw data generated during the conduct of the study.

Report Number: G.1.1. 050/96.

Prepared by: [redacted] 12/23/96 mm/dd/yy

Approved by: [redacted] 12/23/96 mm/dd/yy

Quality Assurance Officer

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INDEX

TITLE OF THE ASSAY

The *Salmonella typhimurium* reverse mutation assay by the chemical product GLIFOS

SUMMARY

A microbial assay was carried out with the product GLIFOS in order to study the possible mutagenic effect of that substance on the strains TA97a , TA98 , TA100 and TA1535 of *Salmonella typhimurium* in systems with and without metabolic activator (microsomal fraction of rat liver induced with AROCLOR 1254). The compound was tested with five concentrations, 0.001; 0.01; 0.1; 1 and 5 mg/plate of product. The positive controls, sodium azide and 2-aminofluorene produced the anticipated increases revertants, particularly after metabolic activation. The product GLIFOS did not produce an increase in reversion to histidine prototropy in the nonactivation and activation assays at any of the concentrations. These results indicate that, under the test conditions, the product GLIFOS did not exhibit genetic activity on the strains of *Salmonella typhimurium* used.

SUMMARY (PORTUGUESE)

Foi conduzido um teste microbiológico de mutagenicidade (Teste Ames) com o produto GLIFOS visando estudar possíveis efeitos genéticos nas cêpas TA97a , TA98 , TA100 e TA1535 de *Salmonella typhimurium* em sistemas com e sem ativador metabólico (fração microsomal de fígado de rato induzido com AROCLOR 1254). O produto foi testado em cinco concentrações, até o máximo de 0,001; 0,01; 0,1; 1 e 5 mg/placa do produto. Os controles positivos de azida de sódio e 2-aminofluoreno apresentaram os aumentos esperados nos números de revertentes, principalmente nos testes com ativador metabólico. O produto GLIFOS não produziu uma elevação no número de revertentes nos testes com e sem ativador metabólico em nenhuma das concentrações utilizadas. Esses resultados indicam que, nas condições do ensaio, o produto não apresentou atividade mutagênica na cêpas de *Salmonella typhimurium*.

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GENERAL INFORMATION

.Test Substance: GLIFOS

.Chemical name: Sal de isopropilamina de N-(fosfonometil)-glicina

.Common name: Glyphosate

.Declared Purity: 360.0 g/L

.Analyzed Purity: 360.0 g/L

.Sponsor: CHEMINOVA AGRO S.A.

.Study	started on:	10/12/96
.Assay without metabolic activator	started on:	12/20/96
	concluded on:	12/23/96
.Assay with metabolic activator	started on:	12/20/96
	concludeded on:	12/23/96
.Final report	concluded on:	12/23/96

.Technical workers: [redacted], Lab. Technician

Total pages: 37

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I. INTRODUCTION

The Salmonella typhimurium (**his**) reversion system is a microbial assay which measures **his-** -----> **his**$^{+}$ reversion induced by chemicals which cause base change or frameshift mutations in the genome of this organism.

DEFINITIONS

MEC = minimal effective concentration. It is the lowest concentration of a product (expressed as micrograms/plate or microliter/plate) that causes reverse mutations in any one of the S. typhimurium strains used.

II. MATERIAL AND METHODS

1. Test substance

The test substance was GLIFOS. One gram of the product was added to 3 mL of sterile distilled water and mixed with a test tube mixer to a homogeneous solution. Appropriate dilutions were carried out in order to have the following levels of the substance per plate: 0.001; 0.01; 0.1; 1 and 5 mg/plate.

2. Organism

Strains TA98 , TA100 , TA97a and TA1535 of Salmonella typhimurium auxotroph to histidine (Ames et al.,1975) were used. Those strains were made histidine dependent (**his**$^{-}$) through base pair substitutions (TA100 and TA 1535) or frameshift mutation (TA98 and TA97a) in the genome of the organism.

3. Principle of the method

The test is designed to detect mutagenic substances that may cause **his$^+$** reversion in the strains through base pair changes or frameshift mutation in the DNA of the organisms. This reverse mutations produce histidine independent strains that are capable of growing in a minimal medium without that amino-acid.

4. Reference substances

Sodium azide (1.5 µg/plate) for TA100 and TA1535 in the assays with and without metabolic activator, and 2-aminofluorene (10 µg/plate) for TA98 and TA97a in the assays with metabolic activator were used as positive controls. Negative controls were included, with the solvent used in the test.

5. Direct plate incorporation method

The sample was mixed with 0.1 mL of an overnight culture (8-12 hours old), that was added to 2 mL of top agar containing traces of histidine and biotin. This mixture was homogenized with a vortex for 2-3 seconds, and poured over the surface of a petri dish containing 30 ml of minimal agar medium containing 2 % of glucose (for strain TA97a, that level was reduced to 0.2 %). The tests with metabolic activation followed the Maron and Ames (1983) protocol with 20 µL/plate of the microsomal fraction of rat liver activated with AROCLOR 1254. This product was reconstituted from the freeze-dried product obtained from MolTox (Molecular Toxicology Inc., Annapolis, MD, U.S.A.). Triplicate plates were poured for each dose of the test substance. Negative controls containing the bacteria, S9 mix (when used) and the solvent, were prepared in order to establish the number of colonies that arise spontaneously for each of the tester strains. After incubation for 72 hours at 37°C, the number of colonies on the plate were counted.

6. Data management.

Data were statistically analyzed with the statistical analysis program, Salmonel (Myers et. al., 1991). The substance is considered mutagenic when the following criteria are attended:

1. A statistically significant dose response curve is obtained at $p= 0.05$.
2. The number of revertants is at least twice the control for strains TA100 and TA97a , or at least three fold the control for strains TA98 and TA1535.

III. RESULTS

The tests of histidine requirements, rfa mutation (permeability of the cell wall), mutation uvrB (UV sensitivity) and resistance to 25 ug/ml of Ampicillin (R-factor), confirmed that the tester strains had the genotypes required for the mutagenicity test. The values of spontaneous reversion of the tester strains to histidine independence were also within the historical values observed in our laboratory.

The positive control with sodium azide promoted a strong increase in the number of revertant of strain TA100 and TA1535 confirming the sensitivity of these strains to that mutagen. On the other hand the positive control with 2-AF (2-aminofluorene) only had mutagenic effect on strains TA97a and TA98 when the metabolic activator S9 was used. This product is not mutagenic in its original form and only its metabolized byproducts have genetic activity.

The results of the assay and the statistic analysis through the program Salmonel (appendix) indicate that the product GLIFOS did not have mutagenic activity within the levels tested.

IV. CONCLUSION

Under the test conditions, the product GLIFOS did not have genetic activity in the strains of Salmonella typhimurium used in the assay.

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V. REFERENCES

1. Ames, B.N.; Mc Cann J. & Yamasaky E. 1975. Methods for detecting carcinogenics and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31:347-364. 1975.

2. Brusick, D.J. & Young, R.R. IERL-RTP. Procedures Manual: Level 1. Env. Ass. Biol. Tests. Washington, EPA, p 138 (EPA 600/8-81-024). 1981.

3. Environmental Monitoring Systems Laboratory, EPA. Data management Systems in genetic toxicology. Salmonel Assay Software, version 2.3 . 1989.

4. IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Manual de testes para a avaliação da ecotoxicicidade de agentes químicos. 1990.

5. Maron, D. & Ames, B. Compatibility of organic solvents with the Salmonella/microsome test. Mutation Research, 88:343-350. 1981.

6. Maron, D. & Ames, B. Revised methods for the Salmonella mutagenicity test. Mutation Research 113: 173-215. 1983.

7. Myers, L.E. , Adams, N., Kier, L., Rao, T.K., Shaw, B. & Willians, L. Microcomputer software for data management and statistical analysis of the Ames/Salmonella test. In: D. Krewisk (Ed.). Statistical Methods in Toxicological Research. Gordon and Breech, New York, pp 265-279. 1991.

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Salmonella Assay

Test Sample Name: GLIFOS, without metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 1 Exp. Date: 12/27/96 Exp. No.: 050/96
Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA100 Activation S9: -
Data File Name: b:\model.sal

Code	Dose mg/L	--	counts		Mean	S.D.	Predicted Linear
	0.00	153	152	169	158.00	9.54	143.14
	0.00	140	167	151	152.67	13.58	143.11
	0.01	130	159	142	143.67	14.57	142.85
	0.10	127	147	152	142.00	13.23	140.28
	1.00	75	121	97	97.67	23.01	114.58
	5.00	1	0	0	0.33	0.58	0.33
N	0.00	0	0	0			
P	0.00	300>	300>	300>			

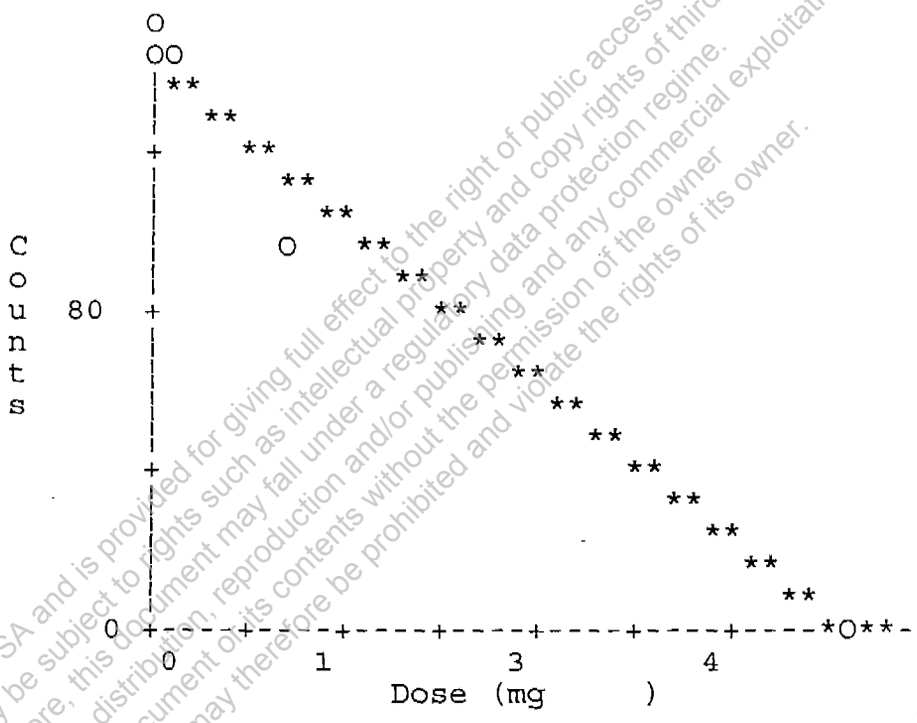
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.561

Estimate of the slope is = -28.561062 .
Standard error of the slope is = 1.440000 .
90% confidence limits for the slope are <-31.127661, -25.994462>.

P-value for the test of the positive dose response
(slope at origin) is 1.000
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 1 Experiment Date: 12/27/96 Experiment No.: 050/96
Test Sample Name: GLIFOS, without metabolic activator.
Tester Strain: TA100



O = Observed; * = Predicted.
The predicted values are based on Linear model.

Salmonella Assay

Test Sample Name: GLIFOS, without metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 2 Exp. Date: 12/23/96 Exp. No.: 050/96
Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA1535 Activation S9: -
Data File Name: b:\model.sal

Code	Dose mg/L	--	counts		Mean	S.D.	Predicted Linear
	0.00	17	8	7	10.67	5.51	9.28
	0.00	8	10	7	8.33	1.53	9.28
	0.01	8	8	11	9.00	1.73	9.29
	0.10	9	13	7	9.67	3.06	9.32
	1.00	9	13	7	9.67	3.06	9.70
	5.00	0	0	0	0.00	0.00	11.35
P	0.00	300>	300>	300>			

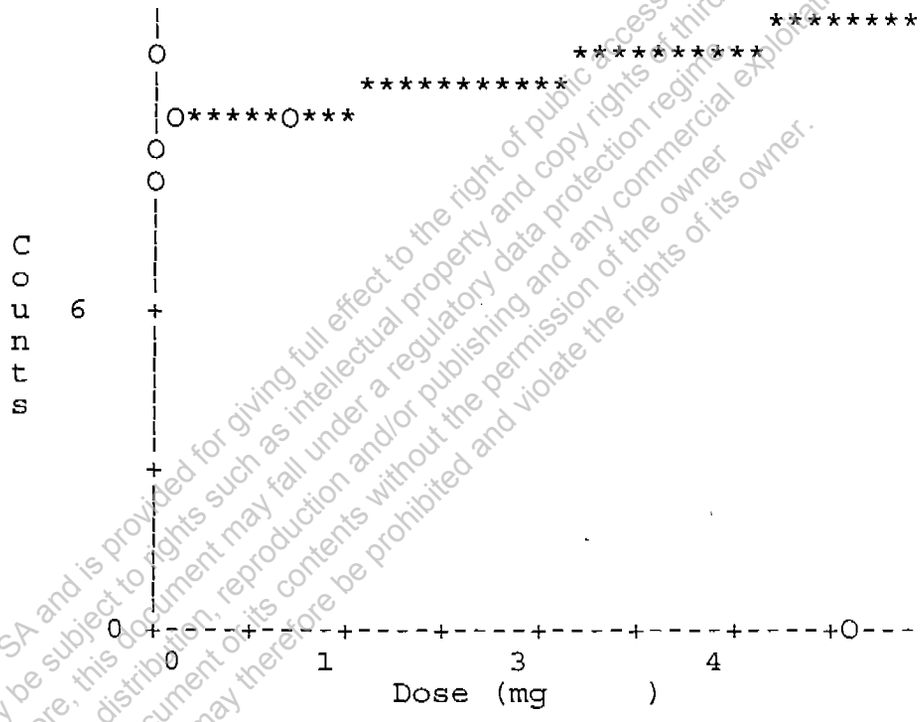
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.947
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.896

Estimate of the slope is = 0.413632 .
Standard error of the slope is = 1.915842 .
90% confidence limits for the slope are <-3.001091, 3.828354>.

P-value for the test of the positive dose response (slope at origin) is 0.416
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 2 Experiment Date: 12/23/96 Experiment No.: 050/96
Test Sample Name: GLIFOS, without metabolic activator.
Tester Strain: TA1535



O = Observed; * = Predicted.
The predicted values are based on Linear model.

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Salmonella Assay

Test Sample Name: GLIFOS, without metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 3 Exp. Date: 12/23/96 Exp. No.: 050/96
Technician:
Assay Type: Plate incorporation,
Strain: TA98 Activation S9: -
Data File Name: b:\model.sal

Code Dose mg/L	--	counts		Mean	S.D.	Predicted Bernstein
0.00	24	34	21	26.33	6.81	20.13
0.00	17	20	16	17.67	2.08	20.12
0.01	16	25	17	19.33	4.93	20.00
0.10	14	20	21	18.33	3.79	18.78
1.00	6	5	9	6.67	2.08	6.65
5.00	1	1	4	2.00	1.73	

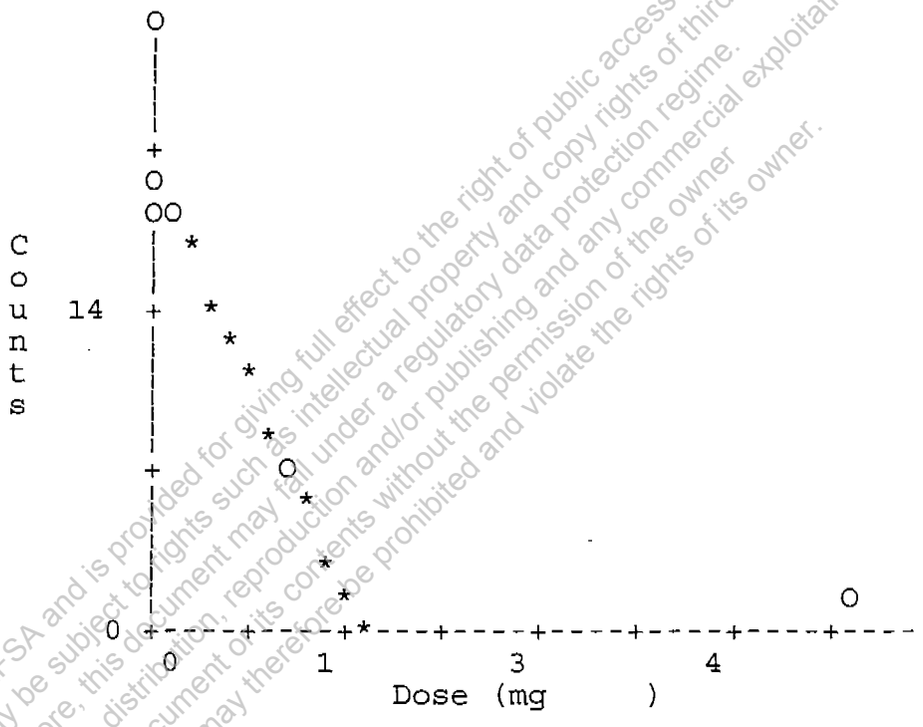
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Bernstein with pval = 0.565
Berstein model used the first 5 doses

Estimate of the slope is = -13.477207 .
Standard error of the slope is = 2.247365 .
90% confidence limits for the slope are <-17.482822, -9.471591>.

P-value for the test of the positive dose response (slope at origin) is 1.000
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 3 Experiment Date: 12/23/96 Experiment No.: 050/96
Test Sample Name: GLIFOS, without metabolic activator.
Tester Strain: TA98



O = Observed; * = Predicted.
The predicted values are based on Bernstein model.

BIOAGRI

Salmonella Assay

Test Sample Name: GLIFOS, without metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 4 Exp. Date: 12/23/96 Exp. No.: 050/96
Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA97A Activation S9: -
Data File Name: b:\model.sal

Code Dose mg/L	--	counts		Mean	S.D.	Predicted Linear
0.00	140	129	131	133.33	5.86	141.65
0.00	131	136	121	129.33	7.64	141.56
0.01	128	157	161	148.67	18.01	140.73
0.10	159	156	141	152.00	9.64	132.49
1.00	60	37	52	49.67	11.68	50.04
5.00	0	0	0	0.00	0.00	-316.38

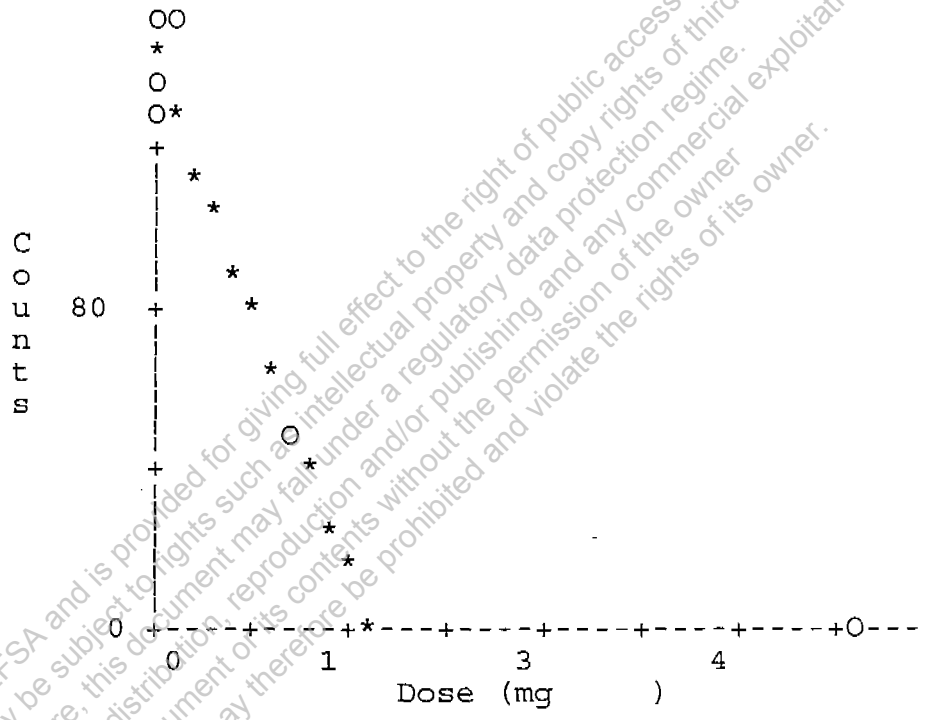
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.094

Estimate of the slope is = -91.605420 .
Standard error of the slope is = 5.500154 .
90% confidence limits for the slope are <-101.408681, -81.802159>.

P-value for the test of the positive dose response (slope at origin) is 1.000
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 4 Experiment Date: 12/23/96 Experiment No.: 050/96
Test Sample Name: GLIFOS, without metabolic activator.
Tester Strain: TA97A



O = Observed; * = Predicted.
The predicted values are based on Linear model.

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Salmonella Assay

Test Sample Name: GLIFOS, with metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 5 Exp. Date: 12/23/96 Exp. No.: 050/96
Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA100 Activation S9: + RAT LIVER AROCLOR 4%
Data File Name: b:\model.sal

Code	Dose mg/L	--	counts		Mean	S.D.	Predicted Linear
	0.00	161	168	151	160.00	8.54	159.34
	0.00	165	179	142	162.00	18.68	159.31
	0.01	160	169	151	160.00	9.00	159.04
	0.10	172	152	160	161.33	10.07	156.30
	1.00	110	133	121	121.33	11.50	128.87
	5.00	6	8	7	7.00	1.00	6.98
P	0.00	300>	300>	300>			

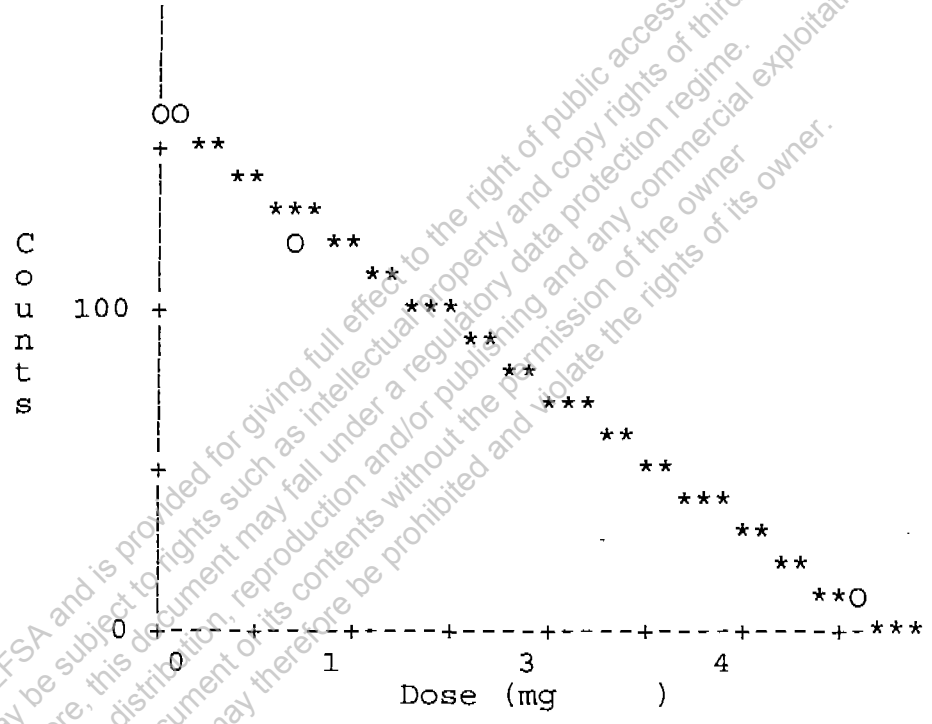
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.674

Estimate of the slope is = -30.472560 .
Standard error of the slope is = 0.657206 .
90% confidence limits for the slope are <-31.643939, -29.301180>.

P-value for the test of the positive dose response (slope at origin) is 1.000
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 5 Experiment Date: 12/23/96 Experiment No.: 050/96
Test Sample Name: GLIFOS, with metabolic activator.
Tester Strain: TA100



O = Observed; * = Predicted.
The predicted values are based on Linear model.

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Salmonella Assay

Test Sample Name: GLIFOS, with metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 6 Exp. Date: 12/23/96 Exp. No.: 050/96
Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA1535 Activation S9: + RAT LIVER AROCLOR 4%
Data File Name: b:\model.sal

[redacted]

Code	Dose mg/L	--	counts		Mean	S.D.	Predicted Linear
	0.00	16	16	14	15.33	1.15	10.26
	0.00	12	4	11	9.00	4.36	10.26
	0.01	14	19	10	14.33	4.51	10.24
	0.10	7	14	9	10.00	3.61	10.09
	1.00	6	5	8	6.33	1.53	8.53
	5.00	1	1	3	1.67	1.15	1.61
P	0.00	300>	300>	300>			

S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.134

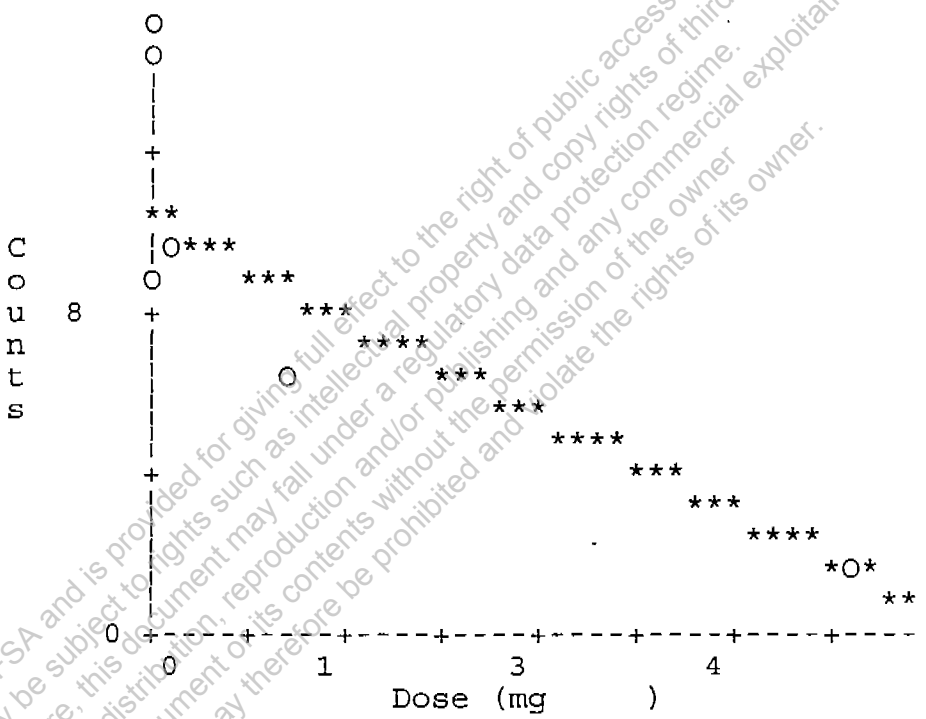
[redacted]

Estimate of the slope is = -1.730787 .
Standard error of the slope is = 0.219199 .
90% confidence limits for the slope are <-2.121480, -1.340095>.

P-value for the test of the positive dose response (slope at origin) is 1.000
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 6 Experiment Date: 12/23/96 Experiment No.: 050/96
Test Sample Name: GLIFOS, with metabolic activator.
Tester Strain: TA1535



O = Observed; * = Predicted.
The predicted values are based on Linear model.

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Salmonella Assay

Test Sample Name: GLIFOS, with metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 7   Exp. Date: 12/23/96   Exp. No.: 111
Technician:
Assay Type: Plate incorporation,
Strain: TA98   Activation S9: + RAT LIVER AROCLOR 4%
Data File Name: b:\model.sal

Code	Dose mg/L	--	counts		Mean	S.D.	Predicted Linear
	0.00	26	23	20	23.00	3.00	13.50
	0.00	14	13	17	14.67	2.08	13.50
	0.01	9	14	21	14.67	6.03	13.47
	0.10	9	10	20	13.00	6.08	13.24
	1.00	7	5	11	7.67	3.06	10.93
	5.00	0	2	0	0.67	1.15	0.65
P	0.01	300>	300>	300>			

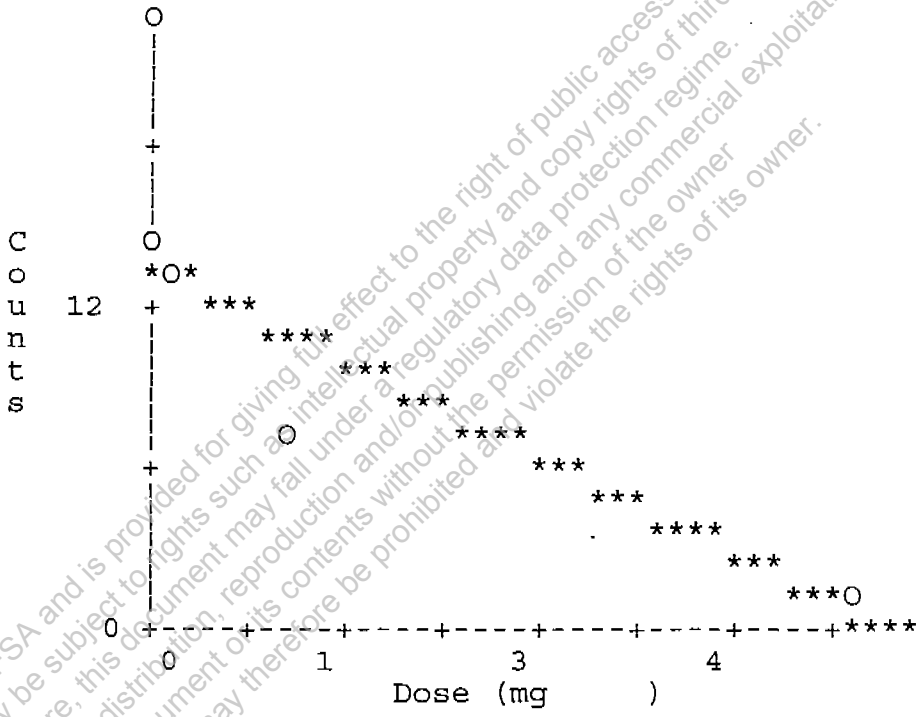
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.300

Estimate of the slope is = -2.570150 .
Standard error of the slope is = 0.333173 .
90% confidence limits for the slope are <-3.163985, -1.976315>.

P-value for the test of the positive dose response (slope at origin) is 1.000
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 7 Experiment Date: 12/23/96 Experiment No.: 111
Test Sample Name: GLIFOS, with metabolic activator.
Tester Strain: TA98



O = Observed; * = Predicted.
The predicted values are based on Linear model.

Salmonella Assay

Test Sample Name: GLIFOS, with metabolic activator.
Source/Batch/Lot: 1
Solvent: DISTILLED WATER
Record No.: 8 Exp. Date: 12/23/96 Exp. No.: 050/96
Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA97A Activation S9: + RAT LIVER AROCLOR 4%
Data File Name: b:\model.sal

Code	Dose mg/L	--	counts		Mean	S.D.	Predicted Linear
	0.00	133	157	143	144.33	12.06	147.60
	0.00	130	139	161	143.33	15.95	147.57
	0.01	149	152	143	148.00	4.58	147.32
	0.10	150	137	151	146.00	7.81	144.82
	1.00	117	140	120	125.67	12.50	119.82
	5.00	0	11	15	8.67	7.77	8.69
P	0.01	300>	300>	300>			

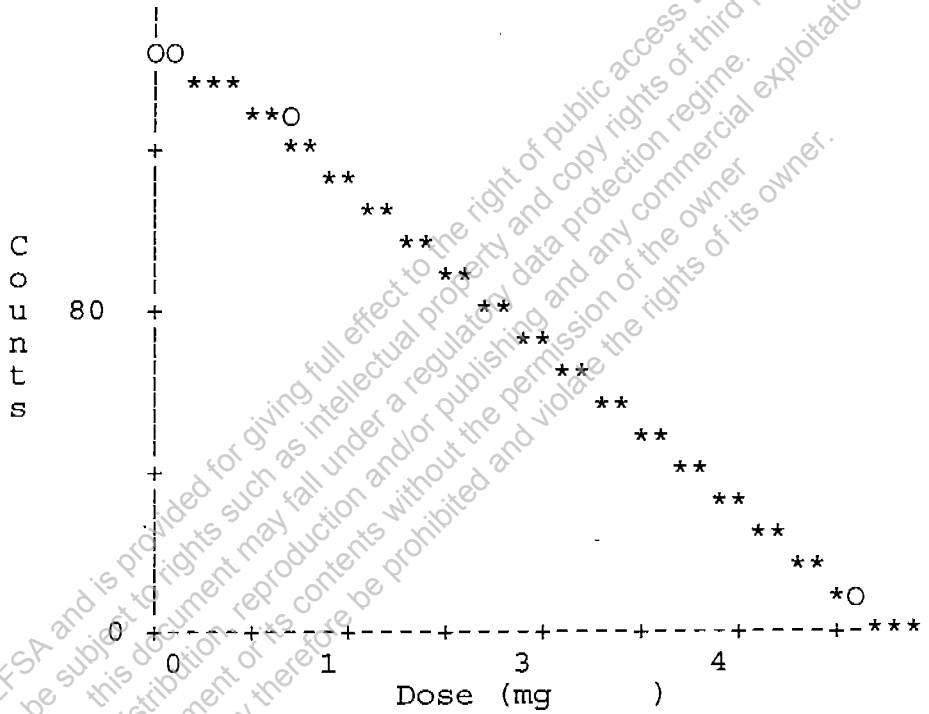
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.990

Estimate of the slope is = -27.782229 .
Standard error of the slope is = 1.558277 .
90% confidence limits for the slope are <-30.559642, -25.004815>.

P-value for the test of the positive dose response (slope at origin) is 1.000
Note: Smaller P-value means more positive dose response

Salmonella Assay

Record No.: 8 Experiment Date: 12/23/96 Experiment No.: 050/96
Test Sample Name: GLIFOS, with metabolic activator.
Tester Strain: TA97A



O = Observed; * = Predicted.
The predicted values are based on Linear model.

MOLTOX™ POST MITOCHONDRIAL SUPERNATANT (S-9)
PRODUCTION & QUALITY CONTROL CERTIFICATE

NO.: 0668	SPECIES: Rat	PREPARATION DATE: 21 May 1996
T NO.: 11-01L	STRAIN: Sprague Dawley	EXPIRATION DATE: 21 May 1998
UME: 2.1ml	SEX: Male	BUFFER: 0.154M KCl
	TISSUE: Liver	INDUCING AGENT(s): Aroclor 1254
ERENCE: Maron, D & Ames, B. Mutat. Res. 113:173, 1983		Monsanto Lot No. KL615 - 500mg/kg

USE: *Reconstitute with 2.1ml sterile purified water.*

CHEMISTRY:

- PROTEIN

36.8 mg/ml

Assayed according to the method of Lowry et al., JBC 193:265, 1951 using bovine serum albumin as the standard.

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By [illegible] date 13.12.96

- ALKOXYRESORUFIN-0-DEALKYLASE ACTIVITIES

[redacted]ctivity	P450	Fold - Induction
EROD	IA1, IA2	238.9
PROD	2B1, 3B2	51.9
BROD	3A, 2B	27.0

Assays for et[illegible] and benzyloxyresorufin-0-dealkylases (PROD, BROD) were conducted using a modification of the methods of Burke et al., Biochem Pharm 34:3337, 1985. Fold-inductions calculated as the ratio of the sample vs. uninduced control specific activities (SA). Control SA's (pmoles/min/mg protein) were 7.71, 3.75 & 50.3 for EROD, PROD & BROD, respectively

ASSAY:

- STERILITY TEST

Samples of S-9 were assayed for the presence of contaminating microflora by plating 1.0ml volumes on Trypticase Soy and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05mM L-histidine and D-biotin) media. Triplicate plates were read after 48 or 72h incubation at 37C. No evidence of contamination was observed.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
[redacted]tBr/ [redacted]A98	CPA/ TA1535
974.6	1267

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., Mutation Res 129:299, 1984. Data were expressed as revertants per ug EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 - 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to intermediates mutagenic to TA100. Assays were conducted using duplicate plates as described by Maron & Ames (Mutat. Res.113:173, 1983.).

	ul S9 per plate/number his+ revertants per plate					
Promutagen	0	1	5	10	20	50
BP (5ug)	85.5	261.5	519.5	615.5	804	820.5
2-AA (2.5ug)	100	516.5	1212.5	1208.5	1224	1177.5

ECULAR TOXICOLOGY, INC.
Gibralter St.
polis, MD 21401
268 7232

BIOAGRI - BIOTECNOLOGIA AGRÍCOLA LTDA.

Laboratório de Mutagenicidade

TESTE AMES (G.1.1.)

Test Code: 50/96

Product Code: 071 / 004 Lot#: 50928-01

Product: Glifos

Sponsor: chemiNova

Aspect of Product: (X) Liquid ( ) Solid

Densety: _____

Solvent: (X) water ( ) DMSO ( ) Others

Lot S9: 0668

Technician: [redacted]

Date: 23/12/96

Checked by: [redacted]

Date: 12/23/96

Test Code: 50/96

Treatment: (I) without activador metabolic
(II) with activador metabolic

Start of test: 20/12/96 Final of test: 23/12/96

Strain: 100

Control I	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	140	167	151
	0.25	40	0.01	180	159	142
	2.5	40	0.1	127	147	152
	25	40	1	75	121	97
	250	20	5	1	0	0
solvent						
negative				153	152	169
positive	0.015 mg	100	1.5 ug	>300	>300	>300

Strain: 100

By [redacted] date 23-12-96

Control II	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	165	179	142
	0.25	40	0.01	160	169	151
	2.5	40	0.1	172	152	160
	25	40	1	110	133	121
	250	20	5	6	8	07
solvent						
negative				161	168	151
positive	0.015 mg	100	1.5 ug	>300	>300	>300

Test Code: 50/96

Treatment: (I) without activador metabolic
(II) with activador metabolic

Start of test: 20/12/96 Final of test: 23/12/96

Strain: 1535

Control I	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	8	10	7
	0.25	40	0.01	8	8	11
	2.5	40	0.1	9	13	07
	25	40	1	4	5	6
	250	20	5	0	0	0
solvent						
negative				17	8	07
positive	0.015 mg	100	1.5 ug	>300	>300	>300

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Strain: 1535

Control II	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	12	9	11
	0.25	40	0.01	14	19	10
	2.5	40	0.1	7	14	09
	25	40	1	6	5	08
	250	20	5	1	1	03
solvent						
negative				16	16	14
positive	0.015 mg	100	1.5 ug	>300	>300	>300

Test Code: 50/96

Treatment: (I) without activador metabolic
(II) with activador metabolic

Start of test: 20 / 12 / 96 Final of test: 23 / 12 / 96

Strain: 98

Control I	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	17	20	16
	0.25	40	0.01	16	25	17
	2.5	40	0.1	14	20	21
	25	40	1	6	5	09
	250	20	5	1	1	04
solvent						
negative				34	34	21
positive	0.015 mg	100	1.5 ug			

Strain: 98

Control II	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	14	13	17
	0.25	40	0.01	9	14	21
	2.5	40	0.1	9	10	20
	25	40	1	7	5	11
	250	20	5	0	2	0
solvent						
negative				26	23	20
positive	~~0.015 mg~~ 0.1	100	~~1.5 ug~~ 0.01 mg	>300	>300	>300

Test Code: 120/96

Treatment: (I) without activador metabolic
(II) with activador metabolic

Start of test: 20 / 12 / 96 Final of test: 23 / 12 / 96

Strain: 97a

Control I	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	131	136	121
	0.25	40	0.01	128	157	161
	2.5	40	0.1	159	156	141
	25	40	1	60	37	52
	250	20	5	0	0	0
solvent						
negative				140	129	131
positive	0.015 mg	100	1.5 ug			

Strain: 97a

By [redacted] date 12 23 96

Control II	stock solution (mg/mL)	Volume/plate (uL)	conc./plate (mg)	Plate 1	Plate 2	Plate3
	0.025	40	0.001	130	139	161
	0.25	40	0.01	149	152	143
	2.5	40	0.1	150	137	151
	25	40	1	117	140	120
	250	20	5	0	11	15
solvent						
negative				133	157	143
positive	~~0.015 mg~~ 0.1	100	~~1.5 ug~~ 0.01mg	>300	>300	>300

CHEMINOVA

Cheminova Agro A/S
P. O. Box 9
DK-7620 Lemvig
Denmark

Phone (+45) 97 83 41 00
Fax (+45) 97 63 45 55
Telex 66514 CHEM.V DK
A/S reg. no. 177.122

ANEXO I

1. *Produto Técnico*

Marca Comercial	:	Glyphosate Technical
Ingrediente Ativo	:	GLIFOSATO (ISO)
Concentração	:	96% glifosato
Tipo de formulação	:	produto técnico
Lote	:	229-JAK-142-6

Identificação da amostra: AC 303,757 87.2% wet cake

2. *Produto Formulado*

Marca Comercial	:	Glifos
Sinonimias	:	CHE 3607, CHE 3690
Ingrediente Ativo	:	GLIFOSATO
Concentração	:	Glifosato como sal de isopropilamina 480 g/litro (ou 360 g/litro como Glifosato puro)
Tipo de formulação	:	solução aquosa
Lote	:	50928-01

Identificação da amostra: AC 303,757 36% SC

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By [redacted] date 12-23-96

BIOAGRI

CERTIFICATE OF ANALYSIS - CA 125/96

Subject:	GLIFOS
Common name:	Glyphosate
Product code (lab):	009/123
Batch no.:	50928-01
Date of analysis:	10/18/96
Quantity:	960 ml

RESULTS OF ANALYSIS

We certify that analysis of the sample of the above product gave the following results:

Content of active ingredient: 360.0 g/l

Content of glyphosate was determined liquid cromatograph using a Hewlett Packard LC Model 1050 at the following conditions: UV detector, stainless steel column, ODS-HYPERSIL, 250mm × 4mm × 5 µm film thickness.

Piracicaba October 18, 1996.

[redacted]
CRQ 04432306

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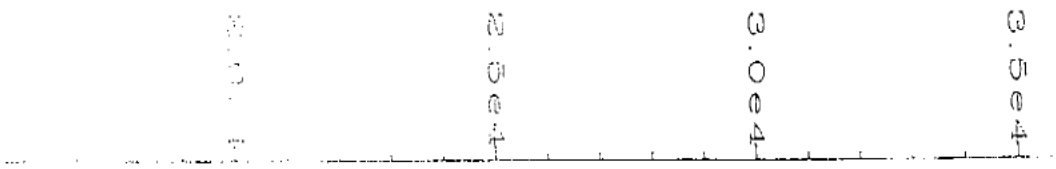
By [redacted] date 12-23-96

[redacted]
Technical Director



--GLIFOSATO 1.247

External Standard Report

File Name	: C:\HPCHEM\2\DATA\GLIFOSAT\AM37.D				
Operator	: [redacted]			Page Number	: 1
Instrument	: HPLC 1050			Vial Number	:
Sample Name	: GLIFOS FORMULADO			Injection Number	:
Sample Bar Code:				Sequence Line	:
Acquired on	: 18 Oct 96 10:08 AM			Instrument Method	: GLIFOSAT.MTH
Data Created on:	18 Oct 96 10:23 AM			Analysis Method	: GLIFOSAT.MTH
Last Recalib on	: 18 Oct 96 10:20 AM			Sample Amount	: 0
Multiplier	: 1			ISTD Amount	:

2 in C:\HPCHEM\2\DATA\GLIFOSAT\AM37.D

Inj	Area	Type	Width	Ref#	ng/ul	Name
247	25609	BB	0.069	1	122.001	GLIFOSATO

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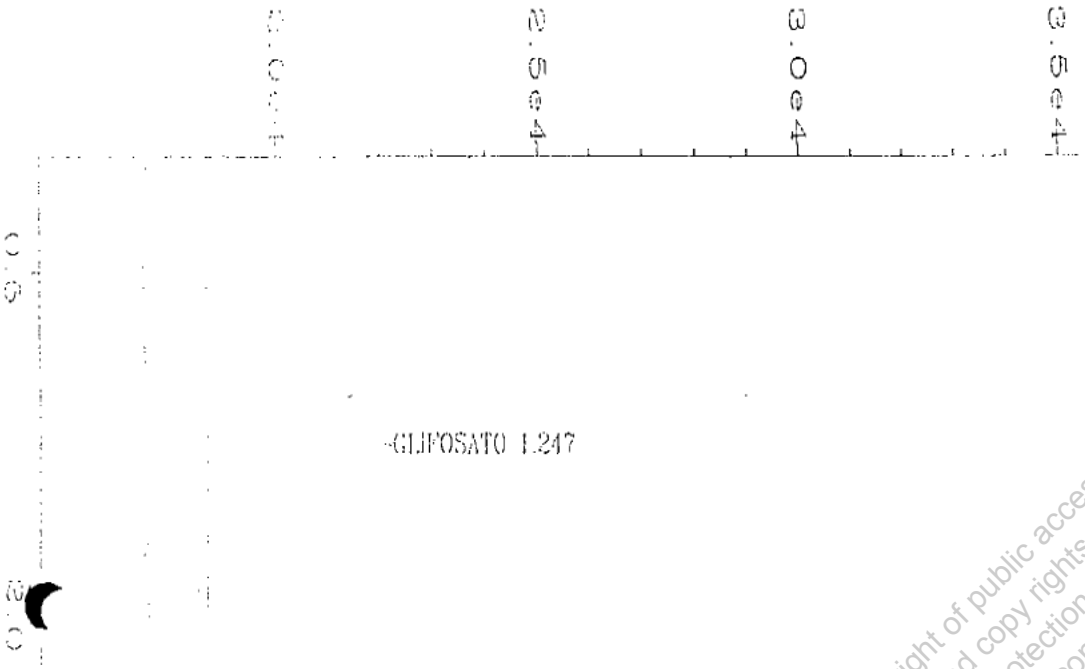
By [redacted] date 12-23-96



External Standard Report

File Name	: C:\HPCHEM\2\DATA\GLIFOSAT\P38.D		
Operator	: [redacted]	Page Number	: 1
Instrument	: HPLC 1050	Vial Number	:
Sample Name	: [redacted]	Injection Number	:
Sample Bar Code:		Sequence Line	:
Acquired on	: 18 Oct 96 10:00 AM	Instrument Method	: GLIFOSAT.MTH
Data Created on:	18 Oct 96 10:21 AM	Analysis Method	: GLIFOSAT.MTH
Recalib on	: 18 Oct 96 10:20 AM	Sample Amount	: 0
Multiplier	: 1	ISTD Amount	:

In C:\HPCHEM\2\DATA\GLIFOSAT\P38.D

Peak#	Area	Type	Width	Ref#	ng/ul	Name
47	23677	BB	0.076	1	112.800	GLIFOSATO

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By [redacted] date 12-23-96