Study title
A micronucleus study in mice for the product
GLIFOS

Data Requirements
Instituto Brasileiro do Meio Ambiente
e Recursos Naturais Renováveis - IBAMA
Portaria Normativa nº139, of December 21th, 1994

Study Completed on
November 18, 1996

Performing Laboratory
BioAgri - Biotecnologia Agrícola Ltda.
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BioAgri Report #
G.1.2 - 60/96

G1206096

AA041271

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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 A/S with the final report. All original raw data were retained at BioAgri - Biotecnologia Agrícola Ltda..

[redacted]

Study Director

11-19-96
mm/dd/yy

[redacted]

Director - BioAgri

11.19.96
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.1.2. - 60/96

Prepared by: [redacted]

Biologist, CRB 14635-01 [redacted] 11-19-96 mm/dd/yy

Approved by: [redacted] c., PhD. 11-19-96 mm/dd/yy

Quality Assurance Officer

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SUMMARY

A mouse bone marrow micronucleus assay was carried out in order to assess the mutagenic potential of the product GLIFOS by measuring its ability to induce chromosome breakage. The product was diluted in water and administered intraperitoneally twice with a 24 hour interval at levels of 68, 137 and 206 mg/kg corresponding to 75%, 50% and 25% of the LD_{50} for mice, respectively. Determination of the LD_{50} for the product as well as the negative and positive controls, used the two applications protocol with 24 hours interval. Negative control mice were treated with water and positive control mice with 25 mg/kg cyclophosphamide. Samplings were carried out 24 hours after the second application, when femurs were removed and smears of bone marrow cells prepared and stained. No increase in the number of polychromatic or normochromatic erythrocytes containing micronuclei was seen in animals treated with GLIFOS when compared to the vehicle control. A statistically significant increase in polychromatic and normochromatic erythrocytes containing micronuclei was observed in animals treated with cyclophosphamide. GLIFOS showed no evidence of mutagenic activity in this study.

SUMMARY (Portuguese)

Foi conduzido o Teste do Micronúcleo para estudar possíveis efeitos mutagênicos do produto GLIFOS em camundongos.O produto foi administrado por via intraperitonial em duas aplicações espaçadas de 24 horas nos níveis de 68; 137 e 206 mg/kg correspondendo a 75%, 50% e 25% da DL_{50} para camundongos, respectivamente. A determinação da DL_{50} bem como os controles positivos e negativos foram também conduzidos com um esquema de duas aplicações espaçadas de 24 horas. O controle negativo utilizou o veículo de diluição e o positivo, 25 mg/kg de ciclofosfamida. As avaliações foram efetuadas 24 horas após a segunda aplicação, quando foram removidos os fêmurs dos animais e esfregaços da medula óssea foram preparados e corados. Não houve aumento no número de eritrócitos policromáticos ou normocromáticos contendo micronúcleos nos animais tratados com o GLIFOS em comparação com o controle negativo. Por outro lado, houve uma elevação estatísticamente significativa nessas variáveis em animais tratados com ciclofosfamida. Os resultados indicam que nas condições do teste o produto GLIFOS não apresentou atividade mutagênica em camundongos.

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GENERAL INFORMATION

.Test Substance: GLIFOS

.Common name: glifosato

.Chemical name: N-(phosphonomethyl) glycine

.Analysed Composition: 360 g/L

.Declared Composition: 360 g/L

.Sponsor: Cheminova Agro A/S

.Study Schedule Experimental Study initiation: 08/Oct/1996
Experimental termination: 14/Nov/1996
Study completion: 19/Nov/1996

.Technical Staff: [redacted], Biologist
[redacted] Lab. Technician
[redacted], Lab. technician

I. INTRODUCTION

The micronucleus test **in vivo** is a method devised primarily for screening chemicals for chromosome breaking effects (Schmid, 1975). The test substances are normally applied sub-acutely to small mammals, and the effect is read in direct smears from bone marrow. Micronuclei arise mainly from chromosome fragments that are not incorporated into daughter nuclei at the time of cellular division, but they may also be generated from whole chromosomes that are not excluded from the telophase nucleus (Salamone & Heddle, 1983). The involvement of chromosomal aberrations, and thus of clastogens in carcinogenesis has been proposed by several authors (Cairns, 1981 ; Kinsella & Radman, 1978) because of the number of loci affected and structural rearrangements have greater consequences than do intragenic or point mutations.

The micronucleus test has been accepted as an indicator of chromosomal damage *in vivo* with about the same level of sensitivity as that of bone marrow metaphase analysis (Miller, 1973 ; Kliesch et al, 1982). This test is based on enumeration of micronuclei of mammalian bone marrow polychromatic erythrocytes (PCE). These cells are readily identifiable and have a relative short and defined life span, so that, any micronuclei they contain must have been generated as a result of recently induced chromosome damage. Other advantages of the test are the higher number of PCE and the consistent low rate of spontaneous micronucleated cells, in the region of three per thousand. The test also reacts positively to very low dosages of standard chromosome-breaking mutagens (Matter & Grauwiller, 1974 ; Von Ledebur & Schmid, 1973).

II. MATERIAL AND METHODS

1. Test substance

The GLIFOS sample was received on 23/July/1996, in good conditions, as a brown liquid in a plastic container. The product was mixed with sterile distilled water at proportions of: 4.53, 9.13 and 13.7 mg/mL and mixed with a test tube mixer to a homogeneous suspension. Appropriates amounts of those suspensions were given to mice in order to have the dosages used for the test: 68, 137 and 206 mg/kg of animal. Those levels correspond to 75% , 50% and 25% of the LD_{50} for mice.

2. Test Animal Management and Treatment.

Swiss albino male and female mice (7-10 weeks) bred in the animal house of our laboratory, were used. The animals were maintained on wood shavings, in propylene rodent cages (five of the same sex per cage) with stainless steel mesh lids. They were fed a commercial pelleted diet (Labina, Purina) and water was available ad libitum. The group of animals for the test was selected within mice of about the same age, and a random selection was carried out for defining groups of animals for each treatment. After treatment, mice were randomized and housed in groups of ten per cage.

3. Determination of the LD_{50}.

The LD_{50} was determined through the Thompson & Weil (1952)method using four levels of the product: 125, 250, 500 and 1000 mg/kg and 5 animals/level. The product was administered by two intraperitonial injections with 24 hours interval. Total mortality number was considered at 10 days after treatment.

4. Test compound exposure.

Three levels of the product were tested: 68, 137 and 206 mg/kg corresponding to 75%, 50% and 25% of the LD_{50} for mice, with ten animals (five male and five female) per level. The animals were dosed twice with intraperitoneal injections in volumes of 0.4 to 0.5 mL/animal within a 24 hours interval, and sacrificed 24 hours after the second injection. Negative control with sterile distilled water and positive control with cyclophosphamide (5 mg/mL in physiological solution, corresponding to 25 mg/kg), were also applied with the two injections protocol. According to Mavournin et al (1990), with that protocol, two sequential cell populations are treated in each animal which then can be sampled simultaneously. MacGregor (1991) demonstrated that a high level steady state frequency of micronucleated cells was established during repeated dosing and concluded that the use of two to four injections of the product in the same animal, and only one sampling time improves the efficiency of the micronucleus test as compared to single dosing, multiple samplings protocol.

5. Bone marrow preparation.

The following protocol is an adaptation of the procedure described by Schmid (1975). Mice were killed by cervical dislocation 24 hours after the second dosing. From the freshly killed animal both femora were removed in total. The bones were then freed from muscle, the distal epiphyseal portion was torn off by gentle traction and the proximal end of the femur was shortened with scissors until a small opening of the marrow was visible. The bone marrow cells were gently flushed out with fetal calf serum (W.L. Imunoquímica). After centrifugation at 1,000 rev./min. for 5 min., the bone marrow cells were resuspended in fetal calf serum and smeared on glass slides which were air dried overnight. The following day, the smears were fixed in ethanol 70 % for 10 min. air dried and stained for 20 min. with Eosin-Methylene Blue solution (Giemsa) diluted to 1:30 with phosphate buffer (PBS), pH 6.8. The smears were then rinsed in PBS followed by rinsing in tapwater.

6. Slide analysis.

The slides were coded and observed with a 1,000X magnification objective in a Olympus microscope. The technicians were not allowed to know the corresponding coding in the slides. For each animal 1,000 polychromatic erythrocytes (PCEs) and 1,000 normochromatic erythrocytes (NCEs) were examined for the presence of micronuclei (MN). The relation PCEs/NCEs were determined in the first 1,000 PCEs or NCEs enumerated.

7. Statistical analysis.

Differences in the incidence per animal of MNPCEs and MNNCEs per 1000 cells and the relation PCEs/NCEs were compared using the Mann-Whitney U test for k independent samples (Kruskal & Wallis, 1952 , cited by Coonover, 1980). All the tests were compared to the negative control. The criteria for a positive response was the detection of a reproducible and statistically significant (p = 0.05) positive response for at least one dose level and the increase in the number of micronuclei to be at least twice the vehicle control. The test is considered valid only if the number of micronuclei in the vehicle control stays within the historic value of the laboratory.

III. RESULTS

The LD_{50} for the GLIFOS was 275 mg/kg. The micronucleus incidence data are presented on table 3. The data were analyzed with and without considering the sex of the animals (tables 1 and 2, respectively). In both cases the Mann-Whitney U test for k independent variables (Kruskal and Wallis, 1952) showed no effect of GLIFOS on micronucleus formation on mice bone marrow erythrocytes when compared to the negative control. There was no effect on the polychromatic and normochromatic erythrocytes ratio when the animals were treated with GLIFOS. A statistically significant increase in micronucleated polychromatic and normochromatic erythrocytes was observed in animals treated with cyclophosphamide.

IV. CONCLUSION

Under the test conditions, the product GLIFOS did not have mutagenic activity in mice.

V. REFERENCES

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Table 1. Effect of GLIFOS and cyclophosphamide on the number of micronuclei in polychromatic and normochromatic erythrocytes in male and female mice bone marrow. Each value is the mean of five animals.

Group	Sex	Micronuclei in 1000 Polychr. erythr.	Normochr. erythr.	Poly-chromatic eryth.	Normo-chrom. erythr.	Polychr./ normochr. ratio
Vehicle	1	0.80000	0.40000	550.20	903.800	0.73556
68 mg/kg	1	0.20000	0.00000	929.80	889.200	1.07919
137 mg/kg	1	0.60000	0.20000	814.40	934.600	0.91584
206 mg/kg	1	0.00000	0.00000	890.40	835.400	1.13639
Cyclophosph.	1	5.20000*	1.20000*	1002.40	957.000	1.04965
Vehicle	2	0.00000	0.00000	1000.00	803.667	1.24633
68 mg/kg	2	0.16667	0.16667	954.33	885.167	1.12713
137 mg/kg	2	0.16667	0.00000	925.33	741.167	1.44039
206 mg/kg	2	0.00000	0.00000	985.75	798.750	1.36099
Cyclophosph.	2	3.16667*	1.33333*	631.33	996.667	0.63940

* Differ statistically from the vehicle control by the Mann-Whitney U test (Kruskal & Wallis,1952) at * for p = 0.05, and ** for p = 0.01.

Sex: 1= male; 2= female

Table 2. Effect of GLIFOS and cyclophosphamide on the number of micronuclei in polychromatic and normochromatic erythrocytes in mice bone marrow. Each value is the mean of ten animals.

Group	Micronuclei in 1000		Poly-chromatic eryth.	Normo chrom. erythr.	Polychr./ normochr. ratio
	Polychr. erythr.	Normochr. erythr.			
Vehicle	0.40000	0.20000	775.100	853.733	0.99095
68 mg/kg	0.18333	0.08333	942.067	887.183	1.10316
137 mg/kg	0.38333	0.10000	869.867	837.883	1.17812
206 mg/kg	0.00000	0.00000	938.075	817.075	1.24869
Cyclophosphamide	4.18333**	1.26667**	816.867	976.833	0.84453

* Differ statistically from the vehicle control by the Mann-Whitney U test (Kruskal & Wallis,1952) at * for p = 0.05, and ** for p = 0.01.

Table 3. Individual erythrocyte differentiation.

Dose	Sex	MNPE	MNNE	PE	NE	PE/NE ratio
0	1	4	2	445	1000	0.44500
0	1	0	0	1000	519	1.92678
0	1	0	0	273	1000	0.27300
0	1	0	0	337	1000	0.33700
0	1	0	0	696	1000	0.69600
1	1	0	0	1000	898	1.11359
1	1	0	0	863	1001	0.86214
1	1	1	0	739	1004	0.73606
1	1	0	0	1000	846	1.18203
1	1	0	0	1047	697	1.50215
2	1	0	0	1000	658	1.51976
2	1	0	0	653	1000	0.65300
2	1	1	0	849	1015	0.83645
2	1	0	0	870	1000	0.87000
2	1	2	1	700	1000	0.70000
3	1	0	0	834	1000	0.83400
3	1	0	0	1000	771	1.29702
3	1	0	0	617	1006	0.61332
3	1	0	0	1000	585	1.70940
3	1	0	0	1001	815	1.22822
4	1	1	1	1000	908	1.10132
4	1	0	0	1000	987	1.01317
4	1	1	0	898	1000	0.89800
4	1	24	5	1000	908	1.10132
4	1	0	0	1114	982	1.13442

SEX: 1= male, 2= female
DOSE: 0= negative control, 1= 68 mg/kg, 2= 137 mg/kg, 3= 206 mg/kg of GLIFOS and 4= positive control (25 mg/kg of cyclophosphamide.
MNPE: number of micronuclei in 1,000 polychromatic erythrocytes.
MNNE: number of micronuclei in 1,000 normochromatic erythrocytes.
PE: Polychromatic erythrocytes.
NE: Normochromatic erythrocytes.
PE/NE ratio: Polychromatic/normochromatic erythrocytes ratio.

(cont.)Table 3. Individual erythrocyte differentiation.

Dose	Sex	MNPE	MNNE	PE	NE	PE/NE ratio
0	2	0	0	1000	811	1.23305
0	2	0	0	1000	839	1.19190
0	2	0	0	1000	761	1.31406
1	2	0	0	1000	826	1.21065
1	2	0	0	803	1001	0.80220
1	2	0	0	1000	580	1.72414
1	2	0	0	923	1000	0.92300
1	2	1	1	1000	981	1.01937
1	2	0	0	1000	923	1.08342
2	2	0	0	1000	374	2.67380
2	2	0	0	1000	805	1.24224
2	2	1	0	769	1000	0.76900
2	2	0	0	1000	684	1.46199
2	2	0	0	1000	584	1.71233
2	2	0	0	783	1000	0.78300
3	2	0	0	1000	458	2.18341
3	2	0	0	1016	873	1.16380
3	2	0	0	1029	849	1.21201
3	2	0	0	898	1015	0.88473
4	2	2	4	577	1051	0.54900
4	2	0	0	1000	929	1.07643
4	2	0	0	180	1000	0.18000
4	2	0	0	436	1000	0.43600
4	2	16	4	862	1000	0.862
4	2	1	0	733	1000	0.733

SEX: 1= male, 2= female
DOSE: 0= negative control, 1= 68 mg/kg, 2= 137 mg/kg, 3= 206 mg/kg of GLIFOS and 4= positive control (25 mg/kg of cyclophosphamide.
MNPE: number of micronuclei in 1,000 polychromatic erythrocytes.
MNNE: number of micronuclei in 1,000 normochromatic erythrocytes.
PE: Polychromatic erythrocytes.
NE: Normochromatic erythrocytes.
PE/NE ratio: Polychromatic/normochromatic erythrocytes ratio.

Anexo II

BIOAGRI - BIOTECNOLOGIA AGRÍCOLA LTDA.

TESTE DE MICRONÚCLEO EM CAMUNGOS G.1.2.

No. do Estudo: 060/96

Empresa: (CYANAMID) CHEMINOVA AGRO

Produto: AC 303,757 - 36% S.C

Solubilidade: Estado Físico: líquido

Solvente utilizado: (∝) H2O ( ) Óleo de milho

D1(∝) D2( ) D3( ) C-( ) C+( )

DOSE: 68 mg/Kg

APLICAÇÕES	QUANT./KG	DATA / HORÁRIO	MORTALIDADE
PRIMEIRA	68 mg	21/10 / 11:20	
SECUNDA	68 mg	22/10 / 11:20	

No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	42	0,63		♂
	02	39	0,59		♂
	03	40	0,60		♂
	04	49	0,74		♂
	05	34	0,51		♂
No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	38	0,57		♀
	02	31	0,47		♀
	03	32	0,48		♀
	04	31	0,47		♀
	05	30	0,45		♀
	06				
F: 0,015	07				

CONCENTRAÇÃO DA SOLUÇÃO: V = DxP / C: 0,45 = 68 x 0,03 / C:

C = 2,04 / 0,45 = 4,53 mg = ml x 15 ml = 68 mg / 15 ml

DILUIÇÃO (PREPARO DA SOLUÇÃO): q.s.p { produto = 68 mg / solvente = 14,33 ml

ASPECTO DA SOLUÇÃO: SOLUÇÃO

PREPARADO POR: [redacted] DATA: 23 / 10 / 96

Anexo II

BIOAGRI - BIOTECNOLOGIA AGRÍCOLA LTDA.

TESTE DE MICRONÚCLEO EM CAMUNGOS G.1.2.

No. do Estudo: 060/96

Empresa: (CYANAMID) CHEMINOVA AGRO

Produto: AC 303,757 - 36% S.C

Solubilidade: Estado Físico: líquido

Solvente utilizado: (∝) H2O ( ) Óleo de milho

D1( ) D2(∝) D3( ) C-( ) C+( )

DOSE: 137 mg/Kg

APLICAÇÕES	QUANT/KG	DATA/HORARIO	MORTALIDADE
PRIMEIRA	137 mg	21/10/11:20	
SECUNDA	137 mg	22/10/11:20	

No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	37	0,56		♂
	02	37	0,56		♂
	03	44	0,66		♂
	04	35	0,53		♂
	05	38	0,57		♂
No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	28	0,42		♀
	02	28	0,42		♀
	03	31	0,47		♀
	04	32	0,48		♀
	05	30	0,45		♀
	06				
F=0,015	07				

CONCENTRAÇÃO DA SOLUÇÃO: V = DxP / C: 0,45 = 137 x 0,03 / C =

C = 4,11

0,45 = 913 mg - ml x = 15 ml = 137 mg / 15 ml

DILUIÇÃO (PREPARO DA SOLUÇÃO): q.s.p { produto → 137 mg / solvente → 14,86 ml }

ASPECTO DA SOLUÇÃO: Boa Visor

[redacted] date 11-18-96

PREPARADO POR: [redacted] By [redacted] DATA: 23 / 10 / 96

Anexo II

BIOAGRI - BIOTECNOLOGIA AGRÍCOLA LTDA.

TESTE DE MICRONÚCLEO EM CAMUNGOS G.1.2.

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Produto: AC 303,757 - 36% S.C

Solubilidade: Estado Físico: líquido

Solvente utilizado: (∝) H2O ( ) Óleo de milho

D1( ) D2( ) D3(∝) C-( ) C+( )

DOSE: 206 mg/Kg

APLICAÇÕES	QUANT./KG	DATA / HORARIO	MORTALIDADE
PRIMEIRA	206 mg	21/10 / 11:20	
SECUNDA	206 mg	22/10 / 11:20	

No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	43	0,65		♂
	02	42	0,63		♂
	03	44	0,66		♂
	04	46	0,69		♂
	05	37	0,56		♂
No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	36	0,54		♀
	02	34	0,51		♀
	03	29	0,44		♀
	04	31	0,47		♀
	05	33	0,50		♀
	06	35	0,53		♀
F= 0,015	07	30	0,45		♀

CONCENTRAÇÃO DA SOLUÇÃO: V = DxP / C: 0,45 = 206 x 0,03/C =

C = 6,18/

DILUIÇÃO (PREPARO DA SOLUÇÃO): q.s.p { produto → 206 mg / Solvente → 14,78 ml

0,45 = 13,73 mg - ml x - 15 ml = 206 mg / 15 ml

ASPECTO DA SOLUÇÃO: Sol Sol

PREPARADO POR: [redacted] DATA: 23 / 10 / 96

30 - 0,45 ♂

42 - 0,63 ♂

Anexo II

BIOAGRI - BIOTECNOLOGIA AGRÍCOLA LTDA.

TESTE DE MICRONÚCLEO EM CAMUNGOS G.1.2.

No. do Estudo: 060/96

Empresa: (CYANAMID) CHEMINOVA AGRO

Produto: AC 303,757 - 36% S.C.

Solubilidade: Estado Físico: líquido

Solvente utilizado: (✓) H2O ( ) Óleo de milho

D1( ) D2( ) D3( ) C-(✓) C+( )

DOSE: 25 ml/Kg

APLICAÇÕES	QUANT./KG	DATA / HORÁRIO	MORTALIDADE
PRIMEIRA	25 ml	21/10 | 14:30	
SECUNDA	25 ml	22/10 | 14:30	

No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	43	0,65		♂
	02	41	0,62		♂
	03	38	0,57		♂
	04	36	0,54		♂
	05	40	0,60		♂
No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	28	0,42		♀
	02	33	0,50		♀
	03	30	0,45		♀
	04	35	0,53		♀
	05	31	0,47		♀
	06				
F=0,015	07				

CONCENTRAÇÃO DA SOLUÇÃO: V = DxP / C: 25 ml de H_2O para cada 30 gramas de peso corpóreo

DILUIÇÃO (PREPARO DA SOLUÇÃO):

ASPECTO DA SOLUÇÃO: solúvel By [redacted] date 11-11-96

PREPARADO POR: [redacted] DATA: 23 / 10 / 96

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Produto: AC 303,757 - 36% S.C

Solubilidade: Estado Físico: líquido

Solvente utilizado: (✓) H2O ( ) Óleo de milho

D1( ) D2( ) D3( ) C-( ) C+(✓)

DOSE: 36,25 mg/Kg

APLICAÇÕES	QUANT./KG	DATA / HORÁRIO	MORTALIDADE
PRIMEIRA	36,25 mg	21/10 / 14:30	
SECUNDA	36,25 mg	22/10 / 14:30	

No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	37	0,56		♂
	02	40	0,60		♂
	03	40	0,60		♂
	04	48	0,72		♂
	05	40	0,60		♂
No. DE ANIMAIS	No.	PESO (g)	VOL. APLIC. (mL)	UNIDADES	SEXO
	01	29	0,44		♀
	02	29	0,44		♀
	03	30	0,45		♀
	04	32	0,48		♀
	05	28	0,42		♀
	06				
F = 0,015	07				

CONCENTRAÇÃO DA SOLUÇÃO: V = DxP / C: 0,45 = 36,25 x 0,031C

C = 1,08/

0,45 - 241 mg x - 15 ml x = 36,25 mg / 15 ml 36,25 mg

DILUIÇÃO (PREPARO DA SOLUÇÃO): de aclofolamida foram solubili
zados em 15 ml de solução salina (0,45 mg de NaCl
P/cada 15 ml de H_2O.

ASPECTO DA SOLUÇÃO:

PREPARADO POR: [redacted] By [redacted] date 11-18-96 23 / 10 / 96

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TESTE DE MICRONÚCLEO EM CAMUNGOS G.1.2.

No. teste 060/96

Empresa CHEMINOVA AGRO

Produto AC 303 757 - 36 % S.C

No. LAM	DOSE	SEXO	MICRON. POLICROM.	MICRON. NORMOCR.	REL. POL/NORM.	DATA
01	D1	♂	0	0	1000-898	06.11.96
02	D3	♂	0	0	834-1000	04.11.96
03	C-	♂	4	2	446-1000	08.11.96
04	D2	♂	0	0	1000.658	05.11.96
05	C-	♀	0	0	1000-811	05.11.96
06	D2	♀	0	0	1000.374	07.11.96
07	D3	♀	0	0	1000-458	06.11.96
08	C+	♀	2	4	577-1051	08.11.96
09	D1	♂	0	0	863.1001	06.11.96
10	C+	♀	0	0	1000-929	05.11.96
11	D1	♀	0	0	1000-826	05.11.96
12	C-	♂	0	0	1000.519	06.11.96
13	D3	♂	0	0	1000.771	05.11.96
14	D1	♂	1	0	739.1004	06.11.96
15	C+	♀	0	0	180-1000	05.11.96
16	C-	♀	0	0	1000.839	05.11.96
17	D2	♂	0	0	653.1000	08.11.96
18	C-	♂	0	0	273.1000	05.11.96
19	D1	♀	0	0	803.1001	04.11.96
20	C+	♂	1	1	1000.908	04.11.96
21	C-	♀				
22	D3	♂	0	0	617.1006	08.11.96
23	C+	♀	0	0	436-1000	08.11.96
24	C+	♂	0	0	1000.987	06.11.96
25	D2	♀	0	0	1000.805	05.11.96

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By [redacted] date 11-18-96

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TESTE DE MICRONÚCLEO EM CAMUNGOS G.1.2.

No. teste 060/96

Empresa CHEMINOVA AGRO

Produto AC 303,757 - 36 % S.C

No. LAM.	DOSE	SEXO	MICRON POLICROM.	MICRON NORMOCR.	REL. POL/NORM.	DATA
26	C-	♀	0	0	1000-761	07.11.96
27	C+	♂	1	0	898.1000	04.11.96
28	D2	♀	1	0	769.1000	05.11.96
29	D2	♂	1	0	849.1015	04.11.96
30	D3	♀	1			
31	D1	♀	0	0	1000.580	07.11.96
32	D3	♀	0	0	1016.873	07.11.96
33	D2	♂	0	0	870.1000	08.11.96
34	C+	♀	16	4	862.1000	04.11.96
35	D1	♀	0	0	923.1000	05.11.96
36	C+	♂	24	5	1000.908	07.11.96
37	D2	♂	2	1	700.1000	08.11.96
38	D1	♂	0	0	1000.846	06.11.96
39	D3	♀	0	0	1029.849	04.11.96
40	D1	♀	1	1	1000-981	04.11.96
41	D3	♀	0	0	898-1016	04.11.96
42	D1	♂	0	0	1047.637	05.11.96
43	C+	♀	1	0	733.1000	05.11.96
44	C-	♂	0	0	337.1000	08.11.96
45	D2	♀	0	0	1000.684	08.11.96
46	D3	♂	0	0	1000.585	06.11.96
47	C-	♂	0	0	696.1000	08.11.96
48	D2	♀	0	0	1000.584	06.11.96
49	C+	♂	0	0	1114.982	07.11.96
50	D3	♂	0	0	1001.815	05.11.96

PREPARADO POR: [redacted] DATA: 14/11/96

REVIDADO POR: [redacted] DATA: 19/11/96

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By [redacted] date 11-11-96

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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 x 4mm x 5 µm film thickness.

Piracicaba October 18, 1996.

[redacted]

CRQ 04432306

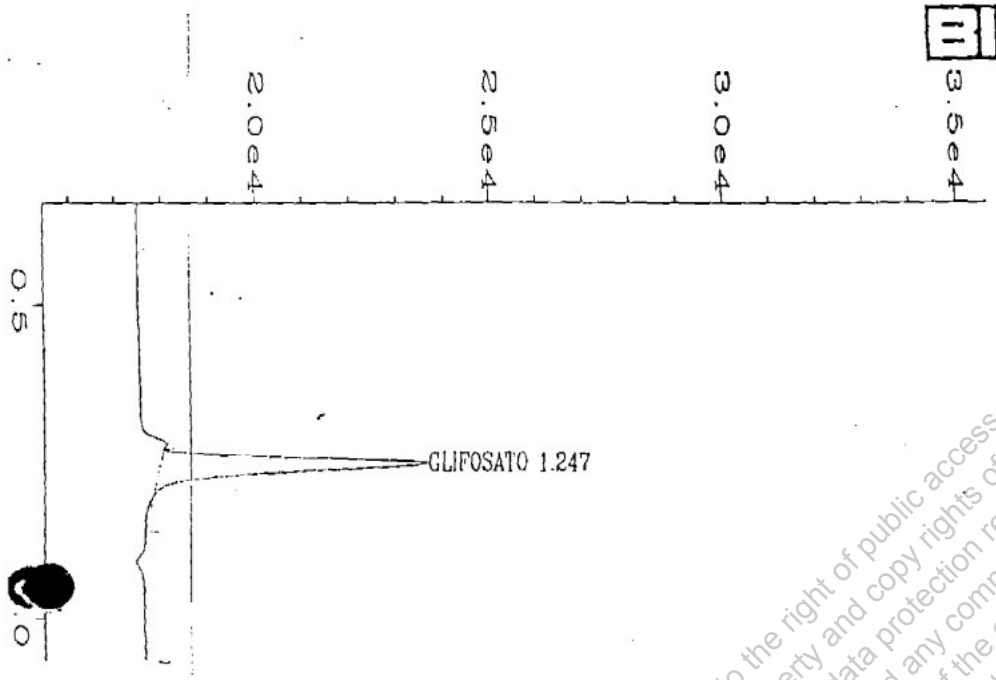
[redacted]

Technical Director

"This is an exact copy of The original document" date 11-18-96

By [redacted]

BIOAGRI BIOTECNOLOGIA AGRÍCOLA
Rodovia Piracicaba/Rio Claro (SP-127) - Km. 24 - Tel.: 019/421-3731 - Fax: 019/421-1106 - CEP 13412-000 - Cx. Postal 573 Piracicaba SP



External Standard Report

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le Name	: GLIFOS FORMULADO	Injection Number	:
Time Bar Code:		Sequence Line	:
ired on	: 18 Oct 96 10:08 AM	Instrument Method	: GLIFOSAT.MTH
rt Created on:	18 Oct 96 10:23 AM	Analysis Method	: GLIFOSAT.MTH
Recalib on	: 18 Oct 96 10:20 AM	Sample Amount	: 0
iplier	: 1	ISTD Amount	:

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

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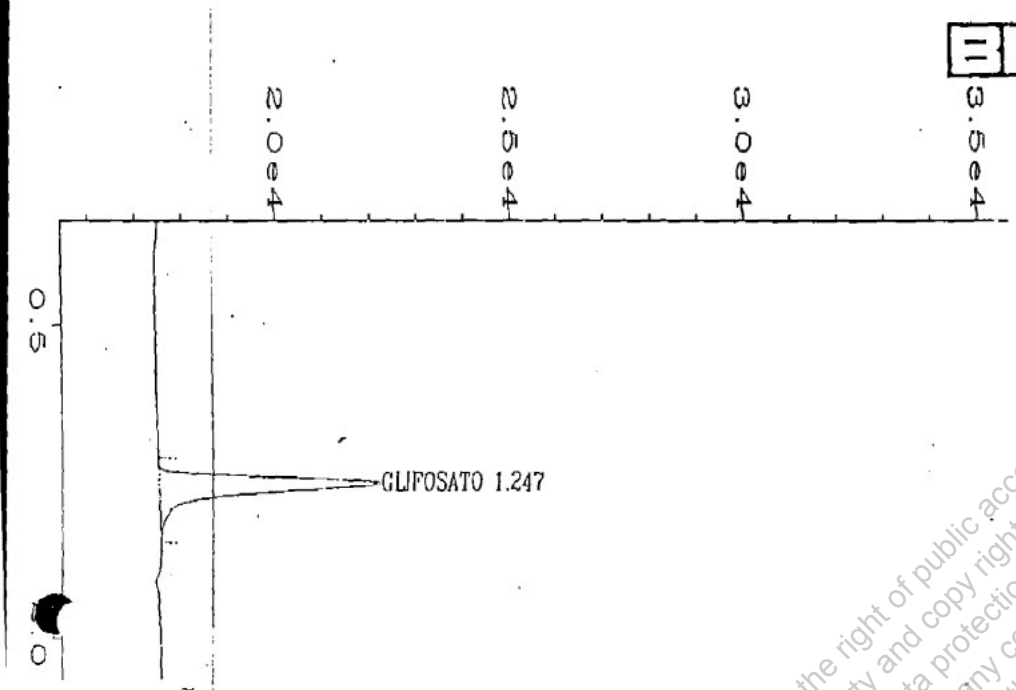
"This is an exact copy of
The original document"

By [redacted] date 11-18-96



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ment	: HPLC 1050	Vial Number	:
Name	: PADRÃO	Injection Number	:
me Bar Code:		Sequence Line	:
ed on	: 18 Oct 96 10:00 AM	Instrument Method	: GLIFOSAT.MTH
Created on:	18 Oct 96 10:21 AM	Analysis Method	: GLIFOSAT.MTH
ecalib on	: 18 Oct 96 10:20 AM	Sample Amount	: 0
lier	: 1	ISTD Amount	:

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

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47	23677	BB	0.076	1	112.800	GLIFOSATO

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By [redacted]

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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]

M.Sc., PhD.
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] PhD. 12/23/96 mm/dd/yy

Quality Assurance Officer

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INDEX

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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.

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

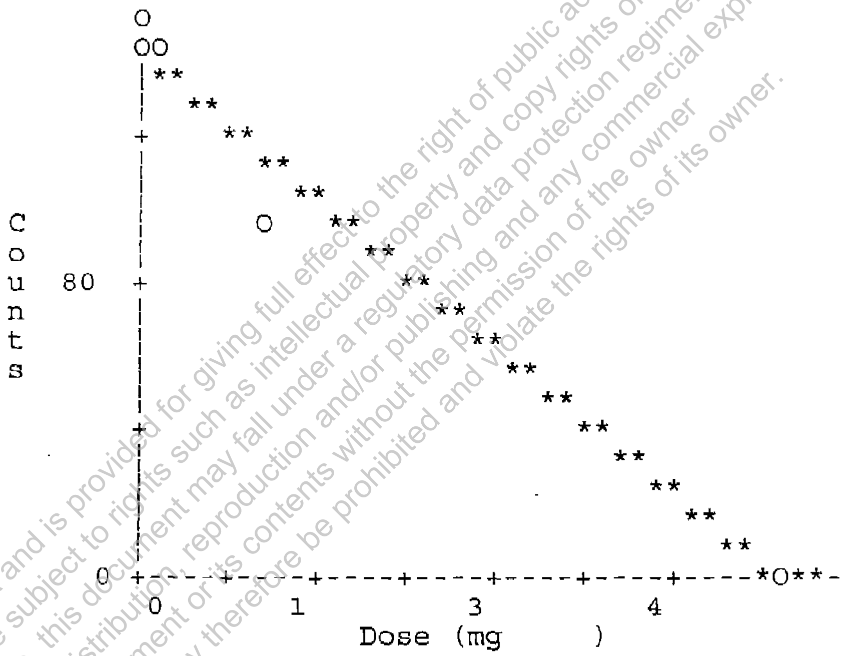
Estimate of the slope is = -28.561062 .
Standard error of the slope is = 1.440000 .
90% confidence limits for the slope are <-31.127661, -25.994462>.

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

BIOAGRI

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.

BIOAGRI

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

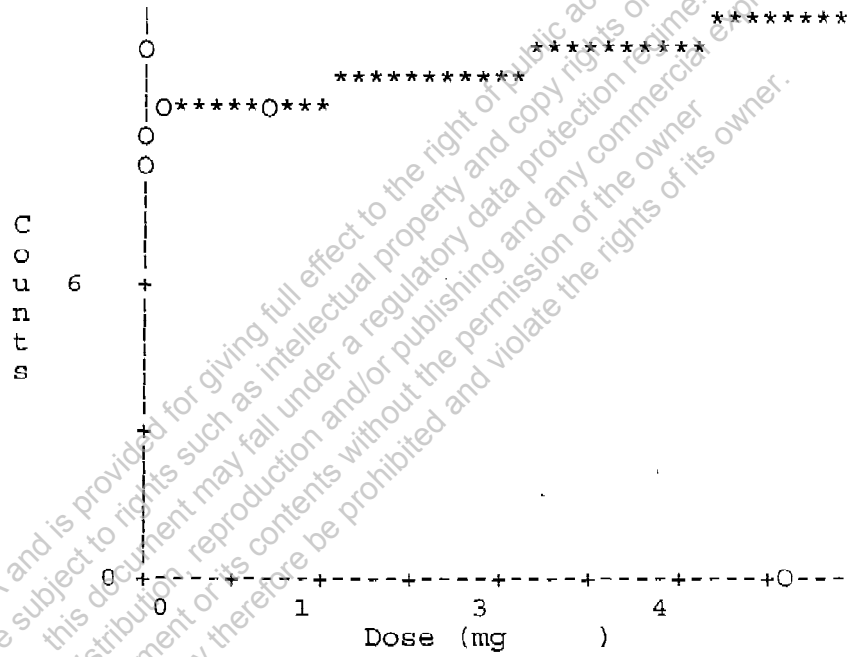
P-value for ANOVA test of dose response is 0.947
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.896

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.

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: [redacted]
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	

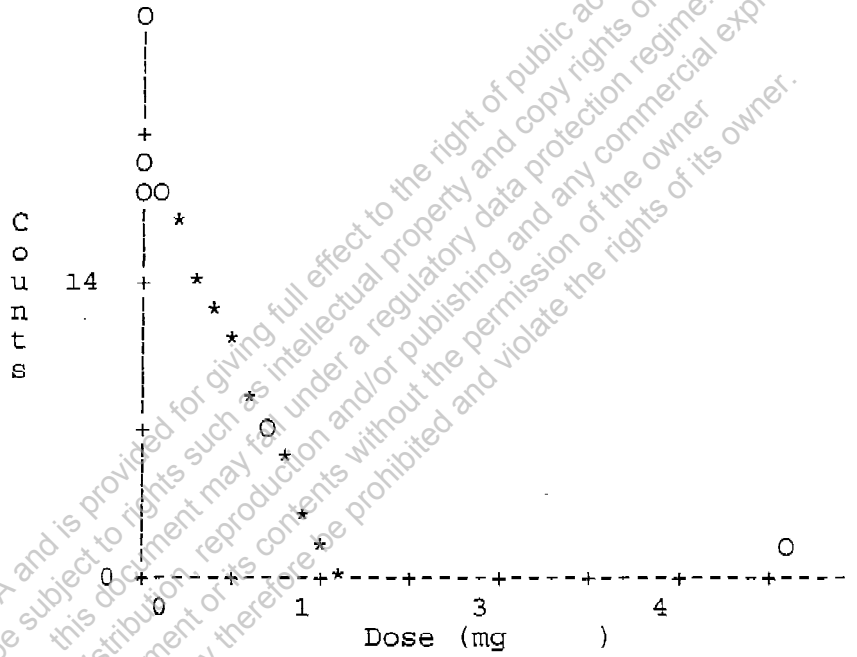
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Bernstein with pval = 0.565
Berstein model used the first 5 doses

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.

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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

[redacted]

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

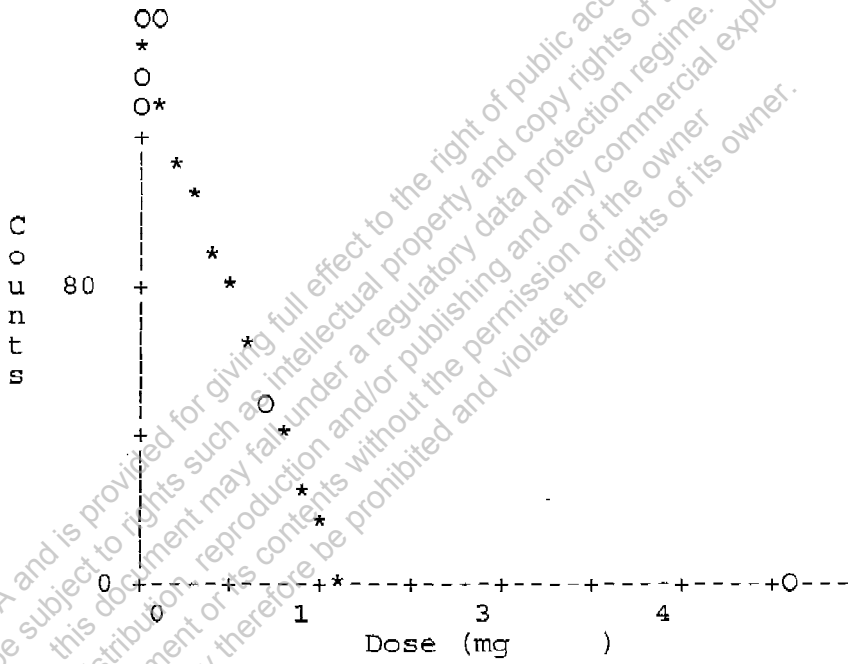
[redacted]

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.

BIOAGRI

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>			

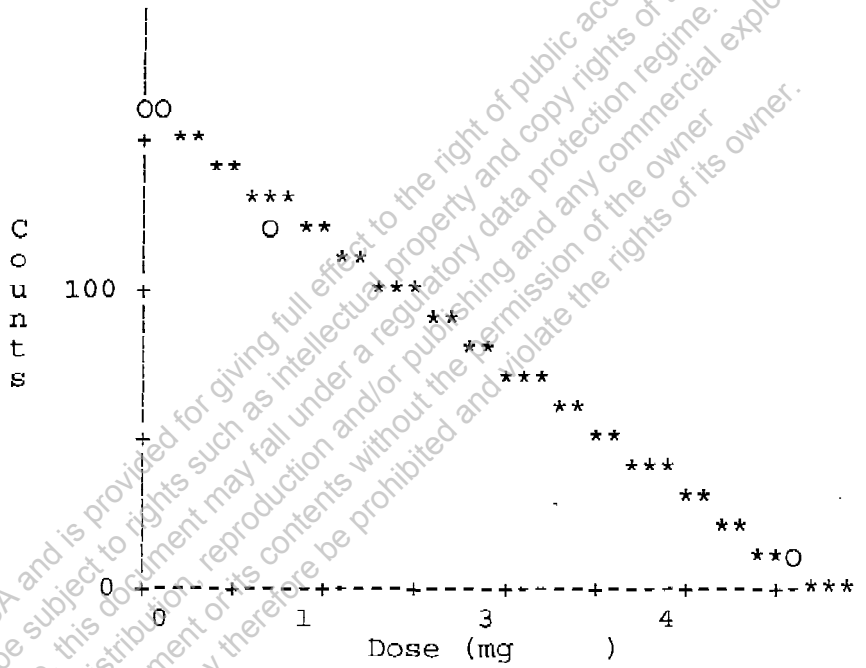
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.674

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.

BIOAGRI

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

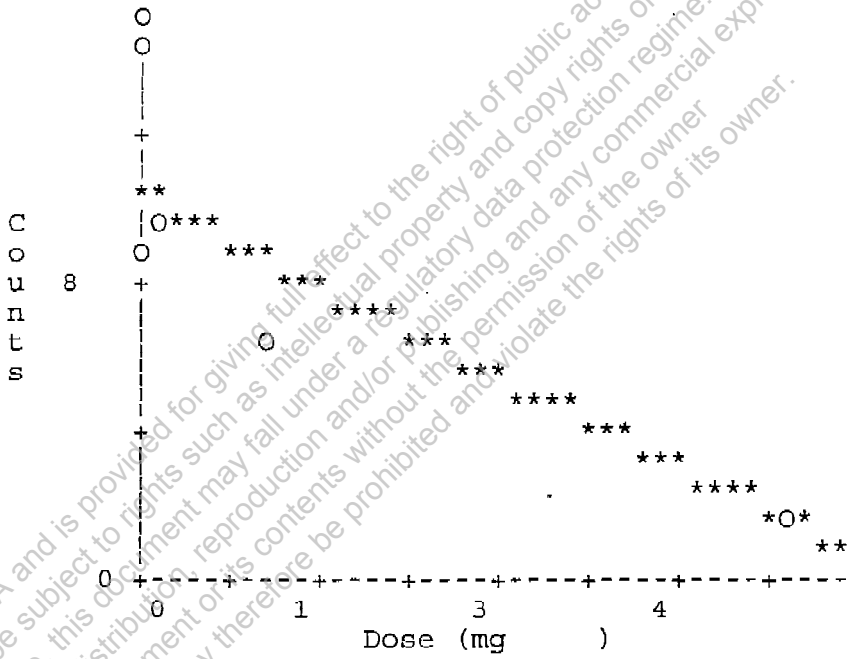
[redacted]

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: [redacted]
Assay Type: Plate incorporation,
Strain: TA98 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	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

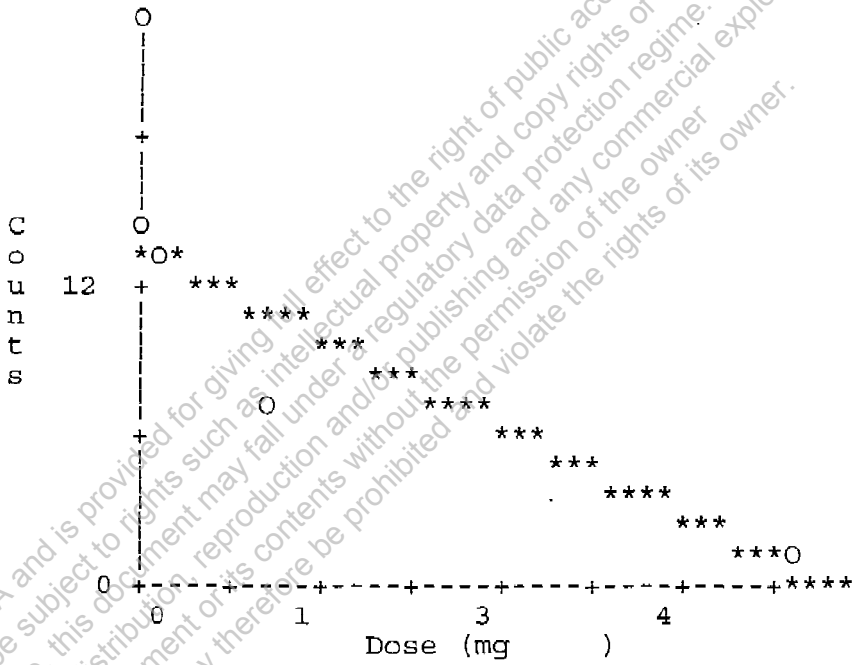
[redacted]

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.

BIOAGRI

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>			

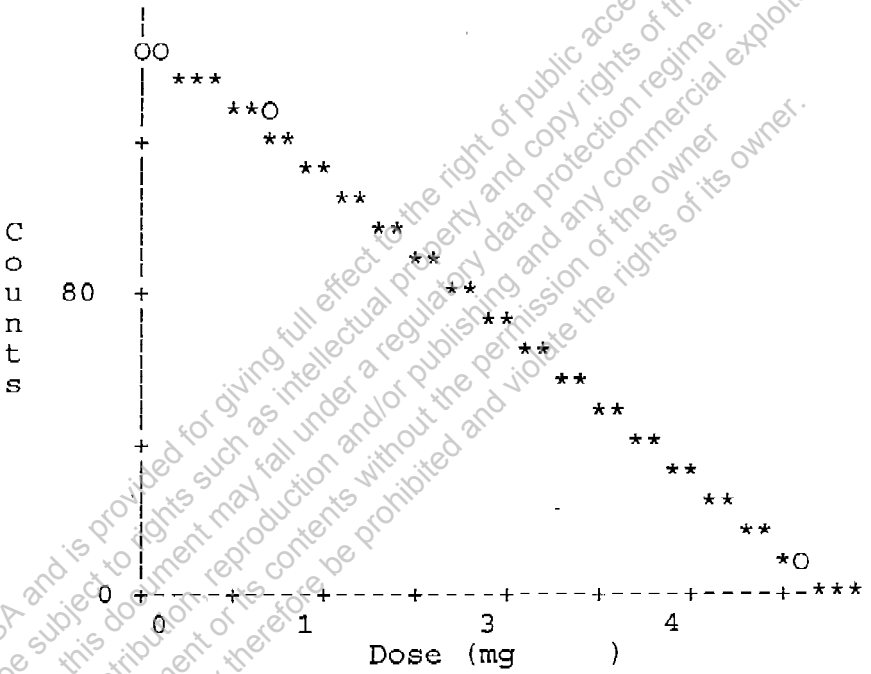
S: Negative control for use in analysis (solvent control)
N: Negative control not used in analysis
P: Positive control not used in analysis
P-value for ANOVA test of dose response is 0.000
An acceptable model is Linear with pval = 0.990

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.

- 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 ethoxyresorufin-0-deethylase (EROD), pentoxyresorufin-0-depentylase 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/ 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

LECULAR 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: 23/12/96

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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

Strain: 1535

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	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~~ 0.01mg	>300	>300	>300

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: 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			

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By [redacted] date 12-23-96

Strain: 97a

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

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Denmark

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Telex 66514 CHEMV 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 x 4mm x 5 µm film thickness.

Piracicaba October 18, 1996.

[redacted]

CRQ 04432306

[redacted]

Technical Director

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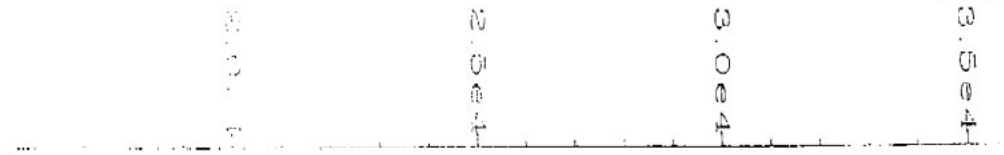
By [redacted] date 12-23-96



External Standard Report

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

Data File C:\HPCHEM\2\DATA\GLIFOSAT\AM37.D

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

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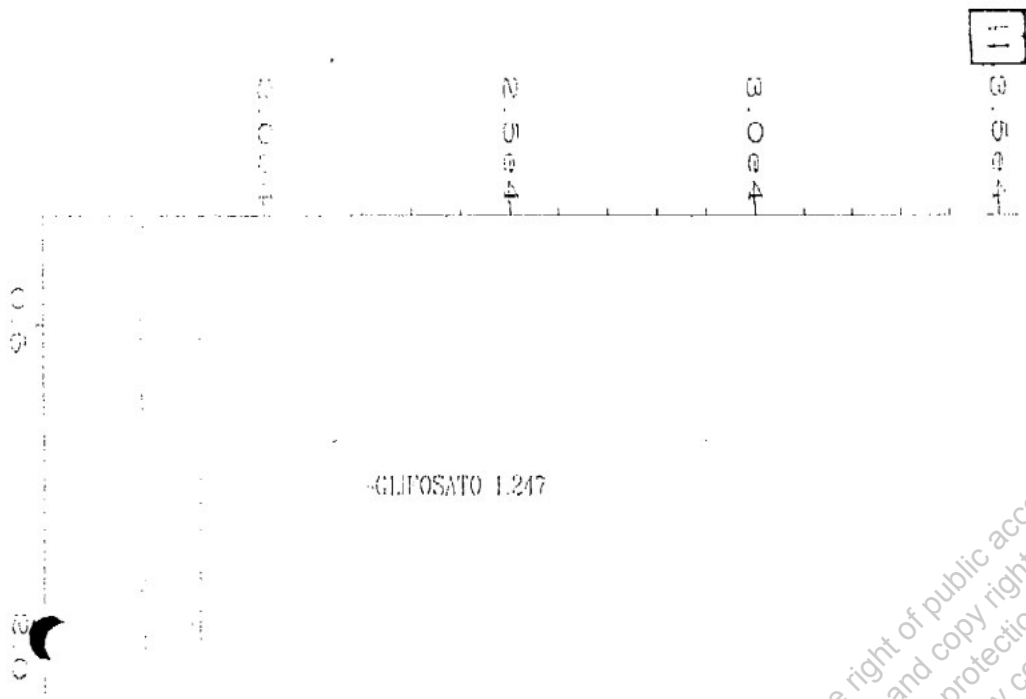
By [redacted] date 12-23-96



GLIFOSATO 1.247

External Standard Report

File Name	: C:\HPCHEM\2\DATA\GLIFOSATO\P38.D		
Operator	: [redacted]	Page Number	: 1
Instrument	: HPLC 1050	Vial Number	:
Sample Name	: PADRÃO	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	:

Data in C:\HPCHEM\2\DATA\GLIFOSATO\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