Study Title

Evaluation of the mutagenic potential of the test substance GLYPHOSATE TECHNICAL by reverse mutation assay in *Salmonella typhimurium* (Ames Test)

Guideline Reference

Organisation for Economic Co-operation and Development. – OECD Guideline for Testing of Chemicals. Genetic Toxicology, 471. 1997 Bacterial Reverse Mutation Test

Study Director

[redacted]

Final Report

15/Sep/2008

Performing Laboratory

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Sponsor

Jingma Chemicals Co., Ltd.
No.50 BaoTa Road, Longyou,
Zhejiang, China

Study #

3996.401.392.07

Study compliance statement

The study described in this final report was performed under my supervision, according to the study plan and procedures described in the Guideline Nº 471 of the Organisation for Economic Co-operation and Development (OECD, 1997) and following the Principles of Good Laboratory Practice (GLP) as established by the OECD - Organisation for Economic Co-operation and Development (revised 1997, ENV/MC/CHEM (98) 17) and INMETRO - Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (NIT-DICLA-035-INMETRO-Dec/2007).

This report represents an accurate and true recording of the results obtained.

Study plan, original raw data, copy of the final report and observations referent to this study are archived at BIOAGRI Laboratórios Ltda.

[redacted]

Study Director
Phone: [redacted]

15 / Sep / 2008
dd mmm yyyy

Final Report approval

The study described in this report was performed according to the Principles of Good Laboratory Practice (GLP) as established by the OECD - Organisation for Economic Co-operation and Development (revised 1997, ENV/MC/CHEM (98) 17) and INMETRO - Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (NIT-DICLA-035-INMETRO-Dec/2007).

The documents and records of this study will be archived at BIOAGRI for a period of 10 years.

15 / Sep / 2008
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Test Facility Manager
Phone: [redacted]

Study code: 3996.401.392.07
Study title: Evaluation of the mutagenic potential of the test substance GLYPHOSATE TECHNICAL by reverse mutation assay in *Salmonella typhimurium* (Ames Test).

Quality Assurance Unit Statement

This study has been audited, and the resulting final report was subsequently reviewed by the Quality Assurance Unit – BIOAGRI. The dates and phases of the audits are given below:

Audit		***Information date***	
Date	Phase	Study director	Test facility manager
04/Sep/2008	Study plan	04/Sep/2008	04/Sep/2008
21/Jul/2008	Draft report	22/Jul/2008	24/Jul/2008
15/Sep/2008	Final report	15/Sep/2008	15/Sep/2008

The most recent process audit prior to the completion of the laboratory phase of this class of study was performed on June 02nd, 2008. This audit has been recorded in the internal QAU document identified as RAS 067/08.

The results and observations presented in this final report are an accurate representation of the raw data generated during the conduct of this study.

[redacted]
Quality Assurance Officer
Quality Assurance Unit
Phone: [redacted]

15/Sep/2008
dd mmm yyyy

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Table index

Summary

The Ames Test, a mutagenic assay in procaryotic cells of *Salmonella typhimurium*, was carried out with the test substance GLYPHOSATE TECHNICAL in order to study its possible genetic effect on the strains TA 97a, TA 98, TA 100, TA 102 and TA 1535. The test was conducted with and without metabolic activator. The test substance was tested at five concentrations: 0.001, 0.01, 0.1, 0.5 and 1.0 mg plate^{-1}. The positive controls produced the expected increases in the number of revertants. The negative control presented a number of spontaneous revertants within the reversion rate for each strain. The test substance GLYPHOSATE TECHNICAL did not produce an increase in the number of revertants in the systems with and without metabolic activator, at any of the studied strains and concentrations when compared with the number of spontaneous revertants of control cultures treated with solvent (DMSO). These results indicate that, under the test conditions, the test substance GLYPHOSATE TECHNICAL did not exhibit mutagenic activity in the strains of *Salmonella typhimurium.*

Summary (Portuguese)

O Teste de Ames, um ensaio mutagênico em células procariontes de *Salmonella typhimurium*, foi conduzido com a substância teste GLYPHOSATE TECHNICAL visando estudar possível efeito mutagênico nas cepas TA 97a, TA 98, TA 100, TA 102 e TA 1535. O teste foi realizado com e sem ativação metabólica. A substância teste foi testada em cinco concentrações: 0,001; 0,01; 0,1; 0,5 e 1,0 mg placa^{-1}. Os controles positivos apresentaram os aumentos esperados nos números de revertentes. O controle negativo manteve o número de revertentes espontâneos dentro da taxa de reversão para cada uma das cepas. A substância teste GLYPHOSATE TECHNICAL não produziu um aumento no número de revertentes nos ensaios com e sem ativador metabólico, em nenhuma das cepas e concentrações estudadas quando comparadas ao número de revertentes espontâneos das culturas padrão tratadas com o solvente (DMSO). Esses resultados indicam que, nas condições do ensaio, a substância teste GLYPHOSATE TECHNICAL não apresentou potencial de atividade mutagênica nas cepas de *Salmonella typhimurium*.

1. General information

Study initiation:	04/sep/2007
Experimental phase initiation:	05/Jun/2008
Experimental phase conclusion:	30/Jun/2008
Draft report:	24/Jul/2008
Final report:	15/Sep/2008

2. Technical staff

Study director:	[redacted]
Laboratory technician:	
Laboratory technician:	
Personnel:	
Personnel:	
Personnel:	
Researcher:	
Laboratory technician:	
Laboratory technician:	

3. Introduction

The Ames Test was developed by [redacted] (AMES *et al.*, 1975) and reviewed by MARON & AMES (1983). CLAXTON *et al.* (1987) contributed with the principal orientations and recommendations in the assay performance as well as in the result evaluations. GATEHOUSE *et al.* (1994) summarized the principal recommendations of international consensus for the assay realization.

The test uses a set of *Salmonella typhimurium* strains, derived from the parental strain LT2, auxotrophic for histidine (*his*). Each standard strain has a different type of mutation in the histidine operon. Besides histidine mutation, the standard strains have other mutations that increase their ability in detecting mutagens. The *rfa* mutation increases the cell permeability to large molecules; the *uvrB* mutation renders the cells more sensitive to ultraviolet light; the strains with *pKM*101 plasmid – R factor are more sensitive to different mutagens and have greater resistence to ampicillin; and the presence of *pAQ*1 plasmid confers resistance to tetracycline. These strains are reverted by different ways and the response to the mutagenic agents depends on the interaction of the test substance with the bacteria DNA. The reversions occur specially by frameshift mutations and/or by base pair substitutions in DNA (MARON & AMES, 1983).

These strains are not capable of growing in a culture medium without histidine except for mutations that are reversions that restore histidine synthesis. The reverse frequency is measured by counting colonies units formed from a cell population that was exposed to a mutagenic substance. In addition to negative (solvent used to solubilize the test substance) and positive (mutagenic substances specific to the mutation sites at each standard strain) controls, the assay includes a system of metabolic activation (S9 mix) for promutagens or indirect mutagens (MARON & AMES, 1983). Those extra activators, like S9 mixture, are prepared using mammal enzymes, needed to detect some substances that are only mutagenic after being metabolized by some metabolic pathway not common to bacteria (EATON *et al.*, 1995).

The numbers of revertants for the test substance are calculated, for each strain, and statistical methods are used to evaluate the results.

4. Definitions

4.1. *rfa* mutation
The rfa mutation induces alterations in the lipopolysaccharide barrier of the bacterial cellular membrane, resulting in a greater permeability to large molecules.

4.2. *uvrB* mutation
The uvrB mutation caused by deletion of a gene responsible for the excision repair system, that prevents the repair of some DNA damages, consequently making the cell more sensible to mutagenic agents; for technical reasons, this mutation extended throught the biotin gene, determining also a requirement to this vitamin as growing factor.

4.3. Mutations by base pair substitutions
The mutations by base pair substitutions are mutations due to mutagens which cause nucleotide changes, by change or substitution, in the DNA molecule. In the reversion assay this change could occur at the site of the original mutation or at a second site in the chromosome.

4.4. Frameshift mutations
The frameshift mutations are mutations that occur due to the action of mutagens which cause addition or deletion of single or multiple base pairs in the DNA molecule.

4.5. Plasmid *pKM*101
The plasmid *pKM*101 is a plasmid that has a gene for ampicillin resistance, responsible for enhancing an error prone DNA repair system, making the bacteria more sensitive to mutagens.

4.6. Plasmid *pAQ1*
The plasmid *pAQ1* is a multicopy plasmid that has a gene for tetracycline resistance.

4.7. *his*⁻
Strain auxotrophic for histidine.

4.8. S9
Metabolic system activation for promutagens or indirect mutagens.

4.9. Ap^R
Strain ampicillin resistance.

4.10. Tt^R
Strain tetracycline resistance.

5. Objective

The aim of the assay is to evaluate the potential of GLYPHOSATE TECHNICAL for inducing mutations in the genome of the *Salmonella typhimurium* strains by measuring *his*⁻ to *his*⁺ reversion, with and without a system of metabolic activation.

6. Material and methods

6.1. Reagents

Reagent	CAS number	Batch number	Brand
Acetonitrile	75-05-8	706204	Tedia
Agar technical	-	7198746	Difco
Ampicillin ($C_{16}H_{19}N_3O_4S$)	69-53-4	106K0484	Sigma
Benzo(a)pyrene	50-32-8	03717JS	ALDRICH
Citric acid ($C_6H_8O_7$. H_2O)	5949-29-1	K91368144742	Merck
D(+)-glucose ($C_6H_{12}O_6$)	492-62-6	K35625437626	Merck
d-Biotin	58-85-5	A0212127001	Acrós
Dimetyl sulfoxide (DMSO) [$(CH_3)_2$ SO]	67-68-5	0702579	Vetec
di potassium hydrogen phosphate (K_2HPO_4)	7758-11-4	A755201646	Merck
di-sodium hydrogen phosphate (Na_2HPO_4)	7558-79-4	F1327886434	Merck

Glucose-6-phosphate (C_6H_{11} $Na_2O_9P.H_2O$)	3671-99-6	017K9152	Sigma
Glyphosate standard solution	-	395-135A	-
L-histidine ($C_6H_9N_3O_2.HCl.$ H_2O)	7048-02-4	026K0367	Sigma
Magnesium chloride hexahydrate ($MgCl.$ $6H_2O$)	7791-18-6	A649033524	Merck
Magnesium sulfate heptahidrate ($MgSO_4.$ $7H_2O$)	10034-99-8	A892986734	Merck
Methanol	-	B08E54	J.T.Baker
Milli-q water	-	LFQ1 038/08	-
NADP (nicotinamide adenide dinucleotide phosphate) ($C_{21}H_{27}N_7O_{17}P_3Na$)	1184-16-3	045K7011	Sigma
Nutrient broth nº2	-	544599	Oxoid
Phosphoric acid	-	30391	Quimex
Potassium chloride (KCl)	7447-40-7	K35183536544	Merck
Potassium hydrogen phosphate (KH_2PO_4)	-	044830	Vetec
Sodium ammonium hydrogen phosphate tetrahydrate ($NaNH_4HPO_4.$ $4H_2O$)	51750-73-3	A670482729	Merck
Sodium chloride (NaCl)	7647-14-5	K35518404601	Merck
Sodium hydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$)	10049-21-5	A629346541	Merck
S9 Fraction	-	2151	Moltox
Tetracycline ($C_{22}H_{24}N_2O_8$)	60-54-8	046K0733	Sigma

6.2. Equipment

Equipment	**Bioagri code**
Mechanical stirrer	AT-01, 21, 22 and 06
Air insufflator	IA-01
Autoclave	AU-02, 07, 08 and 10
Semi-analytic balance	B-34
Analytic balance	B-06, 18, 16 and 30
Water bath	BM-19 and 43
Water bath with shaker plataform	BM-18 and 42
Cryogenic flask	BC-01 and 02
Colony counter	CC-05, 06 and 07
Laminar flow chamber	CF-06 and 07
Incubator chamber	CI-15, 49, 54, 55 and 56
Liquid chromatograph	CL-01
Stove of dry and sterilization	EST-11
Microwave stove	FM-06
Freezer	FR-19
Refrigerator	GE-09 and 18
Micropipets	MA-106,107,151,152,164,165,166,167,127 and 132
pHmeter	P-21
Graduated pipet	PG-06
Glass thermometer	TEI-13 and 15
Thermometer of maximum and minimum	TM-104, 105, 187, 188, 192, 193, 202, 203, 204, 205 and 206
Glass thermometer	TR-91, 154 and 155
Ultrasound bath	US-01
Laboratory general glassware	-

6.3. Test substance information

Test substance:	GLYPHOSATE TECHNICAL
Supplier	Jingma Chemicals Co., Ltd.
Common name:	glyphosate
Chemical name (IUPAC):	*N*-(phosphonomethyl)glycine [1][2]
BIOAGRI code:	AGR-0790/07
Received on:	20/Aug/2007
Physical state	Solid
CAS number:	1071-83-6 [1][2]
Batch number:	20070606
Declared concentration of a.i. (Sponsor):	min. 980.0 g kg^{-1}
Analysed concentration of a.i. (BIOAGRI):	980.5 g kg^{-1}
Analysis certificate:	CA 1302/07
Homogeneity Test:	Homogeneous (TH 189/07)
Chemical formula:	$C_3H_8NO_5P$ [1][2]
Molecular weight:	169.1 [1]
Solubility in water (a.i.):	10.5 g L^{-1} (pH 1.9, 20°C) [1]
Solubility in organic solvents (a.i.):	Practically insoluble in common organic solvents, e.g. acetone, ethanol and xylene. The alkali-metal and amine salts are readily soluble in water. [1]
Stability data (a.i.)	Glyphosate and all its salts are non-volatile, do not photochemically degrade in buffered water and are stable in air. Glyphosate is stable to hydrolysis at pH 3, 6 and 9 (5-35°C). [1]
Test substance sent by:	Jingma Chemicals Co., Ltd.
Source:	[1] TOMLIN, (2006-2007) [2] Information provided by sponsor

6.4. Standard strains – Negative, positive and solvent controls

Tables 1 and 2 show the genetic characteristics, historical data of the laboratory for negative, solvent and positive controls and the historical control range of literature for each standard strain of *Salmonella typhimurium*. The strains used in the study should yield spontaneous revertant colony plate counts within the frequency ranges expected from the laboratory's historical control data and preferible within the range reported in the literature (OECD, 1997 No. 471). The historical control data of literature are used to help the interpretation of the results obtained in the laboratory, and the statistical treatment of the historical data of the laboratoy should be a guide rather than a rule (LOVELL, 1997). In this context, the data obtained in the study, that could be upper or/and lower than the historical data, should be jugded with care, considering the biological mean.

Table 1- Strains that detect mutagens which cause frameshift mutation in the bacterial DNA and respective historical data of spontaneous reversion of laboratory and literature.

Strain	Mutation Site	Genotype	Lower and upper limit control * Historical data of laboratory						Historical data of literature**	
			Negative control		Water		Positive control		Negative control	
			S9		S9		S9		S9	
			+	-	+	-	+	-	+	-
TA 97a	**CG**	*His* D6610, *rfa*, Δ *uvr*B, *bio*-, *pKM*101 (Ap^R)	89-172 (104) [47]	80-165 (106) [46]	84-166 (103) [53]	92-154 (78) [41]	> 344	> 330	100-200	75-200
TA 98	**CG**	*His* D3052, *rfa*, Δ *uvr*B, *bio* -, *pKM*101 (Ap^R)	31-58 (34) [18]	27-49 (29) [15]	31-54 (29) [15]	28-47 (24) [13]	> 116	> 98	20-50	20-50

Key: *His* = mutation of gene responsible for histidine synthesis; *rfa* = permeability of the lipopolysaccharide membrane; Δ *uvr*B = deletion of the gene *uvr*B; *bio*- = biotin mutation; Ap^R = ampicillin resistance; +: with S9, -: without S9.
* Historical data from January to December of 2007; [ ]: mean deviation of upper control limit. ( ): Standard deviation.
** Source: Mortelmans and Zeiger (2000).

Table 2 - Strains that detect mutagens which cause base pair substitutions in the bacterial DNA and respective historical data of spontaneous reversion of laboratory and literature.

Strain	Mutation Site	Genotype	Lower and upper limit control * Historical data of laboratory						Historical data of literature**	
			Negative control		Water		Positive control		Negative control	
			S9		S9		S9		S9	
			+	-	+	-	+	-	+	-
TA 1535	CG	*His* G46, *rfa*, Δ *uvr*B, *bio* -	26-47 (26) [14]	26-46 (25) [13]	25-42 (23) [12]	25-44 (24) [13]	> 141	> 132	5-20	5-20
TA 100	CG	*His* G46, *rfa*, Δ *uvr*B, *bio* -, *pKM*101 (Ap^R)	115-182 (85) [43]	117-188 (90) [55]	114-177 (80) [42]	114-182 (86) [46]	> 364	> 376	75-200	75-200
TA 102	AT	*His* G428, *rfa*, *pKM*101 (Ap^R), *pAQ*1(Tt^R)	233-321 (111) [59]	223-306 (104) [55]	242-311 (88) [46]	234-310 (95) [50]	> 642	> 622	200-400	100-300

Key: *His*= mutation of gene responsible for histidine synthesis; *rfa*= permeability of the lipopolysaccharide membrane; Δ *uvr*B= deletion of the gene *uvr*B; *bio*- = biotin mutation; Ap^R= ampicillin resistance; Tt^R= tetracycline resistence; +: with S9, -: without S9.
* Data from January to December of 2007; ( ) mean deviation of upper control limit; [ ]: Standard deviation.
** Source: Mortelmans and Zeiger (2000).

6.5. Reference substances information – positive controls

Positive controls strain specific used in the tests without metabolic activation:

- Sodium azide:

Chemical name	Sodium azide
Batch number:	609048
Brand:	Merck
CAS number:	26628-22-8
Solvent:	Purified Water
Declared concentration:	99%
Utilized concentration:	1 mg mL^{-1} (0.05 mg $plate^{-1}$)
Standard strains:	TA 100 and TA 1535

- 2-Nitrofluorene:

Chemical name:	2-Nitrofluorene
Batch number:	ES 02408LR/ N1,675-4
Brand:	Aldrich Chem. Co.
CAS number:	607-57-8
Solvent:	DMSO
Declared concentration:	98%
Utilized concentration:	1 mg mL^{-1} (0.05 mg $plate^{-1}$)
Standard strain:	TA 98

- 9-aminoacridine:

Chemical name:	9-Aminoacridine
Batch number:	106F06681/ A-7295
Brand:	Sigma
CAS number:	90-45-9
Solvent:	ethylic alcohol
Declared concentration:	98%
Utilized concentration:	1 mg mL^{-1} (0.05 mg $plate^{-1}$)
Standard strain	TA 97a

- Cumene hydroperoxide:

Chemical name:	Cumene hydroperoxide
Batch number:	89H0823
Brand:	Sigma
CAS number:	80-15-9
Solvent:	Purified Water
Declared concentration:	approx. 80%
Utilized concentration:	1 mg mL^{-1} (0.1 mg $plate^{-1}$)
Standard strain:	TA 102

For the tests with the metabolic activation system, the reference substance used as positive control, with the standard strains TA 97a, TA 98, TA 100, TA 102 and TA 1535, was:

- 2-Aminoanthracene:

Chemical name:	2-Aminoanthracene
Batch number:	77H1867
Brand:	Sigma
CAS number:	613-13-8
Solvent:	DMSO
Utilized concentration:	1 mg mL^{-1} (0.05 mg $plate^{-1}$)

6.6. Test system storage

The strains were acquired from Moltox Toxicology, Inc. and they are usually available in small paper discs where they are adsorbed and sealed in small plastic bags with a little agar. On receipt, they are reactivated and permanent stock cultures are prepared by addition of DMSO 5% (dimethylsulfoxide) as cryoprotective agent, where they are maintained under freezing condition in liquid nitrogen (-196°C). The Master plates are prepared from the permanent stock cultures for routine use and kept up under refrigeration for no more than two months.

The genotypes of each strain are systematically confirmed immediately after receiving the cultures, when a Master plate is prepared from stock cultures or when any genotype characteristics are not found in accordance with the laboratory control.

The genetic characteristics verification of each strain is tested from overnight cultures, that originated Master plate or from new cultures, for the number of spontaneous revertants, histidine dependence, presence of *rfa* mutation by testing sensitivity to the crystal violet, presence of *uvr*B deletion checking the sensitivity to ultraviolet, presence of *pKM*101 and *pAQ*1 plasmids that confer resistance to ampicillin and tetracycline, according to SOP M–0008 (rev. 09), based on MARON & AMES (1983).

6.7. Preparation of the *Salmonella typhimurium* inocula

An aliquot of 120µL of each defrosted stock culture was inoculated in 30 mL of nutrient broth. The inoculated flasks were incubated at 35-37°C for 10-12 hours, under agitation (150-170 rpm), so that the cells density was 10^8 to 10^9 cells mL^{-1}.

The feasibility of each strain is done by counting the formed colony units. (SOP–M 0261, rev. 14), based on MARON & AMES (1983).

6.8. Culture medium

The following culture media were used:

- Nutrient Broth: Oxoid Nutrient Broth N° 2 was prepared at a concentration of 2.5% in purified water and autoclaved prior to use. This was used for the preparation of liquid cultures of the tester strains;
- Nutrient Agar: Difco Nutrient Agar (23g) was added to 1 litre of purified water and autoclaved. The solution was then poured into 9 cm plastic Petri dishes and allowed to solidify and dry before use. These plates were used for the non-selective growth of the tester strains. Incubation on Nutrient Agar were done for approximately 48 or 72 hours;
- Minimal Agar+ glucose + VB: Minimal medium agar was prepared with 1.5% Difco agar technical in Vogel-Bonner Medium E, with 2% D(+) Glucose, except for TA 97a when the agar minimal medium was prepared with 0.4% of glucose, and poured into 9 cm plastic Petri dishes;
- Top Agar: "Top Agar" (overlay agar) was prepared as 0.6% Difco agar technical + 0.5% NaCl in 1000 mL of purified water. This solution was autoclaved and stored. Prior to use 10 ml of a sterile solution of 0.5 mM d-Biotin + 0.5 mM L-Histidine was added to 100 mL of the top agar.

6.9. Preparation of the metabolic activator – S9

The S9 mix – lyophilized microsomal fraction from rat liver activated with Aroclor 1254 (Moltox Toxicology Inc., Annapolis, MD, U.S.A.) cofactor-supplement (NADP and solutions of potassium chloride, magnesium chloride hexahidrate, phosphate buffer and glucose-6-phosphate) was prepared immediately before the test beginning, (following the instructions of SOP–M 0261 (rev. 14) in accordance with MARON & AMES (1983)). The S9 mix was maintained in a ice bath for no more than five hours. Every care of asepsis during preparation and manipulation of the S9 mix were kept up. At the same time, a sterility test was conducted for each solution and S9 fraction used.

Before each batch of S9 was used, its capacity to metabolize a substance was checked making a previous assay with a positive control that caused reversion in presence of S9 fraction. Any available strain of *Salmonella typhimurium* and reference substance can be used. In case of using 2-aminoanthracene, the rate of spontaneous reversion should also be checked with benzo(a)pyrene, because the 2-aminoanthracene is a mutagenic agent that does not need metabolization.

Each new batch of S9 is checked for sterility, taking a flask of S9 prepared by addition of 2 mL sterilized purified water, than plating 0.1 mL in nutrient agar plates that are incubated at 37°C (±1) for 72 hours. After this period the presence of colonies are evaluated. The batch is considered sterile if no growth is observed.

The test was performed using 2 replicates with and without mix-S9 at 4 concentrations each, following the method by plate incorporation in the same way of the test.

The mixture of S9 (5%), tissue fraction, and cofactors was prepared for each 40 mL, as follows:

S9 tissue fraction	2.00 mL
NADP (100 mM)	1.60 mL
Glucose-6-Phosphate (100 mM)	0.20 mL
$MgCl_2$/ KCl	0.80 mL
Phosphate buffer (pH 7.4, 200 mM)	20.00 mL
Distilled Water	15.40 mL
	40.00 mL

6.10. Preparation of the test substance

The first stock solution used in the definitive test was obtained by homogenization of 1.25 gram of the test substance in a volumetric flask at final volume of 10.0 mL with solvent (DMSO). From the first stock solution four other ten fold dilutions were prepared to obtain apropriated concentrations on plates. The final concentrations used on plates were: 0.001, 0.01. 0.1, 0.5 and 1.0 mg plate^{-1}, according to OECD (1997 N° 471) recommendation. A preliminary test, using 2.5 mg plate^{-1} as the higher concentration, was conducted to observe signs of cytotoxicity such as reduction in number of revertant colonies and a clearing of the background lawn.

Stock solutions of the test substance were checked by chemical analyses; deviations from up to 20% were considered within the limits of the allowed variation.

Solutions	Test substance	Final volume	Nominal concentration
1st	1.25 gram	10 mL of solvent	125 mg mL^{-1}
2nd	1 mL of 1st solution	10 mL of solvent	12.5 mg mL^{-1}
3rd	1 mL of 2nd solution	10 mL of solvent	1.25 mg mL^{-1}
4th	1 mL of 3rd solution	10 mL of solvent	0.125 mg mL^{-1}
5th	1 mL of 4th solution	10 mL of solvent	0.0125 mg mL^{-1}

6.11. Verification of the test substance concentration

After the end of the test, the first test solution (125 mg mL^{-1}) and the fifth solution (0.0125 mg mL^{-1}) were taken to determine the test substance concentration. The samples were kept in a refrigerator (approximately 5°C) until the analysis being performed. The test substance concentration in the test solutions was determined by analysis for the active ingredient (glyphosate) with a validated analytical method (VM.040/07). The analyses were carried out using a high performance liquid chromatograph with an ultraviolet absorption detector (HPLC/UV), under the following conditions:

Chromatograph:	HP 1050 (CL#01) POP-E 018 (rev. 04)
Detector:	UV
Wavelength:	195 nm
Column:	Sax (250mm/ 4.6mm / 5µm)
Injected volume:	20 µL

Mobile phase: H_2O/Met/KH_2PO_4 (960 mL + 40 mL + 0,8435g) pH 2.0 Phosphoric acid
Flow of mobile phase: 1.5 mL $minute^{-1}$
Retention time: 4.9 min
Run time: 8 min

The analyses were performed in the Physico-Chemistry Laboratory of the Chemical Division of Bioagri Laboratórios Ltda., SP.

6.11.1. Calculations

The effective concentration of the test substance in a test solution was calculated using the equation 1:

Equation 1:
$C_{ts} = (C_f \times F \times 100/C_{ca}) \times d$

Where:
C_{ts} = effective concentration of the test substance (mg mL^{-1})
C_f = concentration of the a.i. (test substance) determined by HPLC/UV (mg mL^{-1})
F = dilution factor or concentration factor (for some concentrations of the a.i. it was necessary to dilute or concentrate the test solutions prior to analysis by HPLC/UV, so that the concentration following dilution was within the range over which the system response varied linearly with the a.i. concentration)
C_{ca} = concentration of the a.i. in the test substance (%), indicated on the Certificate of Analysis
d = density (g cm^{-3})

The deviation of effective test substance concentration from the nominal concentration was calculated using the equation 2:

Equation 2:
$Dev = (C_{ts}-C_{nom}) \times 100/C_{nom}$

Where:
Dev = deviation of the effective concentration from the nominal concentration (%)
C_{nom} = nominal concentration of the test substance (mg mL^{-1})

6.12. Experimental procedure

A preliminary test was done with TA 100 strain to verify the cytotoxicity of the test substance to bacteria. This test was performed using the following concentrations: 2.5; 1.0, 0.1; 0.01 and 0.001 (mg $plate^{-1}$). Two replicate plates were prepared without metabolic activation. Control treatments were also performed: positive, negative and solvent (DMSO). The results obtained were used to determine the concentrations to be used in the definitive test.

The adopted method in the experimental procedure was the direct plate incorporation. Aliquots of 40, 80 or 200 µL of the test substance stock solutions, depending on the concentration, were incorporated together with 0.1 mL of the cell suspension (overnight culture) and 0.5 mL of phosphate buffer or 0.5 mL of mix S9 (depending on treatment) in a sterile glass tube containing 2 mL of top agar with histidine and biotin traces. This mixture was homogenized with a mechanical stirrer for 2-3 seconds and poured over the surface of a Petri dish containing a selective agar minimal medium with glucose 2%, except for TA 97a when the agar minimal medium was prepared with 0.4% of glucose.

The same procedure was adopted in tests with metabolic activator, performed in accordance with MARON & AMES (1983), using 0.5 mg $plate^{-1}$ of the S9 mix freshly prepared.

For all treatments, controls and test substance, triplicate plates were inoculated. After 72 hours incubation at 36-37°C, the number of revertant colonies was counted (SOP–M 0261, rev. 14).

The control treatments used were:

	Control	Culture strain		
Without S9 mix	Negative	0.1 mL	-	0.5 mL of phosphate buffer
	Solvent	0.1 mL	0.1 mL of solvent	0.5 mL of phosphate buffer
	Positive	0.1 mL	0.05 mL of reference substance*	0.5 mL of phosphate buffer
With S9 mix	Negative	0.1 mL	-	0.5 mL of S9 mix
	Solvent	0.1 mL	0.1 mL of solvent	0.5 mL of S9 mix
	Positive	0.1 mL	0.05 mL of reference substance	0.5 mL of S9 mix

* For strain TA 102 the volume is 0.1 mL.

6.13. Plate incorporation

The components of the assay (the tested strain bacteria, the test substance and S9 mix or phosphate buffer) were added to culture top agar and shaken. The mixture was then poured on the surface of a minimal medium agar plate, and allowed to solidify prior to incubation.

The aliquots of the test substance solutions added were proportional to the diluted solutions obtained from the stock solution, and were related to the following concentrations on plates, respectively:

Concentrations	Aliquots of	mg plate^{-1}
Stock solution 125 mg mL^{-1}	40 µL	5.0
12.5 mg mL^{-1}	200 µL	2.5
12.5 mg mL^{-1}	80 µL	1.0
12.5 mg mL^{-1}	40 µL	0.5
1.25 mg mL^{-1}	80 µL	0.1
0.125 mg mL^{-1}	80 µL	0.01
0.0125 mg mL^{-1}	80 µL	0.001

In addition, plates were prepared to check the sterility of the test substance solutions and the S9 mix; dilutions of the bacterial cultures were plated on nutrient agar plates to obtain the number of bacteria colonies unities of each strain.

6.14. Statistical method

The data were statiscally analysed by the Salmonel Program Assay (MYERS *et al.*, 1991).

The statistical data shows individual plate counts, the average of revertants colonies per plate, the standard deviation and the dose-response relation.

The plate counts of positive and negative controls were not utilized in the statistical analysis, because they are only a strain genetic characteristics of the strains. Only the results of solvent control were used in statistical analysis.

The test substance is considered mutagenic if the following criteria are attained:

1) Dose related response (p=0.05) in the number of induced revertants and the tested concentrations;

2) And for at least one dose, the mutagenicity ratio (MR = number of revertants on test plate (spontaneous revertants plus induced ones)/ number of revertants on control plate (spontaneous revertants)) is higher or equal to 2 for TA 97a, TA 98, TA 100 and TA 102, or higher than 3 for the strain TA 1535.

7. Results

7.1. Analytical results

The results of the analysis of the active ingredient glyphosate in the test solution of GLYPHOSATE TECHNICAL, and the calculated active ingredient's effective concentration in the test solutions and deviations (%) of the mean effective concentrations from the mean nominal concentrations are shown in Table 3. The deviations of the two solutions (0.0125 and 125 mg mL^{-1}) are within the 20% of the maximum tolerated deviation.

The HPLC/UV chromatograms obtained during the analyses of the test solutions are attached to this report (pages 42 to 45).

An example of the calculation of the test substance's effective concentration is shown in Appendix 01.

Table 3. Nominal and effective concentrations of the test substance, and their respective chromatogram, dilution factors, and deviations.

Nominal concentration (mg mL^{-1})	Concentration of a. i. found in the chromatogram (mg L^{-1})	Dilution factor	Effective concentration* (mg mL^{-1})	Deviations* (%)
0.0125	D.L.	-	-	-
125	1994.239	50	101.70	18.64

D. L: Detection limit (1.016143 mg/L)

* Effective concentration and the deviation was determined according to equations (1) and (2), respectively, described on page 16, and exemplified on page 46.

7.2. Preliminary test

A preliminary test was done with TA 100 strain to verify the toxicity of the test substance to bacteria. The following concentrations were tested: 2.5; 1.0, 0.1; 0.01; 0.001 (mg $plate^{-1}$). The average number of revertants were, respectively: 54, 88; 128; 131 and 137 fcu $plate^{-1}$. Counts obtained on negative and positive controls were 121 and 6500 fcu $plate^{-1}$ (Table 4). Considering the cytotoxicity at 2.5 mg $plate^{-1}$ in the preliminary test, the concentrations chosen for the main test were: 0.001, 0.01, 0.1, 0.5 and 1.0 mg $plate^{-1}$.

Table 4. Data of individual counts and average of the preliminary test with strain TA 100.

Control	Dose (mg $plate^{-1}$)	Counts (fcu $plate^{-1}$) *			Average
		Plate 1	Plate 2	Plate 3	
Solvent	0.00	107	108	122	112
	0.001	147	127	-	137
	0.01	114	148	-	131
	0.1	142	114	-	128
	1.0	70	106	-	88
	2.5	55	53	-	54
Negative	0.00	128	110	126	121
Positive	0.05	>6500	>6500	>6500	>6500

*The controls were carried out in triplicate plates and concentrations were carried out in duplicate plates.

7.3. Main test

The results obtained about genetic characteristics of the standard strains confirmed that they had the necessary genotypes for the mutagenic test. The concentration of 0.5 and 1.0 mg plate^{-1} have shown cytotoxicity for the strain TA 1535 with and without metabolic activation and for the strain TA 102 with metabolic activation.

The feasibility of culture strains were also in the expected range of cells per mL (10^8-10^9), in accordance with MARON & AMES (1983): TA 97a = 3.8 x 10^9, TA 98 = 3.6 x 10^9; TA 100 = 2.7 x 10^9; TA 102 = 4.1 x 10^9; TA 1535 = 3.9 x 10^9 formed colonies units mL^{-1}.

The batch of S9 mix used in this test was checked with benzo(a)pyrene and showed the expected results.

The results obtained in the study and their statistical analysis are in the annexes 01 to 10. The positive controls promoted an increase in the number of revertants for all the standard strains, confirming their sensitivity, as observed in all tested strains. The tested concentrations of the test substance did not promote an increase in the number of revertants in any strain, when comparing to the control treatments, with and without metabolic activator. Statistical significance observed with TA 98 and TA 100 with metabolic activator (annex 7 and 8, respectively) were not considered because the MR was not greater than 2 in none of the obtained results.

8. Conclusion

Under the test conditions and the obtained results, the test substance GLYPHOSATE TECHNICAL did not induce mutagenic activity in the strains of *Salmonella typhimurium* used in this assay.

9. References

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Annexes

Annex 1 – Strain TA 97a without S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 1 Exp. Date: 06/25/08 Exp. No.: 392/07 Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA97a Activation S9: -
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Linear
S	0.00	195	126	123	148.00	40.73	133.05
	0.001	168	149	139	152.00	14.73	133.01
	0.01	106	133	133	124.00	15.59	132.72
	0.10	120	110	104	111.33	8.08	129.74
	0.50	123	126	116	121.67	5.13	116.51
	1.00	140	60	99	99.67	40.00	99.97
N	0.00	101	114	106			
P	0.05	758	6500>	2101			

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis 9-aminoacridene
P-value for ANOVA test of dose response is 0.185
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.419

Estimate of the slope is = -33.078420 .
Standard error of the slope is = 15.054592 .
90% confidence limits for the slope are <-59.911140, -6.245700>.

P-value for the test of the positive dose response (slope at origin) is 0.976
Note: Smaller P-value means more positive dose response

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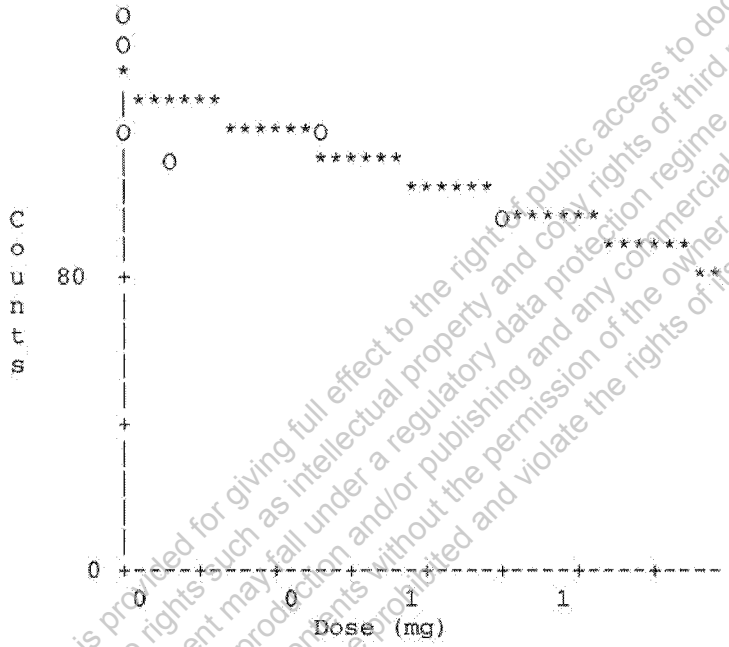
Salmonella Assay

Record No.: 1 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA97a



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

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Annex 2 - Strain TA 98 without S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 2 Exp. Date: 06/25/08 Exp. No.: 392/07 Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA98 Activation S9: -
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Linear
S	0.00	55	48	54	52.33	3.79	36.30
	0.001	45	37	23	35.00	11.14	36.29
	0.01	34	22	43	33.00	10.54	36.22
	0.10	29	38	31	32.67	4.73	35.49
	0.50	36	31	24	30.33	6.03	32.23
	1.00	31	22	35	29.33	6.66	28.16
N	0.00	47	46	42			
P	0.05	825	1365	845			

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis 2-nitrofluorene
P-value for ANOVA test of dose response is 0.123
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.160

Estimate of the slope is = -8.139614 .
Standard error of the slope is = 4.695811 .
90% confidence limits for the slope are <-16.509245, 0.230018>.

P-value for the test of the positive dose response (slope at origin) is 0.946
Note: Smaller P-value means more positive dose response

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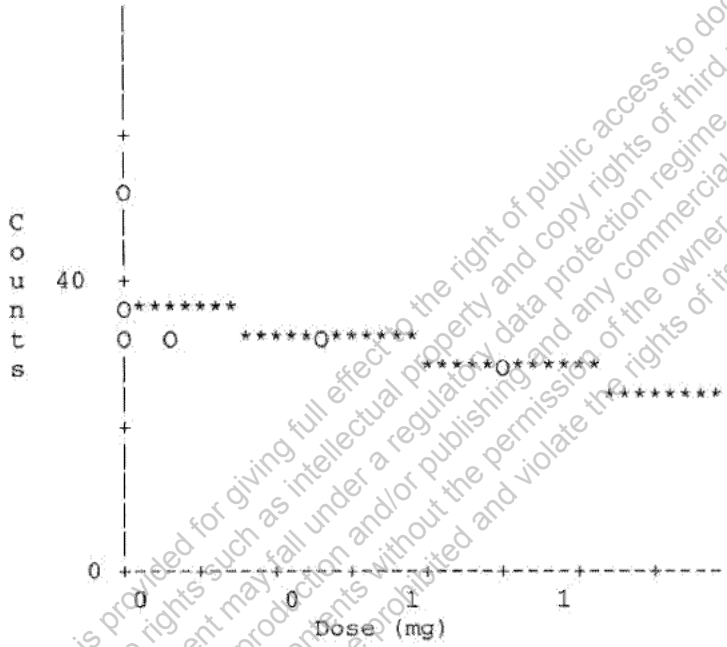
Salmonella Assay

Record No.: 2 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA98



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

[redacted]
SEP 15 2008

Annex 3 - Strain TA 100 without S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 3   Exp. Date: 06/25/08   Exp. No.: 392/07   Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA100   Activation S9: -
Data File Name: a:\model.sal

Code	Dose mg $plate^{-1}$	--	counts	--	Mean	S.D.	Predicted Linear
S	0.00	129	173	173	158.33	25.40	134.77
	0.001	149	117	123	129.67	17.01	134.75
	0.01	139	110	137	128.67	16.20	134.63
	0.10	125	134	128	129.00	4.58	133.38
	0.50	122	104	144	123.33	20.03	127.85
	1.00	131	109	131	123.67	12.70	120.93
N	0.00	169	220	151			
P	0.05	6500>	6500>	6500>			

S: Negative control for use in analysis   DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis   sodium azide
P-value for ANOVA test of dose response is 0.265
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.287

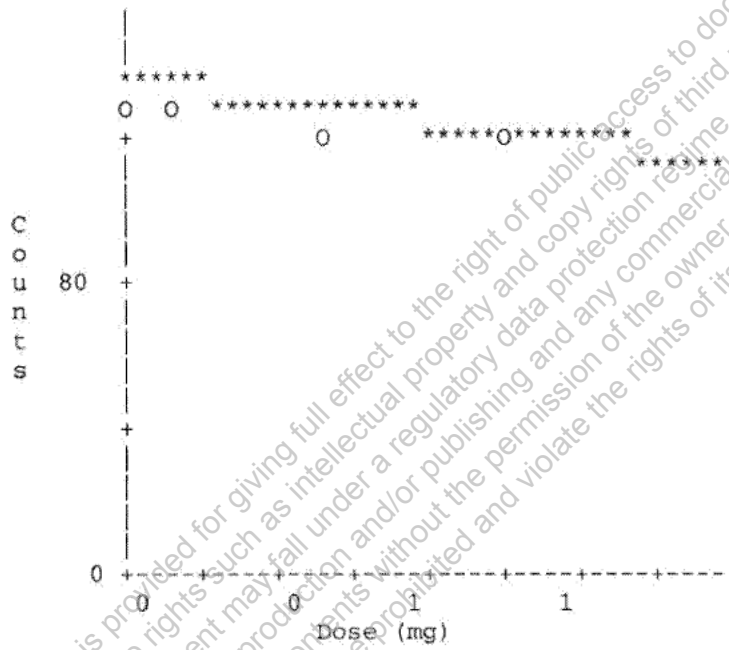
Estimate of the slope is = -13.840304 .
Standard error of the slope is = 10.414411 .
90% confidence limits for the slope are <-32.402545, 4.721937>.

P-value for the test of the positive dose response (slope at origin) is 0.896
Note: Smaller P-value means more positive dose response

[redacted]
SEP 15 2008

Salmonella Assay

Record No.: 3 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA100



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

[redacted]
SEP 15 2008

Annex 4 - Strain TA 102 without S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 4 Exp. Date: 06/25/08 Exp. No.: 392/07 Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA102 Activation S9: -
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	-- counts		--	Mean	S.D.	Predicted Linear
S	0.00	238	236	303	259.00	38.12	283.74
	0.001	327	270	250	282.33	39.95	283.72
	0.01	281	273	270	274.67	5.69	283.59
	0.10	376	301	251	309.33	62.92	282.27
	0.50	240	325	390	318.33	75.22	276.39
	1.00	260	216	281	252.33	33.17	269.05
N	0.00	325	350	281			
P	0.05	6500>	2643	6500>			

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis hydroperoxide cumene
P-value for ANOVA test of dose response is 0.477
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.386

Estimate of the slope is = -14.692130 .
Standard error of the slope is = 27.683385 .
90% confidence limits for the slope are <-64.033919, 34.649659>.

P-value for the test of the positive dose response (slope at origin) is 0.697
Note: Smaller P-value means more positive dose response

[redacted]
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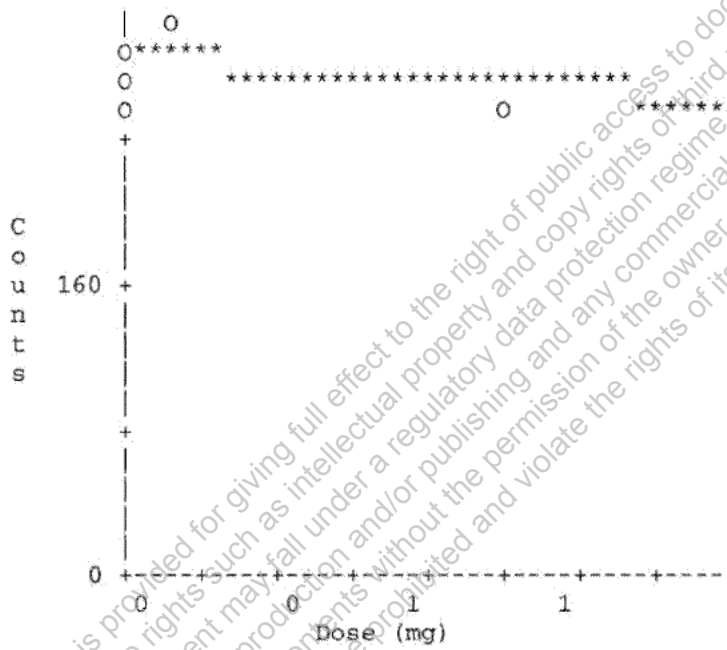
Salmonella Assay

Record No.: 4 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA102



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

[redacted]
SEP 15 2008

Annex 5 - Strain TA 1535 without S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 5 Exp. Date: 06/25/08 Exp. No.: 392/07 Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA1535 Activation S9: -
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Bernstein
S	0.00	25	26	25	25.33	0.58	26.50
	0.001	22	30	20	24.00	5.29	26.49
	0.01	27	37	25	29.67	6.43	26.35
	0.10	24	29	27	26.67	2.52	24.92
	0.50	17	19	19	18.33	1.15	18.56
	1.00	26	27	21	24.67	3.21	
N	0.00	28	31	25			
P	0.05	6500>	6500>	6500>			

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis sodium azide
P-value for ANOVA test of dose response is 0.027
An acceptable model is Bernstein with pval = 0.409
Berstein model used the first 5 doses

Estimate of the slope is = -15.885538 .
Standard error of the slope is = 4.176466 .
90% confidence limits for the slope are <-23.329508, -8.441568>.

P-value for the test of the positive dose response (slope at origin) is 0.999
Note: Smaller P-value means more positive dose response

[redacted]
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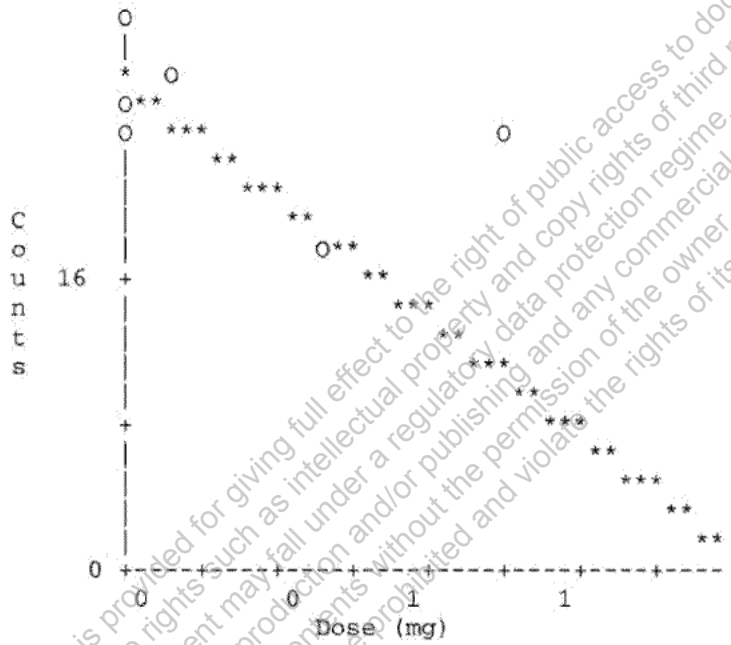
Salmonella Assay

Record No.: 5 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA1535



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

[redacted]
SEP 15 2008

Annex 6 - Strain TA 97a with S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 6 Exp. Date: 06/25/08 Exp. No.: 392/07 Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA97a Activation S9: + rat liver aroclor 0.5mL/plate
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Linear
S	0.00	124	111	106	113.67	9.29	124.58
	0.001	145	147	128	140.00	10.44	124.54
	0.01	143	128	119	130.00	12.12	124.21
	0.10	118	102	119	113.00	9.54	120.87
	0.50	116	94	120	110.00	14.00	106.06
	1.00	195	34	31	86.67	93.83	87.55
N	0.00	110	85	97			
P	0.05	2426	2405	2383			

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis 2-aminoantracene
P-value for ANOVA test of dose response is 0.790
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.968

Estimate of the slope is = -37.022300 .
Standard error of the slope is = 27.324991 .
90% confidence limits for the slope are <-85.725302, 11.680702>.

P-value for the test of the positive dose response (slope at origin) is 0.900
Note: Smaller P-value means more positive dose response

[redacted]
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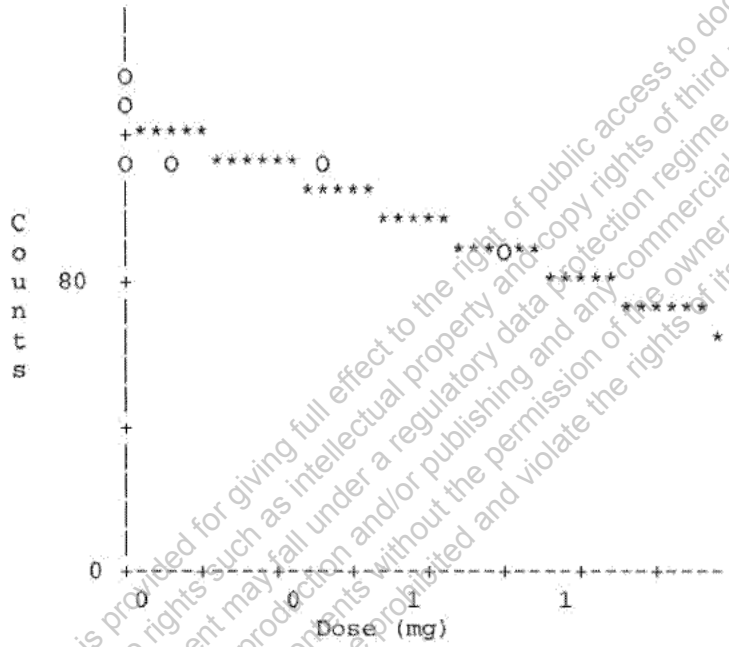
Salmonella Assay

Record No.: 6 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA97a



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

[redacted]
SEP 15 2008

Annex 7 - Strain TA 98 with S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 7 Exp. Date: 06/25/08 Exp. No.: 392/07 Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA98 Activation S9: + rat liver aroclor 0.5mL/plate
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Bernstein
S	0.00	38	38	39	38.33	0.58	42.95
	0.001	61	55	47	54.33	7.02*	45.68
	0.01	77	68	62	69.00	7.55**	70.24
	0.10	45	57	57	53.00	6.93	
	0.50	53	38	47	46.00	7.55	
	1.00	74	37	43	51.33	19.86	
N	0.00	42	38	26			
P	0.05	6500>	2383	2166			

Note: ** = significant at 1%; *significant at 5%

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis 2-aminoantracene
P-value for ANOVA test of dose response is 0.056
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Bernstein with pval = 0.557
Berstein model used the first 3 doses

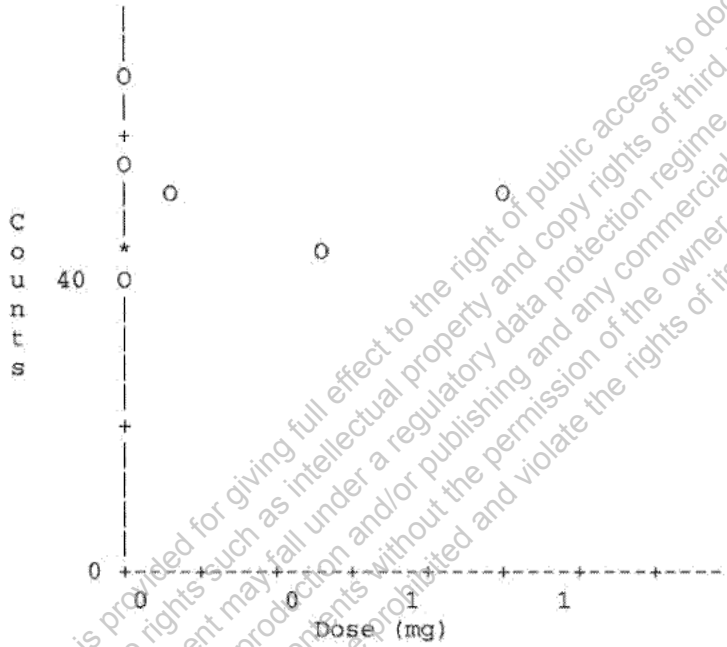
Estimate of the slope is = 2728.920282 .
Standard error of the slope is = 827.994198 .
90% confidence limits for the slope are <1253.135620, 4204.704944>.

P-value for the test of the positive dose response (slope at origin) is 0.003
Note: Smaller P-value means more positive dose response

[redacted]
SEP 15 2008

Salmonella Assay

Record No.: 7 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA98



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

[redacted]
SEP 15 2008

Annex 8 - Strain TA 100 with S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 8   Exp. Date: 06/25/08   Exp. No.: 392/07   Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA100   Activation S9: + rat liver aroclor 0.5mL/plate
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Bernstein
S	0.00	125	136	127	129.33	5.86	134.77
	0.001	138	152	128	139.33	12.06	132.58
	0.01	125	112	100	112.33	12.50	112.82
	0.10	131	130	150	137.00	11.27	
	0.50	155	161	151	155.67	5.03*	
	1.00	133	120	125	126.00	6.56	
N	0.00	100	111	103			
P	0.05	845	6500>	996			

Note: ** = significant at 1%; *significant at 5%

S: Negative control for use in analysis   DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis   2-aminoantracene
P-value for ANOVA test of dose response is 0.003
An acceptable model is Bernstein with pval = 0.675
Berstein model used the first 3 doses

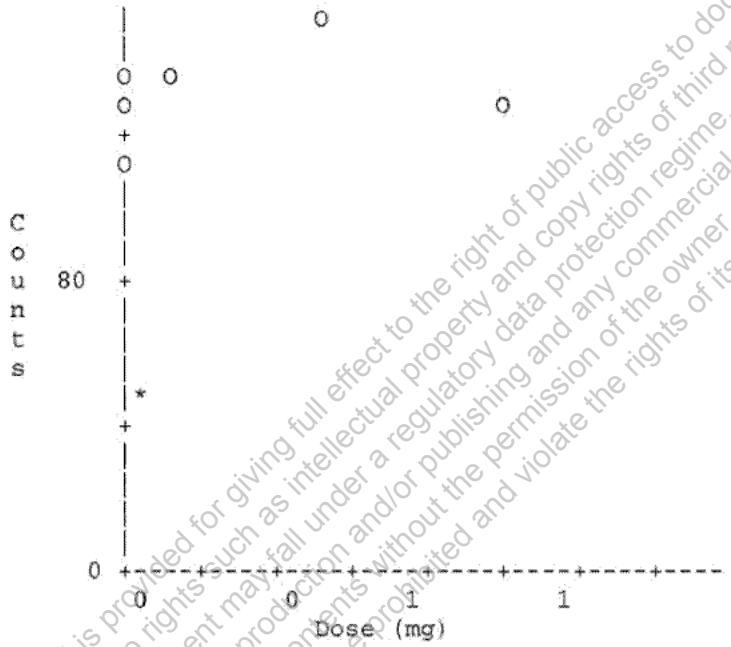
Estimate of the slope is = -2194.912363 .
Standard error of the slope is = 661.163082 .
90% confidence limits for the slope are <-3373.343731, -1016.480995>.

P-value for the test of the positive dose response (slope at origin) is 0.997
Note: Smaller P-value means more positive dose response

[redacted]
SEP 15 2008

Salmonella Assay

Record No.: 8 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA100



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

[redacted]
SEP 15 2008

Annex 9 - Strain TA 102 with S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 9 Exp. Date: 06/25/08 Exp. No.: 392/07 Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA 102 Activation S9: + rat liver aroclor 0.5mL/plate
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Linear
S	0.00	248	368	315	310.33	60.14	278.41
	0.001	234	231	228	231.00	3.00	278.33
	0.01	390	231	368	329.67	86.15	277.57
	0.10	238	260	391	296.33	82.72	269.94
	0.50	216	130	264	203.33	67.89	236.07
	1.00	216	238	173	209.00	33.06	193.73
N	0.00	241	265	260			
P	0.05	1213	1386	848			

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis 2-aminoantracene
P-value for ANOVA test of dose response is 0.093
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.235

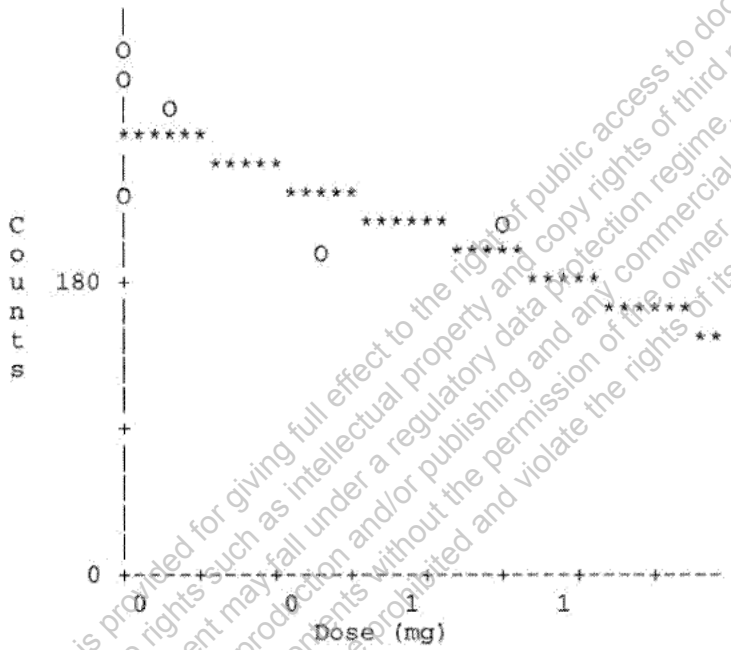
Estimate of the slope is = -84.684358 .
Standard error of the slope is = 34.957963 .
90% confidence limits for the slope are <-146.992072, -22.376644>.

P-value for the test of the positive dose response (slope at origin) is 0.984
Note: Smaller P-value means more positive dose response

[redacted]
SEP 15 2008

Salmonella Assay

Record No.: 9 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA 102



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

[redacted]
SEP 15 2008

Annex 10 - Strain TA 1535 with S9

Salmonella Assay

Test Sample Name: 0790/07
Source/Batch/Lot: 20070606
Solvent: DMSO
Record No.: 10   Exp. Date: 06/25/08   Exp. No.: 392/07   Technician: [redacted]
Assay Type: Plate incorporation,
Strain: TA 1535   Activation S9: + rat liver aroclor 0.5mL/plate
Data File Name: a:\model.sal

Code	Dose mg plate^{-1}	--	counts	--	Mean	S.D.	Predicted Linear
S	0.00	32	32	26	30.00	3.46	27.89
	0.001	25	30	26	27.00	2.65	27.89
	0.01	23	29	26	26.00	3.00	27.82
	0.10	28	34	28	30.00	3.46	27.20
	0.50	21	27	20	22.67	3.79	24.44
	1.00	21	14	30	21.67	8.02	20.99
N	0.00	25	32	33			
P	0.05	736	845	758			

S: Negative control for use in analysis DMSO
N: Negative control not used in analysis
P: Positive control not used in analysis 2-aminoantracene
P-value for ANOVA test of dose response is 0.213
ANOVA test is not significant. Other significant results should be viewed with caution.
An acceptable model is Linear with pval = 0.667

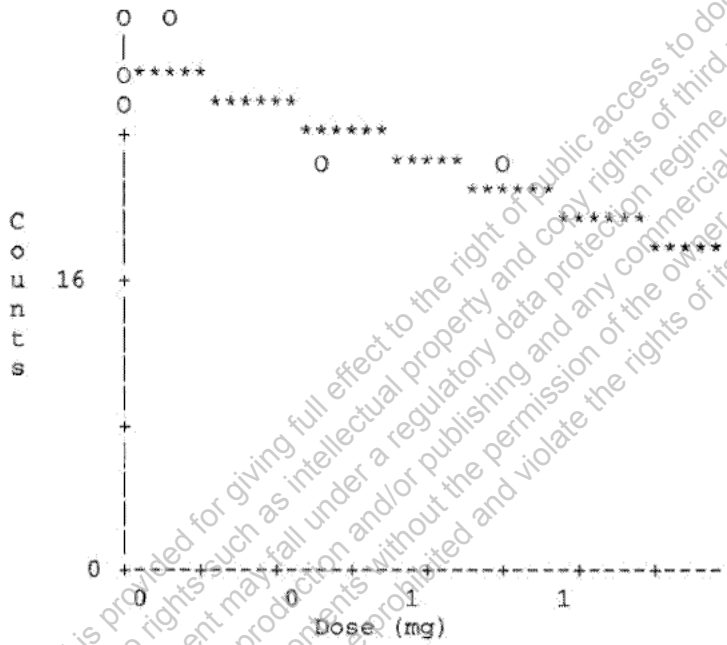
Estimate of the slope is = -6.898463 .
Standard error of the slope is = 2.816111 .
90% confidence limits for the slope are <-11.917789, -1.879137>.

P-value for the test of the positive dose response (slope at origin) is 0.985
Note: Smaller P-value means more positive dose response

[redacted]
SEP 15 2008

Salmonella Assay

Record No.: 10 Experiment Date: 06/25/08 Experiment No.: 392/07
Test Sample Name: 0790/07
Tester Strain: TA 1535



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

[redacted]
SEP 15 2008

Annex 11 - Certificate of analysis

BIOAGRI LABORATÓRIOS

Certificate of Analysis
CA – 1302/07

Company:	Jingma Chemicals Co., Ltd.
Address:	Nº 50, Bao Ta Road, Longyou, Zhejiang, China
Information about the sample:	
Commercial Name:	**GLYPHOSATE TECHNICAL**
Common Name:	glyphosate
Chemical Name (IUPAC):	*N*-(phosphonomethyl)glycine
Declared concentration:	min. 980.0 g/kg
Batch:	20070606
Bioagri code:	AGR – 0790/07
Information about the analysis:	
Start of testing:	03/Sep/2007
Testing completed:	06/Sep/2007
Conclusion of this certificate of analysis:	19/Sep/2007
Methodology used:	SOP M 0118 – Rev.: 06 – 26/Jul/2007
Equipment used:	Liquid Chromatograph (HPLC) Waters (CL#10)

RESULTS OF THE ANALYSIS

Concentration of a.i. glyphosate: 980.5 g/kg

Observations: The results of this analysis refer exclusively to the sample that was submitted for analysis. The results are not valid for other lots or other products.
The documents and records relating to this certificate will be archived at BIOAGRI for a period of 10 years

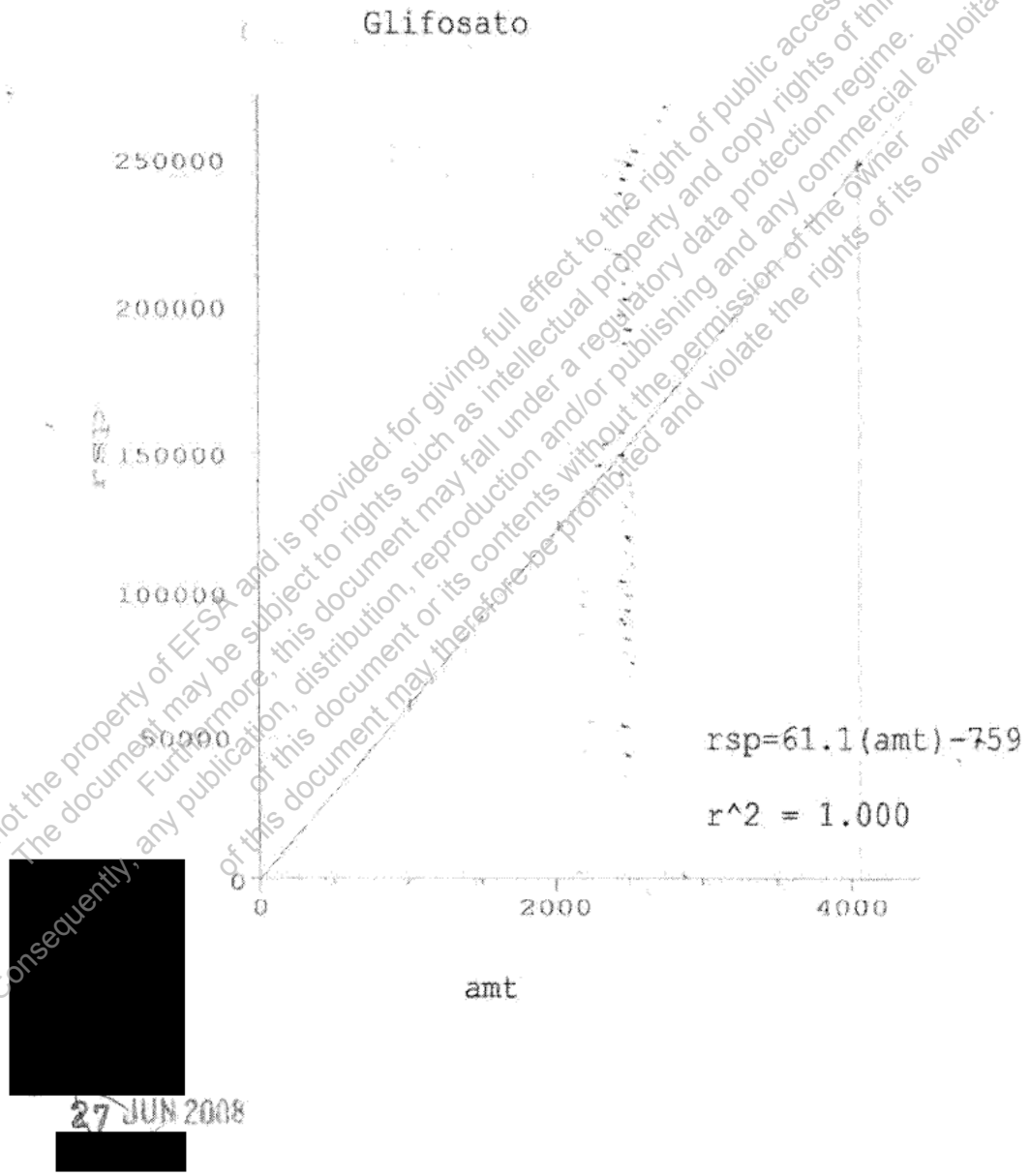
[redacted]
Analyst Responsible

[redacted]
Technician Responsible

SQB 0741 - Register of Quality

Annex 12 – The HPLC/UV chromatograms obtained of the analyses of the test solutions.

a) Linearity;

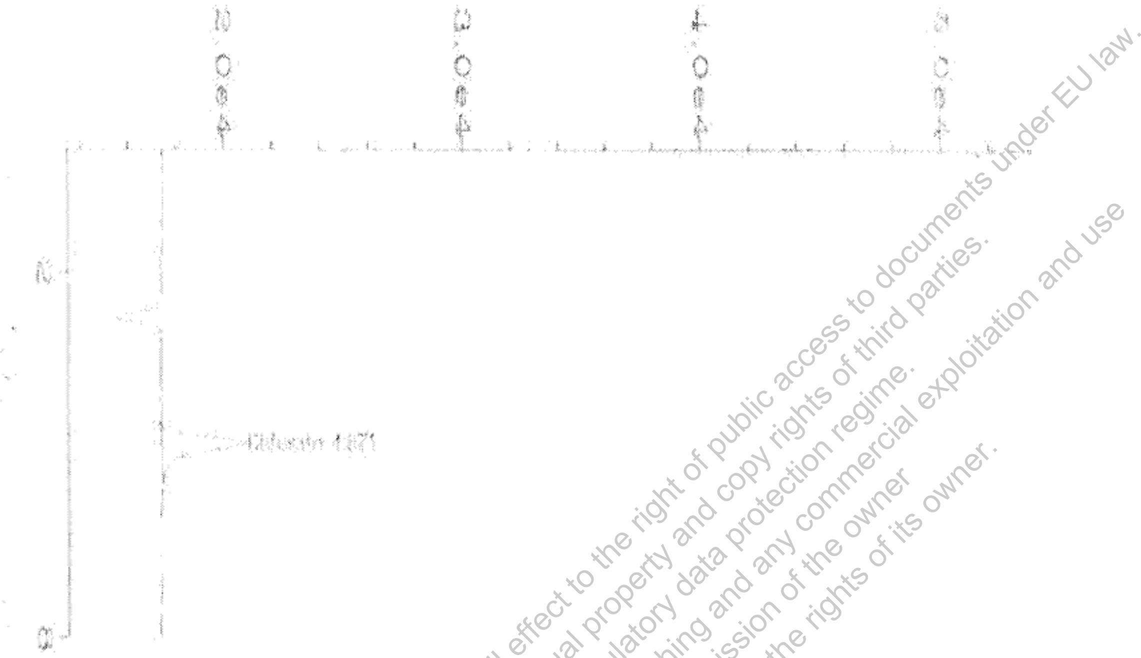


[redacted]

27 JUN 2008

[redacted]

b) Standard;



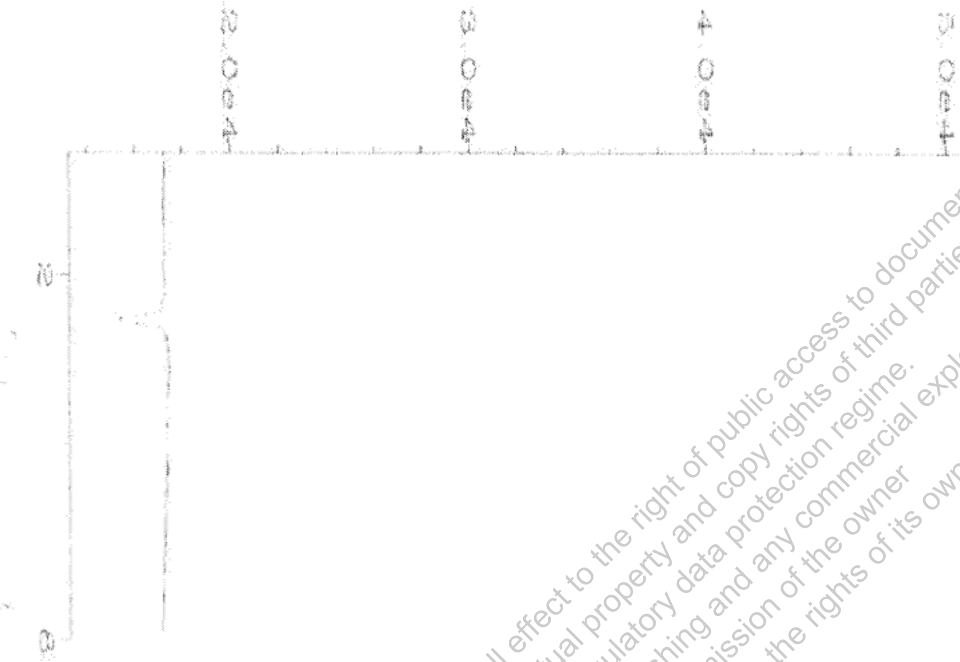
```
External Standard Report

Data File Name   : C:\HPCHEM\2\DATA\JUNHO\27060825.D
Operator         : [redacted]                       Page Number      : 1
Instrument       : HPLC 1050                        Vial Number      : 1
Sample Name      : Pad.1                            Injection Number : 1
Run Time Bar Code:                                  Sequence Line    : 1
Acquired on      : 27 Jun 08  03:03 PM              Instrument Method: GLIFOSAT.MTH
Report Created on: 27 Jun 08  03:35 PM              Analysis Method  : GLIFOSAT.MTH
Last Recalib on  : 27 Jun 08  03:27 PM              Sample Amount    : 0
Multiplier       : 1                                ISTD Amount      :

Sig. 2 in C:\HPCHEM\2\DATA\JUNHO\27060825.D
Ret Time    Area    Type Width Ref#  ng/ul      Name
|-------|----------|----|-----|-----|--------|------------------------------|
   4.871     61022 BBA   0.253  1     1010.430 Glifosato
```

[redacted]

27 JUN 2008

[redacted]

c) Blank;



External Standard Report

Data File Name : C:\HPCHEM\2\DATA\JUNHO\27060822.D
Operator : [redacted]
Instrument : HPLC 1050
Sample Name : controle
Run Time Bar Code:
Acquired on : 27 Jun 08 02:34 PM
Report Created on: 27 Jun 08 03:44 PM
Last Recalib on : 27 Jun 08 03:27 PM
Multiplier : 1

Page Number : 1
Vial Number : 10
Injection Number : 1
Sequence Line : 2
Instrument Method: GLIFOSAT.MTH
Analysis Method : GLIFOSAT.MTH
Sample Amount : 0
ISTD Amount :

Sig. 2 in C:\HPCHEM\2\DATA\JUNHO\27060822.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
4.871 *	not found *			1		Glifosato

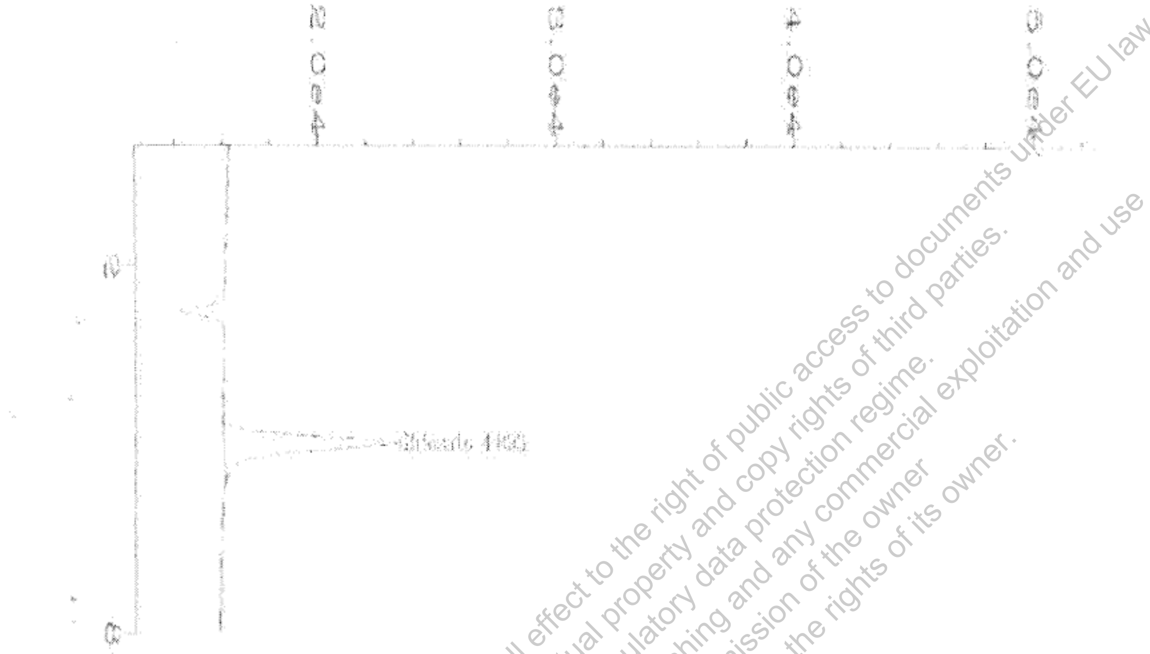
Not all calibrated peaks were found

[redacted]

[redacted] 27 JUN 2008

[redacted]

d) Results of test substance solution (125 mg mL^{-1})



```
=====================================================================
                     External Standard Report
=====================================================================

Data File Name    : C:\HPCHEM\2\DATA\JUNHO\27060824.D
Operator          : [redacted]                Page Number      : 1
Instrument        : HPLC 1050                 Vial Number      : 12
Sample Name       : 125                       Injection Number : 1
Run Time Bar Code:                            Sequence Line    : 2
Acquired on       : 27 Jun 08  02:52 PM       Instrument Method: GLIFOSAT.MTH
Report Created on: 27 Jun 08  03:47 PM        Analysis Method  : GLIFOSAT.MTH
Last Recalib on   : 27 Jun 08  03:27 PM       Sample Amount    : 0
Multiplier        : 1                         ISTD Amount      :

Sig. 2 in C:\HPCHEM\2\DATA\JUNHO\27060824.D
Ret Time     Area    Type Width Ref#  ng/ul              Name
|-------|-----------|----|-----|-----|--------|-------------------------------|
  4.893       121167 BBA  0.248  1    1994.239 Glifosato
=====================================================================
```

[redacted]

27 JUN 2008

[redacted]

Appendix

Appendix 01 - Example of calculation of the test substance's effective concentration.

$C_{ts} = (C_f \times F / C_{ca}) \times d$

Cf = 1994.239 ng μL^{-1} or 1.994239 mg mL^{-1}
F = 50
Cca = 980.5g kg^{-1} or 98.05%
d = 1.0 g cm^{-3}

$C_{ts} = (1.994239 \times 50 / 98.05) \times 1$
$C_{ts} = 101.70$ mg mL^{-1}

Where:
C_{ts} = effective concentration of the test substance (mg mL^{-1})
C_f = concentration of the a.i. (test substance) determined by HPLC/UV (mg mL^{-1})
F = dilution factor or concentration factor (for some concentrations of the a.i. it was necessary to dilute or concentrate the test solutions prior to analysis by HPLC/UV, so that the concentration following dilution was within the range over which the system response varied linearly with the a.i. concentration)
C_{ca} = concentration of the a.i. in the test substance (%), indicated on the Certificate of Analysis
d = density of a.i. (g cm^{-3})

The deviation of the test substance's effective concentration from the nominal concentration was calculated using the following equation:

$Dev = (C_{ts}-C_{nom}) \times 100/C_{nom}$
$Dev = (101.70-125) \times 100/125$
Dev = 18.64 %

Where:

Dev = deviation of the effective concentration from the nominal concentration (%)
C_{nom} = nominal concentration of the test substance (mg mL^{-1})

Appendix 02 – Endorsement of Compliance with the OECD Principles of Good Laboratory Practice

voedsel en waren autoriteit

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC, the conformity with the OECD Principles of GLP was assessed on November 7-10, 2006 at

Bioagri Laboratórios Ltda.
Rodovia Rio Claro/Piracicaba Km 24
Piracicaba - SP - Brazil

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

Den Haag, 19 December 2006

[redacted]

Manager GLP Compliance Monitoring Program

Food and Consumer Product Safety Authority (VWA)
Prinses Beatrixlaan 2, 2595 AL Den Haag
Postbus 19506, 2500 CM Den Haag
The Netherlands

Appendix 03 - INMETRO GLP Accreditation

Federal Republic of Brazil
Ministry of Development, Industry and Foreign Trade
National Institute of Metrology, Standardization and Industrial Quality - INMETRO

(General Coordination for Accreditation)

Accreditation Certificate

Accreditation nº CLA 0002

Initial Accreditation: April 25th, 2000

BIOAGRI LABORATÓRIOS LTDA.
RODOVIA SP, 127 - km 24 - GUAMIUM
PIRACICABA - SP

The General Coordination for Accreditation of Inmetro – CGCRE/INMETRO – grants accreditation to the above-mentioned Laboratory, according to the requirements established in NIT-DICLA-035 (General Requirements for Laboratories, according to the Principles of Good Laboratory Practice - GLP). This accreditation contitutes the formal expression of recognition of the laboratory's competence to carry out studies as described in the scope of Accreditation.

[redacted]

General Coordinator for Accreditation

Issue Date: March 25th, 2008

Valid To: April 25th, 2010