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TECHNICAL GLYPHOSATE:

CHROMOSOME ABERRATION TEST IN CHL CELLS

IN VITRO

SPL PROJECT NUMBER: 434/015

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SPL PROJECT NUMBER: 434/015

QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharm Laboratories is carried out as a continuous process designed to encompass all major phases of each study type once per month. Dates of relevant monthly inspections are given below.

Date(s) of Inspection and Reporting:

04, 05, 19 December 1995

This report has been audited by Safepharm Laboratories Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the procedures followed.

Date of Report Audit:

18 January 1996

..... DATE: 13. MAR. 1996

C.Biol., M.I. Biol.

For Safepharm Quality Assurance Unit

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and data generated.

The work described was performed in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health 1989). These Principles are in accordance with GLP standards published as OECD Environment Monograph No. 45 (OCDE/GD(92)32); and are in conformity with, and implement, the requirements of Directives 87/18/EEC and 88/320/EEC.

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended); and to the Japanese Ministry of Agriculture, Forestry and Fisheries (59 NohSan, Notification No. 3850, Agricultural Production Bureau) - confirmed by an Arrangement between the Ministry and UK Department of Health; the Japanese Ministry of Health and Welfare (Notification No. 313, Pharmaceutical Affairs Bureau - as amended, and Kanpogyo No. 39 Environmental Agency, Yakuhatsu No. 229); and the Japanese Ministry of International Trade and Industry (Chemical Substances Control Law, Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85).

..............................[redacted]...................... DATE: 13 MAR 1996

Study Director
for Safepharm Laboratories

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SUMMARY

STUDY SPONSOR	:	MASTRA INDUSTRIES SDN. BHD.
CO-SPONSOR	:	MARUZEN KAKO CO., LTD.
STUDY TYPE	:	CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*
TEST MATERIAL	:	TECHNICAL GLYPHOSATE

1. Chinese hamster lung (CHL) cells were treated with the test material at six dose levels, in duplicate, together with negative and positive controls, three dose levels were selected for metaphase analysis. Four treatment regimens were used: 6 hours exposure both with and without the addition of an induced rat liver homogenate metabolising system at 50% in standard co-factors; 24 hours continuous exposure and 48-hours continuous exposure without metabolic activation.

   The dose range was selected on the basis of the results of a preliminary toxicity test and a determination of the pH of culture media after the addition of the test material and was 39 to 1250 μg/ml for the 6-hour treatment both with and without S9 and for the 24 and 48-hour continuous treatments. Technical Glyphosate was observed to reduce the pH to an unacceptable level at 2500 and 5000 μg/ml.

2. The vehicle (solvent) controls gave frequencies of cells with aberrations within the range expected for the CHL cell line.

3. All the positive control treatments except cyclophosphamide without S9 gave highly significant increases in the frequency of cells with aberrations indicating the satisfactory performance of the test and of the activity of the metabolising system.

4. The test material, Technical Glyphosate, did not induce any significant increases in the frequency of cells with aberrations in any of the treatment cases. The test material was shown to be toxic to CHL cells *in vitro* in the continuous treatment cases, but only when the pH was reduced to an unacceptable level. Technical Glyphosate was shown to be <u>non-clastogenic</u> to CHL cells *in vitro*.

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TECHNICAL GLYPHOSATE: CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

1. INTRODUCTION

This study was conducted according to Safepharm Standard Method Number JMOL 03A and was designed to assess the potential chromosomal mutagenicity of a test material, on the metaphase chromosomes of the Chinese hamster lung (CHL) cell line.

Numerical and structural chromosome aberrations are implicated in the pathology of neoplasia (Radman *et al*, 1982; Cairns, 1981) and also occur in a high proportion of spontaneous abortions and abnormal live births (Chandley, 1981). Furthermore, most carcinogens are capable of inducing such changes in chromosome fidelity (Ishidate and Odashima, 1977; Ishidate and Sofuni, 1985). Metaphase analysis *in vitro* involves the evaluation of chromosomes of exposed cells for structural damage. Many of these changes are accompanied by more subtle changes (translocations, inversions, small deletions) which are not cell lethal, and therefore represent a hazard. The ability to induce chromosome aberrations also correlates with the induction of gene mutations (Hollstein *et al*, 1979).

This study was performed between 30 August 1995 and 4 January 1996.

2. TEST MATERIAL

Sponsor's identification	:	Technical Glyphosate
Chemical name	:	N-(phosphonomethyl) glycine
Lot number:	:	H95D 161A
Purity	:	95.3% w/w
Date received	:	4 August 1995
Description	:	white powder
Storage conditions	:	room temperature

Data relating to the identity, purity and stability of the test material are the responsibility of the sponsor.

3. METHODS

3.1 Cell Line

The Chinese hamster lung (CHL) cell line, isolated by Koyama *et al* (1970) and cloned by Ishidate and Sofuni (1985), was used. The CHL cell line has an average generation time of approximately 11 hours when grown in the following conditions:

3.2 Cell Culture

Cells were grown in Eagle's Minimal Essential medium with Earle's Salts (MEM), supplemented with 10% foetal bovine serum and antibiotics, at 37°C with 5% CO_2 in air.

3.3 Preparation of Test and Control Materials

The test material was accurately weighed and prepared in Minimal Essential Media (MEM) and appropriate dilutions made. Analysis for concentration, homogeneity and stability of the test material preparations was not a requirement of the test guidelines and therefore was not performed.

An allowance for test material purity was not made when dosing solutions were prepared. When the test material was dosed into media the osmolality was within the 50 mOSM limiting range. However the test material was acidic when dosed into media at 2500 and 5000 µg/ml when compared with the vehicle controls. The pH shift was approximately 1 pH unit or greater (Table 2) and this was considered to be unacceptable because such changes in pH have been shown to induce artefactual responses.

Vehicle and positive controls were used in parellel with the test material. Solvent treatment groups were used as the vehicle controls and the positive control materials were as follows:

Mitomycin C (MMC, Sigma Batch No. 104H2504) 0.05 µg/ml for cultures treated for 24 or 48 hours in the absence of metabolising enzymes.

Cyclophosphamide (CP, Sigma Batch No. 44H0486) 10 µg/ml for cultures treated for 6 hours both with and without S9-mix.

3.4 Preliminary Toxicity Test

A preliminary toxicity test was performed on cell cultures using 24 and 48-hour continuous exposure times without metabolic activation and a 6-hour exposure period both with and without metabolic activation, followed by an 18-hour recovery period in treatment-free media. The dose range used was 19.5 to 5000 μg/ml. Growth inhibition was estimated by counting the number of cells at the end of the culture period on an electronic cell counter (Coulter) and expressing the cell count as a percentage of the concurrent vehicle control value. Slides were also prepared from the cells in order to check for the presence of cells in metaphase.

3.5 Microsomal Enzyme Fraction

Lot No. Aro. S9/11/OCT/95 SPL was prepared in-house at Safepharm Laboratories on 11/OCT/95. It was prepared from the livers of male Sprague-Dawley rats weighing ~ 200g. These had received a single ip. injection of Aroclor 1254 at 500 mg/kg, up to 5 days before S9 preparation. The S9 was stored at -196°C in a liquid nitrogen freezer.

3.6 Culture Conditions

Cultures were established approximately 48 hours prior to treatment, 0.15×10^6 cells were seeded per flask for the 6-hour and 24-hour cultures and 0.075×10^6 cells were seeded per flask for the 48-hour cultures. The cells were exposed to doses of the test material, vehicle and positive controls, both with and without metabolic activation. All treatments were performed in duplicate (A + B). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. The treatment regimes were as follows:

3.6.1 Without Metabolic Activation

i) 24 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 μg/ml.

ii) 48 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 μg/ml.

3.6.2 With Metabolic Activation

i) 6 hours exposure to the test material and S9-mix (0.5 ml per 4.5 ml culture medium of 10% S9 in standard co-factors). A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 μg/ml.

ii) 6 hours exposure to the test material without S9-mix. A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. This group acts as a 'control' for group i). The dose levels selected for assessment were 312.5, 625 and 1250 μg/ml.

3.7 Cell Harvest

Mitosis was arrested by addition of demecolcine (Colcemid 0.1 μg/ml) two hours before the required harvest time. After incubation with demecolcine, the cells were trypsinised to detach them from the tissue culture flask and suspended in 5 ml of culture medium. A sample of the cell suspension from each harvest time was counted to estimate growth inhibition at each concentration. The cells were centrifuged, the culture medium drawn off and discarded, and the cells resuspended in 0.075M hypotonic KCl. After fifteen minutes (including five minutes centrifugation), all but approximately 0.5 ml of hypotonic solution was drawn off and discarded. The cells were resuspended and then fixed by dropping the cell suspension into fresh methanol/glacial acetic acid (3:1 v/v). The fixative was changed several times and the cells stored at 4°C for sufficient time to ensure complete fixation.

3.8 Preparation of Metaphase Spreads, Staining and Coding

The cells were resuspended in fresh fixative before centrifugation and suspension in a small amount of fixative. Several drops of this suspension were dropped onto clean, wet microscope slides and left to air dry. Each slide was permanently labelled with the appropriate identification data. When the slides were dry they were stained in 2% Gurrs Giemsa R66 for 5 minutes, rinsed, dried and coverslipped using mounting medium. After checking that the slide preparations were of good quality, the slides were coded using a computerised random number generator.

3.9 Scoring of Chromosome Damage

Where possible the first 100 consecutive well-spread metaphases from each culture were counted, and if the cell had 23 to 27 chromosomes, any gaps, breaks or rearrangements were noted according to the simplified system of Savage (1976) recommended in the 1983 UKEMS guidelines for mutagenicity testing (Appendix III).

Aberrations recorded by the slide scorer were checked by a senior cytogeneticist. Cells with 28 to 31 chromosomes were scored as aneuploid cells. Cells with greater than 31 chromosomes were classified as polyploid cells and the % incidence of polyploid cells reported. The percentage of cells showing structural chromosome aberrations (gaps, breaks and exchanges) were calculated and reported as both indicating and excluding those with gaps.

4. ARCHIVES

Unless instructed otherwise by the sponsor, all original data and a copy of the final report will be retained in the archives of Safepharm Laboratories Limited for a period of 10 years. After this period the sponsor's instructions will be sought.

5. RESULTS

5.1 Preliminary Toxicity Test

The results of the cell counts of the preliminary toxicity test are presented in Table 1. It can be seen that in all cases except 6 hours with S9, that the test material induced some evidence of cell toxicity. Microscopic assessment of the slides prepared from the treatment cultures showed metaphases present up to 5000 µg/ml in the 6-hour with and without S9-mix treatment cases. The maximum dose with metaphases present was 2500 µg/ml in the 24 and 48-hour continuous exposure treatment case. However, when a pH check was performed on culture media dosed with Technical Glyphosate it was observed that the pH was reduced in a dose-related way. At the maximum two dose levels the pH was reduced by ≥1 unit and this was considered to be unacceptable because alterations in pH have been shown to cause artefactual responses. Therefore the maximum dose level selected for the main study was 1250 µg/ml.

5.2 Chromosome Aberration Test

The results of the cell counts from the cultures after their respective treatments are presented in Table 3. The test material was acidic at 2500 and 5000 µg/ml therefore the toxicity observed in the preliminary toxicity test was not relevant, and 1250 µg/ml was selected as the maximum dose for all treatment groups.

The vehicle control cultures gave values of chromosome aberrations within the expected range (Appendix III).

All the positive control cultures except cyclophosphamide without S9 gave highly significant increases in the frequency of cells with aberrations (Appendix I) indicating that metabolic activation system was satisfactory and that the test method itself was operating as expected.

The test material did not induce a statistically significant increase in the frequency of cells with aberrations at any dose level in any treatment group (Appendix I).

The test material did not induce a significant increase in the numbers of polyploid cells at any dose level in any of the four treatment cases (Appendix I).

6. CONCLUSION

The test material, Technical Glyphosate, did not induce any statistically significant, dose-related increases in the frequency of cells with chromosome aberrations either in the presence or absence of a liver enzyme metabolising system or after various exposure times. Technical Glyphosate is therefore considered to be non-clastogenic to CHL cells *in vitro*.

7. REFERENCES

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TABLES

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TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 1

RESULTS OF PRELIMINARY TOXICITY TEST - CELL COUNTS

24 AND 48-HOUR TREATMENTS

	24-HOUR TREATMENT		48-HOUR TREATMENT	
CONCENTRATION (µg/ml)	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL
0	2.01	100	2.41	100
19.5	2.41	120	2.06	85
39.1	2.48	123	2.37	98
78.13	2.34	116	2.41	100
156.25	2.36	117	2.42	100
312.5	2.53	126	2.42	100
625	2.23	111	2.17	90
1250	2.03	101	2.08	86
2500	1.51	75	1.24	51
5000	3.11	155NM	0.41	17NM

6-HOUR TREATMENT

	6-HOUR WITHOUT S9		6-HOUR WITH S9	
CONCENTRATION (µg/ml)	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL
0	2.53	100	2.00	100
19.5	2.17	86	1.88	94
39.1	1.98	78	2.07	104
78.13	2.18	86	1.96	98
156.25	2.34	93	2.25	113
312.5	2.27	90	2.25	113
625	1.44	57	2.26	113
1250	1.85	73	2.33	117
2500	2.42	96	2.40	120
5000	1.24	49	1.94	97

NM = no scorable metaphases

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TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 2

pH CHECK ON DOSING SOLUTIONS DOSED INTO MEDIA

CONCENTRATION REQUIRED IN FLASK (µg/ml)	pH VALUES	
	20h -S9	20h +S9
0	7.10	7.00
39	7.16	7.06
78.1	7.13	7.07
156.25	7.12	7.05
312.5	7.09	7.01
625	6.96	6.84
1250	6.51	6.54
2500	5.67	6.08
5000	4.64	5.52

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TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 3

RESULTS OF CHROMOSOME ABERRATION TEST - CELL COUNTS

24-HOUR TREATMENT

CONCENTRATION µg/ml	CULTURE A		CULTURE B		MEAN %
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	2.31	100	2.10	100	100
39	2.26	98	2.33	111	105
78.1	2.49	108	2.55	121	115
156.25	2.44	105	2.57	122	114
312.5	1.88	81	2.24	106	94
625	2.48	107	2.59	123	115
1250	2.18	94	2.55	121	108
MMC 0.05 µg/ml	2.01	87	2.26	107	97

48-HOUR TREATMENT

CONCENTRATION µg/ml	CULTURE A		CULTURE B		MEAN %
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	2.56	100	1.84	100	100
39	2.61	102	2.31	126	114
78.1	2.44	95	2.24	122	109
156.25	2.49	97	2.57	140	119
312.5	2.56	100	2.19	119	110
625	2.73	107	1.95	106	107
1250	2.69	105	1.73	94	100
MMC 0.05 µg/ml	2.21	86	1.48	81	84

TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 3 (continued)

RESULTS OF CHROMOSOME ABERRATION TEST - CELL COUNTS

6-HOUR TREATMENT WITHOUT S9

CONCENTRATION µg/ml	CULTURE A		CULTURE B		MEAN %
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	2.40	100	2.03	100	100
39	2.23	93	2.27	112	103
78.1	2.44	102	2.46	121	112
156.25	2.27	95	2.28	112	104
312.5	2.42	101	2.14	105	103
625	2.26	94	2.18	108	101
1250	2.56	107	2.46	121	114
CP 10 µg/ml	2.42	101	2.51	124	113

6-HOUR TREATMENT WITH S9

CONCENTRATION µg/ml	CULTURE A		CULTURE B		MEAN %
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	2.56	100	2.22	100	100
39	2.30	90	2.54	114	102
78.1	2.51	98	2.04	92	95
156.25	2.24	88	2.36	106	97
312.5	2.27	88	2.35	106	97
625	2.38	93	2.15	97	95
1250	2.49	97	2.41	108	103
CP 10 µg/ml	1.62	63	1.54	69	66

APPENDICES

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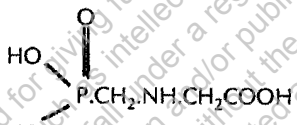
TECHNICAL GLYPHOSATE : METAPHASE ANALYSIS IN CHL CELLS *IN VITRO*

APPENDIX I

REPORT OF RESULTS OF CHROMOSOMAL ABERRATION TEST IN CULTURED MAMMALIAN CELLS (MITI/MHW FORMAT)

[1] GENERAL ITEMS

Name of the new chemical substance (IUPAC nomenclature)	N-(phosphonomethyl) glycine				
Other name	TECHNICAL GLYPHOSATE		Molecular weight		169
Structural formula or rational formula (or outline of manufacturing method, in case both are unknown)			Appearance at ordinary temperature		White powder
			Stability		Stable for >2 years under normal storage conditions
			Melting point		230°C
			Boiling point		
			Vapour pressure		1.94 x 10 $mmHg^{-7}$ at 45°C
Purity of the new chemical substance tested		Physicochemical properties of the new chemical substance	Partition coefficient		
			Solubility		
Name and concentration of impurities			Degree of solubility	Media	Insoluble at 50 mg/ml
				DMSO	Insoluble at 500 mg/ml
				Acetone	Insoluble at 500 mg/ml
				Others (Ethanol)	

[REMARKS] Because physicochemical properties are reference materials, fill in spaces to extent possible

1. **"STABILITY"** - Fill in the stability for water, other solvents, heat, light, etc.
2. **"VAPOUR PRESSURE"** - Fill in the vapour pressure of the test substance at 25°C
3. **"PARTITION COEFFICIENT"** - Fill in the value, the temperature used and the name of solvent used for the measurement.
4. **"SOLUBILITY"** - Fill in such information as water soluble, soluble in oil.
5. **"DEGREE OF SOLUBILITY"** - Fill in the solubility at 25°C for each solvent.

TECHNICAL GLYPHOSATE : METAPHASE ANALYSIS IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

[2] KIND OF A CELL LINE - CULTURE CONDITION

Name of Cell Line	CHL	**Obtained From**		NATIONAL INSTITUTE OF HEALTH SCIENCE - CELL BANK
Species	CHINESE HAMSTER (LUNG)	**Date obtained**		11 MARCH 1988
Medium	EAGLE'S MINIMUM ESSENTIAL MEDIUM	**Manufacturer**		GIBCO LTD.
Serum	10% FOETAL BOVINE	**Manufacturer (lot No.)**		GIBCO LTD.
Doubling Time	12 - 16 hr	**Freezing Condition**		-196°C, 10% DMSO
Passage Number	6: PRELIMINARY STUDY 8: MAIN STUDY	**Culture Condition**	**Container**	25 cm^2 COSTAR TC FLASK
			Temperature	37°C
			CO_2	5% (HUMIDIFIED)
Number of Chromosomes (Mode)	25			
Remarks				

[3] S9 MIX

(1) Source of S9 (Encircle the applicable number & fill in the relevant entries)

(1.) Made in-house	**Prepared on:** Aro. S9/11/10/95
2. Purchase	**Supplier:**
	Prepared on:
	Purchased on:
	Lot number:

(2) Storage Temperature, etc. of S9

Storage Temperature	-196°C	**Name and Model of Storage Apparatus**	STATEBOURNE SXR 34

(3) Preparation of S9 (if purchased material, fill in spaces to extent possible)

Animal Used		**Inducing Substance**	
Species, strain	RAT, SPRAGUE-DAWLEY	**Name**	AROCLOR 1254
Sex	MALE	**Administration method**	SINGLE I.P. INJECTION
Age (in weeks)	7 WEEKS	**Administration period and amount (g/kg weight)**	5 DAYS
Weight	~ 200g		0.5 g/kg

TECHNICAL GLYPHOSATE : METAPHASE ANALYSIS IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

(4) Composition of S9 Mix

Constituents	Amount in 1 ml S9 Mix	Constituents	Amount in 1 ml S9 Mix
S9	0.5 ml	NADPH	- μ mol
$MgCl_2$	8 μ mol	NADP	5.0 μ mol
KCl	33 μ mol	NADH	- μ mol
Glucose-6-phosphate	5.0 μ mol	Buffer (Na phosphate)	30 μ mol
		Others	- μ mol

(5) Treatment Condition of S9 Mix (Encircle the applicable number, and fill in the relevant entries)

	(1.) PLATE METHOD	2. SUSPENSION METHOD
Amount of S9 (final concentration)	5%	-%
Amount of S9 Protein (final concentration)	1.24 mg/ml	- mg/ml
Culture Time	6 hr	- hr
Culture Time After Treatment of the Test Substance	18 hr	- hr
Remark		

[4] CELL GROWTH INHIBITION TEST

(1) Test Condition and Preparation of the Solution of the Test Substance

Period of Experiment		From: 18/9/95	To: 5/10/95
		WITHOUT METABOLIC ACTIVATION	WITH METABOLIC ACTIVATION
Cell	No of cells seeded	0.1×10^6/flask (48 hr) 0.2×10^6/flask (24 hr)	0.2×10^6/flask (6 hr)
	Days after initiation of culture	2 DAY	2 DAY
Plate	Form	RECTANGULAR TC FLASK	RECTANGULAR TC FLASK
	Size	$25cm^2$	$25\ cm^2$
	Manufacturer	NUNC	NUNC
	Number of plates for each concentration	1	1
	Volume of medium	5 ml/plate	5 ml/plate

TECHNICAL GLYPHOSATE : METAPHASE ANALYSIS IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

(1) **Test Condition and Preparation of the Solution of the Test Substance (continued)**

		WITHOUT METABOLIC ACTIVATION	WITH METABOLIC ACTIVATION
Preparation of the solution of the test substance	Kind of solvent	MINIMAL ESSENTIAL MEDIA (MEM)	MINIMAL ESSENTIAL MEDIA (MEM)
	Concentration of the original solution of the test substance	50 mg/ml	50 mg/ml
	Amount of the test substance	312.3 mg	312.3 mg
	Volume of the solvent	6.2 ml	6.2 ml
	Condition of the solution of the test substance (encircle the applicable one)	dissolved / suspended / Others ( )	dissolved / suspended / Others ( )
	Time after preparation	10 minutes	10 minutes
	Method of preservation	None	None
	Method of sterilisation	None	None
Treatment of the test substance	Added volume of the prepared solution	0.05 ml/plate	0.05 ml/plate
	Period of treatment	(48) 24 hr	6 hr
	Added volume of S9 mix		0.5 ml/plate
Method of counting cell number		COULTER COUNTER, GIEMSA	COULTER COUNTER, GIEMSA
Remark	Cell preparation was 48 hours prior to treatment, pH measurements were made on culture media		

[NOTE] **"Method of counting of cell number"** - fill in the method of sample preparation (method of counting, fixation and staining)

(2) **Cell Growth Index** (Fill in the value in order beginning with low concentrations of the test substance, designating the value of the solvent-treated group as (100%)

	CONCENTRATION (μg/ml)	CELL GROWTH INDEX (%)
Without metabolic activation	0, 19.5, 39.1, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000	100, 86, 78, 86, 93, 90, 57, 73, 96, 49
With metabolic activation	0, 19.5, 39.1, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000	100, 94, 104, 98, 113, 113, 113, 117, 120, 97
Without metabolic activation 24 hours	0, 19.5, 39.1, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000	100, 120, 123, 116, 117, 126, 111, 101, 75, 155NM
Without metabolic activation 48 hours	0, 19.5, 39.1, 78.13, 156.25, 312.5, 625, 1250, 2500, 5000	100, 85, 98, 100, 100, 100, 90, 86, 51, 17NM

NM = no scorable metaphases

SPL PROJECT NUMBER: 434/015

TECHNICAL GLYPHOSATE : METAPHASE ANALYSIS IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

[5] CHROMOSOMAL ABERRATION TEST

(1) Test Condition and Preparation of the Solution of the Test Substance

Period of Experiment		From: 16/10/95	To: 4/1/96
		WITHOUT METABOLIC ACTIVATION	WITH METABOLIC ACTIVATION
Cell	No of cells seeded	0.075 x 10^6/flask (48 h) 0.15 x 10^6/flask (24 h)	0.15 x 10^6/flask (6 h)
	Days after initiation of culture	2 DAY	2 DAY
Plate	Form	RECTANGULAR TC FLASK	RECTANGULAR TC FLASK
	Size	25cm^2	25 cm^2
	Manufacturer	COSTAR	COSTAR
	Number of plates for each concentration	2	2
	Volume of medium	5 ml/plate	5 ml/plate
Preparation of the solution of the test substance	Kind of solvent	MEM	MEM
	Concentration of the original solution of the test substance	12.5 mg/ml	12.5 mg/ml
	Amount of the test substance	152.4 mg	152.4 mg
	Volume of the solvent	12.2 ml	12.2 ml
	Condition of the solution of the test substance (encircle the applicable one)	dissolved / (suspended) / others ( )	dissolved / (suspended) / others ( )
	Time after preparation	30 minutes	30 minutes
	Method of preservation	None	None
	Method of sterilisation	None	None
Treatment of the test substance	Added volume of the prepared solution	0.05 ml/flask	0.05 ml/flask
	Period of treatment	(48) 24 hr	6 hr
	Added volume of S9 mix		0.5 ml/plate
Mitotic inhibitor	Name	DEMECOLCINE (COLCEMID)	DEMECOLCINE (COLCEMID)
	Concentration	0.1 μg/ml	0.1 μg/ml
	Period of treatment	2 hr	2 hr
Method of counting cell number		COULTER COUNTER, GIEMSA	COULTER COUNTER, GIEMSA
Remark			

[NOTE] **"Method of counting cell number"** - fill in the method of sample preparation (method of counting, fixation and staining)

TECHNICAL GLYPHOSATE : METAPHASE ANALYSIS IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

[5] CHROMOSOMAL ABERRATION TEST (continued)

(2) Test Results (Test results should be reported on the attached form 1 and form 2)

(3) Judgement of the Results

Judgement (Encircle one)		POSITIVE FALSE POSITIVE (NEGATIVE)
Reason for judgement: Technical Glyphosate induced no statistically significant, dose related increases in the frequency of cells with chromosome aberrations either in the presence or absence of a liver enzyme metabolising system or after various exposure times.		
D_{20} value* Structural	Without metabolic activation With metabolic activation	Not applicable Not applicable
D_{20} value* Numerical	Without metabolic activation With metabolic activation	Not applicable Not applicable

* concentration (mg/ml) of the test substance where 20% of metaphases show structural or numerical chromosome aberrations

(4) Referential Matters

The maximum dose was limited by pH of the test material. Measurements of the pH of culture media after the addition of Technical Glyphosate had shown that at 2500 and 5000 µg/ml the pH was reduced by ≥1 unit. This was considered to be unacceptable because pH change has been shown to induce artefactual responses. MEM was selected as the vehicle because this gave the best doseable solution with the test material. The evaluation criteria are given in Appendix III but in addition the chromosome aberration data were statistically analysed using Fisher's Exact Test.

[REMARK] **"Referential matters"** - fill in the view etc. of the Study Director on the test results

[6] OTHERS

Testing institution	Name	SAFEPHARM LABORATORIES LTD.	
	Address	P.O. BOX 45, DERBY, ENGLAND, UK Tel. 01332 792896	
Study Director	Name Title	[redacted] BSc (Hons), Senior Genetic Toxicologist Signature: [redacted]	
Test Dates	From: 30/8/95	To: 4/1/96	
Protocol authorised	15/5/95	Final report authorised	13 MAR 1996

6. RESULTS

TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

RESULTS OF CHROMOSOME ABERRATION TEST WITHOUT S9

TREATMENT	WITHOUT S9-MIX	CONCEN-TRATION (µg/ml)	NUMBER OF CELLS OBSERVED	NUMBER OF POLYPLOIDS	JUDGE-MENT	GAP g	CHROMATID-TYPE ctb	CHROMATID-TYPE cte	CHROMOSOME-TYPE csb	CHROMOSOME-TYPE cse	OTHERS X	TOTAL -g	TOTAL +g	JUDGE-MENT
SOLVENT (MEM)	24	0	100	0		0	0	0	0	0	0	0	0	
			100	1		0	0	0	1	2	0	3	3	
			200	1(0.5)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	3(1.5)	3(1.5)	
	48	0	100	1		1	0	0	0	1	0	1	2	
			100	0		0	0	1	0	0	0	1	1	
			200	1(0.5)		1(0.5)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	0(0.0)	2(1.0)	3(1.5)	
TEST SUBSTANCE TECHNICAL GLYPHOSATE	24	312.5	100	0	-	2	0	0	0	0	0	0	2	-
			100	1		0	0	0	0	0	0	0	0	
			200	1(0.5)		2(1.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.0)	
		625	100	0	-	0	0	0	0	1	0	1	1	-
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	1(0.5)	
		1250	100	0	-	1	0	0	0	1	0	1	2	-
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	2(1.0)	
	48	312.5	100	2	-	1	1	1	0	2	0	4	5	-
			100	1		1	0	0	1	0	0	1	2	
			200	3(1.5)		2(1.0)	1(0.5)	1(0.5)	1(0.5)	2(1.0)	0(0.0)	5(2.5)	7(3.5)	
		625	100	3	-	1	0	0	1	3	0	4	5	-
			100	0		0	0	0	0	1	0	1	1	
			200	3(1.5)		1(0.5)	0(0.0)	0(0.0)	1(0.5)	4(2.0)	0(0.0)	5(2.5)	6(3.0)	
		1250	100	1	-	0	0	0	0	1	0	1	1	-
			100	0		2	0	0	1	2	0	3	5	
			200	1(0.5)		2(1.0)	0(0.0)	0(0.0)	1(0.5)	3(1.5)	0(0.0)	4(2.0)	6(3.0)	
POSITIVE CONTROL [MMC]	24	0.05	100	1	-	12	13	11	4	0	0	24	29	+
			100	0		0	2	6	1	1	0	8	8	
			200	1(0.5)		12(6.0)	15(7.5)	17(8.5)	5(2.5)	1(0.5)	0(0.0)	32(16.0)***	37(18.5)***	
	48	0.05	100	0	-	4	18	11	5	2	0	28	32	+
			50	0		2	7	15	9	3	1	28	29	
			150	0(0.0)		6(4.0)	25(16.7)	26(17.3)	14(9.3)	5(3.3)	1(0.7)	56(37.3)***	61(40.7)***	

*** = p<0.001 with Fisher's Exact Test

6. RESULTS

TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

RESULTS OF CHROMOSOME ABERRATION TEST WITH AND WITHOUT S9

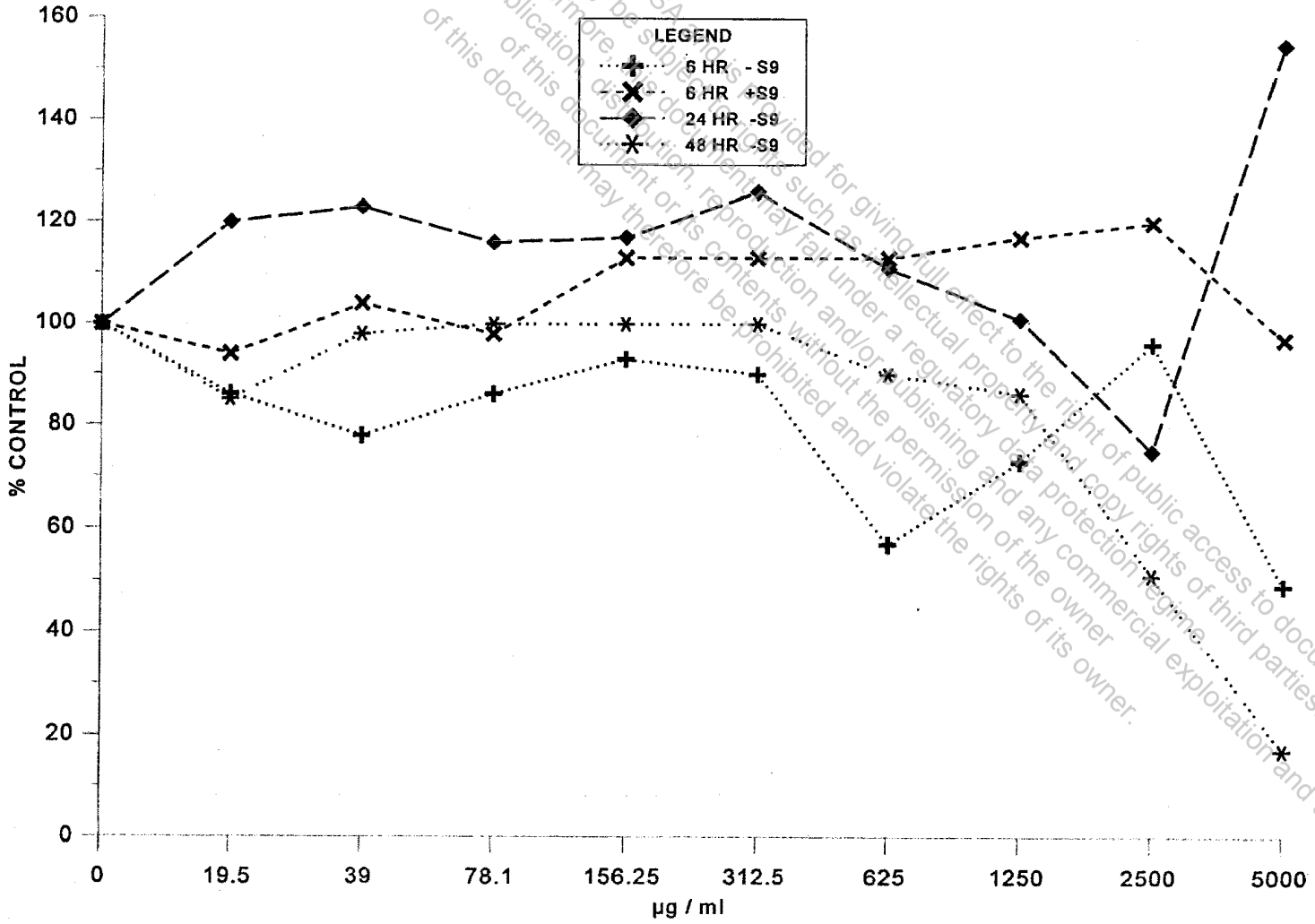
TREATMENT	WITH (+) OR WITHOUT (-) S9-MIX	CONCEN-TRATION (µg/ml)	NUMBER OF CELLS: OBSERVED	NUMBER OF CELLS: NUMBER OF POLYPLOIDS	JUDGE-MENT	GAP g	CHROMATID-TYPE ctb	CHROMATID-TYPE cte	CHROMOSOME-TYPE csb	CHROMOSOME-TYPE cse	OTHERS X	TOTAL -g	TOTAL +g	JUDGE-MENT
SOLVENT (MEM)	-	0	100	0		0	0	0	0	0	0	0	0	
			100	3		0	0	1	0	0	0	1	1	
			200	3(1.5)		0	0	1(0.5)	0	0	0	1(0.5)	1(0.5)	
	+	0	100	0		2	1	0	1	2	0	4	6	
			100	1		0	0	0	0	0	0	0	0	
			200	1(0.5)		2(1.0)	1(0.5)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	4(2.0)	6(3.0)	
6 hr TEST SUBSTANCE TECHNICAL GLYPHOSATE	-	312.5	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		0	0	0	2	0	0	2	2	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	2(1.0)	0(0.0)	0(0.0)	2(1.0)	2(1.0)	
		625	100	0	-	0	0	1	0	0	0	1	1	-
			100	0		0	0	0	0	1	0	1	1	
			200	0(0.0)		0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	0(0.0)	2(1.0)	2(1.0)	
		1250	100	1	-	1	0	0	0	1	0	1	2	-
			100	1		0	0	0	0	0	0	0	0	
			200	2(1.0)		1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	2(1.0)	
6 hr	+	312.5	100	0	-	0	0	0	0	1	0	1	1	-
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	1(0.5)	
		625	100	0	-	0	0	0	0	1	0	1	1	-
			100	0		3	0	0	1	0	0	1	4	
			200	0(0.0)		3(1.5)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	0(0.0)	2(1.0)	5(2.5)	
		1250	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		2	0	0	1	2	0	3	5	
			200	0(0.0)		2(1.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	3(1.5)	5(2.5)	
POSITIVE CONTROL [CP]	-	10	100	0	-	0	0	0	0	1	0	1	1	
			100	0		0	0	0	0	2	0	2	2	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(1.5)	0(0.0)	3(1.5)	3(1.5)	
	+	10	50	0	-	7	9	14	5	2	0	22	25	+
			100	0		10	8	18	3	4	0	26	32	
			150	0(0.0)		17(11.3)	17(11.3)	32(21.3)	8(5.3)	6(4.0)	0(0.0)	48(32.0)***	57(38.0)***	

*** = $p < 0.001$ with Fisher's Exact Test

TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

APPENDIX II

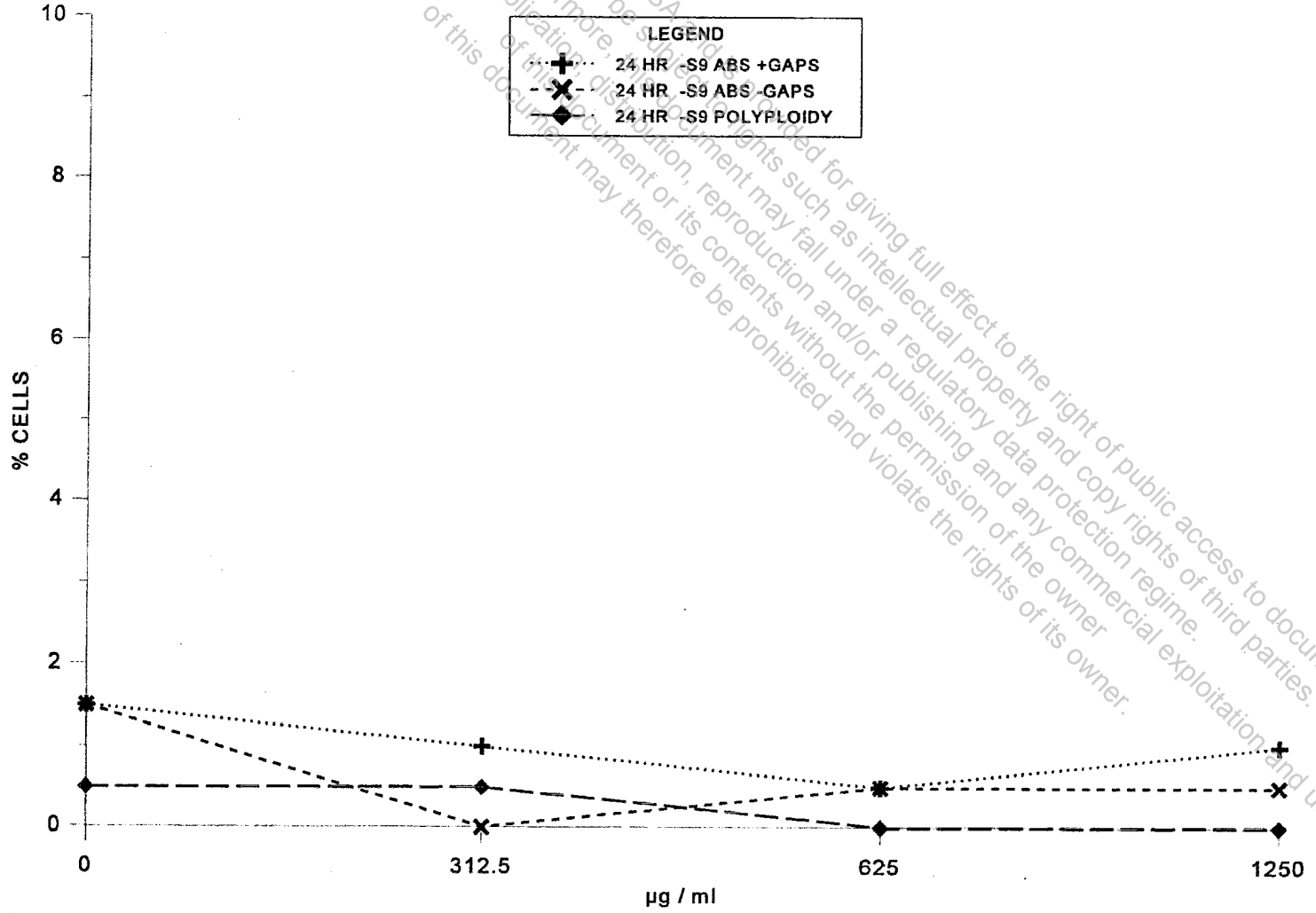
DOSE RESPONSE CURVE: PRELIMINARY TOXICITY TEST



TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST CHL CELLS *IN VITRO*

APPENDIX II (continued)

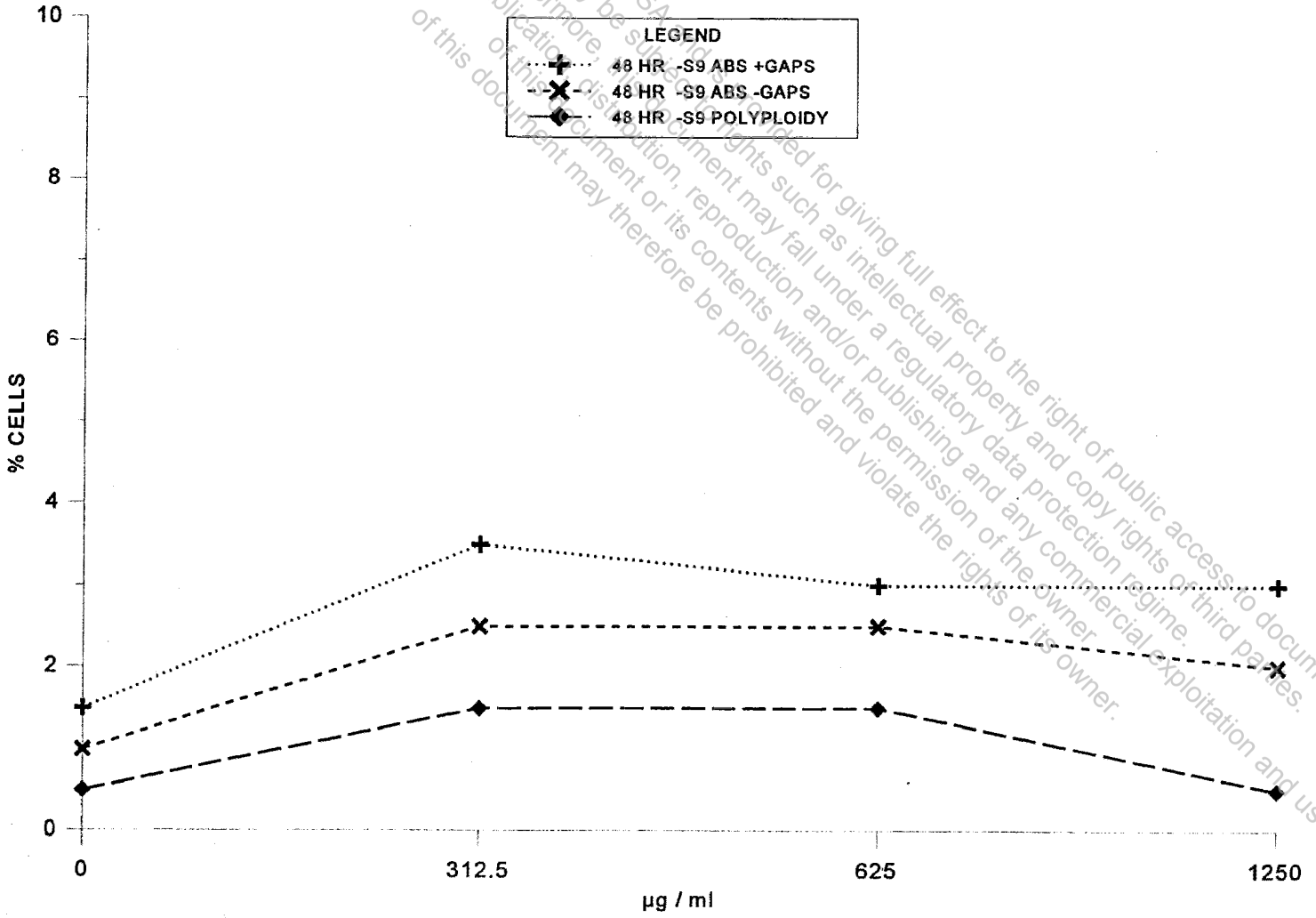
DOSE RESPONSE CURVE: CHROMOSOME ABERRATION TEST



TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST CHL CELLS *IN VITRO*

A P P E N D I X I I (continued)

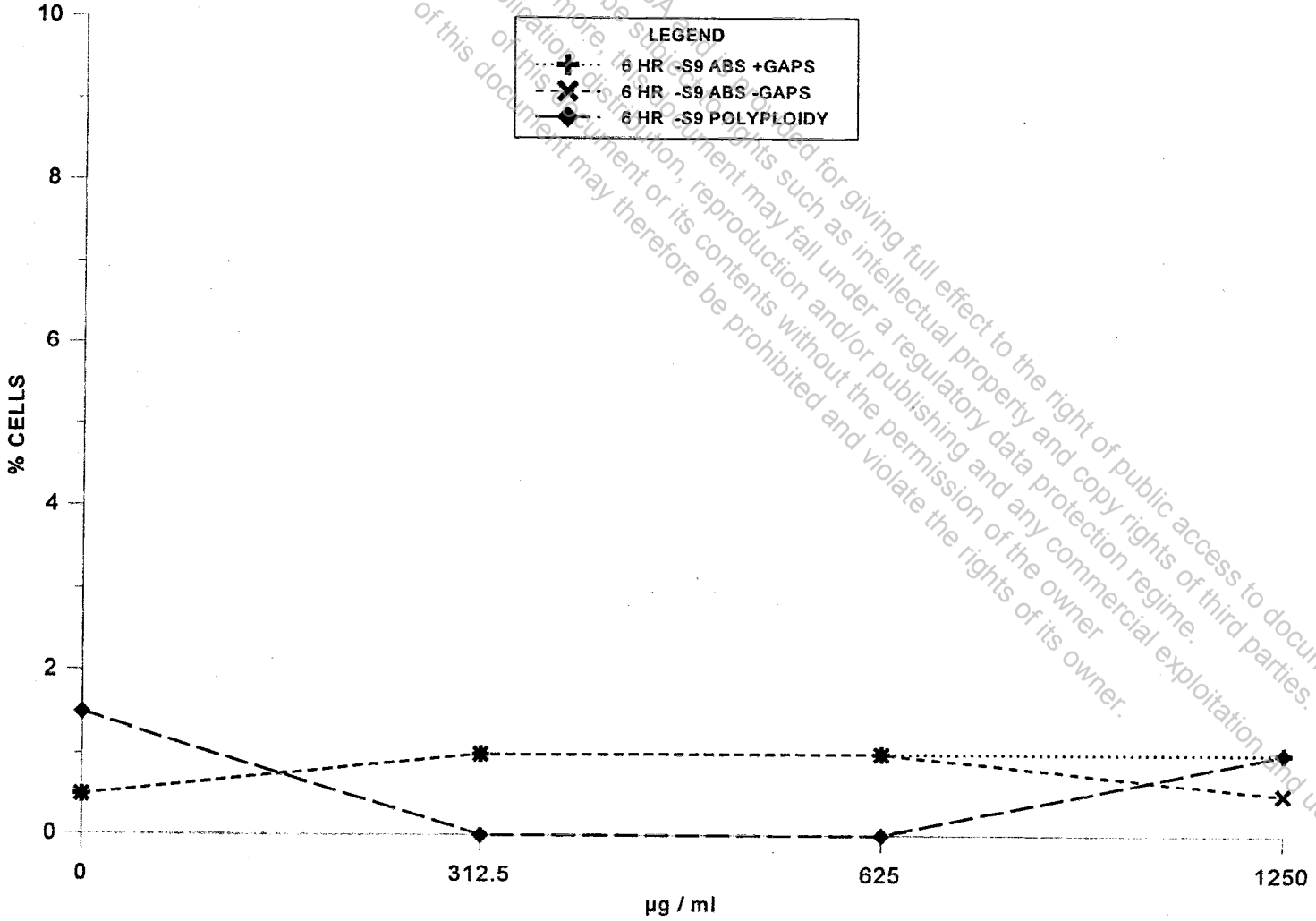
DOSE RESPONSE CURVE: CHROMOSOME ABERRATION TEST



TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST CHL CELLS *IN VITRO*

APPENDIX II (continued)

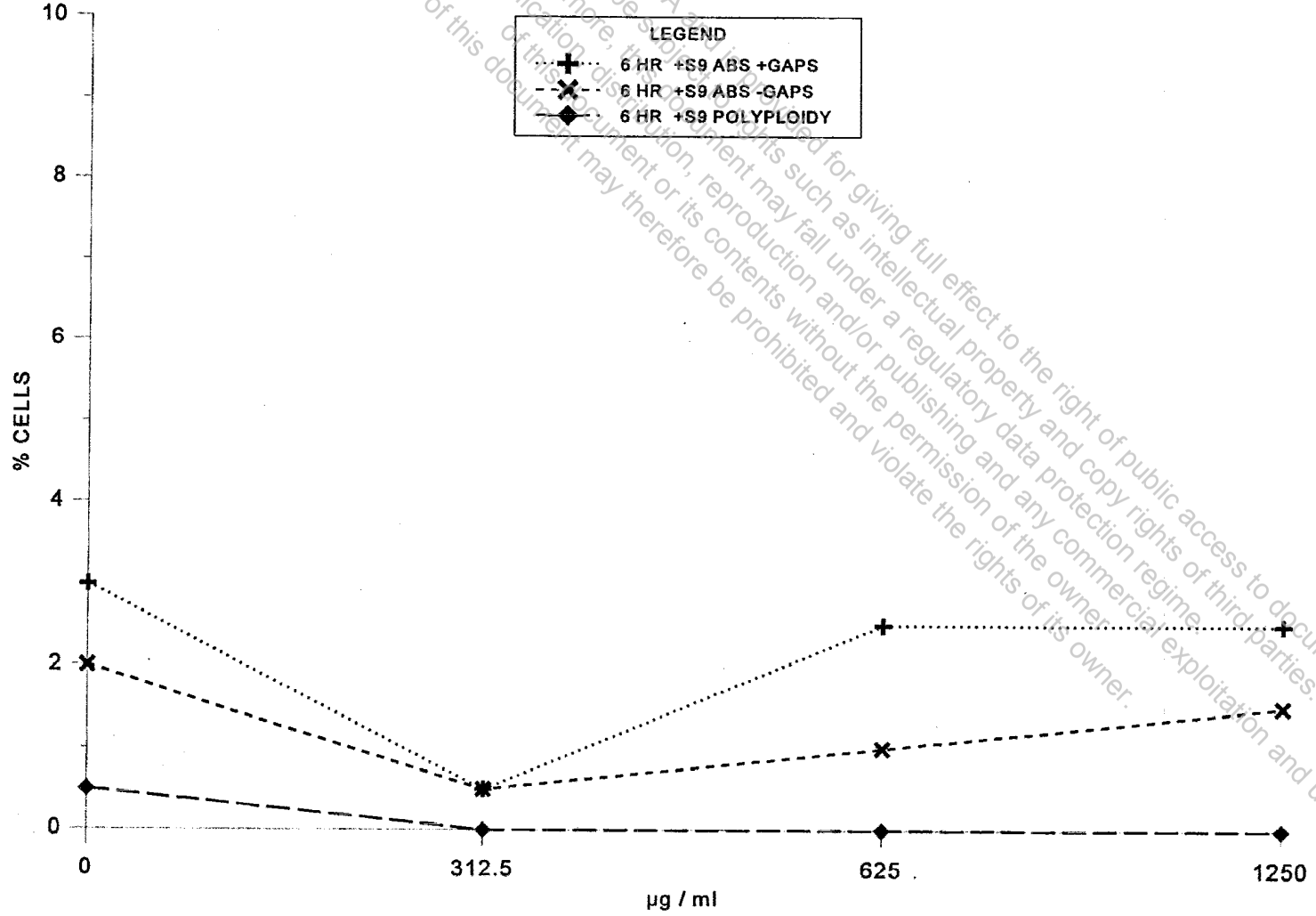
DOSE RESPONSE CURVE: CHROMOSOME ABERRATION TEST



TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST CHL CELLS *IN VITRO*

APPENDIX II (continued)

DOSE RESPONSE CURVE: CHROMOSOME ABERRATION TEST



TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

APPENDIX III

CHROMOSOME STRUCTURAL ABERRATIONS: CLASSIFICATION AND EVALUATION CRITERIA

1. CLASSIFICATION

1.1 Gaps (g)

Gaps are small areas of the chromosome which are unstained. The chromatids remain aligned as normal and the gap does not extend along the chromatid for a distance greater than the width of a chromatid. If the gap occurs on one chromatid only it is a chromatid gap (g). If a gap appears in both chromatids at the same position it is a chromosome gap (G).

1.2 Chromatid Breaks (ctb)

Chromatid breaks (ct) vary in appearance. The chromatid may remain aligned but show a gap which is too large to classify as a gap. Alternatively, the chromatid may be broken so that the broken fragment is displaced. In some cases, the fragment is not seen at all. A chromatid fragment (f) should be scored if the chromosome of origin cannot be identified. Very small fragments are scored as minutes (m).

1.3 Chromosome Breaks (csb)

Chromosome breaks (CS) are breaks in both chromatids of the chromosome. A fragment with two chromatids is formed and this may be displaced by varying degrees. Breaks are distinguished from gaps by the size of the unstained region. A chromosome break is scored if the fragment is associated with a chromosome from which it was probably derived. However, fragments are often seen in isolation and are then scored as chromosome fragments (F). Very small fragments are scored as minutes (M).

1.4 Exchanges (cte and cse)

Exhanges are formed by faulty rejoining of broken chromosomes and may be of the chromosome or chromatid type. Chromatid exchanges (c/c,r) have numerous different forms but are generally not further classified. Where multiple exchanges have occurred each exchange point is counted as one chromatid exchange. Chromosome exchanges generally appear as either a dicentric (D) or a ring (R) form, either of which can be associated with a fragment, which if possible should be scored as part of the exchange.

1.5 Multiple Aberrations

If many aberrations are present in one metaphase, the exact details may not be scorable. This is particularly the case when chromosome pulverisation occurs. If the number of aberrations is 10 or more then the cell is classified as X.

1.6 Chromosome Number

If the chromosome (centromere) number is between 23 and 27 inclusive then it is classified as a diploid cell and scored for aberrations. If less than 23 chromosomes are counted then the cell is ignored under the assumption that some chromosomes may have been lost for technical reasons. If 28 to 31 chromosomes are scored then the count is recorded and the cell classified as an aneuploid cell. If greater than 31 chromosomes are scored then the cell is classified as a polyploid cells. If the chromosomes are arranged in closely apposed pairs, ie. 4 chromatids instead of 2, the cell is scored as endoreduplicated (E).

2. EVALUATION CRITERIA

2.1 Historical Aberration Ranges for Vehicle and Untreated Control Cultures

Many experiments with the CHL cell line have established a range of aberration frequencies acceptable for control cultures, these are commonly in the range of 0 to 3% cells with aberrations (Ishidate, 1987), Data Book of Chromosomal Aberration Test *In Vitro*, (Revised Edition).

A positive response was recorded for a particular treatment if the % cells with aberrations (gaps included) was equal to or exceeded 10%, an equivocal response was recorded for values between 5 and 10% and a negative response for values less than 5%. For polyploid cells, an incidence greater than 10% is generally recorded as positive.

However, consideration is given to a number of factors, such as the frequency of chromosome exchange events which are comparatively rare in control cultures, and the ultimate designation must rely upon experience and sound scientific judgement (UKEMS Guidelines for Mutagenicity Testing, 1983).

SPL PROJECT NUMBER: 434/015

APPENDIX IV

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

SafePharm Laboratories Limited
P O Box No 45
Derby
DE1 2BT

DATE OF INSPECTION

31 January 1994

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities.

[redacted]

16/3/94.

[redacted]
Director
UK GLP Monitoring Unit

SAFEPHARM LABORATORIES LIMITED

AUTHENTICATION OF CHANGE TO FINAL REPORT

TECHNICAL GLYPHOSATE:
CHROMOSOME ABERRATION TEST IN CHL CELLS
IN VITRO
SPL PROJECT NUMBER: 434/015

Amendment to final report at the request of sponsor:

Page 26: Total number of cells observed for Positive Control Treatment should be amended from 100 to 150.

: Statistical method used added to table data.

Page 27: Total numbers of cells observed for Positive Control Treatment should be amended from 100 to 150.

: Judgement for Positive Control Treatment without S9-mix should be amended from positive to negative (-).

: Statistical method used added to table data.

These amendments do not affect the validity or interpretation of the data.

[redacted] .....[redacted].....

STUDY DIRECTOR

DATE 29 MAY 1996

The amended parts of this report (pages 26 and 27) have been audited by Safepharm Quality Assurance Unit and are considered to be an accurate account of the project.

[redacted] .....[redacted].....

QUALITY ASSURANCE MANAGER

DATE -3. JUN 1996

6. RESULTS

TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

RESULTS OF CHROMOSOME ABERRATION TEST WITHOUT S9

TREATMENT	WITHOUT S9-MIX	CONCENTRATION (µg/ml)	NUMBER OF CELLS: OBSERVED	NUMBER OF CELLS: NUMBER OF POLYPLOIDS	JUDGEMENT	GAP g	CHROMATID-TYPE ctb	CHROMATID-TYPE cte	CHROMOSOME-TYPE csb	CHROMOSOME-TYPE cse	OTHERS X	TOTAL -g	TOTAL +g	JUDGEMENT
SOLVENT (MEM)	24	0	100	0		0	0	0	0	0	0	0	0	
			100	1		0	0	0	1	2	0	3	3	
			200	1(0.5)		0(0.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	3(1.5)	3(1.5)	
	48	0	100	1		1	0	0	0	1	0	1	2	
			100	0		0	0	1	0	0	0	1	1	
			200	1(0.5)		1(0.5)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	0(0.0)	2(1.0)	3(1.5)	
TEST SUBSTANCE TECHNICAL GLYPHOSATE	24	312.5	100	0	-	2	0	0	0	0	0	0	2	-
			100	1		0	0	0	0	0	0	0	0	
			200	1(0.5)		2(1.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.0)	
		625	100	0	-	0	0	0	0	1	0	1	1	-
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	1(0.5)	
		1250	100	0	-	1	0	0	0	1	0	1	2	-
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	2(1.0)	
	48	312.5	100	2	-	1	1	1	0	2	0	4	5	-
			100	1		1	0	0	1	0	0	1	2	
			200	3(1.5)		2(1.0)	1(0.5)	1(0.5)	1(0.5)	2(1.0)	0(0.0)	5(2.5)	7(3.5)	
		625	100	3	-	1	0	0	1	3	0	4	5	-
			100	0		0	0	0	0	1	0	1	1	
			200	3(1.5)		1(0.5)	0(0.0)	0(0.0)	1(0.5)	4(2.0)	0(0.0)	5(2.5)	6(3.0)	
		1250	100	1	-	0	0	0	0	1	0	1	1	-
			100	0		2	0	0	1	2	0	3	5	
			200	1(0.5)		2(1.0)	0(0.0)	0(0.0)	1(0.5)	3(1.5)	0(0.0)	4(2.0)	6(3.0)	
POSITIVE CONTROL [MMC]	24	0.05	100	1	-	12	13	11	4	0	0	24	29	+
			100	0		0	2	6	1	1	0	8	8	
			200	1(0.5)		12(6.0)	15(7.5)	17(8.5)	5(2.5)	1(0.5)	0(0.0)	32(16.0)***	37(18.5)***	
	48	0.05	100	0	-	4	18	11	5	2	0	28	32	+
			50	0		2	7	15	9	3	1	28	29	
			100	0(0.0)		6(4.0)	25(16.7)	26(17.3)	14(9.3)	5(3.3)	1(0.7)	56(37.3)***	61(40.7)***	

*** = $p < 0.001$

6. RESULTS

TECHNICAL GLYPHOSATE : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

APPENDIX I (continued)

RESULTS OF CHROMOSOME ABERRATION TEST WITH AND WITHOUT S9

TREATMENT	WITH (+) OR WITHOUT (-) S9-MIX	CONCENTRATION (µg/ml)	NUMBER OF CELLS: OBSERVED	NUMBER OF CELLS: NUMBER OF POLYPLOIDS	JUDGEMENT	GAP: g	CHROMATID-TYPE: ctb	CHROMATID-TYPE: cte	CHROMOSOME-TYPE: csb	CHROMOSOME-TYPE: cse	OTHERS: X	TOTAL: -g	TOTAL: +g	JUDGEMENT
SOLVENT (MEM)	-	0	100	0		0	0	0	0	0	0	0	0	
			100	3		0	0	1	0	0	0	1	1	
			200	3(1.5)		0	0	1(0.5)	0	0	0	1(0.5)	1(0.5)	
	+	0	100	0		2	1	0	1	2	0	4	6	
			100	1		0	0	0	0	0	0	0	0	
			200	1(0.5)		2(1.0)	1(0.5)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	4(2.0)	6(3.0)	
TEST SUBSTANCE TECHNICAL GLYPHOSATE 6 hr	-	312.5	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		0	0	0	2	0	0	2	2	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	2(1.0)	0(0.0)	0(0.0)	2(1.0)	2(1.0)	
		625	100	0	-	0	0	1	0	0	0	1	1	-
			100	0		0	0	0	0	1	0	1	1	
			200	0(0.0)		0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	0(0.0)	2(1.0)	2(1.0)	
		1250	100	1	-	1	0	0	0	1	0	1	2	-
			100	1		0	0	0	0	0	0	0	0	
			200	2(1.0)		1(0.5)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	2(1.0)	
6 hr	+	312.5	100	0	-	0	0	0	0	1	0	1	1	-
			100	0		0	0	0	0	0	0	0	0	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	1(0.5)	1(0.5)	
		625	100	0	-	0	0	0	0	1	0	1	1	-
			100	0		3	0	0	1	0	0	1	4	
			200	0(0.0)		3(1.5)	0(0.0)	0(0.0)	1(0.5)	1(0.5)	0(0.0)	2(1.0)	5(2.5)	
		1250	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		2	0	0	1	2	0	3	5	
			200	0(0.0)		2(1.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	3(1.5)	5(2.5)	
POSITIVE CONTROL [CP]	-	10	100	0	-	0	0	0	0	1	0	1	1	+
			100	0		0	0	0	0	2	0	2	2	
			200	0(0.0)		0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(1.5)	0(0.0)	3(1.5)	3(1.5)	
	+	10	50	0	-	7	9	14	5	2	0	22	25	+
			100	0		10	8	18	3	4	0	26	32	
			200	0(0.0)		17(11.3)	17(11.3)	32(21.3)	8(5.3)	6(4.0)	0(0.0)	48(32.0)***	57(38.0)***	

*** = p<0.001