Table 5.3-11: Overall mean test compound intake (mg/kg bw/day)

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Achieved dose (mg/kg bw/day)	0	125.2	371.9	1262.1	0	156.3	481.2	1686.5

E. OPHTHALMOSCOPIC EXAMINATION

There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group.

Blood clinical chemistry

At termination, low dose males had an significantly decreased mean K value as compared to control animals. Since this finding was restricted to the low dose group, it was not considered to be treatment-related.

High dose males showed a significantly lower ALT value at termination than control males. However, because of the minor degree of change, the absence of a dose response at the other dose levels this findings was considerd to be of no biological relevance. The increased total bilirubin value in high-dose males at termination is also considered to be of no biological relevance.

Table 5.3-12: Intergroup comparison of selected clinical chemistry parameters pre-dose and at termination

	Dietary concentration of glyphosate acid (ppm)							
	Males				**Females**			
Parameter	**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
ALT(U/L)	48	35	41	32*	42	39	35	30
Total bilirubin (mg/dL)	0.3	0.5	0.5	0.6*	0.2	0.3	0.3	0.3
Potassium(mmol/L)	6.9	5.8**	6.2	6.6	5.8	6.2	5.7	6.2
Potassium(mmol/L) (pre-dose)	5.2	5.1	4.8	5.2	5.2	4.6*	4.4**	4.7

* Statistically significant from controls, $p<0.05$)
** Statistically significant from controls, $p<0.01$

G. URINALYSIS

At termination there were marginal increases in the mean scores for ketones, blood, protein and red blood cells (RBCs) in the mid- and high-dose males. It has to be noted that the unusual appearance of ststistical differences among zeros (ketones) or 1 (protein) was the result of rounding (see Table 5.3-13). The number of affected rats, and their respective scores are shown in Table 5.3-14 below. The lower protein value observed in high-dose males was statistically different from control, but still within the normal range. The presence of blood and RBCs is minimally elevated in all treated groups when copmpared to control animals. However, the observation of a few RBCs is common in male rats and this mild degree cannot be attributed unequivocally to the test substance. In mid- and high-dose females the number of rats with 1 RBC/hpf was also increased. Together with the appearance of blood in these groups this change seems to be treatment-related. In addition, there were no indications for microscopic hematuria found during the histopathological examinations. Therefore, at least the changes in urine analysis parameters in the mid-dose group are considered not to be adverse effects.

Table 5.3-13: Urineanalysis at termination – group mean values for selected parameters

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Parameter	0	2000	6000	20000	0	2000	6000	20000
Ketones (mg/dL)	0	0	0*	0	0	0	0	0
Blood (mg/dL)	0	2	1	2**	0	0	1	1
Protein (mg/dL)	1	1	1	1*	0	0	0	0
RBC (cells/hpf)	0	1	1	2**	0	0	1**	1

* Statistically significant from controls, p<0.05)
** Statistically significant from controls, p<0.01

Table 5.3-14: Urineanalysis at termination – affected animals for selected parameters

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Parameter	0	2000	6000	20000	0	2000	6000	20000
Ketones (Score 0)	10/10	10/10	6/10	9/10	10/10	10/10	10/10	10/10
Ketones (Score 1)	4/10	1/10	4/10	1/10	0/10	0/10	0/10	0/10
Blood (score 0)	8/10	3/10	3/10	2/10	8/10	8/10	5/10	7/10
Blood (score 1)	2/10	3/10	3/10	3/10	2/10	2/10	2/10	0/10
Blood (score 2)	0/10	2/10	3/10	1/10	0/10	0/10	2/10	3/10
Blood (score 3)	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Blood (score 4)	0/10	2/10	1/10	4/10	0/10	0/10	1/10	0/10
Protein (score 1)	5/10	6/10	7/10	3/10	1/10	2/10	3/10	0/10
Protein (score 2)	4/10	1/10	2/10	1/10	0/10	0/10	0/10	0/10
RBC (score 1)	3/10	3/10	8/10	6/10	1/10	2/10	8/10	6/10
RBC (score 2)	-	-	-	1/10	-	-	1/10	-
RBC (score 3)	-	-	-	0/10	-	-	-	-
RBC (score 4)	-	-	-	2/10	-	-	-	-

-: no finding

H. NECROPSY

Organ weights

The mean adrenal weights in high- and low-dose males was significantly decreased, whereas the relative adrenal weights were only signicantly decreased high dose males. In females of the high-dose group relative spleen weights were significantly increased when compared to controls.

Table 5.3-15: Results from absolute and relative organ weight determination

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Mean adrenal weight (g)	0.070	0.059*	0.061	0.052**	0.093	0.080	0.086	0.080
Relative adrenal weight (% bw)	0.014	0.012	0.012	0.011**	0.031	0.026	0.029	0.028
Spleen weight (g)	0.785	0.786	0.808	0.752	0.548	0.598	0.589	0.648
Relative spleen weight (% bw)	0.257	0.250	0.279	0.266	0.180	0.192	0.198	0.223**

* Statistically significant from controls, p<0.05)
** Statistically significant from controls, p<0.01

Gross pathology

A few gross lesions were noted at necropsy in all dose groups. The most prevalent abnormality was the swollen, reddened sublingual salivary glands. This lesion was observed in control males, and in one treated male and female of some test substance groups (see Table 5.3-16). Therefore, this finding is considered not to be related to treatment.

Table 5.3-16: Summary of necropsy findings

Finding	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Sublingual salivary glands: enlarged and/or reddened	1/10	0/10	1/10	1/10	0/10	1/10	0/10	1/10

Histopathology

There were no histopathological findings related to treatment. The incidence of findings was low and of a type commonly found in rats of this strain and age

III. CONCLUSION

Glyphosate acid when fed to rats at a level of 20000 ppm resulted in clinical signs of toxicity and slight increases in blood urine and RBC counts in urine. The minor changes in urinalysis seen at 6000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the NOAEL for glyphosate acid in this study.

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/03	[redacted]	1996	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat [redacted] [redacted] Project No.: 434/016 Date: 1996-07-16 GLP: yes unpublished JMMAF 59 NohSan No. 4200

Guideline: (Data from the study report is equivalent to OECD 408.)

Deviations: FOB was not conducted. Some mandatory organs were not weighed/examined.

Dates of experimental work: 1995-08-11 - 1996-01-30

Executive Summary

The test material was administered by dietary admixture to three groups, each of ten male and ten female Sprague Dawley (CD) strain rats, for ninety consecutive days, at dietary concentrations of 1000, 10,000 and 50,000 ppm (equivalent to an estimated mean achieved dose level of male; 79, 730 and 3706 mg/kg/day, female; 90, 844 and 4188 mg/kg/day respectively). A further group of ten males and ten females was exposed to basal laboratory diet to serve as a control.

Clinical signs, bodyweight, food and water consumption were monitored during the study. Haematology, blood chemistry and urinalysis were evaluated for all animals at the end of the study. Ophthalmoscopic examination was also performed. All animals were subjected to a gross necropsy examination and a comprehensive histopathological evaluation of tissues was performed.

At 1,000 ppm no treatment-related effects were noted in any of the investigation conducted.
In the mid dose group statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase was observed in both sexes. Histopathology revealed a mucosal atrophy of the caecum in this group. No other treatment-related findings were observed in this dose group.
Animals treated with 50,000 ppm showed soft faeces/diarrhoea from Day 4 which continued throughout the study period. In addition, body weight gain, food intake and food efficiency in animals of both sexes in the high-dose group was reduced over the first four weeks of treatment when compared with controls. Bodyweight development, food consumption and efficiency recovered in females and were comparable with the control group by the end of the treatment period. In males bodyweight gain showed only a partial recovery, and an adverse effect on dietary intake was still apparent during the remaining treatment period.
Animals of both sexes treated with 50,000 ppm showed a statistically significant reduction in plasma calcium concentration and creatinine levels, as well as an increase in alkaline phosphatase and inorganic phosphorous in comparison with controls. Reductions in total protein and albumin were observed only in high-dose females. Urinalysis revealed increased levels of haemoglobin when compared with controls. Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated with 50,000 ppm. At necropsy high dose animals of both sexes showed an enlarged and fluid-filled caecum, as well as statistically increased liver and kidney weights. Microscopic examination of the caecum revealed changes identified as mucosal atrophy for animals of both sexes treated with 50,000.

Conclusion
Dietary administration of the test material, technical Glyphosate, to rats for a period of 90 consecutive days at concentrations of up to 50,000 ppm, resulted in treatment-related changes at 50,000 and 10,000 ppm. No such effects were demonstrated in the 1,000 ppm treatment group and the "No Observed Effect Level" was, therefore, considered to be 1,000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	H95D161 A
Purity:	95.3%
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	[redacted], UK
Age:	6-7 weeks
Sex:	male and female
Weight at dosing:	♂ 175 - 218 g; ♀ 145 - 195 g
Acclimation period:	7 days
Diet/Food:	Rat and Mouse [redacted] Ground Diet No.1 ([redacted] UK), *ad libitum*

Water:	tap water, *ad libitum*
Housing:	In groups of up to four by sex in polypropylene grid-floor cages.
Environmental conditions:	Temperature: 21 ± 2°C
	Humidity: 55 ± 15%
	Air changes: 15/hour
	12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-08-11 to 1996-01-30

Animal assignment and treatment:
In a 90 day feeding study groups of 10 Sprague Dawley rats per sex received daily dietary doses of 0, 1000, 10000 and 50000 ppm (equivalent to mean achieved dose levels of male; 0, 79, 730 and 3706 mg/kg bw/day, female; 90, 844, 4188 mg/kg bw/day respectively) technical Glyphosate in the diet.
Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for homogeneity and achieved concentration.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded.

Body weight
Individual body weights were recorded on Day 0 (prior to treatment) and at weekly intervals thereafter. Body weights were also determined at necropsy.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group throughout the study.

Water consumption
Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Ophthalmoscopic examination
The eyes of all control and high dose animals were examined before administration of the test and control diets and before termination of treatment (during Week 12). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with 0.5% Tropicamide solution [redacted] UK), detailed examination of the internal structure of each eye using a direct ophthalmoscope.

Haematology and clinical chemistry
Haematological and blood chemical investigation were performed on all animals from each test and control group at the end of the study (Day 90).

Urinalysis
Urinalytical investigations were performed on all animals during Week 12. Urine samples were collected overnight by housing the rats in metabolism cages. Animals were maintained under conditions of normal hydration during collection but without access to food.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, gonads, heart, kidneys, liver, pituitary and spleen.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (at three levels), caecum, colon, duodenum, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

Statistics
Absolute and relative organ weights, haematological and blood chemical data were analysed by one way analysis of variance incorporating 'F-max' test for homogeneity of variance. Data showing heterogeneous variances were analysed using Kruskal-Wallis non-parametric analysis of variance and Mann-Whitney U-Test.
The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$** and $p < 0.05$*.

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:
1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.
The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$**, $p < 0.05$* and $p < 0.1$ (*).

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
Animals of both sexes treated with 50,000 ppm showed soft faeces and diarrhoea from Day 4 which continued throughout the study period.
The remaining observable sign of generalised fur loss was noted in one male and two females treated with 10,000 and 1,000 ppm respectively. This is a commonly reported incidental finding in laboratory maintained rats that, in the absence of any dose-related response, is of no toxicological significance and unrelated to treatment with the test material.

C. BODY WEIGHT
Animals of both sexes treated with 50,000 ppm showed a reduction in bodyweight gain over the first four weeks of treatment when compared with controls (see Table 5.3-17). Female bodyweight development recovered as the study progressed and was comparable with the control group by the end of the treatment period. Male individuals showed only a partial recovery with bodyweight gain remaining slightly lower than the control group values during the subsequent weeks of treatment.
Bodyweight development was unaffected by treatment with the test material at the remaining dose levels.

Table 5.3-17: Group mean weekly bodyweights and standard deviations (sd)

Dietary concentration (ppm)		Bodyweight (g) at Day													
		0	7	14	21	28	35	42	49	56	63	70	77	84	90
		Males													
0	mean	206	269	315	354	382	411	444	457	488	508	523	537	536	551
	sd	8	12	17	24	33	38	45	44	49	52	55	58	56	58
1000	mean	199	260	309	350	377	400	427	446	470	485	497	513	516	528
	sd	11	14	19	21	24	26	30	31	32	32	35	37	36	37
10000	mean	200	257	303	338	364	393	414	429	454	470	483	494	495	506
	sd	12	12	15	21	25	30	35	35	38	38	38	40	39	43
50000	mean	198	215	247	268	283	306	329	335	356	369	382	394	395	408
	sd	8	8	15	21	26	31	33	38	41	43	43	44	42	44
		Females													
0	mean	173	197	214	232	243	256	269	276	284	291	295	306	304	307
	sd	9	11	12	15	16	18	20	19	20	21	24	25	25	27
1000	mean	173	199	218	238	249	261	272	280	286	292	300	308	304	313
	sd	10	13	14	16	16	17	18	19	18	18	19	21	20	20
10000	mean	166	184	201	217	226	237	248	256	262	267	272	277	276	282
	sd	14	18	21	25	24	26	27	27	27	27	27	29	28	29
50000	mean	173	183	197	214	219	231	240	246	251	260	265	271	267	273
	sd	11	12	14	15	14	18	21	21	20	23	23	26	22	25

D. FOOD CONSUMPTION

Animals of both sexes treated with 50,000 ppm showed a reduction in both dietary intake and food efficiency over the first four weeks of treatment when compared with controls (see Table 5.3-18). Female food consumption and efficiency recovered as the study progressed and was comparable with control values by the end of the treatment period. Male food consumption however, remained adversely affected during the subsequent weeks of treatment. A similar prolonged effect on food efficiency was not evident during the same period as male bodyweight gain demonstrated a partial recovery over the corresponding weeks.

Dietary intake and food efficiency were unaffected by treatment with the test material at the remaining dose levels and were comparable with controls.

Table 5.3-18: Group mean weekly food consumption

Dietary concentration (ppm)	Mean food consumption (g/rat/week)												
	1	2	3	4	5	6	7	8	9	10	11	12	13*
	Males												
0	201	199	204	212	208	218	208	222	224	223	24	192	185
1000	200	205	213	211	205	210	211	217	204	219	214	191	180
	(0)	(3)	(4)	(0)	(-1)	(-4)	(1)	(-2)	(-9)	(-2)	(0)	(-1)	(-3)
10000	187	193	199	204	202	198	201	205	211	211	201	185	179
	(-7)	(-3)	(-2)	(-4)	(-3)	(-9)	(-3)	(-8)	(-6)	(-5)	(-6)	(-4)	(-3)
50000	122	183	178	177	183	182	168	187	189	193	188	174	171
	(-39)	(-8)	(-13)	(-17)	(-12)	(-17)	(-19)	(-16)	(-16)	(-13)	(-12)	(-9)	(-8)
	Females												
0	140	131	171	153	149	149	152	152	151	147	155	139	128
1000	143	146	152	156	158	163	157	159	152	154	161	141	137
	(2)	(+11)	(-11)	(2)	(6)	(9)	(3)	(5)	(1)	(5)	(4)	(1)	(7)
10000	123	135	142	144	143	140	143	146	143	143	142	133	131
	(-12)	(3)	(-17)	(-6)	(-4)	(-6)	(-6)	(-4)	(-5)	(-3)	(-8)	(-4)	(2)
50000	128	143	131	148	167	157	148	151	151	151	161	139	139
	(-9)	(9)	(-23)	(-3)	(23)	(5)	(-3)	(-1)	(0)	(3)	(4)	(0)	(9)

( ) - % change compared to control group * - Week 13 comprises six days only

E. WATER CONSUMPTION

There were no treatment-related effects on water consumption for either sex noted during the study.

F. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ocular effects for either sex noted were detected during the study.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

No treatment-related effects were detected in the haematological parameters measured.

Blood chemistry

Animals of both sexes treated with 50,000 or 10,000 ppm showed a statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase (AP) when compared with controls (see Table 5.3-19). A statistically significant increase in inorganic phosphorus and reduction in plasma creatinine were also evident amongst animals of both sexes treated with 50,000 ppm whilst females at this dose level showed statistically significant reductions in total plasma protein and albumin in comparison with controls.

There were no further treatment-related effects.

Table 5.3-19: Group mean blood chemical values and standard deviations (sd)

Dietary concentration (ppm)		Ca^{2+} (mmol/L)	AP (IU/L)	P (mmol/L)	Creatinine (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
		Males					
0	mean	2.74	373	2.33	0.61	–	–
	sd	0.06	101	0.22	0.03	–	–
1000	mean	2.77	404	2.22	0.62	–	–
	sd	0.07	115	0.16	0.05	–	–
10000	mean	2.66*	514*	2.32	0.59	–	–
	sd	0.09	106	0.28	0.04	–	–
50000	mean	2.61*	597***	2.46*	0.57*	–	–
	sd	0.10	150	0.22	0.04	–	–
		Females					
0	mean	2.78	230	1.70	0.69	7.63	3.90
	sd	0.11	54	0.33	0.07	0.45	0.23
1000	mean	2.76	261	1.65	0.69	7.64	3.87
	sd	0.05	71	0.21	0.04	0.29	0.13
10000	mean	2.70*	408**	1.76	0.65	7.41	3.82
	sd	0.07	123	0.23	0.04	0.45	0.20
50000	mean	2.56***	558**	2.12***	0.61**	6.86**	3.47***
	sd	0.10	90	0.15	0.05	0.82	0.39

– no significant changes
* significantly different from control group ($p < 0.05$)
** significantly different from control group ($p < 0.01$)
*** - significantly different from control group ($p < 0.001$)

H. URINALYSIS

Animals of both sexes treated with 50,000 ppm showed an increased level of haemoglobin in the urine when compared with controls (see Table 5.3-20). Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated at 50,000 ppm. This probably represents external contamination, possibly of faecal origin.

There were no treatment-related changes detected at the remaining dose levels.

Table 5.3-20: Urinanalytical findings

Dietary concentration (ppm)	Blood (haemoglobin)							
	Males				Females			
	-	+	++	+++	-	+	++	+++
0	8	0	1	1	10	0	0	—
1000	10	0	0	0	10	0	0	—
10000	7	2	1	0	10	0	0	—
50000	1	5	2	2	4	3	3	—

– - negative
+ - ca. 5-10 × 10^6 ery/L
++ - ca. 50 × 10^6 ery/L
+++ - ca. 250 × 10^6 ery/L

I. NECROPSY

Organ weights

Animals of both sexes treated with 50,000 ppm showed statistically significant increases in both relative liver and kidney weight when compared with controls (see Table 5.3-21).
There were no further direct effects of treatment.

Table 5.3-21: Group mean organ weights and standard variations (sd)

Dietary concentration (ppm)		Relative organ weight (%)			
		Liver		Kidney	
		♂	♀	♂	♀
0	mean	2.9749	2.9734	0.5864	0.6516
	sd	0.2629	0.1558	0.0575	0.0523
1000	mean	2.8868	2.9023	0.5901	0.6257
	sd	0.2552	0.2146	0.0804	0.0375
10000	mean	2.8853	2.9801	0.6070	0.6454
	sd	0.3758	0.1556	0.0552	0.0532
50000	mean	3.2433*	3.1989*	0.6963***	0.7180*
	sd	0.2452	0.2098	0.0436	0.0707

* - significantly different from control group ($p < 0.05$)
*** - significantly different from control group ($p < 0.001$)

Necropsy

Macroscopic abnormalities were detected in the 50,000 ppm dose group with all animals showing enlarged and fluid-filled caecums whilst one female treated with 50000 ppm showed gaseous distension of the stomach at terminal kill.
There were no treatment-related macroscopic abnormalities detected at 10,000 or 1,000 ppm.

Histopathology

Treatment-related changes were observed in the caecum. Atrophy, characterised by flattening of the intestinal mucosa, was observed for five rats of both sexes dosed at 50,000 ppm ($p < 0.05$ for male rats) and for one male and two female rats receiving 10,000 ppm of the test material. The aetiology of this change is uncertain and may represent no more than a stretch atrophy of the mucosa resulting from caecal distension.
There were no further treatment-related changes.

III. CONCLUSION

Dietary administration of the test material, technical Glyphosate, to rats for a period of ninety consecutive days at dietary concentrations of up to 50,000 ppm resulted in treatment-related changes at 50,000 and 10,000 ppm. No such effects were demonstrated in the 1,000 ppm treatment group and the "No Observed Effect Level" was, therefore, considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/04	[redacted]	1995	HR-001 : 13-week Subchronic Oral Toxicity Study in Rats. [redacted] Laboratory Report No.: [redacted]94-0138 Data owner: Arysta LifeScience Date: 1995-07-20 GLP: yes not published

Guideline: Japan MAFF Guidelines 59 NohSan No.4200; 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)

Deviations: None

Dates of experimental work: 1994-12-05 to 1995-07-20

Executive Summary

A subchronic oral toxicity study of HR-001 was conducted in Sprague-Dawley (Crj:CD) rats of both sexes. The test substance was administered to the rats (12 animals/group/sex) by incorporating it into the basal diet at dose levels of 0, 3000, 10000, and 30000 ppm for a period of 13 weeks (91 days).

- 30,000 ppm goup: Body weights of males and females were slightly lower than in the control throughout the treatment period and statistically significant decreases were sporadically observed. The averaged food efficiency in males and females during the treatment period was slightly lower than that in the control. Females showed a significant increase in alkaline phosphatise (ALP) activity. Distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. Both sexes showed significant increases in absolute and relative weighs of the caecum (containing contents). Histologically, there were no abnormalities related to treatment in any tissues including the caecum.
- 10,000 ppm group: At necropsy, 3 males showed distention of the caecum. Organ weight analysis revealed a statistically significant increase (females) or an increasing trend (males) in obth absolute and relative weights of the caecum.
- 3,000 ppm group: There were no abnormalities attributable to the treatment in either sex.

Based on these results, the no-adverse effect level (NOAEL), minimum toxic level, and sure toxic level of HR-001 in Sprague-Dawley (Crj:CD) rats under the conditions of the present study were determined as follows.

	Males	Females
No-adverse-effect level (NOAEL)	3,000 ppm (168.4 mg/kg/day)	3,000 ppm (195.2 mg/kg/day)
Minimum toxic level	10,000 ppm (569 mg/kg/day)	10,000 ppm (637 mg/kg/day)
Sure toxic level	30,000 ppm (1735 mg/kg/day)	30,000 ppm (1892 mg/kg/day)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification:	HR-001		
Description:	White crystal		
Lot/Batch #:	940908-1	941209	T-941209
Purity:	95.68%	95.0%	97.56%
Stability of test compound:	12/12/1994	19/12/1994	26/12/1994

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crj:CD
Source:	[redacted] Japan[redacted]
Age:	5 weeks
Sex:	Male and female
Weight at dosing:	♂ 136-150 g; ♀ 109-121 g
Acclimation period:	1 week
Diet/Food:	[redacted]
Water:	Filtered and sterilized tap-water, *ad libitum*
Housing:	3/cage, sexes separately in stainless steel cages 31.0 x 44.0 x 20.3 cm
Environmental conditions:	Temperature: 24 ± 2°C
	Humidity: 55 ± 15%
	Air changes: 15/hour
	12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1994-12-06 to 1995-03-22

Animal assignment and treatment:
The test substance was incorporated into the basal effect diet and administered on a continuous basis in the basal diet to groups of 24 Sprague-Dawley rats (12 males + 12 females) for a period of 13 weeks. Dietary concentrations were 0, 3000, 10000 and 30000 ppm.

Table 5.3-22: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	12	12
Low	3000	12	12
Mid	10000	12	12
High	30000	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about50 g each) of each does level takent from top, middles and bottom portions of the mixer at

the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weight were recorded for all animals before necropsy.

Food consumption and utilisation
Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumptionby the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.
Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.
Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination
Ophthalmological examinations including observation with a haloen ophtalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.
The following parameters were determined: eyeball, cornea, anterior chamber, pupil, iris, lens/vitreous body, fundus.

Haematology and clinical chemistry
After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC) and differential leukocyte count.

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K) and chloride (Cl).

Urinalysis
At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresg urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was detmerined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology
Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.
The following parameters were determined: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, buccal mucosa of oral cavity, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics
All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths were noted in the control and treated groups of either sex.

B. CLINICAL OBSERVATIONS
There were no abnormalities related to the treatment in clinical signs in the treated groups of either sex. In the 30 000 ppm group, one female showed a poor general condition including emaciation and decreased spontaneous motor activity. The poor general condition seemed to be caused by elongated incisor, malocclusion, or hepatorenal genetic lesions revealed by histopathology. Thus it was not considered to be treatment related.

C. BODY WEIGHT
In the 30 000 ppm group, body weights of males and females were slightly lower (about 5-10% decrease in males and 5% in females) than those in the control throughout the treatment period. Statistically significant decreases in their body weights were sporadically observed during the treatment period (weeks 3, 4 and 11 in males and weeks 10 and 11 in females) when compared to the control.

In the 10 000 and 3 000 ppm groups, body weight changes in males and females were comparable to the control throughout the treatment period.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
In the 30 000 ppm group, males and females showed significant decreases in food consumption at week 1 which were 9 and 14% lower than that of the control, respectively. However, their food consumption were comparable to the control at week 2 and thereafter.
In the 10 000 no significant change was observed while in the 3000 ppm group, significant changes were sporadically observed during the treatment period in females during the weeks 6 and 7. The food consumption recovered from the week 8 up to the end of the study.
The overall food consumption by males and females was comparable to the control and there were no abnormalities considered treatment related.

The overall group mean chemical intakes averaged, calculated from food consumption and nominal concentrations of the test substance, through the treatment period, were:

Dose (ppm)	Chemical Intake (mg/kg/day)	
	Male	Female
3 000	168.4	195.2
10 000	569	637
30 000	1735	1892

E. OPHTHALMOSCOPIC EXAMINATION

In the ophtalmological examination performed on all animals before the start of the treatment and on the animals of the control and 30 000 ppm groups at 13 weeks of treatment, no abnormalities were observed in either sex.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Tere were no abnormalities in any group of either sex.

Blood clinical chemistry

In the 30 000 ppm group, females showed a significant increase in alkaline phosphatase (ALP) activity and a significant decrease in albumin (Alb). There were no abnormalities in males.
In the 10 000 and 3 000 ppm groups, there were no abnormalities in either sex.

G. URINALYSIS

In the 30 000 ppm group, urine pH in males and females was significantly lower than that in the control. Urine protein showed a significant decrease in males and a decreasing trend in females. In addition, females showed a significantly higher urine volume than that of the control, but males showed a decreasing trend in urine volume as compared with the control.
In the 10 000 ppm group, urine pH and protein in males were lower than those in the control. In females, no statistically significant change was observed in any parameter.
In the 3 000 ppm group, no statistically significant changes was observed in either sex.

H. NECROPSY

Organ weights

In the 30 000 ppm group, both sexes showed significant increases in absolute and relative weights of the cecum (containing contents). In addition, females in this group also showed significant increases in relative weights of the brain and liver.
In the 10 000 ppm group, the absolute and relative weight of the cecum showed a statistically significant increase in males and increasing trend in females.
In the 3 000 ppm group, there were no abnormalities attributable to the treatment in either sex.

Gross pathology

In the 30 000 ppm group, distention of the cecum was observed in 9/12 males and 7/12 females with statistical significance. There were no other macroscopic abnormalities attributable to the treatment.
In the 10 000 ppm group, 3/12 males showed distention of the cecum, but there were no macroscopic abnormalities in females.
In the 3 000 ppm group, there were no macroscopic abnormalities attributable to the treatment in either sex.

Histopathology

Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male in the 10 000 ppm group and one female in the 30 000 ppm group showed renal lesion (polycystic kidney) and hepatic lesions (bile ductal

proliferation and cholangiectasis). It is generally regarded that these lesions were caused by genetic disorder and were not considered to be treatment-related.

III. CONCLUSION

Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/05	[redacted]	1995	HR-001: 13-week Subchronic Oral Toxicity Study in Mice. [redacted] Laboratory Report No. [redacted]94-0136 Data owner: Arysta LifeScience Date: 1995-07-24 GLP: yes not published

Guideline: Japan MAFF Guidelines 59 NohSan No.4200, 1985;
U.S. EPA FIFRA Guidelines Subdivision F, 1984
OECD 2408 (1981)

Deviations: None

Dates of experimental work: 1994-12-15 to 1995-07-24

Executive Summary

In order to evaluate the subchronic toxicity of HR-001 in mice, the test substance was administered by incorporating itinto a basal diet to each dose group of 12 males and 12 females of SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm for a period of 13 weeks.

- 50,000 ppm goup: Males showed a depressed body weight gain associated with lowered food consumption and food efficiency throughout the treatment period. Decreased food efficiency was also observed in females. In haematological examinations, females showed decreases in hematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC). Blood chemical exmaniations revealed increases of alkaline phosphatase (ALP) in males and females and inorganic phosphorous (P) in females. At necropsy, males and females revealed increased incidences of distention of the caecum. In organ weight analysis, males and females showed increases of absolute and relative weights of the caecum. Histopathologically, males showed an increase in incidence of cystitis of the urinary bladder.

- 10,000 ppm group: Distention of the caecum was observed in one female at necropsy. In organ weight analysis, increasing tendencies were noted in absolute and relative weights of the cecum.

- 5,000 ppm group: There were no treatment-related changes in either sex in any parameters.

Based on these results, the no-adverse effect level (NOAEL), minimum toxic level, and sure toxic level of HR-001 in ICR (Crj:CD-1) mice under the conditions of the present study were determined as follows.

	Males	Females
No-adverse-effect level (NOAEL)	5,000 ppm (600.2 mg/kg/day)	5,000 ppm (756.0 mg/kg/day)
Minimum toxic level	10,000 ppm (1221 mg/kg/day)	10,000 ppm (1486 mg/kg/day)
Sure toxic level	50,000 ppm (6295 mg/kg/day)	50,000 ppm (7435 mg/kg/day)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification: HR-001
Description: White crystal
Lot/Batch #: T-941209
Purity: 97.56%
Stability of test compound: 26/12/1994

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species: Mouse
Strain: Crj:CD-1
Source: [redacted] Japan, [redacted]
Age: 5 weeks
Sex: Male and female
Weight at dosing: ♂ 27.3-32.7 g; ♀ 22.4-25.8 g
Acclimation period: 9 days
Diet/Food: [redacted]
Water: Filtered and sterilized tap water, *ad libitum*
Housing: 3/cage, sexes separately in stainless steel cages 21.5 x 33.0 x 18.0 cm
Environmental conditions: Temperature: 24 ± 2°C
Humidity: 55 ± 15%
Air changes: 15/hour
12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-01-10 to 1995-04-27

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 24 SPF ICR mice (Crj : CD-1) (12 males + 12 females) for a minimum of 90 days. Dietary concentrations were 0, 5 000, 10 000 and 50 000 ppm.

Table 5.3-23: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	12	12
Low	5000	12	12
Mid	10000	12	12
High	50000	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about50 g each) of each does level takent from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weights were recorded for all animals before necropsy.

Food consumption and utilisation
Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumptionby the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.
Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.
Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination
Ophthalmological examinations including observation with a haloen ophtalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.
The following parameters were determined: Eyeball, cornea, anterior chamber, pupil, iris.

Haematology and clinical chemistry
After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were

withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P).

Urinalysis
At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was detmerined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology
Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.
The following parameters were determined: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics
All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no animals found dead or killed *in extremis* in any groups during the treatment period.

B. CLINICAL OBSERVATIONS
There were no treatment-related abnormalities in clinical signs in the control and treated groups during the treatment period.

C. BODY WEIGHT
In the 50 000 ppm group, mean body weights of males were lower than those of the control form week 2 to the end of the treatment period. Mean body weight of males at week 13 was 91% of that of control. Body weights of females were comparable to the control during the treatment period.
In the groups treated at 10 000 ppm or less, body weights of males and females were comparable to the controls during the treatment period.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

In males of the 50 000 ppm group, a significant depression of food consumption was recorded at week 1. Average food consumption of males during the treatment period was 94% of the control value. Food consumption of females were comparable to the control.
In the groups treated at 10 000 and 5000 ppm, food consumption of males and females was comparable to that of the controls.
The average daily chemical intakes during the treatment are shown in the following table:

Dose level (ppm)	Average chemical intake (mg/kg b.w. / day)	
	Male	Female
5 000	600.2	765.0
10 000	1221	1486
50 000	6295	7435

In the 50 000 ppm group, food efficiency of males and females was lower than that of the controls almost all measuring points during the treatment. Average food efficiency of males and females were stayed at 79% and 88% of the respective control value.
In the groups treated at 10 000 and 5 000 ppm, food efficiency in the treated groups of both sexes was comparable to that in the controls though some significant fluctuations were recorded sporadically.

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophtalmological abnormalities in the animals of both sexes in the highest dose group and the control group.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

The significant changes observed after 13 weeks in the treated groups are summarized in the table below:

Parameter	Sex	Dose group (ppm)		
		5 000	10 000	50 000
Hematocrit (Ht)	female	-	-	↘
Hemoglobin concentration (Hb)	female	-	-	↘
Erythrocyte count (RBC)	female	-	-	↘

↘: decreases of 92% to the control. P< 0.01 (estimated by Dunnett multiple comparison test).

In the 50 000 ppm group, females showed significant decreases in hematocrit (Ht), hemoglobin concentration (Hb) and erythrocyte count (RBC), while males showed no significant differences from the control in any parameters.
There were no significant differences in any parameters between the treated groups of 10 000 ppm or less and the control of either sex..

Blood clinical chemistry

The significant changes observed in the treated groups are summarized in the following table:

Parameter	**Sex**	**Dose group (ppm)**		
		5 000	**10 000**	**50 000**
Alkaline phosphatase (ALP)	Male	No change	No change	↗ 184*
	Female	No change	No change	↗ 150
Glutamic pyrvic transaminase (GPT)	Female	No change	No change	↘

Parameter	Sex	Dose group (ppm)		
		5 000	10 000	50 000
				69
Creatine phosphokinase (CPK)	Female	↗ 361	No change	↗ 943
Blood urea nitrogen (BUN)	Female	No change	↗ 119	No change
Inorganic phosphorus (P)	Female	No change	No change	↗ 128

In the 50 000 ppm group, males and females showed a significant increase in alkaline phosphatase (ALP). In females, creatine phosphokinase (CPK) and inorganic phosphorus (P) were significantly increased, while a significant decrease in glutamic pyrvic transaminase (GPT) was noted.
In the 10 000 ppm group, females exhibited a significant increase in blood urea nitrogen (BUN). There were no significant change in any parameters in males.
In the 5 000 ppm group, females showed a significant increase in CPK, while there were no significant change in any parameters in males

G. URINALYSIS

In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

H. NECROPSY

Organ weights

In the 50 000 ppm group, males and females showed significant increases in both absolute and relative weights of the cecum. The absolute weights of the cecum of males and females were 238% and 187% of that of the respective control. For relative weight, the ratio of the value to the respective control was 263% or 195% in males or females.
In the 10 000 ppm group, absolute and relative weights of the cecum showed increasing tendencies in males and females. The absolute weight of the cecum of males and females were 115% and 122% of that of the respective control. For relative weight, the ratio of the value to the respective control was 111% or 117% in males or females.
In the 5 000 ppm, there were no significant changes in any organ weights of males and females..

Gross pathology

In the 50 000 ppm group, males and females showed a significant increase in incidence of distention of the cecum (12/12 in males and 10/12 in females; 0/12 in males and females of the control group).
In the 10 000 ppm group, distention of the cecum was observed in one female. There were no significant changes in incidence of any macroscopic lesions in males.
In the 5 000 ppm group, there were no treatment-related abnormalities in males and females.

Histopathology

In the 50 000 ppm group, males showed significant increases in incidence of cystitis of the urinary bladder (4/12 ; 0/12 of the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distention of the cecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the cecum.
In the 10 000 and 5 000 ppm groups, there were no significant differences in incidence of histopathological lesions from the control in either sex.

III. CONCLUSION

Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 5000 ppm (equivalent to 600.2 and 765.0 mg/kg bw/day for males and females, respectively).

IIA 5.3.3 Oral 90-day toxicity (dog)

In oral sub-chronic toxicity studies in the dog previously evaluated in the 2001 EU glyphosate evaluation, only unspecific signs of toxicity (decrease in body weight gain and food consumption) were observed at high dose levels. In two dietary dog studies performed at the same laboratory, liver effects of equivocal toxicological significance were observed at low doses (8-29 mg/kg bw/d). However, the previous evaluation found that because these findings were not confirmed in more recent studies using much higher dose levels they were not considered to be compound-related. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/d for glyphosate acid and the IPA salt.

This NOAEL is supported by four recently conducted studies ([redacted] 2007, [redacted] 1999, [redacted] 1996 [redacted] 1996) . All of these studies are summarised below. All studies conform to current guidelines and were performed under GLP. Again the results of these studies are consistent with the studies previously submitted and reviewed, a reduction in body weight gain and food consumption was observed. In the [redacted] 2007) study at 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft feces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) and body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11. Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day. These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item. The relevance of these findings are uncertain given that in this study 1000 mg/kg bw/d clearly exceeded the maximum tolerated dose.

Overall the lowest NOAEL observed was 252.6 mg/kg bw/d. The lowest effect level was 1000 mg/kg bw/d.

Table 5.3-24: Summary of short-term toxicity studies with glyphosate acid

	Reference (data owner)	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.3.3/01 [redacted] 2007 (NUF)	13-week, oral capsule Dog, Beagle	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: Liquid/soft faeces, dehydration, thin appearance, vomiting, pallor, body weight gain ↓ ♂, body weight loss ♀, food consumption ↓, ALT ↑, AP ↓, protein ↓, albumin ↓, adipocytes in sternum ↑, Prostrate and uterine atrophy; slight↑ of absolute and relative adreanal weights of ♂ at 300 mg/kg bw/d (increase was not statistically significant)
	IIA 5.3.3/02 [redacted] 1999 (FSG)	90-day, oral diet, Dog, Beagle	0, 200, 2000, 10000 ppm (0, 5.3, 53.5, 252.6 mg/kg bw/day)	NOAEL: 10000 ppm (252.6 mg/kg bw/day)	No treatment-related effects
	IIA 5.3.3/03 [redacted] 1996 (ALS)	13-week, oral diet Dog, Beagle	0, 1600, 8000, 40000 ppm	NOAEL: 40000 ppm (1015/1014 mg/kg bw/day ♂/♀)	No treatment-related effects
	IIA 5.3.3/04 [redacted] 1996 (SYN)	13-week, oral capsule Dog, Beagle	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	Decreased body weight, clinical chemistry changes

Reference (data owner)		Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.3.2.3.2 Glyphosate Monograph [redacted] 1985 (Luxan / BCL))*	90-day, oral diet, Dog, Beagle	0, 100, 250, 500 mg/kg bw/day)	NOAEL: 250 mg/kg bw/day	500 mg/kg bw/day: reduced body weight gain, reduced food consumption
	Annex B.5.3.2.3.2 Glyphosate Monograph [redacted] 1981* (ALK/MON)	3-month, oral diet Dog, Beagle	0, 200, 600, 2000 ppm	NOAEL: 600 ppm (15/129 mg/kg bw/day ♂/♀)	2000 ppm: Liver: decreased organ weight, congestion, equivocal histological findings

* The study was considered supplementary in the 2001 EU glyphosate evaluation
↓ = decreased; ↑ = increased;

Table 5.3-25: Summary of short-term toxicity studies with glyphosate IPA salt

Reference (data owner)		Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Study from the 2001 evaluation	Annex B-5.3.2.3, Glyphosate Monograph [redacted], 1983	6-month oral capsule Dog, Beagle	0, 10, 60, 300 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	AP ↑; equivocal impact on body weight gain

↓ = decreased; ↑ = increased;

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/01	[redacted]	2007	Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs [redacted] Laboratory Study No.: 29646 [redacted] Data owner: Nufarm Date: 2007-07-15 GLP: yes not published

Guideline:	OECD 409 JMMAF 12 NohSan No. 8147
Deviations:	None
Dates of experimental work:	2005-06-08 - 2005-09-22

Executive Summary
Groups of four Beagle dogs per sex received the test item, glyphosate technical, by daily administration (capsule) at dose-levels of 0, 30, 300 or 1000 mg/kg bw/day for 11/13 weeks. The duration of the treatment period for the high-dose group was shortened to 11 weeks for ethical reasons following the observation of relevant toxic effects.
The animals were checked daily for mortality and clinical signs. Body weight was recorded weekly. Food consumption was estimated daily. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations, as well as urinalysis, were performed before the beginning of the treatment period, in Week 7 and at the end of the treatment period. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.
In the low- and mid-dose groups no treatment-related signs were noted. There were no haematological, blood biochemical, urinary or histopathological effects. Only an slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant.
At 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) or body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11.
Laboratory investigations in the surviving animals demonstrated some abnormalities (higher alanine aminotransferase activity in both sexes and lower alkaline phosphatase activity, as well as lower protein and albumin levels in females) and urinary changes (decrease in specific gravity in both sexes and increase in urinary volume and markedly less colour of urine in females).
Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day, which clearly exceeds the MTD. These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item.
Further major microscopic changes in moribund sacrificed animals were found in the kidneys (bilateral vacuolation of cortical tubules, sometimes with pigment deposits), liver (diffuse macrovesicular vacuolation, acute inflammation and/or pigment deposits), oesophagus, lung, uterus (atrophy) and/or bone marrow (increased number of adipocytes). These findings were associated with numerous changes in laboratory parameters (hemoconcentration, increased urea and creatinine levels, decreased urea, protein, albumin and bilirubin levels, decreased liver enzyme activities).

Conclusion
Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 300 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	White crystalline powder
Lot/Batch #:	H05H016A
Purity:	95.7%
Stability of test compound:	Stable under storage conditions (< 30°C), light protected; Expiry date: 2008-03-25

2. Vehicle and/ or positive control: Empty gelatine capsules, size 12 ([redacted] US)

3. Test animals:

Species:	Dogs
Strain:	Beagle
Source:	[redacted] US
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 6.5 – 8.0 kg; ♀ 6.6 – 7.7 kg
Acclimation period:	14 days
Diet/Food:	[redacted] pelleted diet ([redacted] France), 300 g per day (Following reduced food consumption among some animals standard tinned dog food was distributed instead or in addition.)
Water:	tap water, *ad libitum*
Housing:	Individual housing in pens containing wood shavings.
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 50 ± 20% Air changes: 12/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 2005-06-08 to 2005-09-22

Animal assignment and treatment:
In a 13-week oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 300 and 1000 mg/kg bw/day glyphosate technical by capsule application. The test item capsules were prepared weekly and delivered daily to the animal room, protected from light. As the test item was put into the capsules, no chemical analysis was performed during the study. The purity, characteristics and identification of the test item were indicated on the certificate of analysis that accompanied the test item.

Mortality
Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.

Body weight
The body weight of each animal was recorded twice before group allocation, on the first day of treatment, and then once a week until the end of the study. In addition, the group 4 animals were weighed before final sacrifice on day 75.

Food consumption
The quantity of food consumed was recorded for each animal. Food intake per animal and per day was calculated for 7 days before the beginning of the treatment period and then throughout the study.

Ophthalmoscopic examination
Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry
Haematological and blood chemical and urinalytical investigation were performed on all animals from each test and control group before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3).
Prior to blood sampling the animals were deprived of food for an overnight period of at least 14 hours.
The following parameters were determined: erythrocytes, haemoglobin, MVC, MCHC, MCH, thrombocytes, leucocytes, differential white cell count including morphology, reticulocytes, prothrombin time, activated partial thromboplastin time, sodium, potassium, chloride, calcium, inorganic phosphorous, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, total cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and gamma-glutamyl transferase (GGT).

Urinalysis
Urine samples were collected from all animals of the test and control groups before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3). During urine collection, the animals were deprived of food for an overnight period of at least 14 hours. The following parameters were assessed: appearance, colour, volume, pH, specific gravity, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen, and sediment.

Sacrifice and pathology
On completion of the treatment period (Week 11 or 13), after at least 14 hours fasting, all surviving animals were subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroids with parathyroid and uterus.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur), brain (at three levels), caecum, colon, duodenum, epididymides, oesophagus, eyes, gall bladder, heart, ileum (with Peyers patches), jejunum, kidneys, larynx, liver, lungs (with bronchi), lymph nodes (mandibular and mesenteric), mammary gland, muscle (skeletal), optic nerve, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands (parotid and submandibular), sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, ureters, urinary bladder, uterus (horns and cervix) and vagina.

Statistics
Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
Two unscheduled sacrifices (one male and one female) were noted in animals given 1,000 mg/kg bw/day:
One male was sacrificed on Day 61 on humane grounds. Vomiting was seen once in Week 7 (before dosing) and liquid faeces were noted on many occasions in Weeks 8 and 9. Prior to sacrifice, signs of poor

clinical condition including thin appearance, dehydration, and pallor of lip mucosa, coldness to the touch, hypothermia (34 to 35°C) and hypoactivity were observed. These signs were associated with a body weight loss between Weeks 7 and 9 (-34%) and reduced food consumption from Week 7 (generally only 25 to 50% of this animal's daily ration was consumed), followed by an absence of food intake on the day before death. Medical care (Smecta® and Lactate Ringer®) was given in order to stop the diarrhoea and rehydrate the animal.

One female was sacrificed on Day 72 for humane reasons. This animal showed liquid or soft faeces on many occasions from Week 4 and dehydration from Week 9. Vomiting was observed once in Week 10. These signs were accompanied by a body weight loss between Weeks 8 and 11 (-22%) and decreased food consumption from Week 8 (generally only 25 to 50% of this animal's daily ration was consumed), followed by an absence of food intake on the two days prior to sacrifice. Medical care (Smecta® and lactate Ringer®) was given in many occasions.

B. CLINICAL OBSERVATIONS

No treatment-related clinical signs were noted in control animals or those given 30 or 300 mg/kg bw/day.

The following treatment-related clinical signs were reported in animals given 1000 mg/kg/day (excluding those killed in extremis, which are discussed separately):

- liquid or soft faeces on several occasions in all animals,
- vomiting in 2/3 females on one occasion within 30 minutes or 3 to 5 hours after treatment,
- thin appearance in 1/3 males and all females,
- dehydration in 1/3 males and 2/3 females,
- pallor of ears and mouth in 1/3 females.

C. BODY WEIGHT

No relevant differences in the mean body weight gain were noted between controls and animals given 30 or 300 mg/kg bw/day during the treatment period.

Due to numerous individual body weight losses recorded from Week 4 in males and from Week 1 in females, a marked lower mean body weight was noted in animals given 1000 mg/kg/day at termination.

At the end of the treatment period this resulted in only a slight mean body weight gain in males (+4% vs. +31% in controls) and a mean body weight loss in females (-7% vs. +14% in controls) when compared to their body weight on Day 1. This effect on body weight was considered treatment-related (see Table 5.3-26).

Table 5.3-26: Group mean weekly bodyweights and standard deviations (sd)

Time point	Mean bodyweight and body weight change (kg)						
	Day 1	Week 5	Week 9	Week 11	Change week 1-11	Week 13	Change week 1-13
Dose (mg/kg bw/day)	**Males**						
0	7.4	9.0	9.5	9.7	+2.3	10.4	+3.0
30	7.2	8.5	8.9	9.1	+1.9	9.5	+2.3
300	7.3	8.5	9.0	9.2	+1.9	9.7	+2.4
1000	7.3	8.3	7.7*	7.6*	+0.3	na	na
	Females						
0	7.3	7.8	8.2	8.2	+1.0	8.8	+1.5
30	7.3	8.3	8.7	8.7	+1.6	9.2	+1.9
300	7.4	8.2	8.6	8.6	+1.3	9.2	+1.8
1000	7.2	7.0	6.9	6.9	-0.5	Na	na

* Statistically significant from controls ($p<0.05$).
na not applicable

D. FOOD CONSUMPTION

The food consumption was not affected by the test treatment in animals given 30 and 300 mg/kg bw/day. Reduced food consumption, varying from 25 to 75% of the amount given, was observed on many occasions in animals given 1000 mg/kg bw/day. From Day 62, when tinned dog food was distributed instead of pelleted diet, all animals consumed their full daily ration.

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

The laboratory investigations of the moribund sacrificed male showed the following changes among haematological and blood biochemical parameters when compared to pre-treatment values:

- increase in leucocyte count mainly due to an increase in the neutrophil count,
- increase in haemoglobin level, erythrocyte count and packed cell volume,
- decrease in platelet count,
- decrease in sodium and chloride levels, as well as an increase in potassium and inorganic phosphorus levels,
- increase in glucose, protein, albumin, cholesterol, triglycerides, urea and creatinine levels.

Some of the abnormalities found in the laboratory investigations (such as the increase in red blood cell parameters and in protein and albumin levels) were indicative of haemoconcentration, which was probably secondary to the dehydratation caused by the diarrhoea.

The laboratory investigations performed before sacrifice of the moribund female dog showed the following changes among the blood biochemical parameters when compared to pre-treatment values:

- decrease in sodium, potassium, chloride and inorganic phosphorus levels,
- decrease in urea, protein and albumin levels and increase in total bilirubin level and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities.

The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration).

In the following the results of the laboratory investigations of the surviving animals are summarised.

Haematology

When compared to both pre-dose and control values, no biologically relevant differences were noted in surviving animals of the test item groups in Weeks 7 and 11/13.

Blood chemistry

When compared to control values in Week 13, the following test-substance related differences were noted in animals given 1000 mg/kg bw/day in Week 11 (see Table 5.3-27):

- higher alanine aminotransferase (ALAT) activity in 2/3 males and 1/3 females,
- lower alkaline phosphatase (ALP) activity in 3/3 females,
- lower protein and albumin levels in 3/3 females.

Other changes were not attributed to the test item-treatment.

Table 5.3-27: Group mean blood chemical values and standard deviations (sd) in Week 11/13

Dose (mg/kg bw/d)		ALAT (IU/L)	ALP (IU/L)	Total protein (g/L)	Albumin (g/L)
		Males			
0 (Week 13)	mean	31	–	–	–
	sd	4.8	–	–	–
30 (Week 13)	mean	34	–	–	–
	sd	5.3	–	–	–
300 (Week 13)	mean	30	–	–	–
	sd	8.9	–	–	–
1000 (Week 11)	mean	91	–	–	–
	sd	42.5	–	–	–
		Females			
0 (Week 13)	mean	29	388	61	35
	sd	6.0	168.0	2.1	1.6
30 (Week 13)	mean	31	281	63	34
	sd	10.4	91.5	3.1	1.0
300 (Week 13)	mean	29	330	59	35
	sd	4.1	142.6	2.5	0.6
1000 (Week 11)	mean	122	321	55	30
	sd	163.9	322.0	5.5	2.5

– - no relevant changes

G. URINALYSIS

When compared to both pre-dose and control values, the following findings were noted at 1000 mg/kg bw/day in Week 11:

- decrease in mean specific gravity in 1/3 males and 3/3 females,
- increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females.

As these changes were only noted at the highest dose-level, they were attributed to the test item treatment.

H. NECROPSY

Organ weights

Treatment-related, statistically significant effects were restricted to the prostate.

Gross pathology

Macroscopic pathological examination of the male that was killed moribund demonstrated a reddish mucosa of the colon and rectum appeared, enlarged adrenal glands and thyroids, and reduced size of the spleen and thymus.

In the high-dose female that was killed moribund, the oesophagus, jejunum and ileum presented many greyish/white areas and the colon mucosa showed reddish/purplish foci. The gall bladder was dilated with blackish deposits and the liver was yellowish, enlarged and firm. The kidneys were pale.

All the macroscopic changes noted in surviving animals at termination were considered to be normal variations, when compared to background data, which may be seen in untreated beagle dogs of this age, except for changes in the uterus (reduced in size) for females given 1000 mg/kg bw/day.

Histopathology

The major histopathological findings in the male dog sacrificed moribund were bilateral hyaline degeneration of the cortical tubules in the kidneys with pigment deposits, diffuse acute inflammation in the liver with pigment deposits, acute inflammation of the lamina propria of the oesophagus, bilateral hypertrophy of cortex of the adrenals, diffuse lymphoid atrophy in the spleen, acute inflammation in the lungs with alveolar spaces containing blood and increased number of adipocytes in the sternum.

The bilateral hyaline degeneration of the cortical tubules in the kidneys was considered to be test item treatment-related. However, it is not possible to determine if this lesion, which was associated with increase in urea and creatinine levels, was directly due to the test item action or the result of the dehydration caused by a severe intestinal irritation. The inflammation noted in the liver, oesophagus and

lungs was considered to be test item related and was associated with change in leucocyte count. The increased number of adipocytes in the sternum seen also in the schedule killed animals was considered treatment-related. The abnormalities reported in blood electrolyte levels, glucose, triglycerides and cholesterol levels were not directly attributed to the test item treatment but were considered to be secondary to the poor clinical condition of the animal (diarrhoea, dehydration, changes in the kidneys). The modifications reported in spleen and adrenal glands were not attributed to the test item treatment, as they were non-specific changes that could be found in treated animals housed in laboratories.

At microscopic level, the major findings in the sacrificed female were bilateral vacuolation of the cortical tubules in the kidneys, macrovesicular vacuolation in the liver, diffuse hypoplasia of langerhans islet in the pancreas, severe atrophy of cortex of the thymus, increased number of adipocytes in the sternum and uterine atrophy.
The liver histopathological modification was considered to have resulted from the test item treatment and was correlated with changes in the blood biochemical parameters (i.e. urea, protein, albumin and bilirubin levels as well as liver enzyme activities). The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration). The uterine atrophy and increased number of adipocytes in the sternum, seen also in the schedule killed top dose animals, were considered treatment-related.
The atrophy noted in the thymus is a non specific change that could be found in laboratory housed animals; therefore a relationship to the test treatment was excluded. The other lesions noted (i.e. in the kidneys and pancreas) can be spontaneously observed in untreated beagle dogs of this age and sex. Therefore a relationship to the test treatment was considered unlikely.

No test-substance related histopathological changes were observed in animals of both sexes at and below 300 mg/kg bw/day.

Treatment-related changes observed in surviving animals given 1000 mg/kg bw/day consisted of increased number of adipocytes in the sternum of 2/3 males and 3/3 females, prostate atrophy in 2/3 males and uterine atrophy in 2/3 females.
These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item.
All the other microscopic findings observed in the organs of both male and female animals of the high-dose group were judged to be unrelated to treatment or normal background findings.

III. CONCLUSION

Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 300 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/02	[redacted]	1999	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) [redacted] Data owner: Feinchemie Study No.: 1816 AND 1817-[redacted] Date: 1999-04-17 AND 1997-02-21 GLP: yes not published

Guideline: OECD 409

Deviations: Several organ weights missing: epididymis, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled (gross, lesions. Spinal cord, eyes with optic nerve, trachea and mammary gland.

Dates of experimental work: 1998-03-18 - 1998-06-26

Executive Summary

Three treated groups of four male and four female Beagle dogs received the test item, Glyphosate Technical, at dietary dose-levels of 0, 200, 2,000, or 10,000 ppm (corresponding to 0, 5.3, 53.5 and 252.6 mg/kg bw/day) for 90 days.

The animals were checked daily for mortality and clinical signs. Veterinary examination was carried out before grouping, at start of treatment, monthly throughout the study and at termination. Body weight was recorded weekly. Food consumption was determined weekly. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations were performed before the beginning of the treatment period, after 45 days of exposure and at termination. Urine was analysed at termination. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.

No signs of toxicity or ophthalmoscopical findings were observed in any dose group. Food consumption was significantly reduced in the high dose group initially (week 2) while body weights remained unaffected.

Hematological parameters appeared in general unaffected (clotting time was increased after 45 days of exposure in both sexes, but no effects on this parameter were visible at termination; other parameters attaining statistical point significance fell within historical control). Slight increases on total bilirubin and gamma-glutamyl-transferase were observed in the high dose group. No effects on urine parameters, organ weights or organ histopathology were observed.

Conclusion

In absence of any histopathological correlate, the inconstistent effects described in hematology and clinical chemistry are considered incidental. The No Observed Adverse Effect Level (NOAEL) is considered to be 10,000 ppm, corresponding to 252.6 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	Crystalline solid
Lot/Batch #:	01.12.1997 & 01.06.97
Purity:	> 95%
Stability of test compound:	Expiry dates: 2000-06-01 and 2000-12-01

2. Vehicle and/ or positive control:

Plain diet

3. Test animals:

Species:	Dogs
Strain:	Beagle
Source:	[redacted] India [redacted]
Age:	6 - 8 months
Sex:	Male and female
Weight at dosing:	♂ 10.0 – 12.2 kg; ♀ 8.8 – 11.0 kg
Acclimation period:	6 days
Diet/Food:	[redacted] Pet meal ([redacted] India), was offered daily for one hour *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Individual housing in floor pens.
Environmental conditions:	Temperature: 23 - 29°C
	Humidity: 40 - 70%
	Air changes: no data
	Natural daylight plus fluorescent light from 9 am to 5 pm

B: STUDY DESIGN AND METHODS

In life dates: 1998-03-18 to 1998-06-26

Animal assignment and treatment:
In a 90 day feeding study groups of four Beagle dogs per sex received daily doses of 0, 200, 2000 and 10,000 ppm Glyphosate technical in the diet (corresponding to 5.3, 53.5 and 252.6 mg/kg bw/day).
Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending. This pre-mix was then added to larger amount of basal diet and blended for 20 minutes. The feed was fortified with test compound at weekly intervals.
The stability of the test compound was examined in an additional study (No. 1817-[redacted]). The homogeneity of the test material in diet was determined at start of the study. Three samples from the food fortified with the test compound were taken and analyzed.

Mortality
Each animal was checked for mortality or signs of morbidity daily during the treatment period.

Clinical observations
Each animal was daily checked for signs of toxicity. A more detailed veterinary investigation was performed before start of exposure, monthly throughout the study and before termination.

Body weight
The body weight of each animal was recorded before allocation and start of treatment, weekly throughout the study and before termination.

Food consumption
The quantity of food consumed was recorded for each animal on a weekly basis.

Ophthalmoscopic examination
Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry
Haematological and blood chemical and urinalytical investigation were performed on all animals from each test and control group before the beginning of the treatment period, after 45 days of exposure and at termination from animals fasted since the last feeding.
The following parameters were determined: erythrocytes (RBC), haemoglobin (HB), hematocrit (HCT), MVC, MCHC, MCH, leucocytes (WBC), differential white cell count (Neut, Lymp,Eosi,Mono, Retic), clotting time, glucose, urea, total protein, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine, total bilirubin, albumin, calcium, inorganic phosphorous, total cholesterol, triglycerides, chloride, sodium, potassium.

Urinalysis
Urine was collected from all animals at termination during autopsy. Urinalyis was performed for control and high-dose group animals,
The following parameters were determined: pH, specific gravity, leucocytes, proteins, glucose, ketones, blood, and urobilinogen.

Sacrifice and pathology
On completion of the treatment period, after an overnight fasting, all surviving animals were subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, kidneys, liver (with gall bladder), testes and thyroids with parathyroids.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum), brain, caecum, colon, duodenum, gall bladder, gonads, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), oesophagus, pancreas, pituitary gland, rectum, salivary glands, sciatic nerve, spleen, stomach, thymus, thyroids with parathyroids, urinary bladder, uterus.
These tissues (plus parathyroids) were microscopically investigated for all animals of the control and high dose group

Statistics
Body weights, net body weight gain, food intake, laboratory investigations (haematology and clinical chemistry values of days 0, 45 and 90), organ weights data and organ weight ratios were compared by Bartlett's test for homogeneity of intra group Variances. When the Variances proved to be heterogeneous, the data were transformed using appropriate transformation.
The data with homogeneous intra group variances were subjected to one-way analysis of variance. Following ANOVA, when F was found to be significant, Dunnett's pair wise comparison of means of treated groups with control mean was done individually. Following a significant difference of a test group with the control group, the Dose Response correlation was estimated including the control and all treated groups and tested by 't'-test. All analyses and comparisons are evaluated at 5% probability level.

II. RESULTS AND DISCUSSION

A. MORTALITY

All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed.

C. BODY WEIGHT

Bodyweights remain essentially unaffected from treatment. A slight initial depression of body weight gain might be concluded (and would be in accordance with the food consumption). Definitely no weight loss was observed.

Table 5.3-28: Group mean weekly bodyweights

	Body weight [kg]													
week	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Test item [ppm]	**males**													
0	11.1	11.7	12.2	12.3	12.5	12.7	12.9	13.2	13.3	13.4	13.5	13.6	13.7	13.8
200	11.2	11.6	12.1	12.3	12.8	12.7	13.2	13.5	13.5	13.5	13.6	13.7	13.6	13.5
2'000	11.1	11.7	12.1	12.0	12.6	12.7	12.9	13.2	13.4	13.4	13.3	13.3	13.2	13.2
10'000	11.1	11.4	11.2	11.5	12.4	12.4	12.7	12.8	13.0	13.3	13.3	13.5	13.3	13.6
Test item [ppm]	**females**													
0	10.1	10.4	10.9	10.7	11.0	11.0	11.2	11.4	11.7	11.9	11.7	12.0	11.7	11.8
200	10.2	10.7	11.1	10.9	11.3	11.4	11.5	11.7	11.8	11.5	11.6	11.6	11.6	11.4
2'000	9.8	10.2	10.3	10.2	10.8	10.9	11.0	11.2	11.4	11.3	11.4	11.5	11.6	11.5
10'000	10.0	9.9	10.1	10.1	10.6	10.6	10.7	10.9	11.2	11.3	11.3	11.3	11.3	11.2

C. FOOD CONSUMPTION

The food intake of the high dose group (10,000 ppm) was significantly lower during the second week of treatment only. Except this finding the food consumption of all the treatment groups were comparable to the control group during the study period.

Table 5.3-29: Average weekly food intake

	Food consumption [g/animal/day]												
week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Test item [ppm]	**males**												
0	254	336	342	336	320	346	345	325	312	332	356	368	369
200	287	354	366	376	326	346	350	342	325	343	318	347	312
2'000	305	373	406	347	334	363	358	366	339	321	330	329	339
10'000	262	177*	380	368	342	332	330	324	328	303	341	333	330
Test item [ppm]	**females**												
0	247	263	278	302	295	298	283	297	275	290	295	292	285
200	285	332	324	352	323	321	292	294	294	325	317	290	271
2'000	212	306	338	309	298	286	290	298	284	278	285	303	283
10'000	212	166*	348	327	303	261	288	294	298	262	268	262	256

The calculated mean daily test substance intake is summarised in Table 5.3-30 below.

Table 5.3-30: Group mean compound intake levels

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	200	5.3	5.4	5.3
3 (mid)	2000	53.2	53.8	53.5
4 (high)	10000	252.4	252.7	252.6

* based on actual food intake and body weight data

D. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the beginning and at the end of the treatment period.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

A significant increase in clotting time and GGT-activity was observed in both sexes at the 45-day interim bleed; however, in absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to rather reflect a systemic error during determination than a real effect of the test item.

Table 5.3-31: Summary of results for clotting time and GGT-actvity

	Clotting time [s]							
[ppm]	**0**	**200**	**2'000**	**10'000**	**0**	**200**	**2'000**	**10'000**
	males				**females**			
Pre-exposure bleed	145	150	147	144	154	162	149	131
45 day interim bleed	131	153*	172*	183*	141	161*	173*	182*
90 d final bleed	134	134	136	139	142	142	134	138

	GGT [U/L]							
[ppm]	**0**	**200**	**2'000**	**10'000**	**0**	**200**	**2'000**	**10'000**
	males				**females**			
Pre-exposure bleed	9	10	8	7	9	7	7	11
45 day interim bleed	13	13	16	19*	14	14	14	21*
90 d final bleed	11	12	16	18	17	16	16	29

Total bilirubin seemed affected; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.

Table 5.3-32: Summary of results for total bilirubin

	Total bilirubin [µmol/L]							
[ppm]	**0**	**200**	**2'000**	**10'000**	**0**	**200**	**2'000**	**10'000**
	males				**females**			
Pre-exposure bleed	3.71	3.99	3.71	3.14	3.97	3.51	3.96	4.02
45 day interim bleed	5.25	5.10	5.93	5.97	5.22	5.23	6.49*	6.54*
90 d final bleed	4.21	5.65*	5.95*	6.12*	4.00	6.04*	7.08*	7.18*

F. URINALYSIS

All parameters were in the normal range and comparable between control and treated animals.

G. NECROPSY

Organ weights

No treatment-related effects were observed.

Necropsy

No treatment-related effects were observed.

Histopathology

There were a few incidental findings with equal distribution across control and treated groups – no relation to treatment was observed.

III. CONCLUSION

Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) of Glyphosate Technical in Beagle dogs is considered to be 10,000 ppm (252.6 mg/kg bw/day).

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/03	[redacted]	1996	HR-001: 13-week Subchronic Oral Toxicity Study in Dogs. [redacted] Laboratory Report No.: [redacted]94-0158 Data owner: Arysta LifeScience Date: 1996-09-05 GLP: yes not published

Guideline:	Japan MAFF Guidelines 59 NohSan No.4200, 1985, U.S. EPA FIFRA Guidelines, Subdivision F, 1984 OECD 409 (1981)
Deviations:	None
Dates of experimental work:	1994-12-15 to 1995-03-24

Executive Summary

An oral subchronic toxicity study of HR-001 was conducted in beagle dogs of both sexes. Groups of 4 males and 4 females were given the test substance by incorporating it into a basal diet at a level of 0, 1600, 8000 or 40000 ppm for a period of 13 weeks. Animals were checked daily for general conditions and death, and individual food consumption was also measured daily. Body weights were recorded weekly. All animals were subjected to ophthalmology, urinalysis, haematology, and blood biochemistry periodically. At termination of treatment, the animals were euthanized and subjected to necropsy and organ weight analysis. Histopathological examination was performed on all animals.

- 40,000 ppm goup: Three of the four females showed decrease in urine pH. However, as it was known that test substance was degraded into free acid including acidified urine, the toxicological significance was not indicated in the change.
- 8,000 and 1,600 ppm groups: There were no-treatment related abnormalities in any parameters in either sex.

No significantly adverse effects were observed in beagle dogs of both sexes following the dietary treatment with HR-001 at a concentration as high as 40,000 ppm for 13 weeks. It was determined that the no-observable-effect level of HR-001 was 40,000 ppm (equivalent to 1015 and 1014 mg/kg/day for males and females, respectively).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Glyphosate technical

Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-940308
Purity:	94.61%
Stability of test compound:	Not mentioned in the report

2. **Vehicle and/ or positive control:** Plain diet / none
3. **Test animals:**

Species: Dog
Strain: Beagle
Source: [redacted] (Japan)
Age: ♂ 5 months; ♀ 6 months
Sex: Male and female
Weight at dosing: ♂ 27.3-32.7 g; ♀ 22.4-25.8 g
Acclimation period: ♂ 21 days; ♀50 days
Diet/Food: Solid diet [redacted] restricted at 250 g/dog/day
Water: Filtered and sterilized tap water, *ad libitum*
Housing: Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm
Environmental conditions: Temperature: 24 ± 2°C
Humidity: 55 ± 10%
Air changes: 15/hour
12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-09-20 to 1996-02-08

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 4 males and 4 females Beagle dogs for a minimum of 90 days. Dietary concentrations were 0, 1 600, 8 000 and 40000 ppm.

Table 5.3-33: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	4	4
Low	1600	4	4
Mid	8000	4	4
High	40000	4	4

Homogeneity of the test substance in diet was ascertained for all dose levels using the samples taken from the top, middle and bottom portions of the mixer at the first diet preparation (before initiation of the study). The coefficient of variation of the concentrations of technical glyphosate was 2.3% or less for all test diets and confirmed that the test substance was mixed in the basal diet at good homogeneity.
Concentrations of technical glyphosate in test diets were monitored for all batches of test diets of all dose levels during the study. The overall mean concentrations found in test diets were within a range of 94–101% to the nominal levels and confirmed that the test substance was mixed in the test diets at acceptable concentrations

Mortality
Mortality was expressed weekly as a ratio of the cumulative number of animals found dead or killed in extremis to the effective number of animals per dose group.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. In addition, final body weight of each animal was measured before necropsy.

Food consumption and utilisation
Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

$$Food\ consumption = [Feeding\ amount\ (250g\ diet + 250g\ water) - food\ residue] \div 2$$

Chemical intake (mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmoscopic examination
Prior to initiation of treatment and at week 13, all animals were subjected to ophthalmological examinations with a direct ophthalmoscope.
The following parameters were determined: Eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, and fundus.

Haematology and clinical chemistry
Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to haematological examinations. Blood samples were withdrawn with heparinised syringes from the cephalic vein of the animals following overnight starvation. A part of each sample was transferred to a cup of treated with EDTA and subjected to the haematological examination.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).

Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to biochemical examinations. Plasma from heparinised blood sample from haematological tests were used.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T.Bil.), calcium (Ca), inorganic phosphorus (P).

Urinalysis
Prior to initiation of treatment and at week 13 of treatment, all animals were subjected to urinalysis.
Volume and sediments were determined on urine samples collected for 24 hours using trays. The other parameters were determined on fresh urine samples.
The following parameters were determined: specific gravity, pH, protein, glucose, ketones, accult blood, urobilinogen, bilirubin, appearance urine volume, urinary sediments.

Sacrifice and pathology
All animals were subjected to a complete necropsy and all gross findings were recorded. After 13 weeks of treatment, all animals were anesthetized and euthanized by exsanguinations from the carotid artery before necropsy. At necropsy the organs and tissues except eyes were removed and preserved in neutral-buffered 10% formalin. The eyes were fixed in a phosphate-buffered mixed solution of formalin and glutaraldehyde for about 3 days and transferred to neutral-buffered 10% formalin.

Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: brain, heart, adrenals, thyroids with parathyroids, liver, ovaries, kidneys, prostate, spleen.

The following organs and tissues from all animals were histopathologically examined: brain, spinal cord, peripheral nerve, pituitary, thyroids with parathyroids, thymus, adrenals, tonsil, spleen, bone with marrow, lymph nodes, hearth, aorta, tongue, pharynx, buccal mucosa of oral cavity, salivary glands, esophagus, stomach, liver, gallblader, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, epididymides, penis, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions

Statistics
All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no animals found dead or killed *in extremis* in any groups during the treatment period.

B. CLINICAL OBSERVATIONS
Statistically significant differences in incidence of clinical signs were not observed between the control and treated goups in either sex.

C. BODY WEIGHT
Statistically significant differences in body weights were not observed between the control and treated groups in either sex throughout the treatment.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no significant changes in food consumption and chemical intake in either sex of the treated groups.
The overall group mean chemical intakes (mg/kg/day) over the whole treatment period were calculated from food consumption, body weights, and the nominal dose levels. The results are shown in the table below:

Table 5.3-34: Summary of compound intake

Dose level (ppm)	Overall group mean chemical intake (mg/kg/day)	
	Male	Female
1 600	99.7	39.8
8 000	198	201
40 000	1015	1014

E. OPHTHALMOSCOPIC EXAMINATION
No ocular changes were detected in any dose groups of both sexes.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
Statistically significant changes in haematology parameters were observed in the treated groups as shown in the following table:

Table 5.3-35: Results of haematological examinations

		Dose group (ppm)								
		1 600			8 000			40 000		
Week of treatment		0	7	13	0	7	13	0	7	13
Erythrocyte count (RBC)	Male	-	-	↗ (112)	-	-	↑ (115)	-	-	-
Mean corpuscular volume (MCV)	Male	↘ (89)	-	-	-	-	-	-	-	-
Mean corpuscular hemoglobin concentration (MCHC)	Female	-	-	-	-	-	-	↓ (96)	-	↘ (97)
Lymphocytes (Lym)	Female	↘ (148)	-	-	-	-	-	-		

Statistically evaluated by Dunnett's multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.

Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose dependency was conceived in the changes. A significant decrease in mean corpuscular hemoglobin concentration (MCHC) observed in females of the 40 000 ppm group was considered to be incidental, because the change was also noted for the pre-treatment measurement and was not accompanied with significant abnormalities of erythrocyte count (RBC), Hematocrit (Ht), and hemoglobin (Hb).

Blood clinical chemistry

Statistically significant changes in blood biochemistry parameters were observed in the treated groups and are shown in the following tables.

Table 5.3-36: Results of clinical chemistry examinations

		Dose level (ppm)								
		1600			8000			40000		
Week of treatment		0	7	13	0	7	13	0	7	13
Glucose (Gluc)	Male	-	-	-	-	-	-	↘ (93)	-	-
Chloride (Cl)	Male	-	-	↗ (102)	-	-	-	-	-	↗ (102)
Albumin (Alb)	Female		-	↗ (107)	-	-	-		-	-

Statistically evaluated by Dunnett's multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.

Although there were statistically significant differences in some parameters in the treated group of both sexes, no dose dependency was conceived in the changes. Although significant increases in chloride (Cl) were observed in males of the 1 600 and 40 000 ppm groups at week 13, the changes were considered to be incidental because of no dose dependency and their small degrees of alteration.

G. URINALYSIS

In the 40 000 ppm group, 3 of 4 females showed decrease in urine pH at week 13, although there were no statistically significant differences between the control and treated groups of both sexes in any parameters of urinalysis.

There were no significant changes in urinalysis in males and females treated at 16 000 ppm or less.

H. NECROPSY

Organ weights

There were no gross findings with statistically significant differences in incidence and relationship to the treatment in the treated groups of either sex. Although a statistically significant increase was noted for the relative weight of the adrenals in females of the 1 600 ppm group, the change was considered to be incidental due to the lack of dose-dependency.

Gross pathology

Histopathology

There were no histopathological changes related to the treatment in the treated groups of either sex. A female in the 40 000 ppm group showed cutaneous histiocytoma which is a non-specific lesion in young dogs.

III. CONCLUSION

Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 40,000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/04	[redacted]	1996	First Revision To Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] Data owner: Syngenta Report No.: [redacted]1802 Date: 1996-11-14 GLP: yes not published

Guideline: OECD 409 (1998): OPPTS 870.3150 (1998): 2001/59/EC B.27 (2001)

Deviations: None

Dates of experimental work: 1986-08-04 to 1996-11-14

Excecutive summary

In a subchronic toxicity study glyphosate acid was administered to groups of four male and four female beagle dogs at dose levels of 0 (control), 2000, 10000 or 50000 ppm glyphosate acid in the diet for a period of at least 90 days. The clinical condition and bodyweights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem*. The major organs were fixed, processed and examined microscopically.

Glyphosate acid was palatable to dogs in dietary concentrations up to and including the limit dose of 50000 ppm in the diet.

Toxic effects were confined to dogs given 50000 ppm glyphosate acid, these being small reductions of bodyweight gain. Males also had slightly reduced plasma protein and calcium concentrations.

Liver and kidney weights of males given 10000 and 50000 ppm glyphosate acid were increased. Plasma alkaline phosphatase activity of females given 50000 ppm glyphosate acid was slightly increased. These

effects were not accompanied by a histopathological lesion and are considered to be of no toxicological significance.

There were no haematological, clinical or pathological changes associated with glyphosate acid treatment. The toxicological no effect level of glyphosate acid given in the diet to dogs for 90 days was 10000 ppm, with only minimal effects at 50000 ppm.

An absolute no effect level was 2000 ppm glyphosate acid.

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no effect level for glyphosate acid from this study was 10000 ppm in the diet, equivalent to a dose of more than 300 mg glyphosate acid/kg/day.

I. MATERIALS AND METHODS

A: MATERIALS:

Test Material:	Glyphosate acid
Description:	Technical, white solid (passed through a 75 µm mesh)
Lot/Batch number:	D4490/1, P18
Purity:	99.1% w/w a.i
CAS#:	Not reported
Stability of test compound:	Not reported

Vehicle and/or positive control: Glyphosate acid was administered in diet.

Test Animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	22 - 26 weeks
Source	[redacted] UK.
Housing	Individually in indoor pens, with a floor area of 345 x 115 cm. Each pen consisted of an exercise area and separate sleeping quarters with a heated floor.
Acclimatisation period	4 - 5 weeks
Diet	Laboratory Diet A ([redacted] UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 19 - 22°C Humidity: Not reported Air changes: Approximately 12 changes / hour Photoperiod: 11 hours light / 13 hours dark

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 12 August 1986 End: 19 November 1986

Animal assignment: The study consisted of one control and three treatment groups each containing 4 male and 4 female dogs. The randomisation procedure employed ensured the even distribution of animals across replicates (randomised blocks) and treatment groups, by bodyweight, placing litter mates in different treatment groups. The sexes were randomised separately.

Male dogs received 400 g and females 350 g of the appropriate diet, in the morning between 9 am and 12 noon each day. During the pre-study period, the food was removed 2-5 hours after presentation in an attempt to ensure that the dogs ate the diet rapidly. Several batches of test diets were prepared so that no one batch was fed for longer than 5 weeks.

The clinical condition and bodyweights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem.* The major organs were fixed, processed and examined.

Diet preparation and analysis: All experimental diets were based on expanded, ground Laboratory Diet A.

The glyphosate acid concentration was determined for each occasion diet was mixed. The homogeneity of diets containing glyphosate acid was established by analysis of aliquots of diet taken from each mix of the low and high dose diet on the first occasion on which diets were prepared. The stability of the low and high dose diets was determined over a 39 day period on one mix from the first occasion on which diets were prepared.

Concentration analysis results: The achieved dietary concentrations of glyphosate acid were all within ± 9% of the target concentrations.
Homogeneity results: The homogeneity was considered to be satisfactory with all the mean values from the analysis at the different sampling points being within 6% of the overall mean.
Stability results: Over a period of 39 days, no significant change was seen in the chemical stability at 2000 and 50000 ppm glyphosate acid.

Observations: A detailed clinical examination, which included cardiac and pulmonary auscultation was made on all dogs pre-experimentally and in week 13. In the treatment period, the dogs were observed at least twice during the working day for gross clinical and behavioural abnormalities.

A daily record of faecal consistency was made during the pre-experimental and dosing periods.

Bodyweight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals until termination.

Food consumption: Food residues were recorded daily and were then discarded. These measurements were made usually 4 hours (between 2-5 hours) after presentation of the diet during the pre-experimental period and approximately 24 hours after presentation of the diet during the dosing period.

Ophthalmoscopic examination: The eyes of all dogs were examined by indirect ophthalmoscopy pre-experimentally and in week 13.

Haematology and clinical chemistry: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters measured:

Haemoglobin	mean cell haemoglobin concentration
Haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count
mean cell haemoglobin	blood cell morphology
kaolin-cephalin time	prothrombin time

Bone marrow smears were taken from a femur of all dogs at necropsy, air dried, fixed in absolute methanol and stored but not examined.

Clinical chemistry: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters assessed:.

urea
glucose
albumin
total protein
cholesterol
triglycerides
creatine kinase activity
alkaline phosphatase activity
aspartate aminotransferase activity
alanine aminotransferase activity
gamma-glutamyl transferase activity
calcium
sodium
potassium

Urinalysis: Urine was collected by catheterisation from all dogs, once pre-experimentally and in week 13. Microscopic examination of the centrifuged deposits, from all dogs, was made pre-experimentally and in week 13 on the samples taken for biochemical analysis.

urobilinogen
specific gravity
pH
bilirubin
glucose
ketones
protein
blood

Investigations *post mortem:*
Macroscopic examination: At the end of the 90 days dosing period, all animals were killed and examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands
brain
epididymides
kidneys
ovaries
liver
testes
thyroid glands (with parathyroids)

The left and right components of paired organs were weighed separately.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses
adrenal gland
aorta
brain
bone and bone marrow (rib)
caecum
colon
duodenum
gall bladder
epididymis
eyes
femur (including stifle joint)
heart
ileum
oesophagus
ovary
pancreas
pituitary gland
prostate gland
rectum
salivary gland
spinal cord (lumbar)
skin
spleen
sternum
stomach
testis
thymus

jejunum	thyroid/parathyroid gland
kidney	trachea
liver	urinary bladder
lung	uterus
lymph node - prescapular	voluntary muscle
lymph node - mesenteric	cervix
mammary gland (females only)	nerve - sciatic

Microscopic examination: All processed tissues were examined by light microscopy.

Statistics: Bodyweight gains from the start of the study to each week and final bodyweights were considered by analysis of variance, separately for males and females.

Haematology, blood and urine biochemistry data were considered, at each sampling time after the start of the study, by analysis of co-variance on pre-experimental values. Male and female data were analysed together and the results examined to determine whether differences between control and treated groups were consistent between sexes.

Organ weights at termination were considered by analysis of variance and analysis of co-variance on the last measured bodyweight, separately for males and females. Left and right components of paired organs were considered separately and combined to investigate for any differential effects.

All analyses allowed for the replicate design of the study and were carried out using ***SAS (1982)***. Unbiased estimates of the treatment group means were provided by least square means (LSMEANS option in SAS). Each treatment group was compared to the control group mean using a two-sided Student's t-test, based on the error mean square from the appropriate analysis. Where male and female data were analysed together, these comparisons were made separately.

All data were checked for atypical values and where such values were detected the analyses were repeated omitting these values to determine their influence on the conclusions.

II. RESULTS AND DISCUSSION

Mortality: There were no mortalities.

Clinical observations: The clinical observations noted were of a minor nature, often seen in studies of this duration using this strain of dog, and are considered to be unrelated to treatment with glyphosate acid.

Bodyweight and weight gain: Bodyweight gain of males given 50000 ppm glyphosate acid showed a slight depression throughout the study, but the differences were not statistically significant.

Females given 50000 ppm glyphosate acid showed slightly reduced bodyweight gains throughout the study and these were occasionally statistically significantly different from the controls.

There was no effect on growth in dogs given 2000 or 10000 ppm glyphosate acid.

Table 5.3-37: Intergroup comparison of bodyweight gain (g) (selected timepoints)

	Dietary Concentration ofglyphosate acid (ppm)							
	Males				Females			
week	0	2000	10000	50000	0	2000	10000	50000
Initial wt	10.97	10.60	11.00	10.90	9.70	9.40	9.47	9.47
4	1.00	1.13	1.07	0.65	0.64	0.75	0.85	0.38*
9	2.07	1.92	2.07	1.65	1.31	1.42	1.52	0.97*
Final wt	13.03	13.00	13.37	12.50	11.31	11.13	11.40	10.95

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)

Food consumption and utilisation: All dogs ate all the diet presented during the dosing period. The dose received (in mg glyphosate acid/kg/day) was similar for both males and females. During the study, there was the expected decrease in the dose received, due to the increasing weight of the dogs.

One dog fed 10000 ppm glyphosate acid was given cubed diet for two days in week 5 to prevent it scooping up powdered diet and thereby allowing healing to a wound in its front paw. No glyphosate acid was received by this dog on these two days.

Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:

Table 5.3-38: Mean Dose Received (mg/kg/day)

Glyphosate acid (ppm)	2000	10000	50000
Males	68	323	1680
Females	68	334	1750

Ophthalmoscopic examination: There were no treatment-related ophthalmological findings.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: Male dogs fed 50000 ppm glyphosate acid showed slightly reduced plasma albumin and total protein concentrations, possibly representing the start of an expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Plasma calcium levels were also minimally reduced in these animals, possibly a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid.

Female dogs given 50000ppm glyphosate acid had slightly elevated plasma alkaline phosphatase activities throughout the study.

There were no treatment-related changes in dogs fed 2000 or 10000 ppm glyphosate acid. There were other isolated instances where results were statistically significantly different from control, but these were considered to be unrelated to treatment.

Table 5.3-39: Intergroup comparison of clinical chemistry – selected parameters, selected weeks

		Dose Level of glyphosate acid (ppm)							
		Males				Females			
Parameter	Wk	0	2000	10000	50000	0	2000	10000	50000
Albumin	4	3.70	3.70	3.73	3.43*	3.76	3.65	3.89	3.51*
	8	3.77	3.74	3.69	3.53*	3.72	3.71	3.92	3.63
	13	3.92	3.97	3.77	3.66**	3.84	3.70	3.94	3.78
Total protein	4	5.57	5.42	5.34	5.14**	5.36	5.40	5.42	5.22
	8	5.44	5.49	5.32	5.22*	5.32	5.30	5.52*	5.19
	13	5.60	5.70	5.45	5.38	5.39	5.34	5.65*	5.30
Calcium	4	11.2	11.2	11.1	10.5**	10.9	11.1	11.2	10.7
	8	11.2	11.1	10.9*	10.8**	10.9	11.0	11.2	10.9
	13	10.7	10.5	10.8	10.0**	10.4	10.3	10.6	10.4
plasma alkaline phosphatase	4	182	190	188	193	176	181	182	220**
	8	155	168	164	177	152	155	155	181*
	13	149	165	160	161	140	143	145	166*

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided) Wk – week number

Urinalysis: There were no differences in urine clinical chemistry parameters, nor in urinary sediment examinations, which were considered to be related to treatment.

Sacrifice and pathology:
Organ weights: Kidney weights of males given 10000 or 50000 ppm glyphosate acid were slightly increased above control values, but the increase was not proportional to dose. There was also a small increase in liver weight at these dose levels, but in male dogs only.

Table 5.3-40: Intergroup comparison of liver weight (g) in male dogs (adjusted for bodyweight)

0 ppm	2000 ppm	10000 ppm	50000 ppm
385	409	427*	436**

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

Thyroid weights, adjusted for bodyweight, of females given 2000 or 10000 ppm glyphosate acid were statistically significantly reduced from control values. In the absence of any dose response relationship across all groups this is considered not to be of toxicological significance.

Macroscopic findings: No macroscopic findings were observed attributable to the administration of glyphosate acid.

Microscopic findings: There was no microscopic pathology attributable to the administration of glyphosate acid.

Incidental findings included minor granulomatous/inflammatory lesions in lung, alimentary tract and lymph node associated with ascarid migration. Imperfect spermatogenesis and minimal secretory activity of the prostate were observed in several sexually immature males. Minimal cystitis manifest as infiltration of the mucosa by inflammatory cells and small haemorrhages were found in several animals and were consistent with a subclinical bacterial infection of the lower urinary tract.

III. CONCLUSION

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no effect level for glyphosate acid from this study was 10000 ppm in the diet, equivalent to a dose of more than 300 mg glyphosate acid/kg/day.

IIA 5.3.4 Oral 1 year toxicity (dog)

Oral one year toxicity in the dog was previously evaluated in the 2001 EU glyphosate review. The [redacted] (1991) and and [redacted] (1985) studies have been previously evaluated and like the 90-day studies only non-specific signs of toxicity (slight effect body weight and an increase in clinical signs of soft, liquid stools) were observed at limit dose. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/d for glyphosate acid.

Three additional one year dog studies have been conducted that were not previously reviewed in the 2001 EU evaluation. All 3 studies are considered as confirmatory data and are summarised below. Again non-specific effects of toxicity were observed at doses at or close to limit dose. These effects were characterised as reduction in body weight gain, reduction in urinary pH and minor effects on clinical pathology parameters. An increase in pneumonia in all females dosed at 1259 mg/kg bw/day was observed in the [redacted] (1997) study. In the other female groups, this lesion was observed in only one out of four dogs each. However, the extent of this lesion was very focal and the degree of intensity was slight in all cases. Statistically, no significant differences between control and dose groups were found in the incidence of this lesion.. In this study no treatment-related effects were observed. The lowest dose level where treatment related effects were observed was 926 mg/kg bw/day in the [redacted] (1996) study. The most relevant one year oral dog NOAEL for glyphosate technical is 500 mg/kg bw/day.

Table 5.3-41: Summary of 1 year toxicity studies with glyphosate acid in dogs

Reference (data owner)		Type of study / Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.3.2.3 Glyphosate Monograph [redacted] 1985 (MON)	12 month, oral capsule Dog, Beagle	0, 20, 100, 500 mg/kg bw/day	NOEL: 500 mg/kg bw/day	No treatment-related effects
	Annex B.5.3.2.3 Glyphosate Monograph [redacted] 1991 (CHE)	52-week, oral capsule Dog, Beagle	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: soft, liquid stools (attributable to capsule administration); equivocal impact on body weight gain

	Reference (data owner)	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.3.4/01 [redacted] 2008 (NUF)	52-week, oral capsule Dog, Beagle	0, 30, 125, 500 mg/kg bw/day	NOAEL: 500 mg/kg bw/day	No treatment-related effects
	IIA 5.3.4/02 [redacted] 1997 (ALS)	12-month, oral diet Dog, Beagle	0, 1600, 8000, 50000 ppm (≅ 34.1/37.1, 182/184, 1203/1259 mg/kg bw/day (♂/♀)):	8000 ppm (182/184 mg/kg bw/day ♂/♀)	50000 ppm (≅ 1203/1259 mg/kg bw/day (♂/♀)):: loose stool, retarded body weight gain, reduced body weight at termination without stat. significance, urinary pH ↓, slight anemic changes in ♀, slight focal pneumonia / focal granulomatous pneumonia in the lung of all ♀ (extent of the lesion was very focal and slight in intensity), statistically, no significant differences, blood chemistry changes (Cl ↑, albumin ↓, P ↓♀)
	IIA 5.3.4/03 [redacted] 1996 (SYN)	1-year, oral, diet Dog,	0, 3000, 15000, 30000 ppm	NOEL: ♀: 15000 ppm (447 mg/kg bw/day) ♂: 30000 ppm (906 mg/kg bw/day)	30000 ppm (926 mg/kg bw/day: ♀ only: slight body weight reduction

↓ = decreased; ↑ = increased;

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.3.4/01	[redacted]	2008	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule)in Beagle Dogs [redacted] Data owner: Nufarm Study No.: 29647 [redacted] Date: 2007-07-23 GLP: yes not published

Guideline:	OECD 452 (1981); JMAFF 2-1-14 (2001)
Deviations:	None
Dates of experimental work:	2005-10-03 - 2006-10-17

Executive Summary
The chronic toxicity potential of glyphosate technical was assessed in a 1-year oral toxicity study in male and female beagle dogs. Groups of four dogs per sex received daily doses (capsules) of 0, 30, 125, and 500 mg/kg bw/day for 52 consecutive weeks. (dose level selection was based on the results of a 13 week study run in the same laboratory). Observations covered mortality, clinical signs, body weight, food consumption, ophthalmological examinations, haematology, clinical chemistry, urine analysis, organ weights, necropsy and histopathological examination.
No unscheduled deaths or premature sacrifices occurred during the study. There were no treatment-related effects on clinical signs, eyes, body weight, body weight gain, food consumption, haematology, clinical chemistry and urine analysis parameters in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects.
In conclusion, the NO(A)EL for oral toxicity of glyphosate technical was 500 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification:	Glyphosate tech
Description:	White crystalline powder
Lot/Batch #:	H05H016A
Purity:	95.7%
Stability of test compound:	Expiry date: 2008-03-25

2. Vehicle and/ or positive control: Gelatine capsules size 12 ([redacted] USA)

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted] USA
Age:	Approx. 6 month
Sex:	Males and females
Weight at dosing:	7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:	12 days + 20 days pre-treatment period
Diet/Food:	[redacted] pelleted diet ([redacted] France), approx. 300 g per day. Due to weight loss in three animals the amount for these dogs was increased to 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.
Water:	Tap water, *ad libitum*
Housing:	Individually in pens containing wood shavings for bedding, except when a urine sample was required. The dogs were group-housed once a week, by sex and dose group, after the last recording of clinical signs in the afternoon, until the next morning.

Environmental conditions: Temperature: 20 ± 5°C
Humidity: 50 ± 20%
Air changes: approx. 12/hour
12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 2005-09-27 to 2006-10-17

Animal assignment and treatment:
In a chronic oral toxicity study groups of four beagle dogs per sex received daily doses of 0, 30, 125 and 500 mg/kg bw/day glyphosate technical in gelatine capsules for 52 consecutive weeks. The dose levels were selected based on results of a 13-week oral (capsule) toxicity study in dogs. Dose formulations were prepared weekly by adding the required amount to the capsules. The dosages were calculated based on minimum nominal active substance content of 950 g/kg glyphosate in the test item. Analyses of the test item showed a glyphosate content consistently above 95%. Thus, no adjustment was considered necessary. Since the test item was added under GLP conditions, no additional analyses of dose formulations were deemed necessary.
Administrations of dose capsules were done approximately the same daily time each day. The low and mid-dose animals received one capsule per day, the high-dose and control dogs received three capsules per day. The quantity of dosage form applied to each animal was adjusted weekly based on the most recently recorded body weight.

Clinical observations
Observations for morbidity, and mortality were made twice daily. A check for clinical signs of toxicity was made at least once daily on all animals. In addition, a detailed clinical examination was performed once before start of treatment and weekly thereafter until termination.

Body weight
Individual body weights were recorded three times before group allocation, on Day 1 (prior to treatment) and at weekly thereafter during the conduct of study and at termination.

Food consumption and compound intake
Food consumption of each animal was estimated daily by noting the difference between the amount provided and the remaining amount on the next morning. Food consumption was expressed as percentage of quantity provided. Whenever fasting was required, food was removed at the end of the day and estimation of food consumption as made at that time.

Ophthalmological examination
Ophthalmological examinations were performed on all dogs prior to start and at the end of the treatment period. Pupillary light and blink reflexes were evaluated first. Mydriasis was then induced by adding Tropicamide solution into the eyes and the appendages, optic media and fundus were examined by indirect ophthalmoscopy.

Haematology and clinical chemistry
Blood samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. The following haematological parameters were examined: haemoglobin concentration (HB), erythrocyte count (RBC), mean cell volume (MCV), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes (PLAT), leukocytes (WBC), differential white cell count with cell morphology, neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), monocytes ♂, reticulocytes (RETIC), prothrombin time (PT), and activated partial thromboplastin time (APTT). The following clinical chemistry parameters were examined: alkaline phosphatase (ALP), alanine

aminotransferase activity (ALAT), aspartate amino transferase (ASAT), albumin, albumin/globulin ratio, total bilirubin, glucose, urea, calcium, chloride, total cholesterol, creatinine, γ-glutamyl-transferase (GGT), inorganic phosphorus, total protein, sodium, potassium, and triglycerides.

Urinanalysis
Individual urine samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. Urine was collected in the presence of thymol crystals. The following examinations were made: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen. The sediment was examined microscopically for leukocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals and cells.

Sacrifice and pathology
All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.
Tissue samples were taken from the following organs of all dogs and preserved in 10% buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson's fixative, and testes and epididymides which were preserved in Bouin´s fluid): adrenals, aorta, brain, caecum, colon, duodenum, oesophagus, eyes and optic nerve, epididymides, femur with articulation, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs with bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, skeletal muscle, ovaries, oviducts, parathyroid, pancreas, pituitary, prostate, rectum, salivary glands (parotid and submandibular), skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum with bone marrow, stomach, sciatic nerve, testes, thymus, thyroids with parathyroid, tongue, trachea, urinary bladder, ureters, and uterus (horns and cervix).
A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics
Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortalities or premature sacrifices occurred during the treatment period.

B. CLINICAL OBSERVATIONS
There were no treatment-related clinical signs observed during the study period.
Observed clinical signs consisted of vomiting or soft faeces, thin appearance, hyperactivity, ptyalism, skin findings (scabs and erythema, generally localized on the ear(s)) and nodules on the ears. These clinical observations were seen transiently, and were encountered with a similar incidence in both control and treated animals and/or were independent to the administered dose-level and/or are commonly noted when a test item is given by gavage and/or were already present before the beginning of the treatment period.

C. BODY WEIGHT
There was no treatment-related effect on body weight development. The lower mean body weight recorded in high dose males at the end of the treatment period was due to the lower mean body weight gain during the first month of the study (see Table 5.3-42). Individual body weight changes were within

the range of physiological variations. In addition, such body weight changes were observed in both control and treated dogs.

Table 5.3-42: Mean body weight and body weight changes (kg)

Dose level (mg/kg bw/day)	Males				Females			
	0	30	125	500	0	30	125	500
Mean bw prior to start (day -1)	8.2	8.3	8.3	8.3	7.4	7.4	7.6	7.4
Weeks 1 – 4	+0.6	+0.3	+0.5	+0.2*	+0.3	+0.3	+0.3	+0.3
Weeks 4 – 26	+1.4	+0.9	+1.4	+1.1	+1.2	+1.1	+1.5	+1.6
Weeks 26 – 52	+0.9	+1.4	+1.1	+0.8	+0.6	+0.2	+0.5	+1.1
Weeks 1 – 52/53	+2.8	+2.6	+2.9	+2.0	+2.1	+1.6	+2.3	+3.0
Mean bw in week 52/53	11.2	11.0	11.2	10.5	9.6	9.2	10.0	10.6

*statistically significant from control ($p < 0.05$)

The weight loss of some dogs observed in the control and low-dose group during some periods of the study were resolved when the daily food quantity was increased. Therefore, these changes were considered not test substance related.

D. FOOD CONSUMPTION

There was no treatment-related effect on food consumption noted during the study.
The reduce food consumptions noted during the study was not considered test substance related, since they occurred only on some occasions and in control and treated dogs.
Due to weight loss one male each of the low and mid dose group, and one control female received 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.

E. OPHTHALMOLOGY

There were no ophthalmological findings observed at the end of the study period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related effects noted in the haematological parameters.
The significant differences observed for the activated partial thromboplastin time (↓), MCHC (↓) and eosinophil counts (↓) in the treated animals when compared to control dogs were only slight and not dose-related.

Clinical chemistry

There were no treatment-related effects noted in the clinical chemistry parameters.
The significant differences observed for the inorganic phosphorous (↓), calcium (↓), protein (↓), glucose (↑), albumin/globulin ratio (↑) and AP values (↑) in the treated animals when compared to control dogs were only slight and not dose-related.

G. URINEANALYSIS

There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY

Organ weights

There were no statistically significant differences in organ weights and organ to body weight ratios between control and treated dogs.

The statistically significant lower brain weight (see Table 5.3-43) observed in males at 125 mg/kg bw/day was dose-independent. In addition, there were no macroscopic or histopathological findings noted in this organ. Thus, this finding is considered incidental.

Table 5.3-43: Body/Brain weights and statistics

Dose group (mg/kg bw/day)	0	30	125	500
No of animals	4	4	4	4
Mean final body weight (g)	11165.0	10830.0	11090.0	10255.0
Mean brain weight (g)	87.41	80.06	73.96**	84.09
Mean % of bodyweight	0.78978	0.74484	0.67578	0.82550

**: DUNNETT'S TEST based on pooled variances at 1% (**) level
Assigned control group(s): 1.

Gross pathology
There were no test substance related macroscopic findings observed in any animal of all dose groups.

Histopathology
There were no test substance related microscopic findings observed in any tissue sample of any dose group.

III. CONCLUSION

Based on the study results the NOEL and NOAEL in beagle dogs after 1-year oral exposure to glyphosate technical is 500 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.3.4/02	[redacted]	1997	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs. [redacted] Data owner: Arysta LifeScience Study No.: [redacted]94-0157 Date: 1997-03-20 GLP: yes not published

Guideline:	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 409 (1981)
Deviations:	None
Dates of experimental work:	1996-03-05 to 1997-04-03

Executive Summary
An oral chronic toxicity study of HR-001 was conducted in eagle dogs of both sexes. Groups of 4 males and 4 females each were given the test substance by incorporating it into basal diet at a level of 0, 2000, 10000 and 30000 ppm for a period of 12 months. Animals were checked daily for general conditions. Body weights and food consumption were measured periodically. All animals were subjected to urinalysis at weeks 25 and 51 and to hematology and blood chemistry at weeks 26 and 52. Ophthalmological examinations were performed at week 52. At termination of treatment, animals were euthanized and subjected to organ weight analysis and necropsy. Histopathological examinations were performed on representative organs/tissues from all animal used.
Findings related to the treatment were demonstrated in clinical observation, body weight, urinalysis, hematology and blood chemistry.

- 50,000 ppm group: Loose stool was observed was observed in 3 of 4 males and 4 of 4 females. These animals frequently sowed the clinical sign through the treatment period, whereas in the control group, only one animal in each sex showed the sign at a restricted period during treatment. Body weight gain was retarded gradually with progression of the treatment in both sexes, when compared to the controls. Consequently, the difference in mean body weight between the 50000 ppm and control groups became great with time, although statistical signific ance was not observed. Hematologically, slight anemic changes were noted for females at weeks 26 and 52. Females also showed a significantly increased plasma level of chloride at week 26 and significantly decreased plasma levels of albumin and inorganic phosphorous at week 52. Significantly lowered urine pH values were continuously observed in males and females. However, this finding was not recognized as a toxic change since it is known that the test substance is secreted with little metabolisation into urine, degraded to a free acid in urine, and consequently, make the urine acidic.

- 8,000 and 1,600 ppm groups: There were no treatment-related abnormalities in either sex.

Based on the results, the no-observable-effect level of HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg/day for males and females, respectively).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification: HR-001
Description: White crystals
Lot/Batch #: T-950308
Purity: 94.61%
Stability of test compound: Not mentioned in the report

2. Vehicle and/or positive control: None

3. Test animals:

Species: Dog
Strain: Beagle
Source: [redacted] (Japan)
Age: 5 months
Sex: Males and females
Weight at dosing: 7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period: 23 and 31 days for males and females, respectively
Diet/Food: Solid diet [redacted] restricted at 250 g/dog/day
Water: Tap water, *ad libitum*
Housing: Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm
Environmental conditions: Temperature: 24 ± 2°C
Humidity: 55 ± 10%
Air changes: 15/hour
12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1996-03-05 to 1997-04-03

Animal assignment and treatment:
Groups of 4 males and 4 females Beagle dogs received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 50 000 ppm for a period of 12 months.

Clinical observations
All animals were observed daily for clinical signs.

Body weight
Individual body weights were recorded at initiation of treatment, weekly from weeks 1 to 13, and every 4 weeks from weeks 16 to 52. In addition, final body weight was measured before necropsy.

Food consumption and compound intake
Food consumption of each animal was recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 52. Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

$$Food\ consumption = [Feeding\ amount\ (250g\ diet + 250g\ water) - food\ restdue] \div 2$$

Chemical intake (mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmological examination
Ophthalmological examinations were performed on all dogs prior to start of the treatment period. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, fundus.

Haematology and clinical chemistry
Blood samples were collected from all dogs prior to treatment, in weeks 25 and 52. The following haematological parameters were examined: Hematocrit, Hemoglobin concentration, Erythrocyte count, Mean corpuscular volume, Mean corpuscular hemoglobnin, Mean corpuscular hemoglobin concentration, platelet count, total leukocyte count.

All animals were subjected to blood biochemical examinations at weeks 26 and 52.
The following clinical chemistry parameters were examined alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), Potassium (K), chloride (Cl).

Urinanalysis
Prior to initiation of treatment and at weeks 25 and 51, all animals were subjected to urinalysis on the following parameters: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen.

Sacrifice and pathology
All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.

Tissue samples were taken from the following organs of all dogs and preserved in 10% buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): brain, spinal cord, peripheral nerve, pituitary, thymus, thyroids with parathyroids, adrenals, tonsil, spleen, bone with marrow, lymph nodes, heart, aorta, tongue, buccal mucosa of oral cavity, pharynx, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, penis, epididymides, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions.

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics

Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no deaths in any dose groups of either sex.

B. CLINICAL OBSERVATIONS

In the 50 000 ppm group, loose stool was observed in 3 of 4 males and 4 of 4 females. The animals in the 8 000 and 1 600 ppm groups did not show the clinical sign at all. In the control group, only one animal in each sex showed it. Most of the animals in the 50 000 ppm group frequently showed the sign throughout the treatment period, whereas the occurrence in the suffering animals of the control group was restricted to a limited period.

For other clinical signs observed, the occurrence was sporadic in all dose groups, or the incidence was almost comparable among the dose groups.

C. BODY WEIGHT

In the 50 000 ppm group of both sexes, retarded body weight gain became evident gradually as the study progressed. Consequently, the mean body weights in this group at termination of treatment were 6% in males and 11% in females lower than those in the controls. However, statistically significant differences in mean body weights were not observed throughout the treatment between the control and treated groups including the 50 000 ppm.

D. FOOD CONSUMPTION

Decreased food consumption was noted for one female. In the 1 600 ppm group at weeks 24, 28, and 52 and for another female in the same group at week 32. Consequently, group mean food consumption in this group was decreased at those weeks. However, food consumption in this group recorded at other weeks was comparable to that of the controls. Moreover, the averaged group mean food consumption through the treatment period was almost coparable between the 1 600 ppm and control groups of females.

All males in all dose groups and females except the above 2 animals in the 1 600 ppm group consumed whole amount of diet offered every day.

Group mean chemical intakes were calculated from group mean values of food consumption and body weight, and the nominal dose levels. The overall group mean chemical intakes (mg/kg/day) through the whole treatment period are presented in the table below:

Table 5.3-44: Mean test substance intake

Dose level (ppm)	Test substance intake (mg/kg bw/day)	
	Male	Female
1 600	34.1	37.1
8 000	182	184
50 000	1203	1259

E. OPHTHALMOLOGY

No remarkable ocular changes were detected in animals in any dose groups at week 52.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Statistically significant changes in haematology that were observed in treated groups are presented in the following table:

Table 5.3-45: Results of haematological examination

	Dose level (ppm)	1 600			8 000			50 000		
	Week of treatment	0	26	52	0	26	52	0	26	52
Sex	Female									
Parameter	Hematocrit (Ht)		-	-		-	-	↓	-	↓
	Hemoglobin concentration (Hb)	-		-		-	-	-	↓	↓
	Erythrocyte count (RBC)	-	-			-	-	↓	-	↓

↓: P<0.05 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.

Male groups showed no significant changes in any parameters.
Female in the 50 000 ppm group showed significantly decreased values of hematocrit (Ht), hemoglobin concentration (Hb), and erythrocyte count (RBC) at week 52. Hemoglobin concentration in this group was also significantly lower at week 26. This group had already showed lower values for these 3 parameters than the controls before initiation of treatment (at week 0). In particular, the differences from the control values in hematocrit and erythrocyte count at week 0 were statistically significant. However, the rates of deviation from the control values were, though slightly, augmented in the treatment period when compared to those at week 0.
Females in the 8 000 and 1 600 ppm groups showed no significant changes in hematological examinations

Clinical chemistry

Statistically significant changes in blood biochemistry that were observed in treated groups are presented in the table hereafter:

Table 5.3-46: Results of clinical chemistry examination

Dose level (ppm)	1 600			8 000			50 000		
Week of treatment	0	26	52	0	26	52	0	26	52
Male									
Creatine phosphokinase (CPK)	-	-	-	-	-	↓	-	-	-
Female									
Albumin (Alb)	-	-	-	-	-	-	-	-	↓
Calcium (Ca)	-	-	↓	-	-	-	-	-	↓
Inorganic phosphorus (P)	-	-	-	-	-	-	-	-	↓
Chloride (Cl)	-	-	-	-	-	-	-	↑	-

↓: P<0.05 ; ↑: P<0.01 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.

Females in the 50 000 ppm group showed a significant increase in chloride (Cl) at week 26 and significant decreases in albumin (Alb), calcium (Ca), a,d inorganic phosphorous (P) at week 52. A significant decrease in calcium was also noted for females in the 1 600 ppm group at 52 weeks.

For male groups, the 8 000 ppm group showed a significant decrease in creatine phosphokinase (CPK) at week 52. But this change was not observed in the 50 000 ppm group.

G. URINEANALYSIS

There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY

Organ weights

Males in the 1 600 ppm group showed statistically significant increases in both absolute and relative weights of the pituitary. However, these changes were not observed in the 50 000 or 8 000 ppm groups of males.

In the 50 000 or 8 000 ppm groups, neither males nor females showed statistically significant changes in any organ weights

Gross pathology

The macroscopic lesions observed in the present study were all sporadic in nature and there were no statistically significant differences in the incidence between the control and treated groups.

Histopathology

In the 50 000 ppm group, focal pneumonia / focal granulomatous pneumonia in the lung was observed in all females. In the other female groups including the control group, the lesion was observed in only one of 4 animals each. However, the extent of the lesions was very focal and the degree of intensity was slight in all cases including those of the 50 000 ppm group. Statistically, no significant differences between the control and dose groups were found in incidence of any histological lesions, including the pulmonary lesion.

III. CONCLUSION

Based on the study results the NOEL in beagle dogs after 1-year oral exposure to HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg/day for males and females, respectively).

Annex point	Author(s)	Year	Study title
IIA, 5.3.4/03	[redacted]	1996	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted] Data owner: Syngenta Report No.: [redacted]5079 Date: 1996-09-24 GLP: yes not published

Guideline:	OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30 (1988)
Deviations:	None
Dates of experimental work:	1995-02-28 to 1996-09-24

Excecutive summary

In a chronic toxicity study, groups of four male and four female beagle dogs were fed diets containing 0 (control), 3000, 15000, or 30000 ppm glyphosate acid, for a period of at least 1 year.

Clinical observations and veterinary examinations (including ophthalmoscopy) were made and bodyweights, food consumption and clinical pathology parameters were measured and at the end of the scheduled period, the animals were killed and subjected to a full examination *post mortem.* Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

Mild toxicity was evident at 30000 ppm glyphosate acid, as a slight reduction in bodyweight in females throughout the latter half of the study. This reduction was generally independent of any reduction in food consumption and does not, therefore, reflect a palatability effect. There were no other toxicologically significant effects and the pathological no-effect level was 30000 ppm glyphosate acid.

Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in bodyweight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg/day for males and 926 mg/kg/day for females.

There were no other treatment related findings and the pathological no-effect level was 30000ppm glyphosate acid.

The no-observed effect level (NOEL) for toxicity over 1 year, for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg/day). The no-observed effect level (NOEL) for toxicity over 1 year, for males is 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg/day).

I. MATERIALS AND METHODS

A: MATERIALS:

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	As given in report 95.6% a.i
CAS#:	If available
Stability of test compound:	Confirmed by the Sponsor

Vehicle and/or positive control: The test substance was administered in the diet.

Test Animals:

Species	Dog
Strain	Beagle
Age/weight at dosing	20 – 29 weeks
Source	[redacted] UK.
Housing	Housed by treatment group (sexes separately) in indoor pens. The pens had a sleeping platform with heated floor underneath and interlinking gates which enable the dogs to be separated for feeding and dosing.
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A ([redacted] UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 19 ± 2°C Humidity: 40-70% Air changes: Approximately 15 changes / hour Photoperiod: 12 hours light / 12 hours dark

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 11 April 1995 End: 12 April 1996

Animal assignment: In a chronic toxicity study, groups of four male and four female beagle dogs were fed diets containing 0 (control), 3000, 15000, or 30000 ppm glyphosate acid, for a period of at least 1 year. A randomisation procedure was used which resulted in the even distribution of dogs (16 males and 16 females) to treatment groups according to bodyweight ensuring that litter mates were in different groups. Each morning, male dogs received 400 g and female dogs received 350 g of their appropriate experimental diet.

Table 5.3-47: Study design

Test group	Dietary concentration (ppm)	Dose to animal (mg/kg) Males / females	# male	# female
Control	0		1 – 4	5 – 8
Low	3000	90.9 / 91.1	9 – 12	13 – 16
Mid	15000	440.5 / 447.8	17 – 20	21 – 24
High	30000	906.5 / 926.2	25 – 28	29 – 32

Diet preparation and analysis: The experimental diets were made in 60 kg batches, by direct addition of glyphosate acid (allowing for purity) to ground Laboratory A diet, and mixed thoroughly. Water was then added to each batch and mixed prior to pelleting. The pellets were dried in the residual heat of an autoclave, allowed to cool and were then stored in bins at room temperature.

Samples from all dietary levels (including controls) were taken at approximately two-monthly intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in Lab diet A was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet was determined over a period of up to 10 weeks (69 days) for these same diets.

Samples were extracted with water, portions of the supernatant were diluted with water to give sample solution concentrations within the range of the calibration standards. These were derivitised using 9-

fluorenylmethylchloroformate (FMOCCL) and analysed by High Performance Liquid Chromatography (HPLC).

Concentration analysis results: The mean achieved concentrations of glyphosate acid in analysed dietary preparation were typically within 12% of nominal concentration.
The overall mean concentrations were within 9% of target.
Homogeneity results: The homogeneity of glyphosate acid in diet at concentrations of 3000 ppm and 30000 ppm for a batch size of 60 kg was determined and considered satisfactory; percentage deviations from the overall mean where within 11%.
Stability results: The chemical stability of glyphosate acid in experimental diets (determined at concentrations of 3000 ppm and 30000 ppm) when stored at room temperature, was shown to be satisfactory for 69 days. This covered the period of usage on the present study.

Statistics: All data were evaluated using analysis of variance and / or covariance for each specified parameter using the GLM procedure in SAS (1989).

Observations: All dogs were observed at least three times daily for clinical behavioural abnormalities (at dosing, after dosing and at the end of the working day) and, on a weekly basis, they were given a thorough examination. Individual, daily assessments of gastrointestinal findings were made for up to 5 hours post dosing: any subsequent assessments were made on a group basis. All dogs were also given a full clinical examination by a veterinarian pre-study, during weeks 13, 26, 39 and prior to termination. The examination included cardiac and pulmonary auscultation.

Bodyweight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals until termination.

Food consumption and test substance intake: Food residues were recorded daily, approximately 4 hours after feeding and any residual food was discarded. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.

Ophthalmoscopic examination: The eyes of all dogs were examined pre-study, during weeks 13, 26, 39 and prior to termination.

Haematology and clinical chemistry: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing EDTA or trisodium citrate and the following parameters measured.

haemoglobin	mean cell haemoglobin concentration
haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count
mean cell haemoglobin	red cell distribution width
prothrombin time	activated partial thromboplastin time
blood cell morphology	bone marrow smears (taken but not examined)

Clinical chemistry: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing lithium heparin and the following parameters measured.

urea	alkaline phosphatase activity
creatinine	aspartate aminotransferase activity
glucose	alanine aminotransferase activity
albumin	gamma-glutamyl transferase activity
total protein	calcium
cholesterol	phosphorus (as phosphate)

triglycerides	sodium
total bilirubin	potassium
creatine kinase activity	chloride

Urinalysis: Urine was collected by catheterisation, pre-experimentally, in week 26 and during the week prior to termination. The following parameters were measured and recorded on each urine sample:

volume	glucose
colour (if abnormal)	ketones
specific gravity	protein
pH	bilirubin
	blood

In addition, each urine sample was centrifuged and the sediment stained and examined microscopically to identify the components.

Investigations *post mortem:*
Macroscopic examination: All animals were were killed by exsanguination under terminal anaesthesia induced by intravenous administration of sodium pentobarbitone and examined *post mortem.*

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	kidney
brain	liver
epididymides	testes
thyroid glands	

The left and right components of paired organs were weighed separately.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses	oesophagus
adrenal gland	ovary
aorta	pancreas
brain (cerebrum, cerebellum and brainstem)	parathyroid gland
bone marrow (sternum)	pituitary gland
caecum	prostate gland
colon	rectum
duodenum	salivary gland
epididymis	spinal cord (cervical, thoracic, lumbar)
eyes (retina, optic nerve)	skin
femur (including stifle joint, stored not examined)	spleen
Gall bladder	sternum
heart	stomach
ileum	testis
jejunum	thymus
kidney	thyroid gland
larynx	trachea

liver
lung
lymph node - prescapular
lymph node - mesenteric
mammary gland (females only)
urinary bladder
uterus (with cervix)
voluntary muscle

Microscopic examination: All processed tissues were examined by light microscopy.

II. RESULTS AND DISCUSSION

Mortality: None of the dogs died.

Clinical observations: There were no toxicologically significant findings. Salivation at dosing was observed in individual dogs in all treatment groups throughout the study. The apparent increased incidence in two top dose males and one female, was considered to be related to anticipation of feeding and not to treatment with glyphosate acid. There was also a low incidence of scrotal skin reddening seen in one male in each treatment group; this was considered to be incidental to treatment with glyphosate acid.

There was no increased incidence of faecal abnormalities in dogs treated with glyphosate acid.

Bodyweight and weight gain: There was a slight bodyweight effect evident in females fed 30000 ppm glyphosate acid with a maximum reduction of 11% (compared to controls) in week 51. These dogs showed a gradual reduction in growth rate, compared to the controls, which was consistently statistically significant from week 23 onwards. One female lost 0.6 kg during week 32 but this was related to a loss of appetite during this time. There were no effects in males at any dose level or in females at 15000 ppm but females fed 3000 ppm glyphosate acid also showed slightly poorer growth than the controls, with a maximum reduction of 8% in week 51. However, this effect only achieved statistical significance on occasions during the study and is considered attributable to the poorer growth of two females and not an effect of glyphosate acid, since there was no effect at 15000 ppm.

Table 5.3-48: Intergroup comparison of bodyweights (selected timepoints; adjusted mean values shown for weeks 2-14)

	Dietary Concentration of Glyphosate acid (ppm)							
	Males				Females			
week	0	3000	15000	30000	0	3000	15000	30000
1	11.40	11.53	11.33	11.45	9.60	9.55	9.48	9.58
8	12.66	12.40	12.48	12.37	10.74	10.40*	10.68	10.42*
16	13.35	12.97	13.38	12.95	11.46	11.03*	11.50	10.99*
32	14.19	13.69	13.93	13.69	12.28	11.63*	12.59	11.46**
53	14.57	14.24	14.24	13.85	13.10	12.25	12.94	11.76**

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, $p<0.01$ (Student's t-test, 2-sided)

Food consumption and compound intake: There was no effect on food consumption but 3 dogs left food on occasions which affected the group mean values:

Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:

Table 5.3-49: Mean Dose Received (mg/kg/day)

Glyphosate acid (ppm)	3000	15000	30000
Males	90.9	440.3	906.5
Females	91.1	447.8	926.2

Ophthalmoscopic examination: There was a very low incidence of corneal or lenticular opacities but these were seen in control animals as well as those fed glyphosate acid. There were no treatment related abnormalities.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: There were no toxicologically significant findings.

Plasma cholesterol levels were increased slightly in the treated groups of both sexes at weeks 26 and 52 but there was no evidence of any dose relationship.

Plasma phosphorus levels were lower in the male treated groups at week 52 but this was due, in part, to slightly higher individual control values. Similarly the reduced sodium value in males fed 30000 ppm at week 52 was due solely to one male.

Various animals in all groups (including controls) showed evidence of higher plasma alanine aminotransferase, aspartate aminotransferase and creatine kinase activities throughout the study as well as pre-experimentally, but there was little evidence of any conclusive group effects.

Other statistically significant differences were minor and/or not dose related and were considered to be of no toxicological significance.

Urinalysis: There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.

Sacrifice and pathology:
Organ weights: There were no treatment related effects on any organ weights. Adrenal weights were slightly raised in the male 3000 ppm group but this was exaggerated by a low value for one male in the control group.

Macroscopic findings: Several treated females showed red areas in or diffuse reddening of the urinary bladder mucosa. The incidence was not clearly related to dose and in the absence of a similar effect in males it was considered unlikely that the lesion is related to the administration of glyphosate acid.

Microscopic findings: It was considered unlikely that any of the lesions confined to the treated groups were related to the administration of glyphosate acid as they were either of low incidence or the incidence was not related to dose. The pathological no-effect level for glyphosate acid was 30000 ppm.

III. CONCLUSION

Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in bodyweight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg/day for males and 926 mg/kg/day for females.

There were no other treatment related findings and the pathological no-effect level was 30000 ppm glyphosate acid.

The no-observed effect level for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg/day). The no-observed effect level for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg/day).

IIA 5.3.5 28-day inhalation toxicity (rodents)

One study on sub-acute inhalative toxicity (14 days) in rodents ([redacted] 1985: non-GLP (pre-GLP) study) was reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOEL was 3.8 mg/L. Since glyphosate is non-volatile (VP = 1.31 x 10^{-5} Pa (25°C)) the conduct of 28-day inhalation studies are not needed according to EU requirements. Therefore, no further inhalation studies were conducted.

Table 5.3-50: Summary of short-term toxicity studies with glyphosate acid rats

Reference (data owner)		Type of study Species	Dose levels [mg/L]	NOEL / NOAEL [mg/L]	Targets / Main effects
Study from the 2001 evaluation	Annex B.5.3.3.3.2 Glyphosate Monograph [redacted] 1985 (EXC)*	14-day, inhalation (6h/day) Rat, Wistar	0, 0.28, 0.93, 3.8	NOEL: 3.8	No treatment-related effects

* Study was considered supplementary data in the 2001 EU glyphosate evaluation

IIA 5.3.6 90-day inhalation toxicity (rodents)

90-day inhalation studies are only required for volatile substances (i.e. substances with an vapour pressure (VP) > 10^{-2} Pa). Since glyphoate is non-volatile (VP = 1.31 x 10^{-5} Pa (25°C)), no 90-day inhalation toxicity study was conducted.

IIA 5.3.7 Percutaneous 28-day toxicity (rodents)

The short-term percutaneous toxicity of glyphosate has been investigated in the rat and rabbit. In both Sprague-Dawley (SD) ([redacted] 1993) and Wistar derived ([redacted] 1996) rats, no signs of systemic toxicity were noted following dosing for 21 days at 1000 mg/kg bw/day, the limit dose for this study type. Three studies were conducted in New Zealand White rabbits ([redacted]) and doses ranged from 1000 mg/kg bw/day to 5000 mg/kg bw/day. No signs of treatment related systemic toxicity were noted in any study, the highest NOAEL being 5000 mg/kg bw/day.

An *in vitro* dermal absorption study in the rabbit ([redacted] 2012, see IIA 5.3.7/02) was conducted to estimate how much of a dose of a 5000 mg/kg dermal dose would have been systemically available. A total of 2.66% of the applied dose was concluded to be systemically available and this percentage was used to calculate a systemic equivalent dose for the NOAEL established. An applied dose of 5000 mg/kg bw/day was concluded to equate to a systemic dose of 133 mg/kg bw/day. This systemic dose in rabbits is higher than that used to calculate the AOEL from orally dosed rabbits. This implies that mortality observed in orally dosed rabbits was not due to systemic toxicity, but rather a consequence of the route of administration.

In both the rat and the rabbit no signs of systemic toxicity were evident following repeated application of glyphosate to the skin. The NOAEL for short term percutaneous toxicity was 1000 mg/kg bw/day in the rat and 5000 mg/kg bw/day in the rabbit as previously concluded in the 2001 EU glyphosate evaluation.

Table 5.3-51: Summary of percutaneous 28-day toxicity studies with glyphosate acid

Reference (data owner)		Type of study Species	Dose levels (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Targets / Main effects
Study from the 2001 evaluation	Annex B.5.3.3.1 Glyphosate Monograph [redacted] 1993 (CHE)	21-day, dermal Rat, Sprague-Dawley	0, 1000	1000	Weak dermal irritation at 1000 mg/kg bw/day; no systemic effects
Study not reviewed in the 2001 evaluation	IIA 5.3.7/04 [redacted] 1996 (SYN)	21-day, dermal Rat, Alpk: AP_fSD	0, 250, 500, 1000	1000	No treatment-related effects
Study from the 2001 evaluation	Annex B.5.3.3.1 Glyphosate Monograph [redacted] 1985 (EXC)	21-day, dermal Rabbit, NZW	0, 500, 1000, 2000	2000	No treatment-related effects
	Annex B.5.3.3.1 Glyphosate Monograph IIA, 5.3.7/01 [redacted] 1982 (MON)	21-day, dermal Rabbit, NZW	0, 100, 1000, 5000	5000	Dermal irritation at 5000 mg/kg bw/day; no systemic effects
Study not reviewed in the 2001 evaluation	IIA, 5.3.7/02 [redacted] (2012a)	In vitro rabbit dermal absorption	Equivalent of 5000	133 systemic NOAEL in rabbits	2.42% absorbed 0.243% remaining in dermis 2.66% systemic = 133 mg/kg/day
Study from the 2001 evaluation	Annex B.5.3.3.1 Glyphosate Monograph IIA, 5.3.7/03 [redacted] 1994a (ALK)	28-day, dermal Rabbit, NZW	0, 500, 1000, 2000	2000	No effects

Tier II summaries are only presented for representative studies.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/01	[redacted]	1982	21-Day dermal toxicity study in rabbits [redacted] Data owner: Monsanto [redacted] Report No. [redacted]-81-195 Date: 1982-03-10 GLP: no (pre-GLP study) unpublished

Guideline: Non-stated
Study is in general accordance with OECD guideline 410 (1981)

Deviations: The application area in the high-dose group was about 1.5 – 2 times higher than the recommended 10 % of the body surface area.

Dates of experimental work: Not reported

Executive Summary

The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits on intact and on abraded skin. Doses of 0, 100, 1000 or 5000 mg/kg bw/day were applied five days per week for three consecutive weeks. For application the solid test substance was moistened with an appropriate amount of water and spread evenly over the application site. It has to be noted that the surface areas covered (i.e. 1 - 2 %, 5 – 10 % and 15 – 20% for the low, mid- and high-dose group, respectively) were below and above the area of 10 % recommended by actual guidelines. Due to the higher exposed surface area in the high dose group, it has to be considered that more test substance can be absorbed through the skin and could be therefore systemically available.

There were no mortalities and no treatment-related signs of systemic toxicity. There were also no signs of dermal irritation observed in the control, low- and mid-dose group. At 5000 mg/kg bw/day slight dermal irritation consisting of barely perceptible to slight erythema and oedema was noted. However, this effect is considered not to be of biological significance and no signs of irritation were seen in the histopathological examination.

There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Conclusion

Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment group. There were no treatment-related systemic signs of toxicity. Thus, the "No Observed Adverse Effect Level" is considered to be 5000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	NBP 1992026
Purity:	98.4%

Stability of test compound: No data given in the report.

2. **Vehicle and/ or positive control:** None

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand
Source:	[redacted] USA
Age:	Young, adult
Sex:	male and female
Weight at dosing:	♂ 2359 - 2883 g; ♀ 2344 - 2955 g
Acclimation period:	14-16 days
Diet/Food:	[redacted] Certified Rabbit Chow [redacted] ([redacted] USA), *ad libitum*
Water:	tap water, *ad libitum*
Housing:	Individually in wire mesh cages.
Environmental conditions:	Temperature: Exact range not reported Humidity: Exact range not reported Air changes: Exact value not reported 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1981-07-28 to 1981-08-19

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young adult New Zealand albino rabbits (males and females). Ten rabbits per sex per dose received daily dermal applications of 0, 100, 1000 or 5000 mg/kg bw. The dose groups were further divided in halves. One half received treatment on intact skin, the other half on abraded skin. Abrasion was done twice per week immediately prior to test substance application by producing shallow incisions (not deep enough to cause bleeding) with the blunt end of a scalpel blade.
The day prior to the first application about 30 % of the skin of the back of the animals was clipped free of hair. During the study rabbits were shaved as needed.
For each application the test substance was moistened with an appropriate amount of physiological saline. Each dose was spread evenly over the maximum body surface area possible (see Table 5.3-52), covered with a semi-occlusive dressing. After an exposure period of six hours the dressings were removed and the application site was cleaned with tepid tap water and dried with paper towels. Applications were performed once daily , five days per week for three consecutive weeks. Individual doses were adjusted weekly based on the body weight determined at the beginning of each study week.

Table 5.3-52: Application details for repeated dermal applications

Dose group (mg/kg bw)	Number of animals				Volume of physiological saline used for moistening	Total percent of the body surface covered by test substance
	Intact skin		Abraded skin			
	♂	♀	♂	♀	(mL)	(%)
0	5	5	5	5	--	--
100	5	5	5	5	0.2	1 – 2
1000	5	5	5	5	1.5 – 2.0	5 – 10
5000	5	5	5	5	8.0 – 9.0	15 – 20

It has to be noted that the application area according to current guidelines (OECD and EC) should be about 10% of the body surface. Thus, the body surface covered with test material in the 100 mg/kg bw/day dose group is lower than recommended, whereas the treatment-area in the high dose group is about 1.5 – 2 times higher than recommended. Due to the higher exposed surface area in the high-dose group a higher amount of test substance can be absorbed and would be therefore potentially be systemically available.

Clinical observations
A check for mortality was made twice daily. Observations for clinical signs of toxicity and behavioural changes were made once daily on all animals. The applications sites were assessed for signs of irritation.

Body weight
Individual body weights were recorded at weekly intervals during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed daily for each individual animal by visual inspection.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on 5 rabbits per sex and dose group with intact and abraded skin on Day 21 after an overnight fast.
The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, reticulocyte count, platelet count, total leukocyte count, differential leukocyte count, MCV, MCH, MCHC, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin (calculated), total bilirubin, creatinine, lactate dehydrogenase, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, gonads, heart, kidneys, liver, pituitary and thyroid (with parathyroid).
Tissue samples were taken from the following organs and preserved in buffered formalin: treated and untreated skin (3 samples/each), adrenals, bone & bone marrow (sternum), brain (at three levels), colon, duodenum, eyes with Harderian gland, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs with main stem bronchi, mammary gland, lymph nodes (mediastinal, mesenteric and regional when applicable), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, salivary glands, sciatic nerve, seminal vesicles, spinal cord (cervical), spinal cord and vertebrae (lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and vagina.
Histopathological examinations were performed on the following tissues: treated and untreated skin, liver, kidney, gonads and any gross lesions.

Statistics
Terminal body weights, haematological and clinical chemistry parameters, absolute and relative organ weights were analysed by one-way analysis of variance, Bartlett´s test for homogeneity of variance and appropriate t-test.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
A number of incidental findings were observed in some animals in all dose groups. The most frequent signs were soft stool, diarrhoea, mucoid diarrhoea, and ocular and nasal discharge.

No signs of dermal irritation were observed in the control, low- and mid-dose group. In the high-dose group at 5000 mg/kg bw/day doubtful or barely perceptible to very slight erythema and doubtful or barely perceptible oedema were noted. There were no differences between the animals with intact and abraded skin.

C. BODY WEIGHT
There were no statistical significant differences observed in body weights or body weight gains between the control and treated groups (with abraded and intact skin).

D. FOOD CONSUMPTION
There were no major differences in food consumption between the control and the treated groups.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
No treatment-related effects were detected in the haematological parameters measured.
There were some statistical significance differences in some parameters. However, these were incidental and considered to be biologically insignificant (see Table 5.3.53)

Blood chemistry
There were no treatment-related effects. The incidental significant changes observed were considered not to be biologically significant (see Table 5.3.53)

Table 5.3-53: Group mean haematological and blood chemical values and standard deviations (sd)

Dose level (mg/kg bw/day)		Hb (g/dL)	Haematocrit (%)	Sodium (meq/L)	Glucose (mg/dL)	LDH (IU/L)
		Males				
0	mean	11.3	34.1	143	121	–
	sd	0.68	1.78	1.3	21.6	–
100	mean	12.5*	37.3*	144	134	–
	sd	0.63	1.92	2.1	14.1	–
1000	mean	11.4	34.2	145	149	–
	sd	0.46	1.41	5.1	34.7	–
5000	mean	11.7	35.5	146*	125	–
	sd	0.61	1.13	2.5	6.1	–
		Females				
0	mean	–	–	–	102	189
	sd	–	–	–	16.2	125.9
100	mean	–	–	–	137**	144
	sd	–	–	–	18.7	109.1
1000	mean	–	–	–	125	258
	sd	–	–	–	17.1	204.4
5000	mean	–	–	–	129*	28*
	sd	–	–	–	4.7	6.2

LDH lactate dehydrogenase
– no significant changes
* significantly different from control group ($p < 0.05$)
** significantly different from control group ($p < 0.01$)

I. NECROPSY

Organ weights

Except for the statistically increased relative kidney weight observed in females at 5000 mg/kg bw/day (see Table 5.3-54), there were no treatment-related effects on absolute and relative organ weights noted. Since no histopathological changes were observed in the kidneys of high-dose females, the increase in relative kidney weights is considered to be of no toxicological relevance.

Table 5.3-54: Group mean absolute and relative kidney weights and standard variations (sd)

Dose level (mg/kg bw/day)		Absolute organ weight (g) Kidney		Relative organ weight (%) Kidney	
		♂	♀	♂	♀
0	mean	17.00	16.41	0.58	0.55
	sd	2.381	2.037	0.078	0.040
100	mean	16.38	15.26	0.57	0.54
	sd	1.371	2.260	0.067	0.069
1000	mean	18.15	16.16	0.59	0.54
	sd	2.633	2.449	0.067	0.048
5000	mean	16.77	18.14	0.60	0.63*
	sd	2.016	1.757	0.097	0.072

* - significantly different from control group ($p < 0.05$)

Gross pathology

There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology

There were no treatment-related lesions observed in any dose group.

Microscopic evaluation of treated skin samples demonstrated only mild inflammatory cell infiltration and trace necrosis in the 1000 mg/kg bw/day group. However, in untreated skin samples of three rabbits from the 1000 mg/kg bw/day group from one rabbit of the high-dose group there was also mild necrosis, indicating that this lesion was incidental and unrelated to treatment. The lesions in treated and untreated

skins of the control and test substance groups were similar indicating that the effects were not related to glyphosate treatment.
Trace/mild seminiferous tubule degeneration observed in the testis were not dose related in either incidence or severity and was considered unrelated to treatment. Other lesions observed in kidney, liver, lung, ovary, lymph node and skin (non-application site) were considered incidental or spontaneous (see Table 5.3-55 and Table 5.3-56). In general there were no major differences between the treatment groups of intact and abraded skin.

Table 5.3-55: Histopathological findings in rabbits treated dermally on intact skin*

Dose level	0 (mg/kg bw/day)		100 (mg/kg bw/day)		1000 (mg/kg bw/day)		5000 (mg/kg bw/day)	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Cytoplasmic vacuolation (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Interstitial lymphocytic infiltrates (mild)	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Interstitial inflammation (mild)	0/5	1/5	1/5	0/5	0/5	0/5	2/5	0/5
Infarct (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Mineralisation (trace)	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	1/5	0/5	1/5	1/5
Liver								
Granulome (moderate)	0/5	1/5	0/5	1/5	1/5	0/5	0/5	0/5
Mononuclear cell infiltration (trace)	1/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5
Mononuclear cell infiltration (mild)	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5
Mononuclear cell infiltration (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Necrosis (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Lung								
Abscess (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Lymphocytic infiltration (mild)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Pneumonia (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Pneumonia (moderate)	1/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Congestion (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Congestion (moderate)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Oedema (moderate)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis								
Seminiferus tubule degeneration (trace)	1/5	--	3/5	--	2/5	--	3/5	--
Seminiferus tubule degeneration (mild)	2/5	--	2/5	--	0/5	--	1/5	--
Dilated tubules (moderate)	0/5	--	1/5	--	0/5	--	0/5	--
Ovaries								
Mineralisation (trace)	--	1/5	--	0/5	--	0/5	--	0/5
Salivary gland								
Abscess (moderate)	--	1/5	--	0/5	--	0/5	--	0/5
Skin (non-application site)								
Dermatitis (moderate)	0/5	--	0/5	--	1/5	--	0/5	--
Skin, treated								
Inflammation (trace)	1/5	3/5	2/5	3/5	2/5	5/5	3/5	2/5
Inflammation (mild)	2/5	1/5	2/5	1/5	1/5	0/5	0/5	3/5
Necrosis (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Skin, untreated								
Inflammation (trace)	4/5	1/5	4/5	2/5	3/5	2/5	3/5	2/5
Inflammation (mild)	1/5	3/5	0/5	3/5	1/5	2/5	0/5	3/5
Necrosis (mild)	0/5	0/5	0/5	0/5	3/5	0/5	1/5	0/5

* Number of animals affected / total number of animals; -- not applicable

Table 5.3-56: Histopathological findings in rabbits treated dermally on abraded skin*

Dose level	0 (mg/kg bw/day)		100 (mg/kg bw/day)		1000 (mg/kg bw/day)		5000 (mg/kg bw/day)	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Interstitial inflammation (trace)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (mild)	1/5	0/5	1/5	1/5	1/5	0/5	0/5	1/5
Interstitial inflammation (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5
Infarct (moderate)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	2/5	1/5	0/5	0/5
Liver								
Mononuclear cell infiltration (trace)	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Mononuclear cell infiltration (mild)	1/5	0/5	1/5	2/5	4/5	1/5	1/5	1/5
Necrosis (mild)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Lung								
Pneumonia (moderate)	0/5	--	1/5	--	1/5	--	0/5	--
Lymph node, prefemoral								
Lymphadenitis (mild)	--	0/5	--	0/5	--	0/5	--	1/5
Testis								
Seminiferus tubule degeneration (trace)	2/5	--	1/5	--	2/5	--	2/5	--
Seminiferus tubule degeneration (mild)	2/5	--	3/5	--	2/5	--	2/5	--
Ovaries								
Mineralisation (trace)	--	0/5	--	0/5	--	1/5	--	0/5
Mineralisation (mild)	--	0/5	--	0/5	--	1/5	--	0/5
Atretic follicles (mild)	--	0/5	--	1/5	--	0/5	--	0/5
Skin, treated								
Inflammation (trace)	2/5	2/5	3/5	2/5	4/5	2/5	2/5	1/5
Inflammation (mild)	0/5	3/5	2/5	3/5	0/5	3/5	3/5	4/5
Skin, untreated								
Inflammation (trace)	3/5	3/5	2/5	4/5	2/5	2/5	1/5	3/5
Inflammation (mild)	1/5	1/5	3/5	1/5	3/5	2/5	4/5	2/5

* Number of animals affected / total number of animals

III. CONCLUSION

Repeated dermal application of glyphosate technical to rabbits for a period of 21 days at dose levels of up to 5000 mg/kg bw/day resulted in no systemic treatment-related changes. Only a slight degree of dermal irritation was noted at 5000 mg/kg bw/day. The "No Observed Adverse Effect Level" was, therefore, considered to be 5000 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/02	[redacted]	2012a	Glyphosate acid - In Vitro Absorption through Abraded Rabbit Skin using [^{14}C]-glyphosate [redacted] Study No.: [redacted]2182, Report No.: [redacted]2182-REG Date: 2012-04-x18 GLP: yes Unpublished
Guideline:			OECD 428

Deviations:	None
Dates of experimental work:	2011-12-12 to 2011-12-22

Executive Summary
The purpose of this study was to determine the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin following a 6-hour exposure period and subsequent 18 hour monitoring period. This study was designed to assess the potential dermal penetration of test material through rabbit skin and will be of use in estimating the systemic dose achieved in a previous *in vivo* rabbit dermal toxicity study (see IIA 5.3.7/01, [redacted] 1982). Therefore, the application rate and exposure conditions used in this study were calculated to be equivalent to 5000 mg/kg bw/day as applied to rabbits in the *in vivo* dermal study (IIA 5.3.7/01).

^{14}C-glyphosate was incorporated into a wet cake preparation prior to application. The preparations were applied as a paste to abraded rabbit skin membranes at a rate of 79.8 mg/cm^2 (corresponding to 48.3 mg glyphosate acid/cm^2) and left unoccluded for an exposure period of 6 hours, after which the skin surface was washed. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout a total time-period of 24 hours. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

Conclusion
The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount absorbed after 24 hours was 2.42 %. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 %.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials:

a) Non radio-labelled test substance:

Identification:	MON 77973 (glyphosate acid)
Description:	White wet cake
Lot/Batch #:	GLP-1103-21149-T
Chemical purity:	85.14 % as glyphosate acid (puritiy: 95.93 %)
Stability of test compound:	Expiry date: 2012-03-09

b) Analytical reference standard:

Identification:	Glyphosate acid
Lot/Batch #:	GLP-0810-1915-A
Chemical purity:	Not reported

c) Radio-labelled test substance

Identification:	^{14}C-glyphosate (as glyphosate acid) [phosphonomethylene-^{14}C]-Glyphosate
Lot/Batch #:	4675JJN002-1
Radiochemical purity:	96.7 % (confirmed by analysis)
Specific activity:	48 mCi/mmol; 1776 MBq/mmol; 2523µCi/mL; 9.35 MBq/mL

2. Test skin source:

Species: Rabbit
Strain: New Zealand White Albino
Source: [redacted]
Age: At least 12 weeks
Type: Complete pelt

B: STUDY DESIGN AND METHODS

Preparation of skin samples:
Skin pelts from New Zealand White albino rabbits at least 12 weeks old were obtained from [redacted]. The skin samples were transported on cold blocks and were stored on arrival at -20°C, the day after sacrifice. The skin samples arrived clipped and excised and were examined for scars and blemishes. Any extraneous subcutaneous tissue was removed after defrosting and the pelts clipped further if necessary. The pelts were given an identifying number and individually stored frozen, at approximately -20°C, on aluminium foil until required for use.

Test substance preparation
The doses were prepared, to mimic as closely as possible a 500 mg/kg dose from a previous rabbit *in vivo* study (IIA 5.3.7/01, [redacted] 1982). The dose equivalency was calculated on a dose per unit area of skin basis using an average *in vivo* rabbit weight of 2.78 kg. The doses were prepared as close to the time of application as was practicable.

Radioactive stock solution of ^{14}C-glyphosate
The radiolabelled ^{14}C-glyphosate was supplied as a solution in water.

Trial preparation of the radiolabelled glyphosate acid
Glyphosate acid trial preparation was prepared using the method described below, with the exception that different volumes or smaller amounts of radioactivity or unlabelled material were used, where applicable. Three individual vials were prepared as part of the trial preparation, to assess dosing methodology. The paste like composition of the dose preparation was investigated to ensure that it visually provided good skin contact during application to the membranes.

Preparation of radiolabelled glyphosate acid
Firstly 8008 mg of non-labelled glyphosate wet cake was added to a vial, followed by 4162 µL of radiolabelled glyphosate stock solution, providing a nominal 3.85 mg of glyphosate (40 MBq) radioactivity. 5 mL of water was then added and the preparation mixed thoroughly. The preparation was then freeze dried to remove the water added and the water present in the wet cake. When dry, the glyphosate wet cake preparation was then weighed to confirm the removal of all the water. Approximately 521 mg of the dried wet cake preparation was then added to 8 individual vials together with approximately 300 µL of saline to each vial to create a paste. A final weight of each vial was recorded and the preparation was thoroughly mixed with a spatula into a paste before dosing.

Preparation of non-labelled glyphosate acid
To demonstrate that the dose preparations have a close contact during the application procedure, an additional dose preparation without radiolabel was prepared according to the procedure described above.

Analyses of dose preparations
The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled stock solution of the test substance was determined by thin layer chromatography (TLC) using unlabelled test substance as reference standard.

The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by TLC analyses.

Preparation of diffusion cells

The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm^2 of skin. The cells had a receptor volume of approximately 4.5 mL.

An integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. Non-abraded membranes with a resistance of 1.5 - 5 kΩ were considered having a normal integrity and used for the skin abrasion. Rabbit skin was abraded using a blunt spatula drawn over the skin area approximately six to eight times, in the form of a grid, in order to mimic 'Draize' abrasion as conducted in the in vivo study (IIA 5.3.7/01, [redacted] 1982). After the abrasion a further integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. For abraded skin samples membranes with ER values in the range of 0.7 – 1.0 kΩ were selected for the study.

Cells were selected such that the application rate was represented by eight intact skin samples from five different animals. Physiological saline was chosen as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.

Test substance application and sampling

Prior to dosing a pre-treatment sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.

Each dose formulation was applied to the abraded skin membrane as a dried glyphosate acid wet cake paste and spread over the skin surface using a spatula. The weight of each individual preparation and spatula were recorded before and after dosing to allow the applied dose to be calculated.

Each dose was applied at the nominal rate of 79.8 mL/cm^2 exposed skin area (202.8 mg/cell), corresponding to 48.3 mg glyphoate/cm^2. The applications were left un-occluded for 24 hours.

Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.

After the 6-hour sampling, the skin samples were washed by gently swabbing the application site application site with at least three natural sponges pre-wetted with 3% Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. Two further sponges, pre-wetted with water, were used to further swab the surface.

Terminal procedures

After the last sampling, 24 hours after application the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.

The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3%Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.

The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3% Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.

Due to the fragility of the abraded skin samples tape stripping could not be performed. Instead a heat separation technique was used to separate the epidermis from the dermis.

The skin was carefully removed from the receptor chamber and the flange area cut away and digested in Soluene 350® and aliquots taken for analysis by LSC.

The remaining skin disc was placed dermis side down, on cling film. A second piece of cling film was then used to cover the epidermis side. A 200g weight was placed in a water bath at 65°C for an hour prior to use. The weight was placed onto the epidermal surface with moderate pressure for approximately 90 seconds. The epidermis was peeled away from the dermis using forceps. The dermis was digested in Soluene 350® and aliquots taken for analysis by LSC. The epidermis was digested in Soluene 350® and the whole sample analysed by LSC.

Analysis of samples

The radiochemical purity and stability of the ^{14}C-glyphosate preparations was determined by TLC using silica gel plates and methanol : water : acetic acid (6 : 3 : 0.5, v/v/v). Radioactivity on the TLC plates were measured using a Packard Instant Imager (SOP E003). Unlabelled material was visualised under UV light at 254 nm.

For visualising the test material on the TLC plates a 2 % ninhydrine solution in acetone was used.

In addition, for analyses of dose preparations K2 cellulose plates and a revised solvent system (methanol : water : acetic acid (8 : 1.5 : 0.5, v/v/v) was used.

Liquid samples of the receptor fluid, washing solutions, digested wash sponges and digested dermis and epidermis were measured by LSC using a Packard 3140 TR LSC counter and Goldstar as scintillation fluid.

Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm². The amounts absorbed, rates of absorption (µg/cm²/h) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations.

The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose

The absorbed dose is considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose is regarded as the sum of absorbed dose and the amount recovered from the dermis. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure is considered unabsorbed.

II. RESULTS AND DISCUSSION

A. ANALYSES OF THE ^{14}C-GLYPHOSATE STOCKSOLUTION

TLC analysis of the ^{14}C-glyphosate stock solution confirmed a radiochemical purity of greater than 95 %.

LSC analysis revealed a radioactivity content of 72.1MBq, equivalent to a concentration of 0.924 mg/mL. The stock solution was homogeneous with a 1.31 % deviation between the replicates.

B. ANALYSES OF DOSE PREPARATIONS

LSC analyses confirmed the mean application rate to be 48.3 mg glyphosate/cm².

The dose preparations had low variability between the replicates analysed (1.66%-6.26%) and, considering the physical nature of the preparation, the dose preparations were considered to have acceptable homogeneity.

C. MEMBRANE INTEGRITY CHECK

Based on the ER measurements eight cells with abraded skin samples were selected for the absorption study.

D. DERMAL ABSORPTION OF GLYPHOSATE

Absorption profiles were assessed from eight abraded skin samples. Since one skin sample showed an atypical absorption profile, this was excluded from the calculation of means and SD.

The determined distribution of radioactivity are summarised in Table 5.3-57 below.

Table 5.3-57: Summary of results for dermal absorption of ^{14}C-glyphosate

Dose preparation				
Applied dose "wet cake" [mg/cm^2]	79.8			
Applied dose glyphosate [mg/cm^2]	48.3			
Number of cells assessed	7			
	Distribution of radioactivity (mean values)			
	µg/cm^2	SD	% of applied dose	SD
Surface compartment				
Dermis (after heat separation)	118	19.4	0.243	0.040
Skin wash at 6 hours	42802	3008	87.9	6.30
Skin wash at 24 hours	1159	1224	2.38	2.51
Donor chamber	59.2	56.9	0.121	0.117
Receptor compartment				
Receptor fluid (0-24 h)	1177	244	2.42	0.503
Total absorbed*	**1177**	**-**	**2.42**	**--**
Epidermis (after heat separation)	20.1	9.97	0.041	0.020
Flange area	132	68.6	0.270	0.141
Total potentially absorbable**	**1295**	**-**	**2.663**	**--**
Total recovery	**45468**	**2096**	**93.3**	**4.46**
Absorption rates [µg/cm^2/h] (0-24h)	53.1	10.2	-	--

SD Standard deviation
* Amount in receptor fluid.
** Total potentially absorbable = total absorbed + remaining dermis (after heat separation)

The total recovery of the individual cells was in the range of 87.3 % to 98.2 %, with an overall mean recovery of 93.3 % of applied dose.

The majority of the applied glyphosate acid (mean 87.9%) was washed off the skin at 6 hours, with a further 2.38% washed off at 24 hours. A small proportion (0.041%) of the dose applied was recovered from the epidermis, with 0.243% remaining in the dermis.

The mean rate of absorption of glyphosate acid between 0-1 hours was 47.0 µg/cm^2/h, which increased to 166 µg/cm^2/h between 1-4 hours. The mean absorption rate subsequently slowed to 72.3 µg/cm²/h between 4-10 hours and declined further to 13.3 µg/cm²/h for the remainder of the absorption period (10-24 hours). The overall absorption rate (0-24 hours) was 53.1 µg/cm^2/h.

The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was 1177 µg/cm^2, corresponding to 2.42% of the applied dose.

Considering that the amount found in the remaining dermis after 24 h is potentially available and could further penetrate through the skin, the total amount of glyphosate potentially available was 2.66 % of the applied dose

III. CONCLUSION

The results of this *in vitro* dermal absorption study indicate that the absorption of glyphosate through abraded rabbit skin was generally slow. The vast majority of glyphosate was removed from the skin by the washing procedures after 6 hours exposure.
The total absorbed amount after 24-hour was 2.42 %. The corresponding total potentially absorbable amount, represented by the mean absorbed dose together with the amount in the remaining dermis after 24 hours was 2.66 %.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/03	[redacted]	1994a	Glyphosate technical (Alkaloida, Tiszavasvári): Repeated dose twenty-eight-Day dermal toxicity study in rabbits [redacted] Data Owner: Alkaloida Report No.: [redacted]214/94 (Test Code: GLY-94-410/N Date: 1994-08-30 GLP: yes unpublished

Guideline: Non-stated
Study is in general accordance with OECD guideline 410 (1981)

Deviations: None

Dates of experimental work: 1994-05-23 to 1994-06-20

Executive Summary
The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits. Doses of 0, 500, 1000 or 2000 mg/kg bw/day were applied five days per week for 28 consecutive days. For application the solid test substance was mixed with water resulting in a 50 % (w/v) solution, and spread evenly over the application site.
There were no mortalities and no treatment-related signs of systemic toxicity. Very slight erythema was noted in one high-dose male and one low-dose female. However this effect is not considered biological significant and were not seen in the histopathological examination.
There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.
Conclusion
Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one high-dose male and one low-dose female. There were no treatment-related systemic signs of toxicity. Thus, the "No Observed Adverse Effect Level" is considered to be 2000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	39730494
Purity:	99.6%
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control: water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand
Source:	[redacted] Hungary
Age:	Young, adult
Sex:	male and female
Weight at dosing:	♂ 2200 - 2800 g; ♀ 2100 - 2500 g
Acclimation period:	7 days
Diet/Food:	[redacted] Rabbit Chow, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in wire mesh cages.
Environmental conditions:	Temperature: 18 ± 2 °C Humidity: Exact range not reported Air changes: 10/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1994-05-23 to 1994-06-20

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young adult New Zealand albino rabbits (males and females). Five rabbits per sex per dose received daily dermal applications of 0, 500, 1000 or 2000 mg/kg bw, five days per week for a total of 15 applications.
Two days prior to the first application about 15 % of the skin of the dorsal back of the animals was clipped free of hair. The clipping was repeated weekly thereafter.
For each application the test substance was mixed with water to give a final concentration of 50 % (w/v) of glyphosate. Each dose was spread evenly over about 10 % of the body surface area and covered with a semi-occlusive dressing. After an exposure period of six hours the dressings were removed and the application site was cleaned with hand soap, water and clean, absorbent paper pads. Applications were performed once daily, five days per week for a total of 28 days..

Clinical observations
A check for mortality, clinical signs of toxicity, and general appearance and behaviour, as well as a quantitative assessment of food and water intake was made twice daily. The applications sites were assessed for signs of irritation once daily.

Body weight
Individual body weights were recorded at weekly intervals during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed at weekly intervals during the pre-test and study periods and before sacrifice.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on all rabbits at termination.
The following parameters were measured: haematocrit, haemoglobin, erythrocyte count, platelet count, total leukocyte count, differential leukocyte count, MCV, MCH, MCHC, coefficient of variation of erythrocyte volume (RDW), platetelet volume distribution (PDW), mean platelet volume (MPV), thrombocrit (volume % of platelets), aspartate amino transferase (AST), alanine aminotransferase (ALT),

blood urea nitrogen, total protein, glucose, albumin, total bilirubin, creatinine, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, heart, kidneys, liver, lung, spleen, stomach, thymus, and testes. The organ-to-brain weight ratios were calculated.
Tissue samples were taken from the following organs and preserved in buffered formalin: treated and untreated skin, adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, stomach gl., testes, and thyroid. Histopathological examinations were performed on all collected tissues from the control and high-dose animals, as well as from abnormal tissues of animals from the low- and mid-dose groups.

Statistics
Body weights, haematological and clinical chemistry parameters, absolute and relative organ weights and histopathology data of treated animals were compared with control animals. Body weight, food consumption and haematology and clinical chemistry parameters were analysed by t-test. Histopathology data were analysed by Fisher´s exact test.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
There were no signs of systemic toxicity noted in any animal of any dose group.

Signs of dermal irritation consistent of very slight erythema was observed in one high-dose male and one low dose female. The erythema lasted from day 7 to 20 for the male, and for 5 days for the female.

C. BODY WEIGHT
There were no statistical significant differences observed in body weights or body weight gains between the control and treated groups.

D. FOOD CONSUMPTION
There were statistically significant differences in food consumption between the control and the treated groups. Observed differences were unrelated to treatment.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
No treatment-related effects were detected in the haematological parameters measured.
In females of the mid-and high-dose group MPV and PDW values were significantly lower when compared to controls. However, the values were within the historical control range for female NZW rabbits of this age. Thus, these changes were not considered treatment-related (see Table 5.3-58).

Blood chemistry
There were no treatment-related effects. The incidental significant changes observed urea level in high dose males were within the historical control range of the testing facility (see Table 5.3-58)

Table 5.3-58: Group mean haematological and blood chemical values and standard deviations (sd)

Dose level (mg/kg bw/day)		MPV (fl)	PDW (fl)	Urea (mmol/L)
		Males		
0	mean	7.48	5.80	7.80
	sd	0.28	1.10	0.66
500	mean	7.94	6.80	8.72
	sd	0.62	1.57	1.68
1000	mean	7.64	6.20	7.64
	sd	0.42	1.04	0.50
2000	mean	7.48	5.60	9.82**
	sd	0.04	0.42	1.02
		Females		
0	mean	8.96	9.70	10.24
	sd	0.85	1.86	1.09
100	mean	8.15	7.50	9.70
	sd	0.99	2.71	0.53
1000	mean	7.62*	6.20*	10.58
	sd	0.13	0.57	0.39
2000	mean	7.46*	5.10***	9.96
	sd	0.27	0.65	1.31

MPV mean platelet volume; PDW platelet volume distribution (coefficient of variance of platelets volume)
– no significant changes
* significantly different from control group ($p < 0.05$)
** significantly different from control group ($p < 0.01$)
*** significantly different from control group ($p < 0.001$)

I. NECROPSY

Organ weights

There were no treatment-related effects on mean absolute and relative organ weights noted.

Gross pathology

There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology

There were no treatment-related lesions observed in any dose group.

III. CONCLUSION

Repeated dermal application of glyphosate technical to rabbits for a period of 28 days at dose levels of up to 2000 mg/kg bw/day resulted in no systemic treatment-related changes. Only a slight degree of dermal irritation was noted at one animal each of the low- and high-dose group. The "No Observed Adverse Effect Level" was, therefore, considered to be 2000 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/04	[redacted]	1996	Glyphosate Acid: 21 Day Dermal Toxicity Study In Rats [redacted] Data owner: Syngenta Report No.: [redacted]4985 Date: 1996-06-24 GLP: yes not published

Guideline: OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)

Deviations: None

Dates of experimental work: 1996-01-09 to 1996-06-24

Excecutive summary

In a subchronic toxicity study groups of five male and five female Alpk:APfSD (Wistar-derived) rats received 6 hour dermal applications of 0 (control), 250, 500 or 1000 mg glyphosate acid/kg/day. Glyphosate acid was prepared as a paste using deionised water as the control substance and vehicle. A total of 15 applications were made over a 21 day period (5 applications per week).

Clinical observations were made and bodyweights and food consumption were measured, and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no clinical signs of systemic toxicity at any dose level and no adverse compound related effects on bodyweight, food consumption, haematology, clinical chemistry or organ weights. There was no evidence of toxicity at histopathological examination.

There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg/day.

The no observed adverse effect level (NOAEL) for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg/day in both sexes.

I. MATERIALS AND METHODS

A: MATERIALS:

Test Material: Glyphosate acid

Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6% w/w a.i
CAS#:	Not reported
Stability of test compound:	Not reported

Vehicle and/or positive control: Deionised water

Test Animals:

Species	Rat
Strain	Alpk:APfSD
Age/weight at dosing	6 - 8.5 weeks / males 214-249 g, females 193-227 g
Source	[redacted] UK
Housing	Individually, in cages on multiple rat racks suitable for animals of this strain and weight range expected during the course of the study.
Acclimatisation period	At least 5 days
Diet	Diet ([redacted]) supplied by [redacted] UK *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 21 ± 2°C Humidity: 40-70% Air changes: At least 15 changes/hour Photoperiod: 12 hours light/12 hours dark

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 10 January 1996 End: 1 February 1996

Animal assignment: The study was divided into ten (randomised blocks), each containing one cage per treatment group. The animals were randomly allocated to groups as shown below:

Table 5.3-59: Study design

Test group	Dose level of glyphosate acid (mg/kg/day)	# male	# female
Control	0	5	5
Low	250	5	5
Mid	500	5	5
High	1000	5	5

Preparation and treatment of animal skin: Sixteen to twenty-four hours before application of the test substance, the hair was removed with a pair of veterinary clippers from an area, approximately 10 cm x 5 cm, on the dorso-lumbar region of each animal. The rats were dosed dermally and the amount applied was calculated for each animal according to its weight at the time of dosing. The paste covered by a gauze patch (approximately 7 cm x 7 cm x 4-ply) was applied to the shorn back of each animal and was kept in contact with the skin for approximately 6 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film (7 cm x 7 cm) and was held in position using adhesive bandage (25 cm x 7.5 cm). This was secured by two pieces of PVC tape (approximately 2.5 cm x 20 cm) wrapped around the animal. The control animals were treated in a similar manner except that deionised water only was used. The rats were dosed sequentially in group order at approximately the same time each day.

At the end of each 6-hour contact period, the dressings were carefully removed. The skin, at the site of application, was cleansed using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

A total of 15 six-hour applications was made during a period of 21 days. During this time there were three two-day periods when the animals were not dosed. Following each application there was an 18-hour 'rest' period during which the animals were fitted with plastic collars to prevent oral contamination.

Observations: Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. Detailed clinical observations were recorded daily and after decontamination. Cage-side observations were also made as soon as possible after dosing, and towards the end of the working day.

Bodyweight: The bodyweight of each rat was recorded daily, immediately prior to application of the test substance where applicable and prior to termination on day 22.

Food consumption and test substance intake: Food consumption was recorded continuously throughout the study for each rat and calculated as a weekly mean (g food/rat/day).

Haematology and clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:

haemoglobin	mean cell haemoglobin concentration
haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count
mean cell haemoglobin	blood cell morphology
red cell distribution width	prothrombin time
activated partial thromboplastin time	

Clinical chemistry: Blood was collected at termination by cardiac puncture and the following parameters were examined:

urea	alkaline phosphatase activity
creatinine	aspartate aminotransferase activity
glucose	alanine aminotransferase activity
albumin	gamma-glutamyl transferase activity
total protein	calcium
cholesterol	phosphorus (as phosphate)
triglycerides	sodium
total bilirubin	potassium
creatine kinase activity	chloride

Investigations *post mortem:*
Macroscopic examination: All animals were examined *post mortem.* This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	liver
kidneys	testes

Paired organs were weighed together.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

gross lesions including masses	adrenal gland*
testis*	epididymis*
kidney	treated skin
liver	untreated skin

* Tissues marked were stored and not examined microscopically

Microscopic examination: All selected tissues processed from the control and 1000 mg glyphosate acid/kg/day, together with macroscopic abnormalities from these groups, were examined by light microscopy.

Statistics: Haematology, clinical chemistry, organ weights and weekly food consumption were analysed using Analysis of variance. Bodyweights, on initial (day 1) bodyweight, organ weights on final bodyweight were analysed using analysis of covariance. All data were analysed using ***SAS (1982)***.

II. RESULTS AND DISCUSSION

Mortality: There were no mortalities.

Clinical observations: There were no significant signs of toxicity at any dose level of glyphosate acid. Generally the clinical findings observed were consistent with those commonly seen in dermal studies as a consequence of bandaging and were considered not to be related to treatment with glyphosate acid.

Bodyweight and weight gain: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.

Food consumption: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.

Haematology: A minimal statistically significant increase in haemoglobin levels was observed in females dosed at 1000 mg glyphosate acid/kg/day. A statistically significant decrease compared with control was seen in red cell distribution width in females dosed at 250 and 1000 mg glyphosate acid/kg/day. In the absence of any adverse effects on the red cell parameters these minor changes are considered not to be of toxicological significance.

Table 5.3-60: Intergroup comparison of selected haematology parameters

	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
Parameter	**0**	**250**	**500**	**1000**	**0**	**250**	**500**	**1000**
Haemoglobin	15.2	15.3	15.3	15.0	13.9	13.7	14.1	14.6*
Red cell distribution width	13.1	12.9	12.6	13.4	13.8	12.4**	13.0	12.6*

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p<0.01$ (Student's t-test, 2-sided)

Blood clinical chemistry: Females dosed at 1000 mg glyphosate acid/kg/day showed a minimal, but statistically significant increase in plasma urea levels, but there were no differences seen in the plasma creatinine levels. This minimal change in urea was considered not to be of toxicological significance. A minimal but statistically significant decrease in plasma triglycerides was observed in males dosed at 500 mg glyphosate acid/kg/day and as this did not form part of a dose response relationship was considered not to be treatment related.

Table 5.3-61 Intergroup comparison of selected clinical chemistry parameters

	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
Parameter	0	250	500	1000	0	250	500	1000
plasma urea	8.4	8.2	8.5	8.1	7.6	7.7	6.9	8.6*
plasma trigycerides	1.27	1.01	0.87*	1.27	0.70	0.66	0.69	0.76

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

Sacrifice and pathology:
Organ weights: Testes weights were slightly, but statistically significantly decreased at 500 mg glyphosate acid/kg/day, due to one animal having a very low weight recorded. There were no effects due to treatment with glyphosate acid in the other organs weighed.

Macroscopic findings: A small number of lesions were observed, none of which was related to treatment.

Microscopic findings: A small number of common spontaneous lesions were observed, none of which was related to treatment.

III. CONCLUSION

There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg/day.

The no observed adverse effect level (NOAEL) for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg/day in both sexes.

IIA 5.3.8 Percutaneous 90-day toxicity (rodents)

Due to the low dermal absorption of glyphosate (see II 5.9.9 and IIA 5.3.7/02), as well as the low toxicity observed in percutaneous 28-day studies (see IIA 5.3.7), 90-day percutaneous toxicity studies are not considered necessary, and therefore not provided.

IIA 5.4 Genotoxicity

In the 2001 EU evaluation glyphosate was examined for mutagenicity and clastogenicity in a wide range of test systems covering all relevant endpoints *in vitro* (see IIA 5.4.1 to IIA 5.4.3). Additional studies have been conducted on glyphosate since the last EU review, however, all these studies were negative and are considered confirmatory data. Glyphosate has clearly been proved to have no genotoxcity potential in a wide range of regulatory studies *in vitro*.

IIA 5.4.1 In vitro genotoxicity testing – Bacterial assay for gene mutation

Table 5.4-1: Summary of *in vitro* genotoxicty testing with glyphosate acid

Reference (Owner)		Type of study	Test organism / test system	Dose levels (purity) Metabolic activation	Results
***In vitro* gene mutation tests in bacteria**					
Studies from the 2001 evaluation	Annex B.4.1.1.1 Glyphosate Monograph [redacted] 1995 (Herbex)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538	8.0 – 5000 µg/plate (95%); +/- S9	negative
	Annex B.4.1.1.1 Glyphosate Monograph [redacted] 1995	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	50 – 5000 µg/plate (purity not reported); +/- S9	negative
	Annex B.4.1.1.1 Glyphosate Monograph [redacted] 1991a (CHE)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537	- S9: 160 – 2500 µg/plate + S9: 310 – 5000 (98.6%)	negative
	Annex B.4.1.1.1 Glyphosate Monograph [redacted] 1978 (published MON study by [redacted], 1988)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538 and *E. coli* WP2 hcr	310 – 5000 µg/plate (98.4%); +/- S9	negative
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/01 [redacted] 1995a (ALS)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	156 – 5000 µg/plate (98.68%) +/- S9	negative
	IIA 5.4.1/02 [redacted] 2007a (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test) (95.1%) +/- S9	negative
	IIA 5.4.1/03 [redacted] 2007b (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test) (97.%) +/- S9	negative
	IIA 5.4.1/04 [redacted] 2007c (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test) (95%) +/- S9	negative
	IIA 5.4.1/05 [redacted] 2007 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	648 – 5000 µg/plate (98.01%) +/- S9	negative

	Reference (Owner)	Type of study	Test organism / test system	Dose levels (purity) Metabolic activation	Results
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/06 [redacted] 2008 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	1 – 1000 µg/plate (98.01%) +/- S9	negative
	IIA 5.4.1/07 [redacted] 2009a (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate incorporation and pre-incubation test) (98.8%) +/- S9	negative
	IIA 5.4.1/08 [redacted] 2010 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate incorporation and pre-incubation test) (96.4%) +/- S9	negative
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/09 [redacted] 2010 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate incorporation and pre-incubation test) (97.16%) +/- S9	negative
	IIA 5.4.1/10 [redacted] 2010 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 5000 µg/plate (plate incorporation and pre-incubation test) (98.2%) +/- S9	negative
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/11 [redacted] 1996 (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	0 – 5000 µg/plate (plate–incorporation) (95.3%) +/- S9	negative
	IIA 5.4.1/12 [redacted] 1996 (SYN)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2P *uvrA* and WP2P	100 – 5000 µg/plate (plate-incorporation) (95.6%)	negative
	IIA 5.4.1/13 [redacted] 2009 (SYN)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA* pKM 101 and WP3 pKM 101	33 – 5000 µg/plate (plate-incorporation) (96.3%)	negative

Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/01	[redacted]	1995a	HR-001: Reverse mutation test. [redacted] Data owner: Arysta LifeScience Study No.: [redacted] 94-0142 Date: 1995-04-03 GLP: yes not published

Guideline: U.S. EPA FIFRA Guidelines, Subdivision F

Deviations: None

Dates of experimental work: 1995-02-21 to 1995-03-09

Executive Summary
Reverse mutation tests were performed on HR-001 in *Escherichia coli* WP2 uvrA and four testere strains of *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537). Experiments were carried out with and without metabolic activation system (S9 mix) at dose levels up to 5000 µg/plate. The mean number of revertant colonies did not exceed the factor of 2 above that of the corresponding solvent control in any strain at any dose with or without S9 mix.

Based on the results, HR-001 is non-mutagenic to bacteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification: HR-001
Description: Solid crystals
Lot/Batch #: 940908-1
Purity: 95.68%
Stability of test compound: Not mentioned in the report
Solvent used: Sterile water

2. control materials:

Negative: Sterile water
Solvent/final concentration: Water / > 12 mg/mL
Positive: non-activation and activation

Strain	Positive controls	
	Without S9 (µg/plate)	With S9 (µg/plate)
TA100	AF – (0.01)	2-AA (1)
TA1535	NaN_3 (0.5)	2-AA (2)
WP2 uvra	AF-2 (0.01)	2-AA (10)
TA98	AF-2 (0.1)	2-AA (0.5)
TA1537	9-AA (80)	2-AA (2)

AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide dissolved inDMSO; NaN_3: sodium azide dissolved in sterile water
2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water

3. activation: The enzyme activity measured by mutagenicity was good.
S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10% (v/v) S9 fraction, 8 mM MgCl2, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.

4. test organisms: *Escherichia coli* WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)

5. test concentrations:

(a) Preliminary cytotoxicity assay: One preliminary assay was performed:

Plate incorporation assay: Concentrations up to 5000 µg/plate were evaluated with and without S9 activation in strain TA1535, TA1537, TA98, TA100 and WP2 uvrA. A single plate was used, per dose, per condition.

Pre-incubation assay: As above.

(b) Mutation assays:

Plate incorporation assay: 156, 313, 625, 1250, 2500 and 5000 µg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.

Pre-incubation assay: As above for the plate incorporation assay.

Re-tests: Not concerned

B: TEST PERFORMANCE

1. Preliminary cytotoxicity/plate incorporation mutation assay

Results of the range-finding are presented in table below.

Table 5.4-2: Preliminary dose range finding test

		Dose	Revertants (n° colonies/plate)				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix		Solvent control (H_2O)	124 111 (118)	14 18 (16)	25 21 (23)	17 25 (21)	7 5 (6)
		200	113	16	24	20	7
		500	116	18	23	10	3
		1000	116	16	20	14	4
		2000	82	10	15	5	5
		5000	79	3	19	8	3
+ S9 mix		Solvent control (H_2O)	83 86 (85)	11 9 (10)	21 25 (23)	29 28 (29)	6 10 (8)
		200	99	11	20	28	9
		500	82	7	12	30	6
		1000	97	8	28	25	6
		2000	96	9	18	38	7
		5000	33	4	17	20	5
Positive controls	**- S9 mix**	compound	AF-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	648 724 (686)	583 559 (571)	312 344 (328)	669 708 (689)	798 775 (787)
	+ S9 mix	compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	640 658 (649)	371 372 (372)	610 645 (628)	285 304 (295)	71 81 (76)

( ): average

HR-001 did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.

2. Pre-incubation assay

The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1°C.

3. Statistics

Results were judged without statistical analysis.

4. Evaluation Criteria

The test items were carried out twice. Reproducibility of results was confirmed by two independent experiments. Results were judged positive without statistical analysis when the following criteria are all satisfied:

1. A two-fold or greater increase above solvent control in the mean number of revertants is observed
2. This increase in the number of revertants is accompanied by a dose-response relationship
3. This increase in the number of revertants is reproducible.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

None

B. PRELIMINARY CYTOTOXICITY ASSAY

HR-001 did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.

C. MUTATION ASSAYS

Results are shown in table hereafter

Table 5.4-3: summary data – experiment 1

	Dose	Revertants (n° colonies/plate)*				
		Base-change type			Frameshift type	
	(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix	Solvent control (H_2O)	117	12	21	37	3
	156	119	11	12	40	3
	313	117	11	16	42	4
	625	139	9	15	39	2
	1250	125	9	22	43	5
	2500	106	3	15	38	3
	5000	105	4	20	39	2

		Dose	Revertants (n° colonies/plate)*				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
+ S9 mix		Solvent control (H_2O)	78	9	21	35	7
		156	83	6	19	36	9
		313	77	7	19	31	5
		625	99	6	19	30	8
		1250	93	6	22	37	6
		2500	73	7	16	39	7
		5000	56	3	16	25	4
Positive controls	- S9 mix	compound	AF-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	510	524	305	621	786
	+ S9 mix	compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	606	392	522	360	75

* values are the mean of three plate

Table 5.4-4: summary data – experiment 2

		Dose	Revertants (n° colonies/plate)*				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix		Solvent control (H_2O)	146	9	16	24	5
		156	137	10	18	18	7
		313	140	7	19	20	6
		625	136	8	17	18	3
		1250	136	7	15	15	3
		2500	144	6	18	10	3
		5000	111	10	14	9	4
+ S9 mix		Solvent control (H_2O)	123	8	17	37	7
		156	112	7	15	32	10
		313	125	7	13	29	9
		625	103	8	20	35	9
		1250	107	7	14	28	9
		2500	89	7	19	20	8
		5000	67	4	17	17	4
Positive controls	- S9 mix	compound	AF-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	595	527	252	742	909
	+ S9 mix	compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	768	322	605	327	87

* values are the mean of three plates

III. CONCLUSIONS

A two-fold or greater increase in the mean number of revertant colonies was not observed in any strain at any dose of HR-001 in the reverse mutation tests with or without metabolic activation. It is concluded that HR-001 is non mutagenic for bacteria under the conditions used with this experiment.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/02	[redacted]	2007a	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05068) [redacted] Data owner: Nufarm [redacted] Study No.: 1061401 Date: 2007-03-16 GLP: yes unpublished

Guideline:	The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies, Guideline No.3-1-19-1 >Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9200, on March 16, 2005, English translation by ACIS on October 17, 2005.
Deviations:	None
Dates of experimental work:	January 15, 2007 to January 25, 2007

Executive Summary

This study was performed to investigate the potential of Glyphosate technical (NUP05068) to induce gene mutations in the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.

The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Only in experiment II a minor reduction in the number of revertants, occurred in strain TA 1537 in the absence of metabolic activation at 5000 µg/plate. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical (NUP-05068)
Description:	Crystalline powder White
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Not specified

2. Vehicle/Controls

Vehicle = water

Negative/solvent control:	Concurrent untreated and solvent controls were performed.
Positive control:	without metabolic activation: Sodium azide, NaN3 with metabolic activation: 2-aminoanthracene; 2-AA
Activation:	Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::	Histidine auxotrophic strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.
Culture medium:	8 9 [redacted] Nutrient Broth [redacted]) 5 9 NaCl ([redacted])
Test concentrations::	0, 33, 100, 333, 667, 1000, to 5000 µg/ plate with or without metabolic activation.g

B: STUDY DESIGN AND METHODS

In life dates: January 15, 2007 to January 25, 2007

Study Conduct:
For each strain and dose level including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
- 500 µL 89 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix and S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45°C) was added to each tube. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 hours at 37°C in the dark.

II. RESULTS AND CONCLUSION

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor recuction in the number of revertants was observed at 5000 Iµg/plate without metabolic activation in experiment II. No substantial increase in revertant colony

numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/03	[redacted]	2007b	Salmonella typhimurium and E.coli Reverse mutation assay with Glyphosate technical (NUP-05070) [redacted] Data owner: Nufarm [redacted] Study No.: 1061402 Date: 2007-03-16 GLP: yes unpublished

Guideline:	The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC). Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12Nohsan No. 8147, as partly revised in 16-Shoan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:	None
Dates of experimental work:	January 15, 2007 to January 25,2007

Executive Summary

This study was performed to investigate the potential of Glyphosate technical (NUP05070) to induce gene mutations in the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.

The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Minor toxic effects occurred at 5000 µg/plate in strain WP2 uvrA in the absence of metabolic activation in experiment

I and in strain TA 98 with metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05070) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical (NUP-05070)
Description:	Crystalline powder White
Lot/Batch #:	20060901
Purity:	97.7%
Stability of test compound:	Not specified
2. Vehicle/Controls	Vehicle – water
Negative/solvent control:	Concurrent untreated and solvent controls were performed.
Positive control:	without metabolic activation: Sodium azide, NaN3 with metabolic activation: 2-aminoanthracene, 2-AA
Activation:	Phenobarbital/β-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::	Histidine auxotrophic strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.
Culture medium:	8 9 [redacted] Nutrient Broth ([redacted]) 5 0 NaCl ([redacted])
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

B: STUDY DESIGN AND METHODS

In life dates: January 15, 2007 to January 25,2007

Study Conduct:
For each strain and dose level including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
- 500 µL 89 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL Overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix and S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-

incubation 2.0 mL overlay agar (45°C) was added to each tube. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 hours at 37°C in the dark.

II. RESULTS AND CONCLUSION

The plates incubated with the test item showed reduced background growth at 333 - 5000 and 2500 - 5000 in strains TA 1537 and TA 100, respectively. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Minor toxic effects occurred at 5000 µg/plate in strain WP2 uvrA in the absence of metabolic activation in experiment I and in strain TA 98 with metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was bserved following treatment with Glyphosate technical (NUP-05070) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was not quite reached in the untreated control of strain TA 1535 with and without metabolic activation in experiment II. These minor deviations (10 versus 11 colonies and 9 versus 10 colonies, respectively) are judged to be based on biologically irrelevant fluctuations in the number of colonies and have no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/04	[redacted]	2007c	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05067) [redacted] Data owner: Nufarm [redacted] Study No.: 1061403 Date: 2007-03-16 GLP: yes unpublished

Guideline: The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).
Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No.
8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.

Deviations: None

Dates of experimental work: January 16, 2007 to January 25,2007

Executive Summary
This study was performed to investigate the potential of Glyphosate technical (NUP050067) to induce gene mutations in the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.
The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.
The test item was tested at the following concentrations:
Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Minor toxic effects occurred at 5000 µg/plate in strain WP2 uvrA in the absence of metabolic activation in experiment I and in strain TA 98 with metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05067) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical (NUP05067)
Description:	Crystalline powder White
Lot/Batch #:	0609-1
Purity:	95%
Stability of test compound:	Not specified

2. Vehicle/Controls Vehicle = water

Negative/solvent control:	Concurrent untreated and solvent controls were performed.
Positive control:	without metabolic activation: Sodium azide, NaN3 with metabolic activation: 2-aminoanthracene, 2-AA
Activation:	Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::	Histidine auxotrophic strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, TA100) to prototrophy.
Culture medium:	8 9 [redacted] Nutrient Broth ([redacted]) 5 9 NaCI ([redacted])
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

B: STUDY DESIGN AND METHODS

In life dates: January 16, 2007 to January 25,2007

Study Conduct:
For each strain and dose level including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
- 500 µL 89 mix (for test with metabolic activation) or 89 mix substitution buffer (for test without metabolic activation),
- 100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL Overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45°C) was added to each tube. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 hours at 37°C in the dark.

II. RESULTS AND CONCLUSION

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor reduction in the number of revertants was observed at 5000 µg/plate without metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/05	[redacted]	2007	Bacterial reverse mutation test (Ames Test) for Glifosato Téchnico Helm [redacted] Data owner: HAG Report No.: 3393/2007-2.0[redacted] Date: 2007-12-13 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	30/11/2007 – 03/12/2007

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535 and TA1537. The assay was carried out with and without metabolic activation. Concentrations of test item ranged between 648 and 5000 µg/plate. The mutation rates after 72 hours of incubation were lower than 2. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glifosato Téchnico Helm
Description: Solid
Lot/Batch #: 2007091801
Purity: 980.1 g/kg
Stability of test compound: Stable (CIPAC MT 46, 54 °C, 14 days)

2. Vehicle and/ or positive control:

Negative control: vehicle (DMSO, 100 µl/plate)
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	ICR 191 – Acridine
S9-	TA102	Mitomycin C
S9+	TA98; TA100; TA102; TA1535; TA1537	2-aminoanthracene

S9+ = metabolic activation

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535; TA1537
Source: [redacted] ([redacted] USA)

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. Test concentrations:

Preliminary cytotoxicity test: 8, 40, 200, 1000, 5000 µg/plate
Mutation assay: 648, 1080, 1800, 3000, 5000 µg/plate

B: STUDY DESIGN AND METHODS

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 8.0 and 5000.0 µg/plate.

Mutation study:
The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing 0.1 mL of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or substitution buffer (with / without metabolic activation) and 3.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 648 and 5000 µg/plate. The protein concentration of the S9 fraction was 34.9 mg/mL.

Liability check:
The acceptance criteria of the assay were as follows:

- The presence of background lawn in the test plates.
- Spontaneous revertant colonies of the negative control were in the range reported in the literature (MARON. D.M, & AMES. B.N. Revised methods for the *Salomonella* mutagenicity test. Mutation Research. 113: 173-215, 1983) and established in the laboratory by historical control values.
- Positive controls showed mutagenic activity in all tested strains.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
None of the concentrations tested showed cytotoxic effects.

B. MUTATTION ASSAYS
No significant mutation rate was observed up to a concentration of 5000 µg of test item per plate.

C. LIABILITY CHECK
All acceptance criteria were met.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/06	[redacted]	2008	Evaluation of the mutagenic potential of the test substance Glyphosate Technical by reverse mutation assay in *Salmonella typhimurium* (Ames Test) [redacted] Data owner: HAG Report No.: [redacted]-3996.401.391.07 Date: 2008-09-15 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	05/06/2008 – 30/06/2008

Executive Summary

The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA97a; TA98; TA100; TA102 and TA1535. The assay was carried out with and without metabolic activation. Concentrations of test item ranged between 0.001 and 1.0 mg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: GLYPHOSATE TECHNICAL
Description: Solid
Lot/Batch #: 20070606
Purity: 980.5 g/kg
Stability of test compound: Stable to hydrolysis at pH 4, 6 and 8 (5-35°C)

2. Vehicle and/ or positive control:

Vehicle: DMSO

Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA97a	9-Aminoacridine
S9-	TA102	Cumene hydroperoxide
S9+	TA97a; TA98; TA100; TA102; TA1535	2-Aminoanthracene

S9 = metabolic activation

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA97a; TA98; TA100; TA102; TA1535
Source: [redacted]

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. Test concentrations:

Preliminary cytotoxicity test: 0.001, 0.01, 0.1, 1.0, 2.5 mg/plate
Mutation assay: 0.001, 0.01, 0.1,0.5, 1.0 mg/plate

B: STUDY DESIGN AND METHODS

Preliminary cytotoxicity assay:

A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.001 and 2.5 µg/plate.

Mutation study:
The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing the corresponding volume of test stock solution, of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 0.001 and 1.0 µg/plate.

Liability check:
The concentration of test item in the lowest and highest concentrated sample were determined by HPLC-UV

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
Only the highest concentration of 2.5 mg/plate showed cytotoxic effects. Therefore, the mutation assay was carried out with a maximum concentration of 1.0 mg/plate.

B. MUTATTION ASSAYS
No significant mutation rate was observed up to a concentration of 1.0 mg of test item per plate.

C. LIABILITY CHECK
The concentrations of the lowest and highest concentrations were confirmed.

III. CONCLUSION
The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/07	[redacted]	2009a	Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) [redacted] Data owner: HAG Report No.: [redacted] 23916 Date: 2009-04-30 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	04/02/2009 – 27/02/2009

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535, TA1537. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate TC
Description: Solid, white powder
Lot/Batch #: 20080801
Purity: 988.0 g/kg
Stability of test compound: Stable for two years at ambient temperature

2. Vehicle and/ or positive control:

Negative control: Vehicle (*aqua ad injectabilia*)
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535, TA1537
Source: [redacted]

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. Test concentrations:

Preliminary cytotoxicity test: 0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0, 5000.0 µg/plate
Mutation assay: 31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate

B: STUDY DESIGN AND METHODS

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50%.

Mutation study:
Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48-72 hours. After incubation the revertant colonies per plate

were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was 31.55 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37°C for 20 min prior to mixing with agar and plating as described above.

Liability check:
As quality criteria the genotypes, i.e. histidine and biotin requirement (his^-) (bio^-), deep rough character (rfa^-), UV-sensitivity ($uvr\ B^-$), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.

B. MUTATTION ASSAYS
No mutagenic effect was observed for Glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.

C. LIABILITY CHECK
The genotypes of the 5 strains used were confirmed regularly.

III. CONCLUSION
The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/08	[redacted]	2010	Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) [redacted] Data owner: HAG Report No.: [redacted] 24880 Date: 2010-01-25 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	15/10/2009 – 23/11/2009

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535, TA1537. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between

31.6 and 3160.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate TC
Description: Solid, white powder
Lot/Batch #: 20080801
Purity: 964.0 g/kg
Stability of test compound: Stable for two years at ambient temperature

2. Vehicle and/ or positive control:

Negative control: Vehicle (*aqua ad iniectabilia*)

Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	N-Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535, TA1537
Source: [redacted]

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. Test concentrations:

Preliminary cytotoxicity test: 0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0, 5000.0 µg/plate
Mutation assay: 31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate

B: STUDY DESIGN AND METHODS

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50%.

Mutation study:
Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48-72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was 26.6 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37°C for 20 min prior to mixing with agar and plating as described above.

Liability check:
As quality criteria the genotypes, i.e. histidine and biotin requirement (his^-) (bio^-), deep rough character (rfa^-), UV-sensitivity ($uvr\ B^-$), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.

B. MUTATTION ASSAYS
No mutagenic effect was observed for Glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.

C. LIABILITY CHECK
The genotypes of the 5 strains used were confirmed regularly.

III. CONCLUSION
The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/09	[redacted]	2010	*Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine [redacted] Data owner: HAG Report No.: 1332300 Date: 2010-04-07 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	17/03/2010 – 22/03/2010

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA1535 and TA1537 and the *Escherichia coli* strain WP2 uvrA. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate TC (5000 mg/L) spiked with Glyphosine (32 mg/L)
Description: An aqueous solution of Glyphosate technical grade active ingredient (purity 97.16% w/w), containing 0.69% (w/w) Glyphosine in the technical grade active ingredient
Lot/Batch #: 2009051501 (Glyphosate TC)
Purity: 971.6 g/kg
Stability of test compound: Stable for two years at ambient temperature

2. Vehicle and/ or positive control:

Negative control: Vehicle (deionised water)
Positive controls:

Assay	Strain	Compound
S9-	TA98, TA1537	4-nitro-o-phenylene-diamine
S9-	TA100; TA1535	Sodium azide
S9-	WP2 uvrA	Methyl methane sulfonate
S9+	TA98; TA100; TA1535; TA1537, WP2 uvrA	2-Aminoanthracene

S9 = metabolic activation

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA1535, TA1537

Species: *Escherichia coli*
Strain: WP2 uvrA

Source: [redacted] Germany)

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

5. Test concentrations:

Preliminary cytotoxicity test	3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate
Mutation assay:	3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate

B: STUDY DESIGN AND METHODS

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants.

Mutation study:
Each sample was prepared by mixing 1.0 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 34.3 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37°C for 60 min prior to mixing with agar and plating as described above.

Liability check:
The acceptance criteria of the assay were as follows:

- Regular background growth in the negative and solvent control.
- Spontaneous revertant colonies of the negative control were in the range of historical data.
- Positive controls showed mutagenic activity in all tested strains.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

B. MUTATTION ASSAYS
No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Solution of Glyphosate TC spiked with Glyphosine at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).

C. LIABILITY CHECK
The acceptance criteria were met.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/10	[redacted]	2010	Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with Glyphosate TC [redacted] Data owner: Helm AG Report No.: [redacted] 101268 Date: 2010-04-08 GLP: yes unpublished

Guideline: OECD 471

Deviations: None

Dates of experimental work: 25/03/2010 – 06/04/2010

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535; TA1537. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between 31.6 and 5000.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate TC
Description: Solid
Lot/Batch #: 20090305I
Purity: 982.0 g/kg
Stability of test compound: Stable for two years at ambient temperature

2. Vehicle and/ or positive control:

Negative control: Solvent controls, consisting of solvent or vehicle alone.

Positive controls:

Assay	Strain	Compound
S9-	TA98, TA1537	4-nitro-o-phenylene-diamine
S9-	TA100; TA1535	Sodium azide
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA100; TA102; TA1535; TA1537	2-Aminoanthracene

S9 = metabolic activation

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535, TA1537
Source: [redacted] USA

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

5. Test concentrations:

Preliminary cytotoxicity test: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Mutation assay: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate

B: STUDY DESIGN AND METHODS

Preliminary cytotoxicity assay:
No preliminary cytotoxicity assay was performed.

Mutation study:
Each sample was prepared by mixing 0.2 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 33.0 mg/mL.

The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37°C for 60 min prior to mixing with agar and plating as described above.

Liability check:
The acceptance criteria of the assay were as follows:

- The bacteria demonstrate their typical responses to ampicillin (TA 98, TA 100, TA 102).
- Regular background growth in the negative and solvent control.
- Spontaneous revertant colonies of the negative control were in the range of historical data.
- Positive controls showed mutagenic activity in all tested strains.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY

No preliminary cytotoxicity assay was performed.

B. MUTATTION ASSAYS

In the plate incorporation test toxic effects of the test item were observed in tester strain TA 100 at a dose of 5000 µg/plate (with and without metabolic activation). In tester strain TA 1535 toxic effects of the test item were noted at doses of 2500 µg/plate and higher (with and without metabolic activation). In the preincubation test toxic effects of the test item were noted in tester strains TA 100 and TA 1535 at a dose of 5000 µg/plate (without metabolic activation).

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Glyphosate TC at any concentration level with or without metabolic activation.

C. LIABILITY CHECK

The acceptance criteria were met.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/11	[redacted]	1996	Technical glyphosate: Reverse mutation assay "Ames test" using Salmonella typhimurium and Escherichia coli [redacted] Data owner: Nufarm [redacted] Project No.: 434/014 Date: 1996-02-20 GLP: yes unpublished

Guideline: OECD 471(1983)
Commission Directive (EC) 92/69/EEC (1992), Method B14
US EPA (TSCA) guidelines

Deviations: None

Dates of experimental work: August 10, 1995 to November 13, 1995

Executive Summary

This study was performed to investigate the potential of Technical Glyphosate to induce gene mutations in the Ames plate incorporation method using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.
The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.
The test item was tested at the following concentrations:
0, 50, 150, 500, 1500, and 5000 µg/plate with and withput metabolic activation.
The plates incubated with the test item caused no visible reduction in the growth of the bacterial lawn at any dose level up to maximim recommended dose of 5000 µg/plate either with or without metabolic activation, however a decrease in the frequency of revertant colonies was observed with some bacterial strains.
No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation.
Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.
In conclusion, the test material was found to be non-mutagenic under the conditions of this test.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	H95D 161 A
Purity:	95.3 %
Stability of test compound:	No data given in the report.

2. Vehicle/Controls

Vehicle = sterile distilled water

Negative/solvent control: Vehicle/solvent controls were performed.

Positive control:

Without metabolic activation:

N-ethyl-N`-nitro-N-nitrosoguanidine (ENNG):

- 2 µg/plate for WP2uvrA;
- 3 µg/plate for TA100;
- 5 µg/plate for TA1535.

9-Aminoacridine (9AA) 80 µg/plate for TA1537.

4-Nitroquinoline-1-oxide (4NQO) 0.2µg/plate for TA98.

With metabolic activation:

2-Aminoanthracene (2AA) was used in S9 series of plates in the concentrations:

- 1 µg/plate for TA100;
- 2 µg/plate for TA1535 and TA1537;
- 10 µg/plate for WP2uvrA;
- 0.5 µg/plate for TA98.

Activation: S9 was prepared from the livers of male Sprague-Dawley rats. Each received a single i.p. injection of Aroclor 1254 at 500 mg/kg, 5 days before S9 preparation.

Test organisms: *Salmonella typhimurium* TA1535, TA 1537, TA98, and TA100
Escherichia coli WP2uvrA

Culture medium: Top agar was prepared using 0.6% Dicfo Bacto agar and 0.5% sodium chloride with 5 ml of 1.0 mM histidine/1.0 mM biotin and 1.0 mM tryptophan solution added to each 100 ml of top agar.

Base agar plates were prepared using 1.2% Oxoid Agar Technical No 3 with Vogel-Bonner Medium E and 20 mg/mL D-glucose.

Test concentrations: 0, 50, 150, 500, 1500, and 5000 µg/plate with and withput metabolic activation. In triplicate for each bacterial strain and for each concentration of test material with an without S9-mix.

B: STUDY DESIGN AND METHODS

In life dates: August 19, 1995 to November 13, 1995

Study Conduct:
For each strain and dose level including the controls, three plates were used with and without S9-mix.
The following materials were mixed in a test tube and poured onto the selective agar plates:

- 0.1 ml of bacterial suspension;
- 0.1 ml of test solution at each dose level, vehicle/solvent (negative control) or reference mutagen solution (positive control);
- 2 ml of molten, trace histidine/tryptophan supplemented media;
- 0.5 ml of buffer (for test without metabolic activation) or S9-mix (for test with metabolic activation).

Known aliquots (0.1 ml) of one of the bacterial suspensions were dispensed into sets of sterile test tubes followed by 2.0 ml of molten trace histidine/tryptophan supplemented top agar at 45 °C, 0.1 ml of the appropriately diluted test material or vehicle control or reference mutagen solution (with and without metabolic activation) and either 0.5 ml of the S9 liver microsome mix or 0.5 ml of pH 7.4 buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner agar plates (one tube per plate). Plates were incubated for approximately 48 hours at 37 °C.

II. RESULTS AND CONCLUSION

The plates incubated with the test item caused no visible reduction in the growth of the bacterial lawn at any dose level up to maximim recommended dose of 5000 µg/plate either with or without metabolic activation, however a decrease in the frequency of revertant colonies was observed with some bacterial strains.
No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation.
Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.
In conclusion, the test material was found to be non-mutagenic under the conditions of this test.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/12	[redacted]	1996	Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E.Coli*. [redacted] Data owner: Syngenta Report No.: [redacted]/4874 Date: 1996-02-16 GLP: yes not published

Guideline:	OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC B.13/B.14 (2000)
Deviations:	None
Dates of experimental work:	1995-10-23 to 1996-02-16

Excecutive summary
In a reverse gene mutation assay in bacteria (***Gatehouse et al, 1990***, based on the method of ***Maron and Ames, 1983***), four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and two strains of *Escherichia coli* (WP2P and WP2 *uvrA*) were exposed to glyphosate acid in the presence and absence of rat liver-derived metabolic activation system (S9-mix).

In two separate experiments, glyphosate acid did not induce any significant, reproducible increases in the observed numbers of revertant colonies in any of the tester strains used, either in the presence or absence of S9-mix.

The sensitivity of the test system, and the metabolic activity of the S9-mix, were clearly demonstrated by the increases in the number of revertant colonies induced by positive control substances.

Under the conditions of this assay, glyphosate acid gave a negative, ie non-mutagenic, response in *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P and WP2 *uvrA* in both the presence and absence of S9-mix, when tested to a limit dose of 5000 µg/plate.

I. MATERIALS AND METHODS

A: MATERIALS:

Test Material: Glyphosate acid

Description: Technical; white solid

Lot/Batch number: P24

Purity: 95.6% a.i

CAS#: Not reported

Stability of test compound: Confirmed by Sponsor

Control Materials:

Negative: Dimethylsulphoxide – DMSO

Solvent control (final concentration): Dimethylsulphoxide – DMSO (100 µL/plate)

Positive control: Nonactivation:

2-Acridine mutagen ICR191 TA1537
2-Aminoanthracene TA1537, WP2 uA
Daunomycin hydrochloride TA98
N-Ethyl-N'-nitro-N-nitrosoguanidine WP2P *uvrA*
Mitomycin C WP2P
Sodium Azide TA1535 and TA100

Activation:

2-Aminoanthracene TA1535, TA1537, TA98, TA100, WP2 uvrA and WP2P

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbital		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

The metabolic activation system (S9-mix) used in this study was prepared as a 3:7:20 mixture of S9 fraction, Sucrose-tris-EDTA buffer (250:50:1 mM) and cofactor solution.

The cofactor solution was prepared as a single stock solution Na_2HPO_4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and $MgCl_2$ (12 mM), in sterile deionised water and adjusted to a final pH of 7.4.

Test organisms:

S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		

E. coli strains

X	WP2P (pKM101)	X	WP2P *uvrA* (pKM101)						

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	X	Yes		No

Test compound concentrations used:
Nonactivated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate.
Activated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate.
For all strains triplicate plates were used for all test substance and positive control treatments. For solvent controls 5 plates were used.

B: STUDY DESIGN AND METHODS:

In-life dates: Start:28 November 1995 End: 11 December 1995

TEST PERFORMANCE
Preliminary Cytotoxicity Assay: Not performed.
Type of Bacterial assay:

- X standard plate test (both experiments –S9, initial experiment +S9)
- __ pre-incubation (60 minutes) (second experiment +S9)
- __ "Prival" modification (i.e. azo-reduction method)
- __ spot test
- __ other

Protocol:
Bacterial cultures were prepared from frozen stocks by incubating for 10-12 hours at 37°C.
The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level, solvent and positive controls;
- 500 µL S9 mix or phosphate buffer;
- 100 µL Bacteria suspension;
- 2 mL Overlay agar containing 50 µM histidine or tryptophan as appropriate.

In this assay 100 µL aliquots of an overnight culture of each bacteria strain were stored in bijou bottles at room temperature until required (1-2 hours). 500 µL S9 mix (or buffer) was then added by dispensing syringe to the number of bijou bottles of one strain required for one dose level, followed by 0.1 mL of the appropriate concentration of the test substance solution added by micropipette. Finally, 2.0 mL top agar was added to each bijou, the force of addition was sufficient to mix the contents. The mixture was then rapidly poured onto a prepared Vogel Banner agar plate. After the agar was set the plates were incubated upside down for 64 - 68 hours at 37° C in the dark. For each strain and dose level including the controls, three plates were used.

Following the total incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there should be a background lawn on the negative control plates and on the plates for (at least) the lower doses of test substance, and that the positive controlshad responded as expected.

The plates were counted using an automated colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies.

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:

- a significant, dose-related increase in the mean number of revertants is observed;
- a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations

A negative result in a (valid) individual experiment is achieved when:

- there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and
- in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effects must be consistently reproducible.

II. RESULTS AND DISCUSSION

Mutagenicity assay: In two separate experiments glyphosate acid did not induce any significant increases in the observed numbers of revertant colonies in the four *Salmonella typhimurium* strains (TA1535,TA1538, TA98, TA100) and the two *Escherichia coli* strains (WP2P and WP2 *uvrA*) in either the presence or absence of an auxiliary metabolising system (S9).

The positive controls for each experiment induced the expected responses indicating the strains were working satisfactorily in each case.

III. CONCLUSION

Under the conditions of this assay, glyphosate acid gave a negative, ie non-mutagenic, response in *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* strains WP2P and WP2 uvrA in both the presence and absence of S9-mix, when tested to a limit dose of 5000 µg/plate.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/13	[redacted]	2009	Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay [redacted] Data owner: Syngenta Report No.: 1264500 Date: 2009-12-18 GLP: yes not published

Guideline:	OECD 471 (1997): OPPTS 870.5100 (1998): 2008/440/EC B.13/B.14 (2008)
Deviations:	None
Dates of experimental work:	2009-09-15 to 2009-12-18

Excecutive summary
This study was performed to investigate the potential of Glyphosate technical (via the Nantong Jiangshan (glycine-route)) to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strains WP2 uvrA pKM 101 and WP2 pKM 101 over the range 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate (Experiment I), and 33; 100; 333; 1000; 2500; and 5000 µg/plate (Experiment II).

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with Glyphosate technical at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Positive control chemicals showed appropriate responses in the relevant strains.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Glyphosate technical is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

I. MATERIALS AND METHODS

A: MATERIALS:

Test Material:	Glyphosate
Description:	white solid
Lot/Batch number:	569759
Purity	96.2 % of Glyphosate Acid
Stability of test compound:	Not indicated by the sponsor
Control Materials:	
Negative:	Concurrent untreated and solvent controls were performed
Solvent control (final concentration):	100µl/plate
Positive control:	Nonactivation: Sodium azide 10 µg/plate TA100, TA1535 4-nitro-o-phenylene-diamine, 50 µg/plate TA 1537, 10 µg/plate TA98 methyl methane sulfonate 3 µL/plate WP2 (pKM101), WP2 *uvrA* (pKM101) Activation: 2-Aminoanthracene 2.5 µg/plate TA 1535, TA 1537, TA100, TA98 10 µg/plate WP2 (pKM101), WP2 *uvrA* (pKM101)