Diet/Food:	[redacted] Certified RODENT CHOW [redacted] *ad libitum*
Water:	St. Louis public water, *ad libitum*
Housing:	Housing for premating and gestation (day 0 through 13): individual suspended stainless steel cages over paper bedding; during mating females were housed in the male´s cages Housing for gestation and lactation (from day 14 of gestation through lactation): females housed in double wide cages with solid bottoms and wood shavings for bedding
Environmental conditions:	Temperature: 18 - 26°C Humidity: 40 - 70% 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

Animal assignment and treatment:
In a two-generation reproduction study groups of 30 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 2000, 10000 and 30000 ppm (corresponding to 132-140, 666-711, 1983-2230 mg/kg bw for males and 160-163, 777-804, 2322-2630 mg/kg bw/day for females (calculated from F0 and F1a adults)) glyphosate in the diet. After 11 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis in a male´s cage for 7 days, to produce the F1a litters. If there was no evidence of mating after 7 days (copulatory plug or vaginal smear), the female was co-housed with a male having recorded copulatory activity for additional 7 days, or until copulatory evidence was found. For F0 and F1 generation gestation day 0 was set on the day on which copulatory evidence was found and lactation day 0 the day on which delivery of pups was completed.
At weaning of offspring from the F0 mating phase, groups of 30 males and 30 females offspring from each dose group were selected to form the F1 generation and the mating procedure for F1a adults was conducted in the same way except modifications to exclude sibling matings. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*. F0 males were killed at completion of mating phase. The offspring selected for the F1a generation were dosed for at approximately 14 weeks and then mated to produce the F2a and F2b litters (a second mating of the F1 generation was performed due to reduced litter sizes in pups from F0 of the 30000 ppm dose group). At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.

Diet preparation and analyses
Approximately each week (except in one week when diets were prepared twice the same week and not during the following week) a known amount of glyphosate was mixed with the diet for 10 minutes in a HOBART HCM-450 mixing machine to achieve a batch size of 18 kilograms at each dose level.
The stability and homogeneity of the test substance in the diet were determined by liquid chromatography of duplicate samples from top, middle and bottom of mixer from the lowest and highest dietary levels stored in an open container at ambient temperature for 6 and 14 days or when frozen in a closed container for 35 days.

Clinical observations
A detailed observation for signs of toxicity was performed once weekly for the adult animals and for the offspring on days of weight measurement.

Body weight
Adult male animals of the F0 and F1a generation were individually weighted once weekly. The same was done for the female animals until copulation was confirmed, then females were weighted on days 0, 7, 14, and 21 of gestation and lactation.
Offspring was weighted on days 0, 4 (pre- and post-culling), 14 and 21 of lactation (except F1a males approximately two weeks prior to sacrifice and F1a females for approximately three weeks prior to mating for the F2b generation).

Food consumption and compound intake
Food consumption was recorded weekly for F0 and F1a adult males, except during mating, and also weekly for adult F0 and F1a female animals until mating. After confirmed copulation, the maternal food consumption was monitored for days 0-7, 7-14 and 14-21 of gestation and lactation, but it was not determined for females approximately three weeks prior to mating for the F2b generation and generally not for female animals that did not became pregnant.
Food conversion efficiency was not calculated.

Water consumption
No data on water consumption was given in the report.

Reproduction parameters

Pregnancy and parturition
Data on total paired females, females with confirmed copulation/total paired, pregnant/total paired, pregnant/ confirmed copulation was monitored as well as precoital (for pregnant animals) and gestational length in days. For males, the following items of interest were given: males with confirmed copulation/total paired, males impregnating females/total paired and males impregnating females/confirmed copulation.

Litter data
The following litter data were recorded: litter size, dead pups/litter, mean pup weight (on day 0, 4 (pre-/post-cull), 14, 21) and survival (%).

Physical and sexual development
No details on physical and sexual development of the offspring was reported.

Sacrifice and pathology
All adult animals, which died or were sacrificed in moribund condition were subjected to a gross necropsy and selected tissues were sampled. Pups found dead or culled pups also underwent gross pathology, but no tissues were saved. No organ weights were determined.
All F1a weanlings, that were not selected for mating, F2a and F2b weanling pups as well as females which had littered on or after 21 of lactation were sacrificed as scheduled. Non-pregnant adult females were killed at least 5 days after last expected parturition date and adult males after completion of the mating phase.
External and internal cavities of the dead animals were opened and the organs were examined in place and then removed. Hollow organs were opened and examined. The following organs of F0 and F1a males and females from each dose group that were sacrificed at the end of the study sampled, were weighed: ovaries and testes with epididymides. When present, the following organs from the F0 and F1a adults (unscheduled deaths and scheduled sacrifice) were retained: kidneys, ovaries, prostate, seminal vesicle, skin/mammary gland, testes, epididymis, uterus/vagina and gross lesions (pituitary retained for F1a adults only). Tissues from the F1a weanlings were saved at the discretion of the necropsist. From the F2a and F2b weanlings, which where sacrificed at schedule, the kidneys of 1 pup per sex and litter were saved.

A histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on one F2b weanling/sex/litter (selected at random) as well as on all retained

tissues from unscheduled adult deaths. For preparation, fixed tissues were washed, dehydrated, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examinated under light microscopy.

Statistics

Dunnett´s multiple comparison test (two-tailed) was used to detect statistically significant differences in adult body weights and food consumption between treated animals and their respective control.
Terminal body weights, maternal body weights and food consumption during gestation and lactation, pup weights, precoital length, gestational length, litter size, dead pups/litter, pup survival, absolute organ weights and organ/body weight ratios were evaluated by decision-tree statistical analyses procedures which, depending on the results of tests for normality and homogeneity of variance [Bartlett's Test], were chosed either parametric [Dunnett's Test and Linear Regression] or nonparametric [Kruskal-Wallis, Donckheere's and/or Mann-Whitney Tests] routines to detect differences and analyzed for trend.
The uncorrected Chi-Square test was used to examine fertility indices, e.g. females/males with confirmed copulation/total paired, pregnant/confirmed copulation (females) and males impregnating females/total paired as well as males impregnating females/confirmed copulation.
Fisher´s Exact test with Bonferroni Inequality Procedure was used for statistical analysis of microscopic lesions.
Other statistical routines used for some data included: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample group means could be considered as an estimate of a common population, and Grubb's Test to detect outliers.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The analysis of the test substance stability conducted over the time span of the study indicated that the test material was stable in the diet and homogeneity was adequate for study use. The stability of the test material in the diet was demonstrated at the low and high dose level, stored in an open container at ambient temperature for 6 and 14 days, or when frozen in a closed container for 35 days.
Analysis for achieved concentrations, demonstrated that the test substance-levels in the prepared diet were in the range of 95 to 96.7% of the nominal concentration.

B. TEST COMPOUND INTAKE

The group mean achieved dosages are summarised in the table below.

Table 5.6-17: Group mean compound intake levels during pre-mating periods of F0 and F1

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*			
		F0		F1	
		Males	Females	Males	Females
control	0	0	0	0	0
low	2000	132	160	140	163
mid	10000	666	777	711	804
high	30000	1983	2322	2320	2536

* based on actual food intake and body weight data; values were calculated in the report

C. MORTALITY

There were no treatment-related mortalities.
One female of the F0 generation died early in the study. This animal was never mated and at necropsy changes in bladder in kidneys were observed. Two male animals of the 2000 and 30000 ppm dose groups (F1 generation) died. Necropsy of these animals noted thymus and respiratory changes. One female animal of the F1 generation (2000 ppm) was sacrificed in extremis and another female (same generation, same

dose group) died. Kidney changes and retained foetus; pups in uterus and stomach changes, respectively, were observed in these two females.

Concerning the offspring, dead pup counts at day 0 and survival of all F1a, F2a and F2b treated pups were not adversely affected when compared to the controls.

D. CLINICAL OBSERVATIONS

The only clinical signs that were related to the test substance were soft stool in the animals of the 30000 ppm dose group. Other clinical signs, such as red ocular discharge/laboured respiration/overgrown teeth/piloerection/abrasions/emaciated and dehydrated appearance/misuse of limbs/focal loss of hair/swollen feed, occurred sporadically and were not considered to be treatment-related.

E. BODY WEIGHT

At the highest exposure level of 30000 ppm, reduced body weights were observed in both sexes and in F0 and F1 generation. In the F0 generation, body weights gradually decreased within time to approximately 8% less than controls prior to mating. F0/F1 weaning animals were lighter in weight as their corresponding controls and maintained that weight difference (approx. 10% less than control) until the end of the study (see Table 5.6-18).
No test-substance related body weight effects were observed in the adult animals of the 2000 and 10000 ppm dose groups prior to mating.
During gestation and lactation, maternal body weights in the highest dose group tended to remain lower than in controls, but the animals showed a rather greater body weight gain than the controls during gestation and lactation so that by the end of lactation body weights were approximately the same as those of the controls (see Table 5.6-19 and Table 5.6-20).
Terminal body weights were significantly decreased for both sexes at the highest exposure level (see Table 5.6-18).

Table 5.6-18: Mean group body weights

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day					
			0	72	T#	0	72	T#
			F0 Generation					
			Males			Females		
0 (Control)	30	mean	187.9	494.6	549.56	150.5	276.7	296.31
		sd	11.55	34.86	46.76	6.86	23.85	23.63
2000	30	mean	188.1	497.6	550.19	150.5	272.6	290.64
		sd	11.35	49.87	80.72	7.03	22.86	19.50
10000	30	mean	188.4	484.4	539	150.2	273	290.71
		sd	11.57	42.13	58.13	7.04	27.92	25.35
30000	30	mean	188	455.8**	503.51**	150.3	253.8**	265.91
		sd	11.56	46.46	45.66	7.06	18.46	15.44
			F1 Generation					
			129	219	T#	128	219	T#
0 (Control)	30	mean	118.3	534.7	625.04	99.8	285.8	316.21
		sd	26.11	38.84	53.11	17.44	27.63	37.37
2000	30	mean	115.2	540.3	632.14	96.7	282.1	313.74
		sd	16.2	44.9	74.57	11.47	24.5	30.53
10000	30	mean	114.8	514.1	590.98	97.1	275.9	312.36
		sd	17.42	58.31	70.06	14.18	20.55	26.71
30000	30	mean	104.9*	483.4**	543.40**	88.8*	253.7**	284.72**
		sd	19.79	41.32	58.12	16.32	19.56	18.04

*: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.05$)
**: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.01$)
#T: Termination

Table 5.6-19: Mean maternal body weights during gestation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Gestation) 0	7	14	21
			F0 Generation			
0 (Control)	24	mean	274	301.83	324.41	398.26
		sd	24.26	24.58	22.85	26.12
2000	29	mean	272.72	297.33	319.90	392.86
		sd	20.52	21.71	19.84	24.28
10000	28	mean	271.80	299.22	323.43	395.08
		sd	24.12	26.40	28.44	25.87
30000	28	mean	255.05**	282.44**	305.83**	375**
		sd	16.49	16.27	17.44	24.70
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	285.29	308.95	328.70	392.56
		sd	25.48	26.58	29.48	36.19
2000	29	mean	278.65	304.80	324.15	383.45
		sd	23.42	23.48	25.06	28.18
10000	28	mean	268.89*	297.23	319.68	382.71
		sd	19.24	18.81	19.27	21.77
30000	28	mean	251.80**	276.28**	299.48**	360.46**
		sd	12.42	18.92	19.29	33.31
			F1 Generation (Second Mating)			
			0	7	14	21
0 (Control)	24	mean	324.22	340.99	363.44	428.99
		sd	23.11	27.85	27.98	36.87
2000	29	mean	315.21	338.27	360.35	426.88
		sd	26.06	28.67	28.39	33.67
10000	28	mean	305.27*	333.66	357.50	428.51
		sd	20.26	22.45	24.49	26.17
30000	28	mean	281.46**	308.92**	330.95**	393.67**
		sd	17.79	22.19	22.36	34.88

Table 5.6-20: Mean maternal body weights during lactation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Lactation) 0	7	14	21
			F0 Generation			
0 (Control)	24	mean	299.96	319.59	317.33	313.39
		sd	23.21	23.58	28.96	20.01
2000	29	mean	297.48	317.91	314.53	313.96
		sd	21.10	18.66	25.22	16.63
10000	28	mean	298.78	315.15	312.41	319.10
		sd	20.81	22.04	22.94	18.61
30000	28	mean	285.84*	307.64	304.75	316.68
		sd	13.91	12.48	20.68	15.43
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	299.29	313.60	337.68	318.49
		sd	27.02	26.12	35.71	21.38
2000	29	mean	295.16	308.28	332.10	314.69
		sd	23.58	22.56	23.92	23.95
10000	28	mean	296.63	310.80	328.29	313.14
		sd	19.04	18.64	18.33	14.06
30000	28	mean	277.91**	289.88**	315.88**	306.15
		sd	12.89	17.73	17.47	20.18
			F1 Generation (Second Mating)			
			0	7	14	21
0 (Control)	24	mean	342.78	343.21	353.34	337.16
		sd	32.46	27.11	21.15	17.22
2000	29	mean	340.16	338.62	348.40	331.96
		sd	28.54	16.11	25.89	20.67
10000	28	mean	333.80	342.41	352.70	334.56
		sd	28.33	26.93	20.43	13.82
30000	28	mean	312.39**	324.09*	337.08	329.95
		sd	23.73	20.50	19.09	18.41

F. FOOD CONSUMPTION

Overall, food intake was not notably affected during the study.

All animals of the 30000 ppm dose group consumed about 1 to 2 grams/day less than controls. This effect was mostly pronounced in the first week of exposure and also observed in the F0 dams. Subsequent dams (F1 first and second matings) tended to eat similar or larger amounts of the diet than controls.

No effects on food consumption were observed in the animals of the 2000 and 10000 ppm dose groups.

G. REPRODUCTIVE PARAMETERS

Mating Performance, Fertility, Gestation and Lactation

No effects on mating and fertility rates were observed in the F0 and F1a dams when compared to controls and no effects were observed on precoital length at any treatment level.

H. LITTER DATA

Size and Viability

Day 0 dead pup counts among treated groups were comparable to the control group for all three litters of pups (F1a, F2a and F2b generation).

A slight reduction in the average litter size was observed in the F0 dams of the 30000 ppm dose group. This effect was less pronounced in animals after the first F1 mating. Although the difference was not statistically significant and not accompanied by an increase in dead pups/litter, a treatment-related effect could not be excluded. Therefore a second mating of the F1a adults was performed. In the resulting F2b generation, no dose-related decrease in litter size was observed.

Growth and Development

Birth weights and initial growth rate for pups from the treated dams compared well to the ones of the control, except the pups of the 30000 ppm dose group had reduced body weights on day 21 of lactation (more than 10% difference to controls). The effect was earlier pronounced in the F1 matings (day 14). This effect was reasoned by the titrated uptake of the test substance-containing diet at the end of lactation. In the mid dose group, slight and transient decreases in the body weights of the pups were observed. They were not evident in both sexes from all generations and therefore regarded of questionable toxicological significance.

Table 5.6-21: Mean pup weights

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day 0	Mean group body weight (g) at Day 21	Mean group body weight (g) at Day 0	Mean group body weight (g) at Day 21
			Males		Females	
			F0 Generation			
0 (Control)	24	mean	6.28	53.39	6.06	50.80
		sd	0.49	3.90	0.52	4.39
2000	29	mean	6.27	51.82	5.91	49.47
		sd	0.48	5.26	0.48	5.05
10000	28	mean	6.43	50.42*	6.13	49.16
		sd	0.47	3.66	0.50	3.12
30000	27	mean	6.47	46.30**	6.12	44.99**
		sd	0.62	4.09	0.59	4.34
			F1 Generation (First Mating)			
0 (Control)	28	mean	6.33	55.11	5.95	51.93
		sd	0.80	5.64	0.55	5.07
2000	23	mean	6.20	52.47	5.90	51.42
		sd	0.76	5.15	0.70	4.08
10000	22	mean	6.32	51.53*	5.98	48.49*
		sd	0.74	7.35	0.64	5.93
30000	25	mean	6.50	47.29**	6.05	44.41**
		sd	0.84	4.62	0.74	4.90
			F1 Generation (Second Mating)			
0 (Control)	16	mean	6.48	55.03	6.04	49.35
		sd	0.75	6.38	0.63	10.96
2000	18	mean	6.17	52.74	5.86	50.73
		sd	0.74	6.12	0.83	5.91
10000		mean	6.36	52.29	5.92	49.48
		sd	0.52	3.35	0.47	2.52
30000	23	mean	6.51	44.43**	6.04	43.10**
		sd	0.63	6.86	0.55	3.81

Clinical signs

No clinical signs were observed in the offspring of treated animals.

I. PATHOLOGY

Necropsy

There were no toxicologically significant macroscopic gross lesions attributed to the test chemical administration.

Organ weights

There were no statistically significant organ weight changes, except a slight increase in testes to body weight ratios in F1a adults of the 30,000 ppm dose group. This effect was attributed to their lower terminal body weight.

Histopathology
No treatment-related changes were detected.

III. CONCLUSION

The oral administration of glyphosate to rats via diet at a dose levels of 2000, 10000 and 30000 ppm for two successive generations resulted in possible treatment-related changes at the maximum dose of 30000 ppm. A high incidence of soft stools in adults was accompanied by consistent reduction of body weights of adults and pups at this dose level. Decreases in body weights of the pups obviously occurred at the end of lactation, with the beginning of consuming the test substance-containing diet. Furthermore, slightly but not statistically significant reduced average litter size was noted in F0 dams of the 30000 ppm dose group at first mating.
Therefore the NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations (corresponding to 666-711 mg/kg bw for males and 777-804 mg/kg bw/day for females).
.
The NOAEL for reproductive toxicity, for both generations and offspring was considered to be 30000 ppm.

The NOAEL for developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

IIA 5.6.2 Separate male and female studies
Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

IIA 5.6.3 Three segment designs
Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

IIA 5.6.4 Dominant lethal assay for male fertility
Studies considered not necessary. Information provided in chapter IIA 5.4.6.

IIA 5.6.5 Cross-matings of treated males with untreated females and vice versa
Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

IIA 5.6.6 Effects on spermatogenesis
Studies considered not necessary. Effects on spermatogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

IIA 5.6.7 Effects on oogenesis
Studies considered not necessary. Effects on oogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

IIA 5.6.8 Sperm motility and morphology
Studies considered not necessary. Parameters are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

IIA 5.6.9 Investigation of hormonal activity
Separate studies considered not necessary. The potential hormonal activity is assessed in two-generation and developmental toxicity studies (see IIA 5.6.1, IIA 5.6.10 and IIA 5.6.11).

IIA 5.6.10 Teratogenicity test by the oral route in the rat

The 2001 EU glyphosate review concluded that in rats the lowest relevant NOEL for both maternal and developmental effects was 300 mg/kg bw/day and the lowest effect level was 1000 mg/kg bw/day. The evaluation found there was no evidence of teratogenicity. A summary of the teratogenicity studies conducted in the rat are available in Table 5.6-22. Two additional teratogenicity studies have been performed in the rat that have not been previously reviewed in the 2001 EU glyphosate evaluation. These studies are considered to be confirmatory data and are summarised below.

Maternal toxicity in rats at 1000 mg/kg bw/day was characterised by GI disturbances (loose stool/diarrhoea), reduced body weight gain and noisy respiration and in addition at 3500 mg/kg bw/day maternal toxicity was characterised by mortality, post dose salivation and lower food consumption (Table 5.6-22 Non-specific signs of foetotoxicity were observed at 1000 mg/kg bw/day only in the [redacted] study (1991) which was characterised by a slight increase above the historical control range of foetuses showing skeletal variations. Reduced foetal weight, delayed ossification and a lower number of viable foetuses was observed at the extremely high top dose of 3500 mg/kg bw/day in the [redacted] (1991) and [redacted] (1980) studies. The toxicological relevance of these findings are uncertain because of the clear increase in mortality in the dams at this dose level suggest it was in excess of the maximum tolerated dose.

Table 5.6-22: Summary of developmental toxicity in rats

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL (mg/kg bw/day (ppm)) Maternal	NOAEL Offspring / develop-mental	LOAEL Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.6.2.1.1 Glyphosate Monograph [redacted] 1991b (CHE)	Developmental toxicity, rat, CD	0, 300, 1000, 3500	300	300	Maternal: 3500 mg/kg bw/day: mortality, salivation, loose stool, noisy respiration, reduced body weight, slightly reduced food intake, increased water intake 1000 & 3500 mg/kg bw/day: noisy respiration, reduced body weight gain Developmental: 3500 mg/kg bw/day: reduced mean foetal weight 1000 & 3500 mg/kg bw/day: reduced / delayed ossification, increased incidence of skeletal variations
	Annex B.5.6.2.1.2 Glyphosate Monograph [redacted] 1991g (FSG)	Developmental toxicity, rat, Wistar	0, 1000 (Limit test)	1000	1000	Maternal: no effects Developmental: No treatment related effect
	Annex B.5.6.2.1.2 Glyphosate Monograph [redacted] 1980 (MON)	Developmental toxicity, rat, CD	0, 300, 1000, 3500	1000	1000	Maternal: mortality, diarrhoea, reduced body weight gain Developmental: reduced number of viable foetuses

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL [mg/kg bw/day (ppm)] Maternal	NOAEL Offspring / develop-mental	LOAEL Targets / Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.6.10/01 [redacted] 1996b (SYN)	Developmental toxicity, rat, Alpk: AP_fSD	0, 250, 500, 1000	1000	1000	Maternal: no effects Developmental: no effects
	IIA 5.6.10/02 [redacted] 1995 (ALS)	Developmental toxicity, rat	0, 30, 300, 1000	300	1000	Maternal: slightly loose stool Developmental: No effects

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, 5.6.10/01	[redacted]	1996b	Amendment 001 to Glyphosate Acid: Developmental Toxicity Study in the Rat [redacted] Data owner: Syngenta Report No.: [redacted]4819 Date: 2002-11-20 GLP: yes Not published

Guideline: OECD 414 (2001): OPPTS 870.3700 (1998): 2004/73/EC B.31 (2004)

Deviations: None

Dates of experimental work: 1995-05-17 to 1996-03-26

Excecutive summary

In a developmental study, groups of time-mated female rats of the Alpk:APfSD (Wistar-derived) strain were dosed by gavage with 0, 250, 500 or 1000 mg glyphosate acid/kg/day using deionised water as a vehicle. The day of mating was designated day 1 of gestation. The rats were dosed on days 7-16 (inclusive) of gestation which thus included the period of major organogenesis. On day 22 of gestation the rats were killed and their uteri examined for live foetuses and intra-uterine deaths. The foetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

There was no evidence of maternal toxicity attributable to glyphosate acid as assessed by the clinical condition of the animals during the study, their bodyweight gain and food consumption and the type and incidence of macroscopic findings *post mortem*.

There was no evidence of developmental toxicity attributable to glyphosate acid as assessed by the number, growth and survival of the foetuses. Observation of the external appearance of the foetuses, examination of the viscera and assessment of the skeletons revealed no treatment-related findings.

The dose level of 1000 mg glyphosate acid/kg/day was the no observed effect level in this study for both maternal and developmental effects.

I. MATERIALS AND METHODS

Materials:

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6% w/wa.i
CAS#:	Not reported
Stability of test compound:	Confirmed

Vehicle and/or positive control: Deionised water

Test Animals:

Species	Rat
Strain	Alpk:APfSD Wistar-derived
Age/weight on arrival	Approximately 11 weeks / 210 - 303 g
Source	[redacted] UK.
Housing	Individually
Acclimatisation period	Not applicable
Diet	CRL diet ([redacted] UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 21 ± 2°C Humidity: 40 - 70% Air changes: 25 – 30 changes / hour Photoperiod: 12 hours light / 12 hours dark

B: STUDY DESIGN AND METHODS:

In-life dates: Start: Not reported End: Not reported (QA audits conducted between May 1995 and March 1996)

Mating procedure: Virgin female rats were paired overnight (at the Breeding Unit) with males of the same strain. On the following morning, vaginal smears from these females were examined for the presence of sperm. The day when spermatozoa were detected was designated day 1 of gestation and, on this same day, successfully mated females were delivered to the experimental unit at [redacted].

Animal assignment: A total of 96 mated females was supplied over a two week period. Twelve female rats were supplied on each of eight days. The study was divided into twenty four replicates (randomised blocks) with each replicate containing one rat from each group. Animals were randomly assigned to test groups as shown in the following table.

Table 5.6-23: Animal numbers and treatment groups

Dose level of Glyphosate acid (mg/kg bw/day)			
0 (control)	**250**	**500**	**1000**
1 - 24	25 - 48	49 - 72	73 - 96

Dose selection rationale: The dose. levels selected for this study were based on a dose range finding study in the pregnant rat. The highest dose level of 1000 mg/kg/day is the limit dose for this type of study.

Dose preparation and analysis: Glyphosate acid was administered in deionised water and the concentration was adjusted to give a constant volume of 1 mL/100 g bodyweight for each dose level. An appropriate amount of deionised water was added to a weighed amount of test substance (adjusted for purity) to provide each preparation. One preparation per concentration (ie 25, 50 and 100 mg/mL) was made. Each preparation was thoroughly mixed before being subdivided into aliquots. The control substance was also dispensed into aliquots. The aliquots were stored at room temperature and fresh aliquots were used for each day of the study.

A sample of each preparation was analysed prior to the start of dosing to verify the achieved concentrations of glyphosate acid in deionised water. Samples of the lowest concentration of dosing formulation was analysed to confirm the homogeneity of glyphosate acid in deionised water. The homogeneity of the 100 mg/mL formulation was not determined as part of this study and the data have been obtained from a preliminary study ([redacted] *1995*) for which the method of preparation of the dosing formulations was the same. The chemical stability of glyphosate acid in deionised water was determined by re-analysis of the lowest and highest concentrations of dosing formulation after an interval of 26 days.

Concentration analysis results: The achieved concentrations of glyphosate acid in deionised water were within 5% of nominal concentrations.
Homogeneity results: The homogeneity of glyphosate acid in deionised water at concentrations of 25 mg/mL and 100 mg/mL was within 5% of the overall mean.
Stability results: The stability of the 25 mg/mL and the 100 mg/mL formulations was satisfactory over a period of 26 days which exceeded the period of use in this study.

Dosage administration: All animals were dosed once daily from days 7 – 16 (inclusive) of gestation with 1mL of dosing formulation per 100 g bodyweight using a disposable syringe and a plastic nelaton catheter. The volume given to each animal was adjusted daily according to bodyweight. Control animals received the appropriate volume of deionised water. Dosing was performed in group order with all animals receiving the same dose level being dosed sequentially.

Observations:
Maternal observations: All animals were observed on arrival to ensure that they were physically normal externally and were subsequently observed at least twice each day. Any changes in behaviour or clinical condition were recorded daily during the study.

Bodyweight: The bodyweight of each animal was recorded on arrival and on days 4, 7-16 (inclusive) and on days 19 and 22 of gestation.

Food consumption: The amount of food consumed by each animal over three day periods was measured by giving a weighed quantity of food contained in a glass jar on days 1, 4, 7, 10, 13, 16 and 19 and calculating the amount consumed from the residue on days 4, 7, 10, 13, 16, 19 and 22, respectively.

Terminal investigations: One rat requiring euthanasia was killed by over-exposure to halothane Ph. Eur. vapour and given a macroscopic examination *post mortem*.

On day 22 of gestation the animals were killed by over-exposure to halothane-Ph. Eur. vapour and a macroscopic examination *post mortem* was performed. The uterus from any animal without clear evidence of implantation was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred.

For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries and uterus were then examined and the following data recorded:-

Number of *corpora lutea* in each ovary
Number and position of implantations subdivided into:
a) live foetuses
b) early intra-uterine deaths (decidual or placental tissue only)
c) late intra-uterine deaths (embryonic/foetal tissue plus placental tissue)
Individual foetal weights

The implantations were assigned letters of the alphabet to identify their position *in utero* starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. In addition, each foetus was weighed and individually identified within the litter by means of a cardboard tag. After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 ml of 200 mg/mL pentobarbitone sodium solution.

Percentage pre-implantation loss and percentage post-implantation loss were calculated.

$$\% \text{ pre-implantation loss} = \frac{\text{number of } \textit{corpora lutea} - \text{number of implantations}}{\text{number of } \textit{corpora lutea}} \times 100$$

$$\% \text{ post-implantation loss} = \frac{\text{number of implantations} - \text{number of live foetuses}}{\text{number of implantations}} \times 100$$

Foetal observations: An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70% industrial methylated spirits. After approximately 24 hours the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70% industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed. The individual bones of the *manus* and *pes* were assessed and the result converted to a six point scale.

The observations were classified as major (permanent structural or functional deviations considered likely to be incompatible with survival or rarely seen) or minor defects or variants (small, generally transient deviations considered compatible with survival). The difference between the minor defect and variant classification is the frequency of occurrence in the control population of rats of this strain.

Statistical analyses: Data relating to animals which were non-pregnant, totally resorbed their litters or died intercurrently were excluded from the statistical analysis.

Maternal bodyweight during the dosing and post-dosing periods was considered by analysis of covariance on initial (day 7) bodyweight.

Maternal food consumption during the dosing and post-dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by analysis of variance.

Maternal-performance data (excluding the animal with undetermined pregnancy status), the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test.

Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external/visceral defects, minor external/visceral defects, external/visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:-

- Percentages were analysed by analysis of variance following the double arcsine transformation of ***Freeman and Tukey (1950)***
- the proportion of foetuses affected and with the exception of male foetuses the proportion of litters affected were considered by Fisher's Exact Test.

All analyses were carried out in ***SAS (1989)***. For Fisher's Exact Test the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.

All statistical tests were two-sided.

RESULTS

Maternal toxicity:
Mortality and clinical signs: One control animal was killed on day 7 as a result of being misdosed. Excess watery fluid in the thoracic cavity and dark red areas on the surface of the lung lobes were observed at examination *post mortem*. The pregnancy status of the animal was not determined.

There were no changes in the clinical condition of the animals given glyphosate acid which were considered to be treatment-related.

Bodyweight: There was no effect of glyphosate acid on maternal bodyweight.

Table 5.6-24: Intergroup comparison of maternal bodyweight (g) (selected timepoints, adjusted means for days 8 and 22)

	Dose level of glyphosate acid (mg/kg/day)			
day	**0 (control)**	**250**	**500**	**1000**
1	255.6	255.5	253.5	252.8
8	288.2	288.1	288.0	287.5
22	406.4	410.1	411.1	408.6

Food consumption: There was no adverse effect of glyphosate acid on maternal food consumption. The amount of food consumed by the animals given 1000 mg glyphosate acid/kg/day was marginally lower during the dosing period but differences from the controls were not statistically significant.

Table 5.6-25: Intergroup comparison of food consumption (g/day) (selected timepoints)

day	Dose level of glyphosate acid (mg/kg/day)			
	0 (control)	250	500	1000
1-4	23.9	24.6	24.6	23.2
13-16	33.2	33.4	33.7	31.9
19226	29.5	31.6*	30.5	30.5

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

Sacrifice and pathology:
Gross pathology: There were no macroscopic findings which were considered to be related to the administration of glyphosate acid.

Developmental Toxicity: There was-no effect of glyphosate acid on the number, growth or survival of the foetuses *in utero*.

Table 5.6-26: Intergroup comparison of maternal performance

Observation	Glyphosate acid (mg/kg/day)			
	0 (control)	250	500	1000
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	23	24	23	24
#pregnancy status not determined (intercurrent death)	1	0	0	0
Gravid uterus weight (g)	89.7	87.2	91.3	89.9
#Intercurrent deaths	0	0	0	0
#aborted	0	0	0	0
# totally resorbed at termination	0	0	0	1
Corpora Lutea/Dam	15.8	15.7	15.5	15.5
Implantations/Dam	14.4	12.9*	14.1	13.6
Total # Litters (viable)	22	24	23	23
Live Foetuses/Dam	12.9	12.4	13.1	12.9
Early (Proportion of litters affected)	8.7	3.4**	6.2	5.5
Late (Proportion of litters affected)	1.3	0.5	1.6	0.3
Litter Weight (g)	62.4	61.2	64.3	63.6
Mean Foetal Weight (g)	4.86	5.02	4.95	4.96
Sex Ratio (% Males per litter)	51.9	54.1	53.3	51.0
Preimplantation Loss (%)	8.7	18.0**	8.8	12.0
Postimplantation Loss (%)	9.9	4.0**	7.8	5.8*

* 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)

Major defects: The incidence of foetuses with major defects was 1/284, 1/297, 1/301 and 2/296 in the control and 250, 500 and 1000 mg glyphosate acid/kg/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The defects were dissimilar in type and of single incidence.

Minor defect: The proportion of foetuses with minor external/visceral defects and the proportion of foetuses with minor skeletal defects were similar for all groups. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Variants: The proportion of foetuses with external/visceral variants and the proportion of foetuses with skeletal variants were lower in the glyphosate acid treated groups than in the control group. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Manus and pes assessment: There was no effect of glyphosate acid on the ossification of the *manus* or *pes*.

III. CONCLUSION

The dose level of 1000 mg glyphosate acid/kg/day was the no observed effect level in this study for both maternal and developmental effects.

Annex point	Author(s)	Year	Study title
IIA, 5.6.10/02	[redacted]	1995	HR-001: Teratogenicity Study in Rats. [redacted] Data owner: Arysta LifeScience Study No.: [redacted]94-0152 Date: 1995-07-21 GLP: yes not published

Guideline: Japan MAFF Guidelines 59 NohSan No.4200, 1985
U.S. EPA FIFRA Guidelines Subdivision F, 1984

Deviations: None

Dates of experimental work: 1995-01-23 to 1995-06-26

Executive Summary

A teratogenicity study was conducted to evaluate the potential maternal and developmental toxicity of HR-001 in rats. The test substance was suspended in 0.5% aqueous solution of sodium carboxymethylcellulose and was administered orally with stomach tube to 24 copulated Crj:CD (SD) female rats per group at dose levels of 0, 30, 300 or 1000 mg/kd/day from days 6 to 15 of gestation.
No adverse effects related to test substance treatment were observed for maternal rats in the 30 and 300 mg/kg groups. In the 1000 mg/kg group, 20 of 22 pregnant females showed slightly loose stool during the dosing period and/or on the following day of last dosing, and the incidence was statistically significantly high. No treatment related changes were observed in the body weights and body weight gains. Food consumption in this group was significantly decreased at an interval of days 6-9 of gestation (early dosing period).
Observation at cesarian section revealed no treatment related adverse effects in any of the parameters tested, i.e. gross pathology findings, gravid uterine weights, numbers of corpora lutea, implants and live foetuses, percent incidences of resorptions and foetal deaths, foetal sex ratio, foetal body weights and placenta weights.
In the teratological examination foetuses, no treatment related malformations or variations were noted in any of the treated group.

Based on the results, the No-Observable Adverse Effect level (NOAEL) was set at 300 mg/kg/day for maternal rats, and that 1000 mg/kg/day is not teratogenic to SD rat foetuses.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

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

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	SPF Crj:CD (SD)
Source:	[redacted] Japan, [redacted]
Age:	13 weeks
Sex:	Males and females
Weight at dosing:	Males: 380 – 450 g, females: 267 – 322 g
Acclimation period:	11 days
Diet/Food:	Certified diet [redacted], *ad libitum*
Water:	Filtered and sterilized water, *ad libitum*
Housing:	By pair in aluminium cages with wire-mesh floors for mating period; Individually for copulated females in aluminium cages with wire-mesh floors.
Environmental conditions:	Temperature: 24 ± 2°C
	Humidity: 55 ± 10%
	Air changes: 12/hour
	12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-03-23 to 1995-06-26

Animal assignment and treatment:
Vaginal smears were taken from females for microscopic examination. Females showing proestrus or estrus vaginal smears were paired overnight with males on a 1:1 basis. The females were examined next morning for the presence of vaginal plugs and sperm in vaginal smears and considered to copulate when vaginal plugs and/or sperm were observed. These mating procedures were repeated for 4 consecutive days. Four test groups were set. The test substance was administered orally with a stomach tube to 10 copulated Crj:CD (SD) female rats per group at dose levels of 0, 300, 1000 mg/kg/day from day 6 to 15 of gestation.

Clinical observations
Each female was observed for clinical signs and mortality at least once daily during the pre-dosing and post-dosing periods and at least twice daily during the dosing period.

Body weight
Individual body weights were recorded on days 0, 6-15 (daily during the dosing period) and 20 of gestation. Adjusted body weight gains were calculated by subtracting the gravid uterine weight from the body weight value on day 20 of gestation.

Sacrifice and pathology
All surviving females were euthanized by overdosage of ether inhalation and cesarian section was performed on day 20 of gestation. Each female was necropsied. The ovaries and uterus were removed and the gravid uterine weight and numbers of copora lutea and implants were recorded. Then the uterus was opened and the numbers of live and dead foetuses were recorded with their positions in the uterine horns. Resorbed embryos or dead foetuses were classified into implantation sites, placental remnants or macerated foetuses (including dead foetuses) according to developmental stage in which resorptions or deaths occurred. When no uterine implants were grossly apparent, the uterus was stained with 10% ammonium sulphide solution to detect very early resorptions. The weights of each live foetus and of each placenta were determined and recorded. Live foetuses were sexed and were euthanized by an intraperitoneal injection of pentobarbital sodium solution for examination of external abnormalities. The eyes were examined for alterations after removing the palpebral skin. Then the foetuses were examined for visceral and skeletal abnormalities.

Statistics
Variance analysis using Bartlett's test was evaluated for body weights, adjusted body weights, body weight gains and food consumption of maternal rats, numbers of corpora lutea, implants and live foetuses, and weights of gravid uteri, foetuses and placentas.

II. RESULTS AND DISCUSSION

A. CLINICAL OBSERVATIONS
During the pre-dosing period, clinical observation revealed no abnormalities in any groups.
During the dosing period, no abnormalities were observed in maternal rats of the control group. In the 30 and 300 mg/kg groups, one of two maternal rats had hair loss or scabs on the skin which have been usually observed in the historical control rats. In the 1 000 mg/kg group, 20 out of 22 pregnant females showed slightly loose stool and the increase in its incidence was statistically significant.
During the post-dosing period, slightly loose stool was also observed on the following day of last dosing (day 16 of gestation) in 9 out of 20 females that showed this finding during the dosing period in the 1 000 mg/kg group. Another finding observed during this period was hair loss in 1-2 maternal rats in each treated group.
No deaths occurred during the study in any groups.

B. BODY WEIGHT
No significant differences were found in the mean body weights and the mean adjusted body weights of maternal rats between the control groups and any of the treated group.
No significant differences were found in the mean body weight gains of maternal rats between the control group and any of the treated groups.

C. FOOD CONSUMPTION AND COMPOUND INTAKE
No significant differences were found in the mean food consumption of maternal rats between the control group and any of the 30 and 300 mg/kg groups. In the 1 000 mg/kg group, lower and higher values were observed in the mean food consumption at intervals of days 6-9 of gestation (early dosing period) and days 15-20 of gestation (post-dosing period), respectively, and the differences from the corresponding controls were statistically significant.

D. NECROPSY

Gross pathology at cesarian section

Gross pathological examination of maternal rats at cesarean section revealed several findings such as hair loss and pelvic dilatation in the kidney in 1-2 animals in all groups including the control group. These findings were not considered to be due to test substance treatment.

Ovaries and uterus

Out of 24 copulated females, 23, 24, 24 and 22 were proved to be pregnant in the control, 30 mg/kg, 300 mg/kg and 1 000 mg/kg groups, respectively.

No significant differences were found in the mean gravid uterine weights and the mean numbers of corpora lutea and implants between the control group and any of the treated group.

E. FOETUSES

Number of live foetuses and percent incidences of resorptions and foetal deaths

There were no significant differences in the mean number of live foetuses and the mean percent incidence of resorptions and foetal deaths between the control group and any of the treated groups.

Sex ratio, fetal body weights and placental weights.

There were no significant differences in the foetal sex ratio, the mean foetal body weights and the mean placental weights between the control group and any of the treated group.

Findings in external, visceral and skeletal examination

External malformations observed were short tail in a foetus of the 30 mg/kg group and microphthalmia in a foetus of the 1 000 mg/kg group.

Visceral examination revealed two types of malformations: right aortic arch in a foetus of the 300 mg/kg group and ventricular septal defects in a foetus of each of the 300 and 1 000 mg/kg groups.

Visceral variations were observed in all groups including the control group. The types and number in fetuses were thymic remnant in the neck, dilatation of the renal pelvis and left umbilical artery in 16-26, 1-2 and 0-3, respectively.

Skeletal examination revealed three types of malformations: splitting of the ossification centers of the thoracic vertebral bodies in 2, 1 and 2 foetuses in the control, 300 mg/kg and 1 000 mg/kg groups, respectively, asymmetry of the sternebrae with sterno-costal joint displacement in a foetus of the 300 mg/kg group, and fusion of the sternebrae in a foetus of the 300 mg/kg group, and fusion of the sternebrae in a foetus of the 1 000 mg/kg group.

Skeletal variations were observed in all groups including the control group. The types and the number in foetuses were cervical ribs shortening of the 13th ribs, lumber ribs, sacralization of the lumber vertebra and asymmetry and/or splitting of the sternebrae in 0-1, 0-1, 1-11, 0-1 and 3-5, respectively

III. CONCLUSION

Based on these results, no observable effect level and minimal toxic level in the teratogenicity study with technical glyphosate in SD rats were established as follows.

	Maternal rats	Foetal rat
No observable effect level	300 mg/kg/day	1000 mg/kg/day
Minimal toxic level	1000 mg/kg/day	-

It is also concluded that the highest dose level of 1000 mg/kg/day of HR-001 is not teratogenic to SD rat foetuses.

IIA 5.6.11 Teratogenicity test by the oral route in the rabbit

The 2001 EU glyphosate review concluded that the NOEL for developmental effects was 350 mg/kg bw/day ([redacted] 1980) and that effects on the foetuses were only observed in the presence of marked maternal toxicity. Overall the previous evaluation determined that glyphosate was not teratogenic in rabbits. Three additional studies have been included in this submission. The results from these studies are consistent with the data that has been previously reviewed, the pattern of maternal toxicity is consistent and effects on the foetuses were only observed in the presence of maternal toxicity.

In rabbits, glyphosate exposure via oral gavage led to clinical signs of toxicity in does consistent with gastro-intestinal disturbances. Rabbits were more sensitive to oral gavage dosing than other species. Clinical signs observed included diarrhoea/soft faeces, reduced faecal output, reduced body weights, reduced food consumption and increased mortality. Table 5.6-22 details maternal toxicity observed following glyphosate treatment via oral gavage. These effects are consistent with gastro-intestinal stasis (ileus) likely caused by the mucosal membrane irritation potential of glyphosate acid. Rabbits (caecotrophs) are particularly sensitive to disruption of the gastro-intestinal tract. Stress and other environmental factors can lead to the normal muscular contractions of the stomach and intestines being greatly diminished, which in turn leads to disruption of the normal intestinal/caecum bacterial flora. It is likely that the mucosal membrane of the rabbit gastro-intestinal tract is irritated by bolus administration of glyphosate acid. Consequently the associated stress leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies by [redacted] (1995); [redacted] (1996) and [redacted] (1996), such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. This finding appears to be relevant to only hindgut fermenters as it is not seen in rats or dogs following administration of an oral bolus dose.

Further evidence, that these findings are related to gastro-intestinal disturbance comes from the [redacted] (2012) study that measured dermal absorption *in vitro* through rabbit skin. This study demonstrated that systemic exposure to glyphosate acid following percutaneous administration in the [redacted] study (1982) was equivalent to the systemic exposure following oral gavage administration in many of the rabbit developmental toxicity studies where clinical signs of soft faeces, reduced faecal output, reduced body weights and mortality were observed.

Table 5.6-27: Summary of maternal toxicity in glyphosate acid developmental toxicity in rabbits

Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality#	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
Japanese White ([redacted] 1995)	300	1/18	1/17	0/17	Lower than control	Erosion in the stomach, hair bolus in stomach, watery contents in large intestine/caecum	15/18
NZW [redacted] 1996)	400	2/18	0/16	2/18	Initial loss and then statistically significant lower bodyweight gain than control	Fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended.	16/18
	200	1/18*	0/16	2/18	Lower body weight gain than control	-	16/18

Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality#	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
NZW ([redacted] 1996)	300	2/20	19/20	9/20	Reduction in maternal body weight gain	Hair-like substance in the stomach	17/20
	175	2/20	11/20	9/20	Reduction in maternal body weight gain	-	17/20
Dutch Belted ([redacted] 1980)	350	10/16	16/16	not recorded	No effect	-	6/16
	175	2/16	slight increase in incidence	not recorded	No effect	-	11/16
NZW ([redacted] *al.*, 1991)	450	1/20	13/20	12/20	No effect	-	13/20
	150	0/16	5/16	11/16	No effect	-	15/16
NZW ([redacted] 1993)	500	8/15	12/15	0/15	Statistically significant lower body weights than controls	-	6/15
NZW ([redacted] 1989)	500	0/15	0/15	0/15	Statistically significant lower body weight gain	-	12/15

- x/y: number of animals affected/total number of animals in group
* - due to mal-dosing.

Foetotoxicity/developmental toxicity occurred at or above doses that caused maternal toxicity. Most indications of developmental toxicity were reduced ossifications of skull, phalangeal and sternebral bones, which are typically seen in the litters of pregnant animals that do not eat well and lose weight during pregnancy (see expert statement of [redacted] 2012[6]). The importance of this observation should not be misconstrued to mean that maternal toxicity in those cases was the proximate agent that injured the fetus, but rather that if exposures to the causative agent are kept below the doses that cause maternal toxicity, the developing offspring are protected. The lowest observed effects on the foetuses occurred at 300 mg/kg bw/day and were characterized by delayed ossification and decreased foetal weights ([redacted] 1996). The relevant NOAEL for foetotoxicity is 250 mg/kg bw/day.

A report from an independent source ([redacted] 2011) has claimed that congenital malformations, especially of the cardiovascular system, were caused by glyphosate exposure in this same series of studies. A variety of malformations were reported across the database of glyphosate studies; these included:

- Dilated aorta/narrow pulmonary artery
- Narrow aorta/dilated pulmonary artery
- Interventricular septal defect
- Cardiomegaly
- Single ventricle
- Retro-esophageal right subclavian artery
- Interrupted aorta
- Right subclavian artery arising from aortic arch
- "Seal-shaped" heart

[6] [redacted] 2012:Review and evaluation of the rabbit developmental toxicity database on glyphosate, with particular attention to cardic malformations, [redacted] 2012-04-30, 1200798.000.A0T0.0412.AW01

If glyphosate does cause congenital heart defects, it would be anticipated that the prevalence of congenital heart defects would be increased and one would expect the malformation rate to increase with increasing dose until the pregnant does would become intoxicated or the fetuses would die. The malformations occurred at a low incidence across all dose groups; they did not exhibit a positive dose-response; and often clusters of the malformations occurred in the same fetuses.

The incidence of aorticopulmonary septum-related defects in the combined control groups was 1/879 (0.1%); in the combined glyphosate-treated groups the incidence was 12/2250 (0.5%). One half of the malformed fetuses was found in litters exposed to the highest doses (450 and 500 mg/kg/day), which also experienced severe maternal toxicity including maternal deaths, abortions, and weight loss. If these groups are not considered because of the potential confounding factor introduced by maternal health issues, the incidence of the defects is 6/2049 (0.3%). These data show that the overall incidence of aorticopulmonary septum-related defects in offspring from mothers exposed to glyphosate at doses below those that cause severe maternal toxicity is similar to that seen in non-exposed rabbits.

The other prominent cardiovascular malformation is dilated heart. All observations of this finding (among both control and treated groups) occurred in a single laboratory ([redacted] 1993). This study has several weaknesses including a small number of litters available for examination due to low pregnancy rates and maternal deaths in the mid- and high-dose groups. None of the other six studies reported dilated hearts. Neither the criteria used to diagnose dilated heart nor measurements of the hearts were provided, so it is not possible to directly compare the dilated heart findings to the hearts of the more than 2800 fetuses in the other studies. It is possible that the observation of dilated hearts is due to overly stringent inspection compared to criteria used by other laboratories.

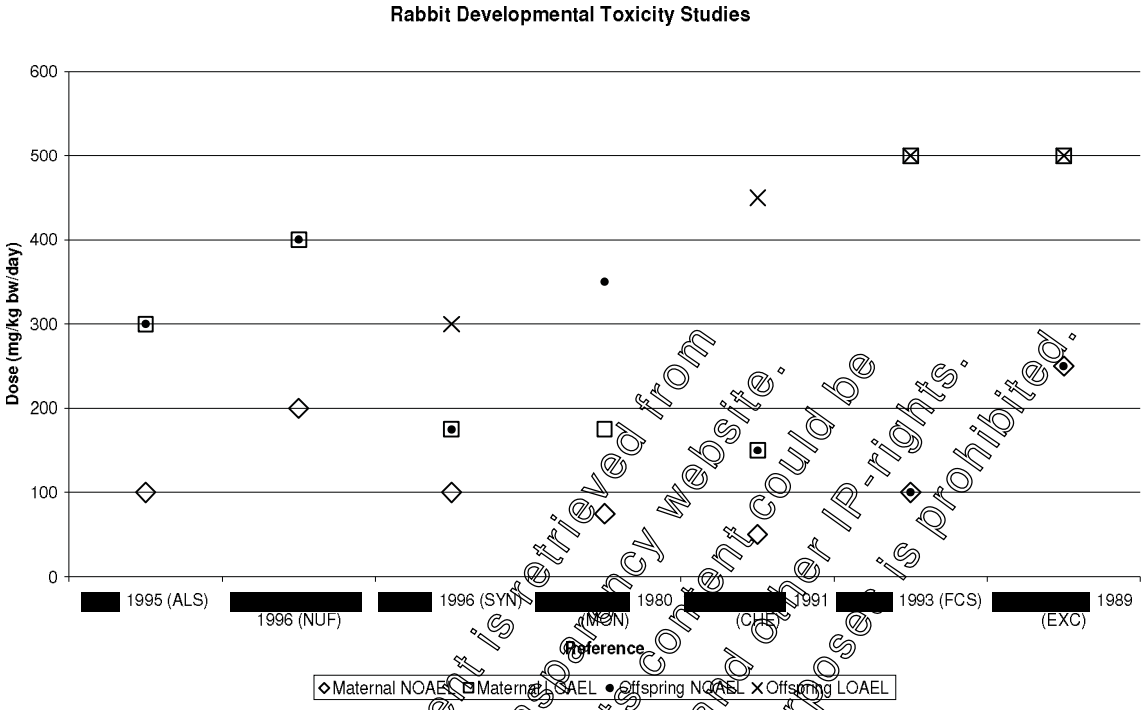
Taken together, overall data regarding potential cardiovascular malformations in the seven rabbit developmental toxicology studies do not support the contention that there is a clear compound related effect on the foetal heart. A comprehensive review of all GTF rabbit developmental toxicity studies was conducted by experts in the field of developmental toxicology and this report is referenced in Doc L and submitted in Doc K (see expert statement of [redacted] 2012b).

Table 5.6-28: Summary of developmental toxicity in rabbits

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL [mg/kg bw/day (ppm)]		LOAEL Targets / Main effects
				Maternal	Offspring / developmental	
Studies not reviewed in the 2001 evaluation	IIA 5.6.11/01 [redacted] 1995 (ALS)	Developmental toxicity, gavage, rabbit, Japanese White	0, 10, 100, 300	100	300	Maternal: defecation of loose stool and subsequent abortion or premature delivery and reduced body weight Offspring: no effects
	IIA 5.6.11/02 [redacted] 1996 (NUF)	Developmental toxicity, gavage, rabbit, NZW	0, 50, 200, 400	200	400	Maternal: increased mortality, reduced body weight and diarrhoea (at the high dose), clinical signs (reduced faecal output, scours), reduced body weight at the mid-dose Offspring: no treatment-related effects

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL [mg/kg bw/day (ppm)]		LOAEL Targets / Main effects
				Maternal	Offspring / developmental	
Studies not reviewed in the 2001 evaluation	IIA 5.6.11/03 [redacted] 1996a (SYN)	Developmental toxicity, gavage, rabbit, NZW	0, 100, 175, 300	100	175	Maternal: diarrhoea, reduced faecal output, staining of genital area at the high and mid-dose dose Offspring: delayed ossification, decreased body weights at the high dose
Studies from the 2001 evaluation	Annex B.5.6.2.2.2 Glyphosate Monograph IIA 5.6.11/04 [redacted] 1980 (MON)	Developmental toxicity, gavage, rabbit, Dutch Belted	0, 75, 175, 350	75	350	Maternal: diarrhoea and soft stool, and increased mortality at the high dose Offspring: No treatment-related effects
	Annex B.5.6.2.2.2 Glyphosate Monograph IIA 5.6.11/05 [redacted] 1991a (CHE)	Developmental toxicity, rabbit, NZW	0, 50, 150, 450	50	150	Maternal: reduced food consumption, soft/liquid faeces, reduced body weight Developmental: increased embryo/foetal deaths and post-implantation loss
	Annex B.5.6.2.2.1 Glyphosate Monograph IIA 5.6.11/06 [redacted] 1993 (FSG)	Developmental toxicity, rabbit, NZW	0, 20, 100, 500	100	100	Maternal: increased mortality, soft stool/ liquid faeces Developmental: general signs of secondary toxicity (e.g. incomplete ossification)
	Annex B.5.6.2.2.2 Glyphosate Monograph IIA 5.6.11/07 [redacted] 1989 (EXC)	Developmental toxicity, gavage, rabbit, NZW	0, 125, 250, 500	250	250	Maternal: body weights and food consumption signify-cantly reduced; 2 abortions were noted in the high dose group Developmental: mean number of viable im-plants significantly reduced, mean number of external, visceral and skeletal malfor-mations, and mean number of variations significantly increased.

Figure 5.6-1: Results of developmental toxicity studies in rabbits



Tier II summaries are presented for all available studies on developmental toxicity in the rabbit to allow for a robust weight of evidence evaluation of endpoints.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/01	[redacted]	1995	HR-001: A Teratogenicity Study in Rabbits [redacted] Data owner: Arysta Life Sciences Study no.: [redacted] 94-0153 Date: 1995-07-21 GLP: yes not published
Guideline:			OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations:			None
Dates of experimental work:			1995-03-31 to 1995-06-09

Executive Summary

HR-001 was administered by gavage to three groups of 18 artificially inseminated Japanese White rabbits each, at doses of 10, 100 and 300 mg/kg bw/day from Gestation Day 6-18 (artificial insemination = Day1). A further group of 18 animals was exposed to the vehicle to serve as control.
No adverse effects related to test substance treatment were observed in any animals of the low and mid dose group. In the high dose group, defecation of loose stool and subsequent abortion or premature delivery was considered to be related to test substance treatment with regard to the results of the preliminary study.

Mean maternal body weights and body weight gains on Days 16-24 of gestation in the high dose group decreased slightly although the differences from controls were not statistically significant.
Examinations at caesarean sectioning demonstrated no significant differences in the gravid uterine weights and the numbers of corpora lutea and implants between the control and the treated groups. The mean number of live foetuses, mean percent incidences of resorptions and foetal deaths, foetal sex ratios, mean foetal body weights, and mean placental weights in the treated groups were comparable to those in the control group.
Teratological examinations demonstrated no test substance treatment-related external, visceral and skeletal abnormalities in any foetuses in any treated groups.

Conclusion:
The oral administration of HR-001 to artificially inseminated rabbits by gavage Gestation Day 6-18 resulted in treatment-related changes at 300 mg/kg bw/day. Therefore the NOAEL was considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for offspring was considered to be 300 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical; Code: HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: 0.5% carboxymethylcellulose

3. Test animals:

Species:	Rabbit
Strain:	Japanese White rabbits Kbl:JW, SPF
Source:	[redacted]
Age:	18 weeks (females); 5-50 month (males)
Sex:	Males and females
Weight at dosing:	3.32 – 4.08 kg
Acclimation period:	10 days
Diet/Food:	[redacted], *ad libitum* (females) / *120 g/day* (males)
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium cages with wire-mesh floors.
Environmental conditions:	Temperature: 22 ± 2°C Humidity: 55 ± 10% Air changes: 15/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-03-31 to 1995-06-09

Animal assignment and treatment:
In a teratogenicity study groups of 18 Japanese White female rabbits received doses of 0, 10, 100 and 300 mg/kg bw/day test substance in carboxymethylcellulose by gavage from Gestation Day 6-18 after artificial insemination performed on 12 or 16 females each day for 5 consecutive days. The dose levels were chosen based on results of a preliminary teratogenicity study.

Diet preparation and analyses
For each dose level, dosing solutions were prepared two times during the study by suspending the test substance in purified water with the aid of 0.5% sodium carboxymethylcellulose. For each dose level dosing solutions were analyzed for concentration of the test substance before use.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on Day 0, 6-18, 24 and 27 of gestation. Body weight gains were calculated by subtracting the body weight value on Day 0 of gestation from each value determined on Days 6 through 27 of gestation. Adjusted weights were also calculated by subtracting the gravid uterine weight from the body weight on Day 27 of gestation.

Food consumption
Food consumption of females was determined on alternate days from Day 0 to Day 26 of gestation and on Days 26- 27 of gestation. In each interval, daily food consumption (g/rabbit/day) was calculated for each female by dividing values of total food consumption by the number of days.

Sacrifice and pathology
Females were euthanatized by an injection of an overdose of a pentobarbital sodium solution into the auricular vein on Day 27 of gestation and subjected to caesarean sectioning.
The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. When uterine implants were not grossly apparent, the uteri were stained with 10% ammonium sulfide solution to detect very early resorptions. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters
Live foetuses and their placentas were individually weighed. Live foetuses were uniquely identified by litters. Then they were euthanatized by an intraperitoneal injection of a pentobarbital sodium solution and examined for external abnormalities. The eyes were examined for alterations after removing the palpebral skin. The sex of the foetuses was determined by observation of the gonads.
After these examinations, each foetus was examined for visceral abnormalities. Then the thoracic and abdominal organs were removed and preserved in 10% neutral- buffered formalin along with the ovaries and placentas. The remaining skeletons were fixed in 70% isopropanol, stained with alizarin red S and cleared in 70% glycerin for examination of skeletal abnormalities. After examination, skeletal specimens were stored.

Statistics
The following statistical tests were used to estimate significance of differences between the control group and the treated groups. The data on body weights, adjusted body weights, body weight gains, and food consumption of maternal rabbits, numbers of corpora lutea, implants, and live foetuses, and weights of gravid uteri, foetuses and placentas were evaluated as follows: Equality of variances was first evaluated by Bartlett's test. When group variances were homogeneous, a parametric analysis of variance in one-way classifications was used to determine if any statistical differences exist among groups. If the analysis of variance was significant, Dunnett's t-test or Scheffé's multiple comparison test was performed to detect

any statistically significant differences between the treated groups and their corresponding controls. When Bartlett's test indicated that the variances were not homogeneous, Kruskal-Wallis test was used for detecting any statistical differences among groups and if significant, Dunnett-type mean rank test or Scheffé-type mean rank test was performed to detect statistical differences between the treated groups and their corresponding controls. Fisher's exact probability test was used for the data on the incidences of clinical and gross pathological findings in maternal rabbits, incidences of maternal rabbits having foetuses with malformations and variations, incidences of foetal malformations and variations, and foetal sex ratio, and Mann-Whitney's U-test for the data on the percent incidences of resorptions and foetal deaths.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The test substance was detected at levels of 95-105% of the target concentrations in each dosing solution.

B. FOOD CONSUMPTION

Mean food consumption in the treated groups was comparable to that in the control group throughout the study period.

C. MORTALITY

One rabbit in the high dose group died on Day 20 of gestation without showing any clinical signs.

D. CLINICAL OBSERVATIONS

During the treatment period one animal each showed hair loss (forelimb) and scab on the auricle, respectively in low and mid dose group, respectively (see Table 5.6-29). In the high dose group four animals showed loose stool and two showed soiled fur in the perianal region that was considered to be an alteration caused by defecation of loose stool. The incidence of loose stool was significantly high when compared with the control.

During the post-dosing period, two and one animal in the control group showed loose stool and red material on the tray, respectively. In the low dose group, hair loss (forelimb) was found in one animal and loose stool in another. Besides these findings, one dam aborted on Day 20 of gestation, and another one prematurely delivered on Day 27 of gestation. In the mid dose group only one animal showed hair loss in the lower abdominal region. In the high dose group, two animals out of four, that had shown loose stool during the dosing period, still showed this alteration, and one animal out of these two aborted on Day 26 of gestation. Although loose stool disappeared from the two other dams, the first prematurely delivered on Day 27 of gestation and the second had hair loss (dorsal region).

Considering the results of the preliminary study, defecation of loose stool and subsequent abortion or premature delivery observed in the highest dose group were considered to be related to test substance treatment.

Table 5.6-29: Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group[#]			
	Control (0 mg/kg/day)	Low (10 mg/kg/day)	Mid (100 mg/kg/day)	High (300 mg/kg/day)
No abnormalities detected	18/18 (0)	16/17 (1)	15/16 (2)	13/17 (0)
Hair loss	0/18 (0)	1/17 (0)	0/16 (0)	0/17 (0)
Scab on the auricle	0/18 (0)	0/17 (0)	1/16 (0)	0/17 (0)
Soiled fur in the perianal region	0/18 (0)	0/17 (0)	0/16 (0)	2/17 (0)
Loose stool	0/18 (0)	0/17 (0)	0/16 (0)	4/17 (1)*

[#] x/y: number affected / total number of animals in group

* Significantly different from control at $p < 0.05$.

Figures in parentheses represent the number of animals having no grossly observable conceptus. These animals were excluded from statistical evaluation.

E. BODY WEIGHT

Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, although differences from controls were not statistically significant, the mean values on Days 16-24 of gestation were somewhat lower than those in the control group.

F. PATHOLOGY

Necropsy

Necropsy of maternal animals aborted, prematurely delivered or found dead on the study noted no abnormalities in the rabbits in the low dose group. In the high dose group, the aborted rabbit had yellow-coloured adipose tissue, a hair bolus in the stomach, watery contents in the large intestine and accentuated lobular pattern in the liver. The prematurely delivered rabbit in the high dose group had soiled fur in the perianal region, erosion in the stomach, a hair bolus in the stomach, and watery contents in the caecum. In the dead rabbit, pale liver and ascites (red) in the abdominal cavity were found; however, the cause of death was not known.

Gross pathological findings observed in animals which survived to termination of the study were: hair loss in the lower abdominal or dorsal region in one animal in each of the mid and high dose groups; hair bolus in the stomach in one animal each of the control and low dose groups. The occurrence of these gross pathological findings was low, and considered to be unrelated to test substance treatment.

Observations on the ovary and uterus

In the control, low, mid and high dose groups, 18, 16, 18, and 15 females, respectively, survived to termination of the study and were proven to be pregnant. However, one, two and one females in the low, mid and high dose group, respectively, had no grossly observable conceptus while implantation sites were detected by uterine staining with a 10% ammonium sulfide solution, indicating very early resorptions;. all data from these females were excluded from subsequent calculations.

Examinations of uterine contents demonstrated no abnormalities in all groups including the control. Mean gravid uterine weights and mean numbers of corpora lutea and implants were comparable between the control and the treated groups.

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

No statistically significant differences were noted in the mean number of live foetuses and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

Sex ratio, foetal body weights and placental weights

No statistically significant differences were noted in the sex ratios, mean foetal body weights, mean placental weights, mean number of live foetuses, and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

External, visceral and skeletal examination

No statistically significant differences were noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the number of litters with malformations was significantly higher than that in the control group (see Table 5.6-30). This increased malformation rate was due to an increase in skeletal malformations, as no external or visceral malformations were noted in foetuses from the high dose group. This was considered to be a sporadic alteration rather than the test substance treatment-related alteration because the types of skeletal malformations observed were inconsistent. Further, a dose-response in the number of foetuses showing skeletal malformations was not evident across dose groups.

With regard to variations, the incidence of total no. of litters with skeletal variations in the 100 mg/kg bw/day group was significantly higher than that in the control group (see Table 5.6-30). This high value was due to a significantly high incidence (87.5% of litters, 27.3% of the foetuses) of lumbar ribs in this dose group when compared with the control (72.2% of litters, 16.4% of foetuses). The total litter incidence for skeletal variations in the 100 mg/kg/day group was 100%. However, the increased incidence of lumbar ribs in the 100 mg/kg/day group was considered to be a sporadic alteration because the value was within

the historical control range (8.1-35.0% of examined foetuses), and because no such increase was observed in the 300 mg/kg bw/day group (13.4%).

Table 5.6-30: Incidence of fetal malformations and variations in rabbits treated with HR-001

Foetal findings	Dose level (mg/kg bw/day)			
	0	10	100	300
Malformations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	1	3	3	5*
Percentage of litters with malformations (%)	5.5	20.0	18.8	35.7
Skeletal malformations				
Fusion of the frontal/parietal bones	0	1	0	2
Fissure of the parietal bone	0	0	3	0
Hypoplasia of the interparietal bone	0	1	0	0
Splitting of the parietal bones	0	0	3	1
Shortening of the nasal/frontal/mandibular bones	0	0	0	0
Hemivertebra	1	0	0	2
Unilateral ossification centre of the thoracic/lumbar vertebral bodies	0	1	0	0
Bifurcation of the ribs	1	0	0	0
Sternal cleft	0	0	1	0
Splitting of the sternebrae with sternocostal joint displacement	1	2	0	0
Total no. of foetuses with skeletal malformations	1	4	6	5
Percentage of foetuses with skeletal malformations (%)	0.7	3.1	4.0	4.5
Total no. of litters with skeletal malformations	1	3	2	5
Percentage of litters with skeletal malformations (%)	5.5	20.0	12.5	29.4
Variations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	16	14	16	8*
Percentage of litters with variations (%)	88.9	93.3	100	57.1
Skeletal variations				
No. of foetuses examined	140	130	150	112
27 presacral vertebrae	4	1	4	3
27 presacral vertebrae with 13th ribs	12	9	15	12
Cervical ribs	1	3	1	1
Lumbar ribs	23	19	41*	15
Extra ossification centre anterior to the 1st sternebra with costal cartilage joining	0	0	0	1
Total no. of foetuses with skeletal variations	40	32	61*	31
Total no. of litters with skeletal variations	16	12	16	8
Percentage of litters with skeletal variations (%)	88.9	80	100	57.1

* Significantly different from control at $p < 0.05$.

III. CONCLUSION

The oral administration of HR-001 to artificially inseminated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 300 mg/kg bw/day. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for offspring was considered to be 300 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/02	[redacted]	1996	Glyphosate technical: Oral gavage teratology study in the rabbit [redacted] [redacted] UK Data owner: Nufarm [redacted] project no.: 434/020 Date: 1996-07-04 GLP: yes not published

Guideline:	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations:	None
Dates of experimental work:	1995-10-13 - 1995-12-12

Executive Summary
Glyphosate technical was administered by gavage to three groups of 18 mated New Zealand White rabbits each, at doses of 50, 200 and 400 mg/kg bw/day from gestation Day 7-19 (mating = Day 0). A further group of 18 animals was exposed to the vehicle to serve as control.
Individual clinical observations, bodyweight and food consumption were recorded during the study. The females were killed on Day 29 of gestation, examined macroscopically for external and internal malformation. The uteri were examined number of corpora lutea, implantation number, position and type, foetal weights, external appearance and internal visceral anomalies/abnormalities were recorded. All live foetuses were preserved, processed and subsequently examined for skeletal anomalies with the heads of half the offspring preserved and examined for visceral anomalies.
At the high dose level there was evidence of treatment-related effects resulting in one treatment-related death. Clinical signs of toxicity, particularly scours, reduced faecal output and diarrhoea, reduced bodyweight gain and reduced food consumption were seen. At the intermediate dose level similar, but less severe, effects were seen on bodyweight gain. At the low dose level, no treatment related effects were observed. There were no treatment-related effects on the uterine or foetal parameters examined in any dose group.

Conclusion:
The oral administration of glyphosate technical to time-mated rabbits by gavage from Gestation Day 7-19 resulted maternal toxicity at 400 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level. Therefore the NOAEL was considered to be 200 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 400 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	H95D161A
Purity:	95.3%
Stability of test compound:	not reported

2. **Vehicle and/ or positive control:** 1% carboxymethyl cellulose

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted] UK
Age:	17 - 19 weeks
Sex:	Females (time-mated)
Weight at dosing:	2.2 - 4.1 kg
Acclimation period:	At least 4 days
Diet/Food:	[redacted] Standard Rabbit Diet ([redacted] UK) *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with grid floor
Environmental conditions:	Temperature: 20 ± 3°C Humidity: 50 ± 20% Air changes: 15/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-10-13 - 1995-12-17

Animal assignment and treatment in the preliminary study:
Twenty-four time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 0 of gestation. The females were delivered to [redacted] at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 6 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1% carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19. The dose levels were chosen based on results of a preliminary dose finding study with 6 female nulliparous rabbits, where administration of 500 or 1000 mg/kg bw resulted in toxicity signs (scours, fluid filled caecum, stomach ulceration, body weight loss, reduced food consumption). Based on these findings dose levels of ≥ 500 mg/kg bw were considered to be too high for a prolonged study.

Animal assignment and treatment in the main study:
Seventy-two time-mated females were supplied as described for the preliminary study (see above). Groups of 18 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1% carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19.

Dose formulation and analysis
For each dose level, the test material was suspended daily in 1% carboxymethyl cellulose by weighing the required amount into a glass jar and adding vehicle to make the appropriate final volume. Homogeneity was assured by mixing the formulation with a homogeniser. The concentration, stability and homogeneity of the test material were analyzed. The formulation was stable for at least 1 h.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on Day 3, 7, 10, 13, 16, 19, 22, 25 and 29 of gestation.

Food consumption
Food consumption of females was recorded on Days 3 to 7, Days 7 to 10, Days 10-13, Days 13-16, Days 16-19, Days 19-22, Days 22-25 and Days 25-29 of gestation.

Sacrifice and pathology
Females were euthanatized by an i.v. injection of an overdose of sodium pentobarbitone into the auricular vein on Day 29 of gestation, examined for macroscopic abnormalities and subjected to caesarean sectioning. The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters
The foetuses were killed by intrathoracic injection of sodium pentobarbitone. All foetuses were dissected and examined for visceral abnormalities macroscopically. The heads of alternate foetuses were removed and identified using an indelible marker and placed in Bouin's fixative. After a minimum of 14 days, the heads were transferred to 90% industrial methylated spirits (IMS) in distilled water and examined for visceral anomalies under a low power binocular microscope (Van Julsingha and Bennett 1977). All foetuses were identified using colour coded wires and placed in 70% IMS in distilled water. The foetuses were eviscerated, processed and the skeletons stained with alizarin red (Dawson 1926). The foetuses were examined for skeletal development and anomalies.

Statistics in the main study
Female bodyweight change (relative to Day 7 of gestation) and food consumption were analysed statistically by one-way analysis of variance with the Bonferroni multiple comparison test followed by pair wise analysis of control values against treated group values using Students 't' test where appropriate. All foetal parameters, skeletal development, group incidence of specific visceral and skeletal anomalies were analysed statistically by Kruskall-Wallis non parametric analysis of variance followed by pair wise analysis of control values against treated values using the Mann-Whitney U - test where appropriate.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The test substance was detected at the levels of 81-102% of the target concentrations in each dosing solution.

B. FOOD CONSUMPTION
In the preliminary study, significantly reduced food consumption was observed while administering in the high dose level of 400 mg/kg/day (Days 7 to19 of gestation). This observation was confirmed in the main study. At the high dose level, there was a reduction in food consumption during the dosing period compared to controls (Days 10 to 13, $p < 0.05$; Days 13 to 19, $p < 0.01$). No other significant changes were observed in the remaining groups during the main study.

C. MORTALITY
In the preliminary study, two does were killed in extremis in the high dose group, one had aborted foetuses and the other was bleeding from the vagina. No mortalities occurred at any dose up to 400 mg/kg/day in the preliminary study.

In the main study, two rabbits were found dead or moribund at the high dose level. One female was found dead prior to dosing on Day 19 of treatment. One female was killed *in extremis* on Day 20 of treatment. Clinical observations noted at this time included hunched posture, lethargy, ptosis, hypothermia and blood

on the litter tray. At the intermediate dose level, one female was found dead after dosing on Day 16 of treatment. Necropsy findings of reddened lungs, a fluid filled thorax and test material in thoracic cavity are consistent with mal-dosing. At the low dose level, no mortalities occurred. One female was found dead two minutes after dosing in the control group. Necropsy findings of blood in thorax, inflated appearance of lungs and a large area of congestion on the right caudal lobe are consistent with mal-dosing.

D. CLINICAL OBSERVATIONS

In both the preliminary and the main study, the clinical signs were in general the same. There was a toxicologically significant increase in the incidence of clinical observations, particularly scours, reduced faecal output and diarrhoea at the high dose level (400 mg/kg bw/day). Observations of lethargy, ptosis, hunched posture, hypothermia and blood on tray were noted for one animal of the main study killed *in extremis*.

At 200 mg/kg bw/day, vaginal bleeding and blood on tray were noted for one animal of the main study. Scours were also noted in animals at 200 and 50 mg/kg bw/day as well as in the control group, but the incidence and duration were not as severe as at the high dose level (see Table 5.6-31). No other treatment-related observations were evident.

Thus, for the findings observed at doses below 400 mg/kg bw/day, a clear dose-response could not be established.

Table 5.6-31: Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group[#]			
	Control (0 mg/kg/day)	Low (50 mg/kg/day)	Intermediate (200 mg/kg/day)	High (400 mg/kg/day)
Scours	5/14 (4)	10/18 (3)	7/16 (2)	16/16 (2)
Reduced faecal output	0/14 (4)	1/18 (0)	2/16 (2)	2/16 (2)
Diarrhoea	0/14 (4)	1/18 (0)	0/16 (2)	10/16 (2)
Diuresis	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)
Blood on tray	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Noisy respiration	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Lethargy	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Ptosis	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hunched posture	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hypthermia	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Anal staining	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Subdued behaviour	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Vaginal bleeding	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)

[#] x/y: number affected / total number of animals in group
Figures in parentheses represent the number of animals having no grossly observable conceptus.

E. BODY WEIGHT

In the preliminary, study a toxicologically significant decrease in body weight gain from Day 13 to 19 *post coitum* was evident at the high and intermediate dose levels.

Likewise a reduction in group mean bodyweight gain from Days 9 to 29 *post coitum* was observed in the high dose level group during the main study. The difference in group mean bodyweight change compared to controls was statistically significant ($P<0.05$ to 0.01) from Days 13 to 29 *post coitum*. Also in the intermediate dose level group a slight reduction (although not statistically significant) in group mean bodyweight gain from Day 9 to Day 29 *post coitum* was noted. In the low dose level group bodyweight gain was comparable to controls throughout the study period (see Table 5.6-32).

Table 5.6-32: Mean body weight gain during gestation

Dose level (mg/kg bw)	No. of animals	Body weight change (g) at Day (relative to Day 7)						
		10	13	16	19	22	25	29
0 (Control)	14	29	95	202	260	314	375	409
50	18	12	75	158	223	278	325	395
200	15	-11	54	143	198	263	309	294
400	15	-33	-45*	11**	21**	96**	153**	250*

* Significantly different from control at $p < 0.05$.
** Significantly different from control at $p < 0.01$.

F. PATHOLOGY

Necropsy

The macroscopic necropsy findings of the two does of the high level dose group that died or were killed *in extremis* included fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended. These findings indicate that the test material may affect the gastrointestinal tract. The animal killed *in extremis* at this level also had both uterine horns containing blood and dead foetuses in the uterus; this may be a result of maternal toxicity. All other necropsy findings were not treatment-related.

Observations on the ovary and uterus

No treatment related effects were evident in both the preliminary and the main study.

In the control, low, intermediate and high dose level groups 14, 18, 16 and 16 females, respectively, survived to termination of the main study and were proved to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

The litter size at caesarean section was comparable in all treatment groups. In the high dose level group, there were slight, but not statistically significant, increases in late foetal deaths and post implantation loss, mainly due to one animal that had nine late deaths, resulting in a post implantation loss of 69.2%. This was therefore considered not to be a treatment-related effect. At 200 mg/kg bw/day, there were statistically significant increases ($p<0.05$) in total foetal deaths and post implantation loss. These increases were caused by a slight, but not statistically significant, rise in early foetal deaths. As at this dose level, there was no rise in late foetal deaths, as seen at the high level; the effect on early foetal deaths was considered not to be treatment-related.

Foetal body weights

No statistically significant differences were noted in the mean foetal body weights between the control group and the treated groups. Mean total litter weights were comparable in all treatment groups.

External, visceral and skeletal examination

At the high dose level, there was one litter with one foetus with major malformations. This foetus was found to have spina bifida and clubbed and malrotated hind limbs. At the intermediate dose level, two foetuses of two different litters had major malformations. One foetus had retinal infolding and a haemorrhage in the retinal layer, the other acephaly, small kinked tail, bilateral forelimb flexure, interrupted aorta and an intraventricular septal defect. At skeletal examination, this foetus was found to have multiple rib and vertebral column abnormalities. At the low dose level, three foetuses of two different litters had major abnormalities. In one litter, one foetus had forked ribs with a displaced vertebral centrum. In another litter, one foetus had a small eye with retinal infolding and aphakia. A second foetus from this litter had nostrils close together, and a thin nasal septum not attached at posterior pole near the front of the nasal passages. In the control group, there were two foetuses from two different litters with

major abnormalities. One foetus had gastroschisis and the other foetus had an extra vertebral arch resulting in scoliosis.
These findings were considered to be within the range of normal variation for this species. There were no treatment-related effects on the degree of skeletal development.

Table 5.6-33: Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	Dose level (mg/kg bw/day)			
	0	50	200	400
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
Skeletal malformations				
Total no. of foetuses with skeletal malformations	1	0	1	0
Total no. of litters with skeletal malformations	1	0	1	0
Percentage of litters with skeletal malformations (%)	7.1	0.0	6.7	0.0
Skeletal variations				
Total no. of foetuses with skeletal variations	43	48	39	49
Total no. of litters with skeletal variations	13	18	15	15
Percentage of litters with skeletal variations (%)	92.8	100	100	100
External and visceral findings				
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
No of litters with anomalous foetuses	2	3	2	3
Percentage of litters with anomalous foetuses (%)	14.3	27.8	13.3	20
No. of litters with major malformations	02	2	2	1
Percentage of litters with malformed foetuses (%)	14.3	11.1	13.3	6.7

III. CONCLUSION

The oral administration of glyphosate technical to pregnant rabbits by gavage from gestation Day 7-19 resulted maternal toxicity at 400 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose levels. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 200 mg/kg bw/day for maternal toxicity. The 'No Observed Adverse Effect Level' (NOAEL) for developmental toxicity was considered to be 400 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/03	[redacted]	1996	Glyphosate acid: Developmental toxicity study in the rabbit [redacted] Data owner: Syngenta Report No.: [redacted]5009 Date: 1996-07-02 GLP: yes not published

Guideline:	OECD 414 (1981), EEC B.31 (1988),US-EPA 83-3
Deviations:	None
Dates of experimental work:	1996-01-01 to 1996-02-09

Executive Summary
Glyphosate acid was administered by gavage to three groups of 20 mated New Zealand White rabbits each, at doses of 100, 175 and 300 mg/kg bw/day from gestation Day 8-20 (mating = day 1). A further group of 20 animals was exposed to the vehicle (deionised water) to serve as control.

Individual clinical observations, bodyweight and food consumption were recorded during the study. The females were killed on Day 30 of gestation, examined macroscopically for external and internal malformation. The uteri were examined number of corpora lutea, implantation number, position and type, foetal weights, foetal sex, external appearance and internal visceral anomalies/abnormalities were recorded. All live foetuses were preserved, processed and subsequently examined for skeletal anomalies with the heads of half the offspring preserved and examined for visceral anomalies.

Administration of 175 or 300 mg/kg bw/day was associated with dose-related maternal toxicity. This toxicity was manifested as signs of diarrhoea, reduction in faecal output as a result of reduced food consumption and a corresponding reduction in body weight. There was no maternal toxicity attributable to the administration of 100 mg/kg bw/day. At the high dose level, when maternal toxicity was seen, a reduction in mean foetal weight and very minor alterations in foetal ossification were evident. There was no effect on the number of foetuses or their survival and there was no evidence of teratogenicity.

Conclusion:
The oral administration of glyphosate acid to time-mated rabbits by gavage from Gestation Day 8-20 resulted in maternal toxicity at 175 and 300 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore, the NOAEL was considered to be 100 mg/kg bw/day for maternal toxicity, and 175 mg/kg bw/day for offspring.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	Y04404/034
Purity:	92.6%
Stability of test compound:	The stability of the test substance was confirmed for the study period.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted] UK
Age:	Not reported
Sex:	Females (time-mated)
Weight at dosing:	approximately 3.8 kg
Acclimation period:	At least 4 days
Diet/Food:	[redacted] rabbit diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in mobile rabbit units
Environmental conditions:	Temperature: 17 ± 2°C Humidity: 55 ± 15% Air changes: 25-30/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1996-01-01 to 1996-02-09

Animal assignment and treatment:
Eighty time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 1 of gestation. The females were delivered to [redacted] at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 20 time-mated New Zealand white female rabbits received 0, 100, 175 or 300 mg/kg bw/day test substance by gavage (2 mL/kg bw) from gestation Day 8-20. The dose levels were chosen based on results of a preliminary dose finding.

Dose formulation and analysis
For each dose level an appropriate amount of deionised water was added to a weighed amount of glyphosate acid (adjusted for purity). Each preparation was thoroughly mixed and subdivided into aliquots. Fresh aliquots were used for each day of the study. Two preparations were made per concentration (i.e. 0, 50, 87.5 and 150 mg/mL). The dosing preparations were stored at room temperature.
Representative samples of each dosing preparation were analysed prior to being used for dosing to verify the achieved concentration of glyphosate acid in the vehicle. Samples were taken for the determination of homogeneity at 50 and 150 mg glyphosate acid/mL (low and high dose levels).
The chemical stability of glyphosate acid in the vehicle was determined by re-analysis of the lowest and highest concentrations of the dosing preparations after an interval of 40 days.
Dose formulations were shaken prior to dosing, and during dosing as required.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on arrival, on Day 4, prior to dosing on Days 8 to 20 and on Days 23, 26 and 30 of gestation.

Food consumption
Food consumption of females was recorded on Days 4-8, Days 8-11, Days 11-14, Days 14-17, Days 17-20, Days 20-23, Days 23-26 and Days 26-30 of gestation.

Sacrifice and pathology
All rabbits at scheduled termination on day 30 and any requiring euthanasia during the study were killed by an overdose of 200 mg/mL sodium pentobarbitone solution given as i.v. injection. All animals were subjected to an examination post mortem. This involved an external observation and an examination of the thoracic and abdominal viscera. The pregnancy status of each animal was determined. Where there was no clear evidence of implantation, the uterus was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred. For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries, uterus and contents where then examined. Number of corpora lutea, number and position of implantations, number of live foetuses, foetus weight and early and late intrauterine deaths were determined for each sacrificed doe.

Developmental parameters
After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution. An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70% industrial methylated spirits. After approximately 24 h the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70% industrial methylated spirits for

subsequent processing and staining with Alizarin Red S. The remaining stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed.

Statistics

Data relating to those animals which were non-pregnant and animals that died intercurrently were excluded from the statistical analysis. Maternal bodyweight during the dosing and post dosing periods was considered by analysis of covariance on initial (Day 8) bodyweight. Maternal food consumption during the dosing and post dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by ANOVA. Maternal performance data, the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test. Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external visceral defects, minor external/visceral defects, external visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:

1) Percentages were analysed by ANOVA following double arcsine transformations of Freeman and Tukey (1950),

2) the proportion of foetuses and, with the exception of male foetuses, the proportion of litters affected were considered by Fisher' s Exact Test.

All analyses were carried out in SAS (1989). For Fisher's Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-square mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.

All statistical tests were two sided.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The concentrations of glyphosate acid in the dosing formulations were within 12% of the target concentrations. The homogeneity and stability of the test substance in the dosing formulations was satisfactory.

B. FOOD CONSUMPTION

During the dosing period, does receiving 175 or 300 mg/kg bw/day showed reduced food consumption compared to the controls.

C. MORTALITY

The incidence of intercurrent deaths was 1, 2, 2 and 2 in the control, 100, 175 and 300 mg/kg/ bw/day groups, respectively.

In the post-dosing period, one doe in the control group showed weight loss, reduced food consumption, signs of diarrhoea, mucus in the faeces, few faeces and staining in the genital area. This animal aborted on Day 30. Changes in the stomach and caecum were observed post mortem.

In the low dose level group, one doe showed slight loss of bodyweight and reduced food consumption between Days 4 and 8 (i.e. prior to the onset of dosing) and this response continued into the dosing period, until the animal aborted its litter on Day 19. Examination post mortem noted the presence of a mass in the right inguinal region of the abdominal cavity. A second animal in this group aborted its litter on Day 25 having shown weight loss and reduced food consumption from Day 14.

At the intermediate dose level, one doe was killed for humane reasons on Day 23 having shown loss of bodyweight and reduced food consumption from Day 4 on. By Day 23, the animal had become thin and

subdued and all uterine implantations were found to be dead. A second animal in this group aborted its litter on Day 22 having shown slight weight loss from Day 14 and reduced food consumption from Day 4. At the high dose level, two animals aborted their litters on Days 24 and 23, respectively. Both animals showed a reduction in food consumption from Day 11 and bodyweight loss from Day 11/13. A hair-like substance was found in the stomachs of both animals at examination post mortem.

D. CLINICAL OBSERVATIONS

In the high dose level group, there was an increased incidence of animals producing few faeces, with signs of diarrhoea or with staining in the genital area, in comparison with the control group. The production of few faeces and signs of diarrhoea were also of increased incidence in does of the intermediate dose group. There were no clinical effects observed in rabbits treated at a dose level of 100 mg/kg bw/day (see Table 5.6-34).

Table 5.6-34: Observed clinical signs during the dosing period

	Number of rabbits affected in dose group			
Clinical sign	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (175 mg/kg/day)	High (300 mg/kg/day)
Blood on tray	0	2		1
Cold	0		1	0
Dry sores 1 or more areas	0	1	0	0
Ears torn	0	2	1	1
Eye opaque	0	1		0
Few faeces on tray	3	3	9	9
Mucus in faeces		0	0	0
No faeces on tray	0	1	2	3
Scabs in 1 or more areas	4	6	3	3
Signs of diarrhoea	4	5	11	19
Staining in genital area	2	2	3	11
Subdued behaviour	0	0	1	0
Thin	0		1	2
Urine coloured		1	1	0
Wet sores in 1 or more areas	2	0	1	0

E. BODY WEIGHT

Administration of 300 mg/kg bw/day was associated with a reduction in maternal body weight gain. The statistical different observed body weight development at 175 mg/kg bw/day was due to differences in body weights at the begin o the study. All animals except one of the high dose group showed signs of recovery in the post-dosing period. The reduction in food consumption was therefore accompanied by a corresponding reduction in body weight. In the low dose level group, bodyweight gain was comparable to controls throughout the study period (see Table 5.6-35).

Table 5.6-35: Mean body weight development (in g) during gestation

		Dose level in mg/kg bw/day			
		0 (Control)	100	175	300
Animals per group		17	18	17	17
Day of gestation	8	3924	3771	3822	3815
	9	3845	3837	3834	3823
	10	3857	3863	3856	3830
	11	3885	3873	3874	3854
	12	3894	3879	3877	3856
	13	3917	3905	3902	3880
	14	3942	3932	3930	3875
	15	3975	3982	3939	3896
	16	4020	4031	3959	3907*
	17	4049	4053	3982	3923*
	18	4063	4051	3990	3914**
	19	4085	4061	4005	3927**
	20	4088	4059	3995	3926**
	23	4177	4118	4049*	3951**
	26	4236	4210	4169	4093*
	30	4313	4294	4256	4183

* Significantly different from control at $p < 0.05$.
** Significantly different from control at $p < 0.01$.

F. PATHOLOGY

Necropsy

There were no macroscopic findings that were considered to be related to the administration of glyphosate acid.

Observations on the ovary and uterus

No treatment related effects were evident in the study.

In the control, low, intermediate and high dose level groups 17, 18, 17, and 17 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

The proportion of foetuses that were male was statistically significantly increased in the intermediate dose level group, in comparison with the control group. In the absence of a dose-related trend, this finding was considered incidental to the administration of glyphosate acid. There was no adverse effect of glyphosate acid on the number or survival of the foetuses in utero.

Foetal body weights

There was a statistically significant reduction in mean foetal weight in the high dose level group, in comparison with the control group. This difference was considered attributable to two litters for which the mean pup weight was particularly low.

External, visceral and skeletal examination

The number of foetuses with major defects was 3/143 (2/17 litters), 1/147 (1/18 litters), 0/135 (0/17 litters) and 2/144 (2/17 litters) in the control, 100, 175 and 300 mg/kg bw/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The proportion of foetuses with minor external visceral defects was similar for all groups, including the

control. There were no significant differences in litter incidences for minor external/visceral defects noted. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid (see Table 5.6-36 and Table 5.6-38).

The proportion of foetuses with minor skeletal defects was statistically significantly increased in the 100 and 300 mg/kg bw/day groups, in comparison with the control group, but not in the 175 mg/kg bw/day group. Evaluation of the specific defects noted an increased incidence of foetuses in the high dose level group with partially ossified transverse processes on the 7th cervical vertebra (8 foetuses in 2 litters), unossified transverse processes on the 7th lumbar vertebra (14 foetuses in 4 litters) or partially ossified 6th sternebra (16 foetuses in 7 litters). None of the specific minor defects were statistically significantly increased in the low or intermediate dose level groups. None of the foetuses were found to have an external/visceral variant.

The proportion of foetuses with skeletal variants was statistically significantly increased in the high dose level group, in comparison with the control group. Evaluation of the specific variants noted a slight, but not statistically significant, increase in the incidence of foetuses in this group with partially ossified odontoids (62 foetuses in 15 litters) or with 27 pre-sacral vertebrae (37 foetuses in 12 litters).

The slightly higher mean *manus* score observed in the high dose level group, in comparison with the control group, was due to a slight reduction in ossification as shown by the increase in incidence of foetuses scoring 4 or 5. A similar response was apparent from the *pes* scores.

Table 5.6-36: Summary of the type and incidence of major defects

Major foetal defects	Number of foetuses affected in dose group*			
	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (175 mg/kg/day)	High (300 mg/kg/day)
Heart single ventricle, ventricle walls thickened, aorta enlarged, pulmonary artery reduced	0/143	0/147	0/135	1/144
Encephalocoele (gross malformation of the skull)	0/143	0/147	0/135	1/144
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, aorta enlarged, persistent truncus arteriosus	1/143	0/147	0/135	0/144
Shortened upper and lower jaws, cleft lip, cleft palate, nares absent, forepaws flexed (right extremely, left slight)	1/143	0/147	0/135	0/144
Reduced number of lumbar vertebrae (25 pre-sacral vertebrae)	1/143	0/147	0/135	0/144

* number affected / total number

Table 5.6-37: Summary of the type and incidence of major defects (litter incidences)

Major foetal defects	Number of litters affected in dose group*			
	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (175 mg/kg/day)	High (300 mg/kg/day)
Heart single ventricle	0/17	1/18	0/17	1/18
aorta enlarged	1/17	1/18	0/17	1/18
pulmonary artery reduced	0/17	1/18	0/17	1/18
Encephalocoele (gross malformation of the skull)	0/17	0/18	0/17	1/18
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, Shortened upper and lower jaws, cleft lip, cleft palate, nares absent	1/17	0/18	0/17	0/18
persistent truncus arteriosus	1/17	0/18	0/17	0/18
forepaws flexed (right extremely, left slight)	1/17	0/18	0/17	0/18
Reduced number of lumbar vertebrae, 25 pre-sacral vertebrae	1/17	0/18	0/17	0/18

* number affected / total number

Table 5.6-38: Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	Dose level (mg/kg bw/day)			
	0	100	175	300
No. of litters examined	17	18	17	17
No. of foetuses examined	143	147	135	144
Skeletal malformations				
Total no. of foetuses with major defects	3	0	0	1
Total no. of litters with major defects	2	0	0	1
Percentage of litters with major defects (%)	11.8	0.0	0.0	5.9
Total no. of foetuses with minor defects	58	82*	59	79*
Total no. of litters with minor defects	16	18	16	17
Percentage of litters with minor defects (%)	94.1	100	94.1	100
Skeletal variations				
Total no. of foetuses affected	119	129	116	132*
Total no. of litters affected	17	18	17	17
Percentage of litters affected (%)	100	100	100	100
External and visceral findings				
No. of foetuses with major defects	2	1	0	2
No of litters with foetuses with major defects	2	1	0	2
Percentage of litters with foetuses with major defects (%)	11.8	5.6	0.0	11.8
No. of foetuses with minor defects	12	7	9	11
No of litters with foetuses with minor defects	8	5	8	7
Percentage of litters with foetuses with minor defects (%)	47.1	27.8	47.1	41.2

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

III. CONCLUSION

The oral administration of glyphosate acid to time-mated rabbits by gavage at a maximum dose level of 300 mg/kg bw/day from Gestation Day 8-20 resulted maternal toxicity at 175 and 300 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the 'No Observed Effect Level' (NOEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The 'No Observed Effect Level' (NOEL) for developmental toxicity was considered to be 175 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/04	[redacted]	1980	Technical Glyphosate: Teratology study in rabbits [redacted] [redacted] Report No.: [redacted]79-018 Date: 1980-02-29 GLP: no (pre-GLP study) not published

Guideline:	Not stated. (pre-guideline; satisfies in general the requirements of OECD 414 (1981), but not of OECD 414 (2001))
Deviations:	Not applicable
Dates of experimental work:	1979-04-16 to 1979-05-11

Executive Summary
Glyphosate acid was administered by gavage to groups of 16 pregnant Dutch Belted rabbits each, at doses of 75, 175 and 350 mg/kg bw/day from gestation Day 6-27 (insemination = Day 0). A further group of 16 animals was exposed to the vehicle (0.5 % aqueous Methocel®) to serve as control.
Individual clinical observations and bodyweights were recorded during the study. The animals were either killed on Day 28 of gestation, or after they had aborted during the study period. All sacrificed animals or animals found dead were subjected to a gross necropsy. The uteri were examined for number of corpora lutea, early and late resorption, and total implantations. Foetuses were weighed, sexed and examined for external and internal malformations, as well as internal visceral anomalies/abnormalities.
At 175 mg/kg bw/day, a slight increase of soft stool and diarrhoea was observed in dams. At 350 mg/kg bw/day, definite signs of maternal toxicity were observed; this toxicity was manifest as signs of soft stool and/or diarrhoea in all high dose animals at least once, nasal discharge, as well as an increase in the number of dams that died. There was no maternal toxicity attributable to the administration of 75 mg/kg bw/day. In addition, there were no signs of developmental effects noted in any dose group.

Conclusion:
The oral administration of glyphosate acid to pregnant rabbits by gavage from Gestation Day 6-27 resulted in maternal toxicity at 175 and 350 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore, the NOAEL was considered to be 75 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 350 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XHJ-64
Purity:	98.7%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: 0.5 % aqueous Methocel®

3. Test animals:

Species:	Rabbit

Strain:	Dutch Belted
Source:	[redacted] USA
Age:	Approx. 7 month
Sex:	Females
Weight at dosing:	2.533 – 3.234 kg
Acclimation period:	At least 30 days
Diet/Food:	[redacted] Rabbit Chow [redacted], *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended wire mesh cages
Environmental conditions:	Temperature: Exact values not reported
	Humidity: Exact values not reported
	Air changes: Exact values not reported
	Light controlled

B. STUDY DESIGN AND METHODS

In life dates: 1979-04-10 to 1979-05-11

Animal assignment and treatment:
Sixty-four female Dutch Belted rabbits were artificially inseminated and randomly assigned to treatment groups of 16 animals. The day of insemination was designated Day 0 of gestation. The rabbits received daily doses of 0, 75, 175 or 350 mg/kg bw/day test substance by gavage (1 mL/kg bw) from gestation Day 6 to 27. Individual doses based on individual body weights determined on gestation Day 6.

Dose formulation
For each dose level an appropriate amount of grounded technical glyphosate was suspended in 0.5 % aqueous Methocel® solution and homogenised. The dose solutions were prepared daily.

Clinical observations
A check for mortality or behavioural changes was made once daily prior to treatment. During the treatment and post-treatment period all rabbits were observed once daily for clinical signs of toxicity, mortality or behavioural changes.

Body weight
Individual body weights of dams were recorded on gestation Days 0, 6, 12, 18, 24 and 28.
These time points for body weight determination differ from the requirements of the current OECD guideline 414 (2001) (i.e., body weights should be determined on gestation day 0 and at 3-day intervals thereafter). Although the time-intervals were longer than required, the time points for body weight determination are considered to be sufficient to evaluate the body weight development of the pregnant animals.

Sacrifice and pathology
Dams
All rabbits at scheduled termination on day 28 were sacrificed, the uterus was excised and weight and the foetuses were removed. The number and location of viable foetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs were examined for gross pathological changes.
Rabbits that died during the study were necropsied to determine the cause of death.

Foetuses
All foetuses were weight, sexed and examined for external malformation and variations, as well as for visceral malformations and variations. The carcasses were then fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for skeletal examination.

Statistics
All statistical analyses compared the treatment groups to the control group with a level of significance at $p < 0.05$. Foetal sex distribution and number of litters with malformations were analysed using the Chi-square test with Yates correction and/or Fisher´s exact probability test. Ther number of early and late resorption and post-implantation losses were compared by the Mann-Whitney U-test.
Mean numbers of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by ANOVA (one-way), Bartlett´s test for homogeneity and appropriate t-test.

II. RESULTS AND DISCUSSION

A. MORTALITY
There was an increased incidence of mortalities in the high dose group (see Table 5.6-39).

Table 5.6-39: Mortalities of dams

	Control (0 mg/kg/day)	Low (75 mg/kg/day)	Intermediate (175 mg/kg/day)	High (350 mg/kg/day)
Spontaneous deaths*	0/16	1/16	2/16	10/17
Time of death (gestation day)	-	26	32, 25	3 to 21
% mortality	0.0	6.3	12.5	58.8
Sacrificed after abortion	2	0	1	1
Sacrificed on gestation day	22	23	27	23

* dead animals / total animals in group

For five of the rabbits that died spontaneously, the cause of death was attributed to pneumonia, respiratory disease, enteritis or gastroenteritis. For one rabbit of the mid-dose group and the other 7 rabbits of the high dose group, the cause of death could not be determined.
The mortality rates in the intermediate- and especially in the high-dose groups were greater than 10%, which exceeds the OECD guideline 414 (2001) suggestion of no more than approximately 10% maternal mortality.

D. CLINICAL OBSERVATIONS
Clinical signs consisting of soft stool and diarrhea were noted in all dose groups during the treatment period. In the 175 mg/kg bw/day dose group, the incidence of this finding was slightly increased when compared with the control group. At 350 mg/kg bw/day, either soft stool, diarrhea or both were observed in each animal at least once during the treatment period. Also in the high dose group, there was an increased incidence of animals with nasal discharge in comparison with the control group.

E. BODY WEIGHT
There were no treatment-related effects on maternal body weights and body weight gain.

F. PATHOLOGY
Necropsy
There were no macroscopic findings in dams that were considered to be related to the administration of glyphosate technical.

Observations on the ovary and uterus
No treatment-related effects were evident in the study.

Table 5.6-40: Maternal observations

	Historical control	Control (0 mg/kg/day)	Low (75 mg/kg/day)	Intermediate (175 mg/kg/day)	High (350 mg/kg/day)
Surviving dams at caesarean section*	27/28	14/16	15/16	13/16	6/17
Pregnant rabbits	24/28	12/16	15/16	11/16	6/17
Non-pregnant rabbits	3/28	2/16	0/16	2/16	0/17
Abortions	1/28	2/16	0/16	1/16	1/16

* number of surviving animals / total animals in group

G. DEVELOPMENTAL PARAMETERS

There were no statistically significant differences in the mean numbers of early or late resorptions, total implantations, corpora lutea, foetal body weights or foetal sex ratio in any of the test substance groups when compared to control. The number of viable foetuses was slightly, but statistically, significantly, increased in the low-dose group at 75 mg/kg bw/day. However, this finding was considered incidental and not related to the test substance.

The mean foetal body weights were slightly decreased in the test substance groups as compared to control. However, the mean foetal body weights in all test substance groups were comparable to the historical control data (i.e. 30.9 g) (see table below).

Table 5.6-41: Mean litter data at caesarean section

	Historical control	Control (0 mg/kg/day)	Low (75 mg/kg/day)	Intermediate (175 mg/kg/day)	High (350 mg/kg/day)
Pregnant dams[#]	24	12	15	11	6
Viable foetuses/dam	6.4	5.3 ± 2.73	7.6* ± 1.84	5.9 ± 2.77	6.3 ± 2.25
Post implantation loss/dam[##]	0.8	0.7 ± 0.89	0.4 ± 0.63	0.2 ± 0.40	0.8 ± 1.33
Total implantations /dam[##]	7.5	5.9 ± 2.39	8.0 ± 1.81	6.1 ± 2.84	7.2 ± 2.93
Corpora lutea/dam[##]	10.1	8.6 ± 2.13	10.1 ± 1.64	10.5 ± 3.45	8.5 ± 1.87
Foetal sex distribution (males/females)[#]	83/77	28/33	53/61	32/33	17/21
Mean foetal body weight (g)[##]	30.9	33.4 ± 7.27	30.9 ± 4.43	29.9 ± 7.21	29.3 ± 4.82

[#] Total number
[##] Number ± SD; historical control without SD
* Statistically significant difference compared to control ($p < 0.05$)

It should be noted that, in all dose groups, the number of pregnant dams were less than the number of pregnant dams required by the current OECD guideline 414 (2001); i.e., 16. Therefore, the evaluation of the developmental parameters may be limited.

Skeletal and visceral examination

The percentages of foetuses with skeletal malformations were 0.0, 2.6, 3.1 and 5.3 in the control, 75, 175 and 350 mg/kg bw/day groups, respectively. Although malformations were observed in the test substance groups, neither the type nor incidence of the malformations provided evidence for an adverse effect of glyphosate acid. There were no visceral malformations observed in any of the dose groups including control. There were no statistically significance differences in the variation observed in the test substance group when compared to the control group (see Table 5.6-42).

Table 5.6-42: Summary of foetal malformations and variations

	Hist. contr.	Control (0 mg/kg/day)		Low (75 mg/kg/day)		Intermediate (175 mg/kg/day)		High (350 mg/kg/day)	
Number of litters examined		12		15		11		6	
	%	x/y	%	x/y	%	x/y	%	x/y	%
Skeletal malformations		**0/63**	**0.0**	**3/114**	**2.6**	**2/65**	**3.1**	**2/38**	**5.3**
Exencephaly	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Acrania	--	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Scoliosis with associated rib anomalies	0.6	0/63	0.0	2/114 (2/15)	1.8	0/65	0.0	0/38	0.0
T1 rib absent	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Carpal flexure	0.6	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Fused cervical vertebral centra	0.6	0/63	0.0	1/114 (1/15)	0.9	0/65	0.0	0/38	0.0
Visceral malformation	--	**0/63**	**0.0**	**0/114**	**0.0**	**0/65**	**0.0**	**0/38**	**0.0**
Total malformations		**0/63**	**0.0**	**3/114**	**2.6**	**2/65**	**3.1**	**2/38**	**5.3**
Variations									
27 presceral vertebrae	8.7	6/63 (5/12)	9.5	7/114 (3/15)	6.1	9/65 (4/11)	13.8	7/38 (5/6)	18.4
13th rudimentary rib(s)	3.7	5/63 (3/12)	7.9	14/114 (6/15)	12.3	3/65 (3/11)	4.6	3/38 (3/6)	7.9
13th full rib(s)	8.1	3/63 (3/12)	4.8	10/114 (4/15)	8.8	5/65 (2/11)	7.7	6/38 (3/6)	15.8
Hyoid arches bent	--	--	--	2/114 (1/15)	1.8	1/65 (1/11)	1.5	--	--
Hyoid body unossified		6/63 (3/12)	9.5	3/114 (2/15)	1.8	6/65 (3/11)	9.2	--	--
Parietals reduced in ossification	0.6	1/63 (1/12)	1.6	--	--	1/65 (1/11)	1.5	--	--
Sternebrae #5 and/or #6 unossified	5.6	6/63 (2/12)	9.5	13/114 (7/15)	11.4	13/65 (5/11)	20.0	4/38 (2/6)	10.5
Pubis unossified	--	4/63 (1/12)	6.3	1/114 (1/15)	0.9	4/65 (1/11)	6.2	--	--
Talus unossified		3/63 (1/12)	4.8	--	--	5/65 (3/11)	7.7	--	--
Extra ossification center, cervical area	--	--	--	--	--	1/65 (1/11)	1.5	--	--
Major vessel variations	8.7	11/63 (6/12)	17.5	14/114 (8/15)	12.3	14/65 (5/11)	21.5	6/38 (4/6)	15.8

x/y: number of foetuses affected / total number of foetuses examined
(a/b): number of litters affected / total number of litters

III. CONCLUSION

The oral administration of glyphosate acid to pregnant rabbits by gavage from Gestation Day 6-27 resulted maternal toxicity at ≥175 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the NOAEL was considered to be 75 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 350 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/05	[redacted]	1991	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations) [redacted] Data owner: Cheminova Study/Project No.: [redacted]45 & 39 & 40/901303 Date: 1991-10-14 GLP: yes not published

Guideline: OECD 414, US EPA 83-3

Deviations: None

Dates of experimental work: 1989-12-14 to 1990-03-02

Executive Summary

In a developmental toxicity study, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate acid in 1% methylcellulose once daily by gavage at dose levels of 0 (vehicle control), 50, 150 or 450 mg/kg bw/day from Day 7 to Day 19 of pregnancy (mating = Day 0). All animals were observed daily for clinical signs and mortality, and body weights and food consumption were measured on Days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation. On Day 29 of gestation, the does were sacrificed and a gross necropsy was performed. The ovaries and uteri were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic and /foetal deaths, individual foetal weights and foetal abnormalities. All live foetuses were examined for external, visceral and skeletal abnormalities.

Observations recorded included one death at 450 mg/kg bw/day following abortion, reduced food intake during the treatment period and reduction in body weight gain from Days 11 – 19 of pregnancy. Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and slight reductions in food consumption and body weight at 150 and 450 mg/kg bw/day.

Glyphosate was not teratogenic in this developmental toxicity study in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw/day based on clinical signs of toxicity including reduced food consumption and bodyweight gain and soft/liquid faeces during the dosing period. The NOAEL was 150 mg/kg bw/day for foetotoxicity and was 450 mg/kg bw/day for teratogenicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	206-JAK-25-1
Purity:	98.6%
Stability of test compound:	Stable over the duration of the study

2. Vehicle and/ or positive control: 1% methylcellulose

3. Test animals:

Species: Rabbit

Strain:	New Zealand White
Source:	[redacted] UK
Age:	11-24 weeks (on delivery)
Sex:	Female
Weight at dosing:	Females: 3582 – 3709 g (mean values)
Acclimation period:	7 days
Diet/Food:	[redacted] Standard rabbit diet ([redacted] UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage
Environmental conditions:	Temperature: 19 ± 1 °C Humidity: 49 ± 15% Air changes: Not recorded Natural lighting supplemented with artificial lighting from 07 – 21:00 hours

B: STUDY DESIGN AND METHODS

In life dates: 14 -12-1989 – 02-03-1990

Animal assignment and treatment:
In a developmental toxicity study, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate in 1% methylcellulose (dose volume 5 mL/kg) once daily by gavage at dose levels of 0 (vehicle control), 50, 150 or 450 mg/kg bw/day from Day 7 to Day 19 of pregnancy. Dose volumes were calculated for individual animals on day 7 and adjusted according to body weight on Days 9, 11 and 15. The day of mating was considered as Day 0. Dose levels were based on the findings of a preliminary study.

Dosing formulations were prepared daily and administered within 3 hours of preparation.

Observations
All animals were regularly handled and observed daily for overt changes or signs of reaction to treatment. Animals that died or were killed for animal welfare reasons were weighed and subjected to post-mortem examination.

Body weight
Individual body weights were recorded Days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation.

Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology
On day 29 of pregnancy all surviving does were subjected to post-mortem examinations for congenital abnormalities and gross pathological changes in maternal organs.
The ovaries and uteri were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic and /foetal deaths, individual foetal weight and foetal abnormalities. Embryonic/foetal deaths were classified as Early, Late or Abortions.

Litter parameters
Live young were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were killed by intrathoracic injection of pentobarbitone sodium then weighed and dissected for examination of visceral abnormalities. Where appropriate, suspected abnormalities were further examined by alternative procedures such as microdissection and histopathology to clarify initial observations. Pups were fixed in industrial methylated spirit, the heads sliced along the line of the frontoparietal suture and the brain examined for abnormalities before clearing and staining by the modified Dawson technique of the carcasses for skeletal examination. Structural changes were presented as malformations, anomalies or variants.

Statistics
Two-tailed tests for significance were performed on litter data only, and significance at 1% and 5% were reported. Mean values of litter size, pre and post-implantation loss, litter weight, mean foetal weight and the incidence of anomalous offspring were analyzed by the Kruskal-Wallis test. Intergroup comparisons were made by the non-parametric equivalent of the Williams' test following a significant h-statistic. Where 75% of the values for a given variable consisted of one value, a Fisher's exact test was used.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The analysis of the dosing formulations taken at the first dosing showed the mid- and high-dose group to be within 6% of the nominal dose whilst the low-dose group was 19% below the nominal dose; however, a renalysis on Day 19 showed the concentration to be 3% above the nominal dose.

B. FOOD CONSUMPTION
During the dosing period, females receiving 150 and 450 mg/kg bw/day showed reduced food consumption compared to the controls. A slight reduction was evident from Days 11 – 19 at 150 mg/kg bw/day (approximately 12% compared with controls) and throughout the treatment period for the 450 mg/kg bw/day dose group (6-17% during Days 7-19) (see Table 5.6-43).

Table 5.6-43: Summary of mean food consumption (g/rabbit/day)

	Dose Group (mg/kg bw/day)			
	0 (control)	**50**	**150**	**450**
Mated females	19	19	16	20
No. of animals included in assessment	18	12	15	13
Food consumption (g/rabbit/day) during				
Days 1-6	142	143	141	152
Days 7-8	143	154	150	135
Days 9-10	146	148	148	132
Days 11-14	153	149	134	129
Days 15-19	148	151	131	123
Days 20-23	142	154	149	149
Days 24-28	131	143	153	166

C. MORTALITY

There was one death in the 450 mg/kg bw/day dose group on Day 20 following signs of abortion on Day 19 and signs of gastrointestinal disturbance, manifested as soft/liquid faeces, severe reduction in food consumption and bodyweight loss from the onset of treatment. Two other deaths (a broken hind leg and an incidence of congenital abnormality) were unrelated to the treatment and were eliminated from the study assessment.

D. CLINICAL OBSERVATIONS

Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and signs of lack of appetite (off feed/reduction in food consumption) at 150 and 450 mg/kg bw/day (see Table 5.6-44).

Table 5.6-44: Summary of relevant clinical signs in does

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20
Not pregnant	0	1	1	5
Number of does with live young or litters at Day 29	18	12	15	13
Clinical signs#				
Off-feed	8	6	10	9
Reduced faecal output	9	8	11	12
Soft/liquid faeces	0	2	5	13

Only animals with live young included

E. BODY WEIGHT

A slight reduction in bodyweight gain was noted from Day 11 of pregnancy to termination of treatment in the 150 and 450 mg/kg bw/day dose groups, which coincided with the reduction in food consumption during the same period (see Table 5.6-45).

Table 5.6-45: Summary of body weight data (group means)

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20
No of animals included in assessment	18	12	15	13
Bodyweights (g) at				
Day 1	3538	3524	3568	3658
Day 7	3582	3604	3624	3709
Day 9	3589	3639	3637	3732
Day 11	3601	3653	3661	3743
Day 15	3742	3804	3779	3833
Day 20	3770	3831	3775	3835
Day 24	3844	3927	3849	3965
Day 29	3999	4084	3975	4103

F. PATHOLOGY

Necropsy

Gross examination of does at post-mortem did not identify any treatment-related effects.

Observations on the ovary and uterus

A total of 18, 12, 15 and 13 pregnant females survived to termination and 163, 104, 112 and 95 foetuses were recorded for the 0 (control), 50, 150 and 450 mg/kg bw/day dose groups respectively. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss was recorded for one female

of the 450 mg/kg bw/day dose group which aborted on Day 19 and died and also for one female at 50 mg/kg bw/day. One female at 150 mg/kg bw/day aborted 1/9 foetuses.

There were no significant intergroup differences in the numbers of corpora lutea, implantations, pre-implantation loss, foetal sex ratios or foetal weights (see Table 5.6-46). There was a statistically significant increase in embryo/fetal death and post-implantation loss at all exposure levels. The study investigators questioned the biological significance of these findings for several reasons: 1) No dose-response pattern was evident, 2) the control value was at the lower end of the historical control range, while those of the exposed groups were at the higher end, and 3) the values in all groups were within or slightly above the historical control range. The latter two statements are supported by the historical control data provided in the study report (page 32) (see Table 5.6-46). Although embryo/foetal death was within the historical control range, post-implantation loss was above the historical control values in the high-dose group, and both of these parameters were statistically significant ($p<0.01$) at the high dose.

Table 5.6-46: Summary of the maternal and litter parameters (group mean values)

Parameter	Dose Group (mg/kg bw/day)				Historical control range (mean value)
	0(control)	50	150	450	
No. of mated females	19	19	16	19	--
No. not pregnant	0	1	1	6	--
No. of does with live young or litters at Day 29	18	12	15	13	--
Corpora lutea	11.5	12.4	11.7	11.3	9.0 – 12.9 (11.2)
Implantations	9.7	10.5	9.0	9.2	7.0 – 11.1 (9.5)
Pre-implantation loss	14.6	15.4	23.4	18.8	2.3 – 26.1 (15.1)
Early embryonic deaths	0.4	0.9	0.8	0.5	0.3 – 1.1 (0.6)
Late embryonic deaths	0.2	0.9	0.5	1.3**	0.1 – 1.3 (0.7)
Abortions	0.0	0.0	0.1	0.0#	0.0 – 0.1 (0)
Total embryonic deaths	0.6	1.8*	1.5*	1.8**	0.6 – 2.0 (1.2)
Post-implantation loss (%)	5.7	19.5*	15.3	21.0**	6.5 – 17.5 (12.9)
Live young	9.1	8.7	7.6	7.3	6.1 – 9.5 (8.3)
Litter weight (g)	389.5	370.6	320.5	315.0	281.9 – 402.2 (352.9)
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--

* Statistically significant by Kruskal –Wallis 'H' test $P < 0.05$
** Statistically significant by Kruskal –Wallis 'H' test $P < 0.01$
\# Fisher exact test follow-up by intergroup comparison with control was not statistically significant $P > 0.05$

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

There were 18, 12, 15 and 13 viable litters at 0, 50, 150 and 450 mg/kg bw/day, respectively. The concurrent control showed low mean values for embryonic deaths and post implantation losses when compared with historical control values. When compared with these historical data as noted above, mean values in the treated groups were within the expected range; therefore, it was concluded that no adverse effect on foetal survival was attributed to glyphosate.

Foetal body weights

There was a dose-related reduction in mean foetal weight on a litter basis in all treated groups (not statistically significant) compared with the control; however, the mean individual foetal weight was not affected.

External, visceral and skeletal examination

Malformations were slightly increased in the 150 and 450 mg/kg bw/day dose groups compared to controls and appeared to be associated with an apparent increase in malformations of the thoracic region.

However, neither the incidence nor the percentage of malformed foetuses was outside the historical control range and the values were not statistically different from concurrent control values. Several of the cardiovascular malformations that were observed, particularly in the high-dose group, occurred in the same animals and are related to a single morphogenetic mechanism (i.e., displacement of the developing aorticopulmonary septum), which is likely to adjust during the first two weeks of postnatal life. These related findings, which often cluster together, included dilated/narrow aorta and narrow/dilated pulmonary artery; interventricular septal defect; and disproportionately sized right and left ventricles. These findings were observed (often in clusters) in the historical control data that were provided by the conducting laboratory. Individual presentation of these malformations in tables when the malformations occurred together in the same foetus and are due to the same mechanisms and artificially inflates the sense that there is a much stronger cardiac effect than is actually present.

The cardiac malformation observed with greatest frequency in this study was interventricular septal defect. The number of foetuses and litters with ventricular septal defects were 1, 1, 1 and 4 in the 0, 50, 150 and 450 mg/kg bw/day dose groups, respectively. Comparison of the historical control data (see Table 5.6-47) shows that the heart findings (when presented on a percent individual and/or litter incidence basis) were slightly outside of the historical background range from 13 studies conducted during the same period. However, the disparity in values is a consequence of the small numbers of litters in the study report. If the data are displayed as a fraction (rather than a percentage), then the number of litters affected were 1/18, 1/12, 1/15, and 4/13 in the 0, 50, 150, and 450 mg/kg/day dose groups, respectively. The historical control range is 0/19 – 3/13. Thus, the findings at the high dose are barely outside of the historical control range. Further, they were observed in conjunction with clear signs of maternal toxicity (reduced food consumption, body weight gains and increased clinical signs).

The other cardiovascular finding found in this study not related to the morphogenetic mechanism involving formation of the spiral septum is retroesophageal right subclavian artery. This finding was also observed regularly throughout the historical period. It is not uncommon and is oftentimes an inconsequential anatomical difference in vascular arrangement. At autopsy this condition is found in 0.5 – 2.0% of subjects.

The malformations of the cranial region, the lumbar and the lumbar/sacral regions did not show any treatment-related trend and are considered to be incidental. The incidences of anomalies and variants did not suggest any treatment relationship. The incidence of foetuses with reduced ossification did not show any dose-relationship; however, lower foetal weights were observed for the 450 mg/kg bw/day dose group with reduced ossification.

The observed foetal malformations and anomalies are summarised in the following (Table 5.6-47).

Table 5.6-47: Summary of foetal parameters

Parameter	Dose Group (mg/kg bw/day) 0(control)	50	150	450	Historical control range or x/y ◊ (mean)
Number of does with live young or litters at Day 29	18	12	15	13	--
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--
Malformations					--
Total number of foetuses examined	163	104	112	95	1511
No. of malformed foetuses	3	3	5	6	51
%	1.9	5.8	4.3	5.9 (F)	0.7 – 5.9 (3.8)
Number of Affected Litters	3	3	3	5	43/188
%	16.67	25	20	38.5	22.9
Thoracic region malformations					--
No. of foetuses with interventricular septal defect	1	1	1	4	10/1511
%	0.6	1.0	0.9	4.2	0.66
Litter incidence	1	1	1	2	10/188
%	5.56	8.3	6.67	20.8	5.32
Foetuses with enlarged left, reduced right ventricles	0	0	0	2	2/1511
%	0.0	0.0	0.0	2.1	0.13
Litter incidence	0	0	0	2	2/188
%	0	0	0	15.4	1.10
Foetuses with retro-oesophageal right subclavian artery	0	0	3	2	7/1511
%	0.0	0.0	2.7	2.1	0.46
Litter incidence	0	0	1	1	7/188
%	0	0	6.6	7.6	3.72
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1	1	1	3	8/1511
%	0.6	1	0.9	3.2	0.52
Litter incidence	1	1	1	3	8/188
%	5.56	8.3	6.67	23.1	4.25
Anomalies					--
Total number of foetuses examined	160	101	107	89	--
No. of foetuses with gross/visceral anomalies	8	14	14	6	--
%	5.4	19.5	12.9	9.6 (K)	--
No. of foetuses with skeletal anomalies	21	13	14	11	--
%	11.7	17.7	12.5	10.1 (K)	--
No. of foetuses with reduced ossification	7	4	5	4	--
%	4.4	4.0	4.7	4.5	--
Mean foetal weight of foetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

◊ number affected / total number examined
Malformed foetuses are excluded
(F) Fisher's exact test applied, not statistically significant (P > 0.05)
(K) Kruskal-Wallis 'H' statistic, not significant (P > 0.05)
-- no data

III. CONCLUSION

Glyphosate was not teratogenic in this developmental toxicity study in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw/day based on clinical signs of toxicity including reduced feed consumption and bodyweight gain and soft/liquid faeces during the dosing period. The NOAEL for

foetotoxicity was 150 mg/kg bw/day based on statistically significantly increased embryo/foetal deaths and post-implantation loss. The NOAEL for teratogenicity was 450 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/06	[redacted]	1993	Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990) [redacted] Data owner: Feinchemie Schwebda GmbH Study No.: [redacted]884-TER-RB Date: 1993-01-17, amended 1994-06-18 GLP: not published

Guideline:	OECD 414 (1981)
Deviations:	None
Dates of experimental work:	1991-12-24 to 1992-03-06

Executive Summary

This developmental toxicity study in rabbits is not clearly or accurately documented and does not provide appropriate interpretation of the study findings. Groups of presumed mated female New Zealand White rabbits were administered once daily by gavage, glyphosate (batch no. 60, purity 96.8 %) in 0.5 % carboxymethylcellulose (dose volume 2 ml/kg) at dose levels of 0 (vehicle control), 20, 100 or 500 mg/kg bw/day from Day 6 to Day 18 of pregnancy (mating = Day 0). Dose volumes were calculated for individual animals from day 6 and adjusted according to body weight daily. The day of mating was considered as Day 0. Dose levels were based on the findings of preliminary studies. All animals were observed twice daily for onset and duration of signs of toxicity and for mortality. All animals in the experiment that died, were killed moribund or killed at termination on day 28 were subjected to post-mortem gross pathological examination. Individual body weights of dams were recorded on Days 0, daily from Days 6 – 18 and on Day 28 of gestation.

The ovaries and uteri were examined to determine the number of corpora lutea, implantations, the number of dead/abnormal/live foetuses, the number and distribution of embryonic and /foetal resorptions. Foetuses were examined for individual foetal weight and foetal abnormalities, sex and visceral organ malformations by foetal necropsy/modified Wilson's technique.

There were 4/16 and 8/15 mortalities at dose levels of 100 and 500 mg/kg bw/day, respectively. The deaths at 500 mg/kg bw/day were accompanied by clinical signs, including the increased incidence of soft liquid/diarrhoea or mucoid faeces, reduction in feed consumption and reduction in body weight gain during the treatment period. However, clinical signs were not evident for the 100 mg/kg bw/day dose group and only one incidence of soft faeces was recorded at 100 mg/kg bw/day. Further, a number of lung and tracheal findings in the dams at gross necropsy indicated possible gavage errors to which the deaths at this dose may be attributed. Thus, the NOAEL for maternal toxicity was set to 100 mg/kg bw/day.

Glyphosate technical was not considered to be teratogenic in this developmental toxicity study in rabbits. The incidence of one visceral effect, dilated heart, was increased at the highest test dose and was present at lower dose levels, but there were too few foetuses present in the high dose group to corroborate a dose-response relationship. Further, foetal findings at the highest test dose were observed in the presence of extensive maternal toxicity. Mortality and clinical signs of toxicity at 500 mg/kg bw/day included reduced feed consumption and soft faeces and reduced bodyweight gain during the dosing period one incidence of

complete resorptions. The NOAEL for foetotoxicity and teratogenicity was 100 mg/kg bw/day based on occurrence of general signs of secondary toxicity (incomplete ossification and similar).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	Odourless white crystals
Lot/Batch #:	60
Purity:	96.8%
Stability of test compound:	Stable over 2 years at ambient temperature.

2. Vehicle and/ or positive control: 0.5 % w/v carboxymethylcellulose

3. Test animals:

Species:	Rabbit
Strain:	Female New Zealand White
Source:	[redacted] India.
Age:	Approximately 6 months and above (at the start of study)
Sex:	Males and females
Weight at dosing:	Females: >2500 g (mean values)
Acclimation period:	At least 10 days.
Diet/Food:	Pelleted rabbit diet, supplied by [redacted] (composition and feed analysis reports were provided) was provided *ad libitum*
Water:	Protected deep bore well drinking water, treated via activated charcoal filter and UV in Aquaguard on-line water filter-cum-purifier, provided *ad libitum*
Housing:	Individually in 3-tier all aluminium cages with wire mesh bottom and common self-draining litter trays.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40 – 70 % Air changes: 10 – 15/h Natural lighting supplemented with fluorescent lighting 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: not reported

Animal assignment and treatment:
In a developmental toxicity study, groups of presumed mated female New Zealand White rabbits were administered once daily by gavage, glyphosate (batch no.: 60, purity 96.8 %) in 0.5 % carboxymethylcellulose (dose volume 2 ml/kg) at dose levels of 0 (vehicle control), 20, 100 or 500 mg/kg bw/day from Day 6 to Day 18 of pregnancy. Dosages for individual animals were calculated from day 6

and adjusted daily according to body weight. Dose levels were based on the findings of preliminary studies.

In a preliminary dose-range finding study, one male rabbit/dose group was administered by gavage glyphosate technical dissolved in 0.5% carboxymethylcellulose (dose volume 2ml/kg bw) at dose levels of 0(control), 10, 20, 50, 500 or 1000 mg/kg bw/day for 13 days. Doses of ≥ 500 mg/kg bw/day resulted in loss in body weight and in feed intake and the 1000 mg/kg bw/day test animal died on Day 9 of treatment.

In a second dose-range finding study, one pregnant rabbit was administered 500 mg/kg bw/day glyphosate from day 6 to 18 of gestation and the findings compared with that of 20 historical control animals. Caesarean section and terminal necropsy was performed on day 28. There were no signs of toxicity from the treatment; body weight gain was greater (26% more than the historical control mean) but notable apparent treatment-related changes were substantial reduction in feed intake (34% of historical control mean) and reduced litter size in the test female (4) compared with the historical control mean (7).

Observations
All animals were observed twice daily for onset and duration of signs of toxicity and for mortality. All animals in the experiment that died, were killed moribund or killed at termination were subjected to post-mortem gross pathological examination. Tissues with gross lesions were preserved for histopathological examination as necessary.

Body weight
Individual body weights were recorded on Days 0, daily from Days 6 – 18 and on Day 28 of gestation.

Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology
On day 28 of pregnancy, all surviving dams were subjected to post-mortem examinations and pups were delivered by Caesarean section. The ovaries and uteri were excised and weighed and maternal and foetal data were recorded. The maternal data determined were pregnant/non-pregnant, uterine weight, the number of corpora lutea, the number of implantations, the number of embryonic and foetal resorptions. The foetal data recorded were the number of dead/abnormal/live foetuses, individual foetal weight and sex.

Litter parameters
All the foetuses were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were euthanized with ether and visceral organs examined by a modified Wilson technique. Skeletal assessments were performed after appropriate preparation including staining in Alizarin Red. Structural changes were presented as variants, minor and major malformations.

Statistics
Statistical methods employed included the following. Maternal body weight and weight gain, feed intake, number of corpora lutea, number of implantations and mean foetal weight were analyzed by Bartlett's test followed by ANOVA and Dunnett's test. Day '0'and absolute body weight data were compared by the Paired Student's ´t` test. The number and percent embryonic resorptions and foetal resorptions, the number of dead foetuses, the number of abnormal foetuses and percentage pre-implantation and post-implantation loss by Mann Whitney test. Litter size was by Student 't' test. The sex ratio, number of dams with any resorptions, number of dams with all resorptions and incidence of malformations were analysed by Chi-square test.

The statistical analysis and comparison of individual treatment groups with control value were done at 5% probability level and the results were designated as significantly higher (+) / lower (-) than control value at $P \leq 0.05$.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Not reported.

B. FOOD CONSUMPTION

During the dosing period, feed consumption was significantly reduced (31%) in females receiving the 500 mg/kg bw/day dose compared to the controls (see Table 5.6-48). Feed consumption during the post-treatment period did not show significant intergroup differences.

Table 5.6-48: Summary of food consumption

Parameter	Historical positive control#	Dose Group (mg/kg bw/day)			
		0 (control)	20	100	500
Food consumption (g/rabbit/day)					
No of dams included in assessment	7	20	13	12	6
Day 0 – 6 (Pre-treatment)	105	114	88*	125	118
Day 6 – 19 (Treatment)	70*	103	109	102	71*
Day 19 – 28 (Post treatment)	129	109	135	107	105
Day 0 - 28	96	107	118	108	92

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw (treatment: Day 6-18; post-treatment: Day 18-28)
* Significantly lower than controls by Dunnett's test $P \leq 0.05$

C. MORTALITY

The four and eight deaths observed in the mid- and high-dose group were considered to be treatment-related by the study director (see Table 5.6-49 below). However, the two confirmed misdosings in the control, the absence of signs of toxicity at 100 mg/kg bw and the absence of mortality in this dose range in the considerably high number of parallel studies shed serious doubt on a relation to treatment at this dose level. Further, various findings at gross necropsy were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups; these findings suggest possible gavage errors, which could be responsible for some of the deaths observed at these doses and are not appropriately discussed in the report.

Table 5.6-49: Summary of mortality in dams

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	20	100	500
Mated females	26	17	16	15
Dead during treatment	1*	0	4	5
Died post-treatment	1*	0	0	3
Total number of deaths	2	0	4	8
% mortality	7.7	0.0	25.0	53.3

* Animal died due to wrong gavaging

D. CLINICAL OBSERVATIONS

Signs of toxicity were observed at the 500 mg/kg bw/day dose group and were predominantly gastrointestinal effects, which included soft stool/liquid faeces and soft stool with mucus. Further signs of toxicity were rales, weakness, dyspnoea and ocular discharge.

Table 5.6-50: Summary of relevant clinical signs in dams

Parameter / clinical sign	Dose Group (mg/kg bw/day)			
	0 (control)	20	100	500
Mated females	26	17	16	15
Pregnant at termination	20	13	12	6
Rales	1	0	0	3
Soft stool with mucus	0	0	0	2
Soft stool/liquid faeces	0	0	1	12

Parameter / clinical sign	Dose Group (mg/kg bw/day)			
	0 (control)	20	100	500
Weak	0	0	0	2
Ocular discharge	0	0	0	1
Dyspnoea	0	0	0	1

E. BODY WEIGHT

No treatment-related and dose-related significant changes were observed in maternal body weight and body weight gain between the control, low- and mid-dose groups. In the high-dose group, initial body weight and body weights at the different time intervals were significantly lower than in the control group.

Table 5.6-51: Summary of maternal body weight data

Parameter	Historical positive control#	Dose Group (mg/kg bw/day)			
		0 (control)	20	100	500
Number of dams pregnant at termination		20	13	12	6
Mean bodyweights (kg)					
Day 0		3.1	2.8	3.0	2.6*
Day 6		3.2	3.0	3.0	2.8*
Day 18		3.2	3.0	3.1	2.8
Day 28		3.3	3.3	3.3	3.0*
Day 28 (bodyweight – uterine weight)	2.7*	3.0	3.0	2.9	2.7
Mean bodyweight gain (kg)					
Day 0 – 6 (Pre-treatment)	0.2	0.1	0.2	0.0	0.1
Day 6 – 18 (Treatment)	-0.1	0.1	0.1	0.1	0.0
Day 18 – 28 (Post treatment)	0.2	0.1	0.2	0.2	0.2
Day 0 – 28 (Throughout gestation)	0.3	0.2	0.5**	0.3	0.3

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw/day
* Significantly lower than controls by Dunnett's test $P \leq 0.05$
** Significantly higher than controls by Dunnett's test $P \leq 0.05$

F. PATHOLOGY

Necropsy

Gross examination of dams at post-mortem did not identify any treatment-related effects. However, various findings were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups which suggest possible gavage errors and issues with animal husbandry.

Observations on the ovary and uterus

A total of 20, 13, 12 and 6 pregnant females survived to termination and 134, 80, 78 and 28 foetuses were recorded for the 0 (control), 20, 100 and 500 mg/kg bw/day dose groups, respectively, and were included in the assessment. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss (complete resorptions) was recorded for one female in the 500 mg/kg bw/day dose group; otherwise, the incidence of dams with any resorptions did not show any treatment-related differences.

There were no significant intergroup differences in the mean numbers of corpora lutea, pre-implantation and post-implantation losses and resorptions (embryonic and foetal) (see Table 5.6-52).

Table 5.6-52: Summary of maternal observations

Parameter	Historical positive control#	Dose group (mg/kg bw/day)			
		0 (control)	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2	0	4	8
Pregnant at termination	7	20	13	12	6
Mean number of corpora lutea	9	11	10	10	9
Mean number of implantations	8	8	8	9	6
Total number of embryonic resorptions (%)	6 (11)	10 (7)	11 (11)	11 (11)	9 (24)
Total number of foetal resorptions (%)	2 (4)	8 (5)	7 (7)	13 (13)	1(3)
Total number of pre-implantation loss (%)	10 (19)	72 (48)	28 (29)	20 (20)	14 (37)
Total number of post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of dams with any resorptions (%)	2 (29)	12 (60)	11 (85)	9 (75)	2 (33)
Dams with complete resorptions (%)	1 (14)	0 (0)	0 (0)	0 (0)	1 (17)

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

Because of the large number of maternal deaths at 500 mg/kg/day (and thus the reduced number of total litters), the total number of foetuses was substantially less in this dose group compared to the other dose groups. However, the mean litter size, the mean numbers of abnormal, dead or live foetuses and the sex ratios of foetuses did not show any significant treatment-related differences. Glyphosate also did not cause an increase in the number of foetal deaths in utero (see Table 5.6-53).

Foetal body weights

Although foetal body weights in the 20 and 100 mg/kg/day dose groups were reported to be significantly different from control, the weights were increased, the changes were less than 10% of control values and no dose-response across treatment groups was evident. Thus, the foetal body weight differences observed in these two dose groups are biologically inconsequential with respect to adverse effects (see Table 5.6-53).

Table 5.6-53: Mean litter data at caesarean section

	Historical positive control#	Dose group (mg/kg bw/day)			
		0	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2	0	4	8
Pregnant at termination	7	20	13	12	6
Number of litters	6	20	13	12	5
Total number of foetuses	46	134	80	78	28
Mean litter size	8	7	6	7	6
Abnormal foetuses (%)	0 (0)	1 (1)	2 (3)	0	0
Dead foetuses (%)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)
Post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of live foetuses	46	133	77	77	28
Mean weight of live foetuses (g ± SD)	29 ± 1.4	32 ± 5.3	35 ± 3.7*	35 ± 2.4*	33 ± 4.9
Sex ratio (Male : Female)	1 : 1.3	1 : 0.7	1 : 1.2	1 : 1.2	1 : 1.8

# Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw
SD = standard deviation
* Significantly higher than controls by Dunnett's test $P \leq 0.05$

External, visceral and skeletal examination

The incidence of major external malformations did not identify any treatment-related differences; further, none of the external malformations occurred in the highest dose group.

Visceral examination noted no significant treatment-related incidences of minor malformations or variants. Major visceral malformations primarily affected the heart, but occurred in single incidences and showed no dose-response (see Table 5.6-54). The exception was dilated heart, which was reported in four foetuses of 3 litters in the 20 mg/kg bw/day dose group, 4 foetuses (3 + 1) from 2 litters of the 100 mg/kg bw/day dose group and all foetuses (4) of one litter and one foetus of another litter at the 500 mg/kg bw/day (Statistically significant $P \leq 0.05$). The terminology used to describe the heart malformations in this study is different than that typically employed in teratology research (e.g., dilated heart, seal-shaped heart). Consequently, what is meant by the description "dilated heart" is not well defined and not documented with photographs or retained tissue sections or slides.How this malformation might relate to others reported in the heart (i.e., dilated left or right ventricle, seal-shaped heart, cardiomegaly) is not clear. Further, because too few foetuses were available for examination in the high dose group, it cannot be determined whether these defects exhibited a true dose-related increase. It is important to note, however, that only 2 litters exhibited major visceral malformations in the high dose group. Additionally, these findings were found in the presence of extensive maternal toxicity, evidenced by reduced food consumption and body weight gains in the few animals that survived this dose level, clinical signs, and substantial deaths.

Major, minor and skeletal malformations did not show any clear treatment-related findings and appeared to be incidental

Table 5.6-54: Summary of relevant external, visceral and skeletal findings (litter data)

Foetal findings	HC Data[#]	Dose level (mg/kg bw/day) 0	20	100	500
No. of litters examined	24	26	13	12	5
No. of foetuses examined	46	153	79	77	28
Minor external malformations					
Percentage of small foetuses (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Major external malformations					
Percentage of foetuses with upper cleft palate (%)	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with forelimb arthrogryposis	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with multiple malformations	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with major malformations (%)	0	1.5	2.5	1.3	0
Litter incidence (%)	--	10	7.7	8.3	0
Major visceral malformations					
Percentage of foetuses with dilated heart (%)	--	0	5.1	5.2	17.9
Litter incidence (%)	--	0	23.1	16.7	40.0
Percentage of foetuses with anencephaly (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with heart–seal shaped (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with cardiomegaly & sealed heart (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (left) (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (right) (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with persistent truncus arteriousus (%)	--	0.8	0	0	0
Litter incidence (%)	--	5.0	0	0	0
Percentage of foetuses with gallbladder absent (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with liver (median) haematoma (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20

Foetal findings	HC Data#	Dose level (mg/kg bw/day) 0	20	100	500
Minor skeletal malformations					
No. of foetuses with extra 13th rib		0	1	2	1
Percentage of foetuses with extra 13th rib	8.7**	0	1.3	2.6	3.6*
Litter incidence (%)	--	0	7.7	16.7	20
Major skeletal malformations					
Percentage of foetuses major malformations (%)	10.9	8.3	6.3	0*	3.6
Litter incidence (%)	50	20	23.1	0	20

Historical positive control data (--: no data available)
* Significantly different from control at $p < 0.05$.
** Significantly different from control by Contingency test ($P \leq 0.05$)

III. CONCLUSION

Glyphosate technical was not considered to be teratogenic in this developmental toxicity study in rabbits. The incidence of one visceral effect, dilated heart, was increased at the highest test dose and was present at lower dose levels, but there were too few foetuses present in the high dose group to corroborate a dose-response relationship. Further, foetal findings at the highest test dose were observed in the presence of extensive maternal toxicity that exceeded guideline recommendations for a high dose. The NOAEL for maternal toxicity was 100 mg/kg bw/day based on mortalities at dose levels of ≥ 100 mg/kg bw/day. Mortality and clinical signs of toxicity including reduced feed consumption and soft faeces and reduced bodyweight gain during the dosing period one incidence of complete resorptions at the 500 mg/kg bw/day dose level. The NOAEL for foetotoxicity and teratogenicity was 100 mg/kg bw/day based on occurrence of general signs of secondary toxicity (incomplete ossification and similar) at the high dose.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/07	[redacted]	1989	Rabbit Teratology Study with Glyphosate Technical [redacted] Data owner: Excel Study no.: [redacted] Project No. 1086 Date: 1989-11-03 GLP: no not published
Guideline:			OECD 414 (1981)
Deviations:			no uterine weight, no maternal necropsy findings
Dates of experimental work:			1989-07-03 to 1989-11-02

Executive Summary

This developmental toxicity study in rabbits is limited in its extent of documentation. Glyphosate Technical was administered by gavage to three groups of 15 successfully mated New Zealand albino rabbits each, at doses of 125, 250, and 500 mg/kg bw/day from Gestation Day 6-18 (mating = Day 1). A further group of 15 animals was exposed to the vehicle to serve as control.

No adverse effects related to test substance treatment were observed in any animals of the low and mid dose group. Mean maternal body weights and food consumption were lower in the high dose group, however clinical signs of systemic toxicity were not observed at any dose. In the high dose group, two cases of total abortion were considered to be related to test substance treatment.

The mean number of viable implants (foetuses) per litter was lower in the high dose group and the mean number of external, visceral and skeletal malformations as well as the mean number of variations was higher in the high dose group compared to the control group. No differences in the examined

developmental parameters were found in the low and mid dose groups, although a dose-related increase in the numbers of malformations and variations was noted.

Conclusion:
The oral administration of glyphosate to successfully mated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. The NOAEL for reproductive and non-reproductive toxicity was considered to be 250 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White amorphous powder
Lot #:	38
Purity:	95%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: 0.1% gum acacia in water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	24 - 28 weeks
Sex:	Females
Weight at dosing:	1.50 – 2.00 kg
Acclimation period:	6 days
Diet/Food:	Pelleted rabbit feed supplied by [redacted] India
Water:	Tap water, *ad libitum,* supplied in polypropylene bottles by [redacted]
Housing:	Individually in stainless steel cages equipped with food and water dispensers and stainless steel grate at bottom
Environmental conditions:	Temperature: 20 ± 3°C Humidity: 30 to 70% Air changes: not reported 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1989-07-03 to 1989-11-02

Animal assignment and treatment:
In a teratogenicity study groups of 15 New Zealand White female rabbits received doses of 0, 125, 250 and 500 mg/kg bw/day test substance in 0.1% gum acacia in water by gavage from Gestation Day 6-18 after successful mating with adult vigorous males. The day of mating was taken as the 1st day of pregnancy.

Diet preparation and analyses
For each dose level, dosing solutions were prepared in 0.1% gum acacia in water as vehicle.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy). Changes in body weight were calculated and recorded as group maternal weight changes for the periods of Days 0-6 (pre-exposure), 6-12, 12-18, 18-23, 23-29 and 18-29 (post-exposure observation period).

Food consumption
Food consumption was recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy).

Sacrifice and pathology
Females were euthanatized by carbon dioxide asphyxiation on day 29 of gestation and examined for any abnormalities that would affect pregnancy.
The ovaries and uteri were removed, the uteri were weighed, and the ovaries were examined for the number of corpora lutea and uteri for the number and position of implants and dead or live foetuses. Uteri from non-gravid females were placed in 10% ammonium sulfide solution for detection of early resorptions.

Developmental parameters
Each rabbit foetus was removed from the uterus and was killed by injection of pentobarbitone. All live foetuses were weighed and examined for external malformations including cleft palate and variations. All live foetuses were examined for thoracic and visceral abnormalities and each foetus was sexed. Following visceral examination, all foetuses were eviscerated and processed for skeletal staining with Alizarin Red SAll foetuses were decapitated and heads were fixed in Bouin's solution for examination of craniofacial structures.

Statistics
Not reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The analytical purity of test substance was stated to be 95%.

B. FOOD CONSUMPTION
Mean food consumption in the low and mid dose groups was comparable to that in the control group throughout the study period. Significantly lower food consumption (~17% lower mean food consumption compared to control, low or mid dose group) was observed in the high dose group starting with the day of treatment throughout the rest of the observation period.

C. MORTALITY
None of the rabbits died during the study period.

D. CLINICAL OBSERVATIONS
No toxic symptoms were observed in any of the animals during the study.

E. BODY WEIGHT
Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, the mean maternal weight increase was lower for each of the observation

periods between Days 12-29 compared to controls, but no stastical comparison was provided in the report.

F. PATHOLOGY

Necropsy

No abnormalities that could affect pregnancy were reported at maternal necropsy.

Observations on the ovary and uterus

Two animals of the high dose group aborted (see Table 5.6-55).

Table 5.6-55: Gestational parameters in rabbits treated with glyphosate

Gestational parameter	Dose level (mg/kg bw/day)			
	0	125	250	500
No. of pregnant females	15	15	15	15
No. of early deliveries	0	0	0	0
No. of abortions	0	0	0	2
No. of females with no live foetuses	0	0	0	2
No. nonpregnant at termination	2	1	1	3
No. of litters	13	14	14	12
Mean no. of corpora lutea per doe	10.0	10.1	10.3	9.8
Mean no. of total implants per litter	9.0	9.3	9.4	8.5
Mean % pre-implantation loss	21.3	14.0	14.7	13.1
Mean no. of viable implants per litter	7.3	8.0	8.0	5.2
Mean no. of non-viable implants per litter	0.07	0.13	0.27	1.4
Mean no. of early resorptions per litter	0.7	1.1	1.0	1.9
Sex ratio (% males)	44.4	49.2	49.7	50.1
Mean foetal body weight per litter	40.6	47.1	47.5	48.7

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

The mean number of viable implants (foetuses) per litter was lower in the high dose group, and accordingly, the mean number of non-viable implants (foetuses) per litter was greater in the high dose group (see Table 5.6-55), but no statistical comparisons were provided in the report.

Sex ratio, foetal body weights and placental weights

No differences were noted in the sex ratios, mean foetal body weights, mean number of corpora lutea per dose, mean number of total implants per litter, mean percentage of pre-implantation loss, and mean number of early resorptions between the control and the treated groups. In the high dose group, two dams had no live foetuses due to abortions (see Table 5.6-55). However, stasticial analyses were provided in the report.

External, visceral and skeletal examination

No difference was noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the incidences of external, visceral and skeletal malformations were higher than that in the control group (see Table 5.6-56). With regard to the heart malformations, 0, 1, 1, and 2 interventricular septal defects were observed in the 0, 125, 250, and 500 mg/kg bw/day dose groups.

A similar pattern was seen in the variations observed externally, viscerally and skeletally; in the high dose group, the total number of observed variations was higher than those of the control, low or mid dose groups. The increase in malformations and variations observed in the high dose group occurred in the presence of maternal toxicity (reduced food consumption and body weight gains). Further, this was at a dose (500 mg/kg bw/day) that caused significant toxicity, including mortality, in another rabbit developmental study. However, stasticial analyses were provided in the report.

Table 5.6-56: Incidence of foetal malformations and variations in rabbits treated with glyphosate

Foetal findings	Dose level (mg/kg bw/day)			
	0	125	250	500
Malformations				
No. of litters examined	**13**	**14**	**14**	**12**
No. of foetuses examined	**109**	**113**	**120**	**78**
No of litters with malformations	**3**	**6**	**10**	**12**
% of litters with malformations	**23.08**	**42.86**	**71.43**	**100**
No. of foetuses with malformations	**3**	**6**	**10**	**20**
% of foetuses with malformations	**2.75**	**5.31**	**8.33**	**25.64**
Number of foetuses (litters) with external malformations				
Tail abnormal	1 (1)	1 (1)	2 (2)	3 (2)
Low-set ears	0 (0)	1 (1)	1 (1)	2 (1)
Total external malformations	1	2	3	3
Total external malformations (%)	0.92	1.77	2.50	3.85
Number of foetuses (litters)with visceral malformations				
Ventricular septal defect	0 (0)	1 (1)	1 (1)	2 (2)
Postcaval lung lobe absent	0 (0)	1 (1)	1 (2)	4 (3)
Kidney(s) absent	1 (1)	2 (2)	3 (2)	6 (4)
Total visceral malformations	1	4	5	12
Total visceral malformations (%)	0.92	3.54	4.17	15.38
Number of foetuses (litters) with skeletal malformations				
Rudimentary rib (no. 14)	1 (1)	0 (0)	2 (2)	5 (2)
Total skeletal malformations	1	0	2	5
Total skeletal malformations (%)	0.92	0.00	1.67	6.41
Variations				
No. of foetuses examined	**109**	**113**	**120**	**78**
Total no. of observed variations	**36**	**30**	**49**	**93**
Number of foetuses (litters) with external variations				
Tail blunt tipped	1 (1)	0 (0)	3 (2)	5 (4)
Number of foetuses (litters) with visceral variations				
Irregular rugae on palate	0 (0)	2 (1)	3 (2)	2 (2)
Lateral ventricles of cerebrum dilated	0 (0)	2 (2)	2 (2)	6 (4)
Right ventricle small than normal	1 (1)	3 (2)	3 (2)	5 (3)
Globular heart	2 (2)	0 (0)	3 (2)	5 (4)
Incomplete separation of lung lobes	1 (1)	2 (1)	2 (1)	4 (2)
Parietal foetal atelectasis	0 (0)	1 (1)	1 (1)	1 (1)
Liver irregular shape	0 (0)	2 (1)	2 (2)	6 (4)
Kidney(s) globular shape	0 (0)	0 (0)	2 (1)	5 (3)
Number of foetuses (litters) with skeletal variations				
Cervical centra 1-3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extra thoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	0 (0)	3 (2)	4 (3)
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

III. CONCLUSION

The oral administration of glyphosate to mated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. Therefore the NOAEL for reprotoxic and non-reprotoxic effects was considered to be 250 mg/kg bw/day. Considering the significantly reduced food consumption and gain in body weight at 500 mg/kg bw/day, the maternal NOAEL is 250 mg/kg bw/day.

IIA 5.7 Neurotoxicity

The 2001 glyphosate evaluation concluded that there was no evidence of neurotoxicity in acute, subchronic or chronic studies in rodents and dogs. Glyphosate is often erroneously called an organophophate pesticide. However, it is important to note that glyphosate is not an organophosphate ester but a phosphonoglycine, that does not inhibit cholinesterase activity. Therefore, studies for delayed neurotoxicity are not considered essential. Despite this, two studies for delayed neurotoxicity in hens ([redacted] 1987 and 1988) have been conducted and were reviewed during the 2001 EU glyphosate evaluation confirmed the absence of neurotoxic effects. The NOAEL derived from these studies is 1000 mg/kg bw/day.

An acute neurotoxicity in rats was performed by [redacted] (1996a) that was not reviewed during the 2001 glyphosate evaluation. Administration of glyphosate acid produced clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) at approximately 6 hours after dosing on day 1 in 3/10 females only, which received 2000 mg/kg. One of these females was subsequently found dead on day 2. These clinical signs were considered to reflect general toxicity associated with the administration of high dose levels of glyphosate acid. Quantitative assessment of landing foot splay, sensory perception, muscle weakness and locomotor activity revealed no changes indicative of neurotoxic potential. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals receiving 2000 mg/kg.

The no-observed effect level (NOEL) for neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg and the NOEL for systemic toxicity was 2000 mg/kg.

In addition a sub-chronic neurotoxicity study was also performed by [redacted] (1996b). In this study administration of glyphosate acid produced no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system revealed no evidence of any changes which could be attributed to administration of glyphosate acid. The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm (equivalent to 1547/1631 mg/kg bw/day in males and females respectively). There was a treatment-related effect on growth and food utilisation in males receiving 20000 ppm (1547 mg/kg bw/day). In this study the NOEL for systemic toxicity was 8000 ppm (617 mg/kg bw/day) in males and 20000 ppm (1631 mg/kg bw/day) in females.

In a study performed by [redacted] (1996, see IIA 5.10/03) *ex vivo* investigations with isolated rat gastrocnemius muscle were performed. Evaluation of innervated muscle response showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline (12 mg/mL), did not cause any neuromuscular blocking activity.

Overall, across a wide database of both specific neurotoxicity and repeat dose studies there is no evidence that glyphosate has neurotoxic potential.

Table 5.7-1: Summary of neurotoxicity studies with glyphosate acid

	Reference (Data owner)	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.7 Glyphosate Monograph [redacted] 1987 (EXC)	21-day, oral diet Hen, White leghorn	0, 250, 500, 1000 mg/kg bw/day	Toxicity: 500 mg/kg bw/day Neurotoxicity: 1000 mg/kg bw/day	1000 mg/kg bw/day: slight ataxia in 1 hen; all hens appeared hunched, lethargic, red liquid and mattening of feathers in anogenital region, body weight and food consumption ↓, haematological changes
	Annex B.5.7 Glyphosate Monograph [redacted] 1988 (EXC)	21-day, oral diet Hen, White leghorn	0, 400, 800, 1600 mg/kg bw/day	Toxicity: 800 mg/kg bw/day Neurotoxicity: 1600 mg/kg bw/day	1000 mg/kg bw/day: slight ataxia in 1 hen; all hens appeared hunched, lethargic, red liquid and mattening of feathers in anogenital region, body weight and food consumption ↓, haemato-logical changes, reduced egg number
Studies not reviewed in the 2001 evaluation	IIA 5.7.1/01 [redacted] 1996a (SYN)	Acute, oral gavage, Rat Alpk:AP$_f$SD	0, 500, 1000, 2000 mg/kg bw/day	Toxicity: 1000 mg/kg bw/day Neurotoxicity: 2000 mg/kg bw/day	2000 mg/kg bw/day: on day 1 subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia in females only
	IIA 5.7.4/01 [redacted] 1996b (SYN)	13-week oral diet, Rat Alpk:AP$_f$SD	0, 2000, 8000, 20000 ppm (≅ 0, 155.5, 617.1 and 1546.5 mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females)	Toxicity: 617.1 / 1630.6 mg/kg bw/day (♂/♀) Neurotoxicity: 1546.5 / 1630.6 mg/kg bw/day (♂/♀)	20000 ppm (1546.5 mg/kg bw/day) males: reduced body weights and food utilisation

↓ = 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.

IIA 5.7.1 Acute neurotoxicity – rat

Annex point	Author(s)	Year	Study title
IIA, 5.7.1/01	[redacted]	1996a	Glyphosate acid: Acute neurotoxicity study in rats [redacted] Data owner: Syngenta Report No. [redacted]4866 Date: 1996-03-01 GLP: yes unpublished

Guideline: No guideline stated in the report but in general compliance with OECD 424 (1997)

Deviations: None

Dates of experimental work: Not reported. The study was conducted during May and June 1995

Executive Summary

Groups of 10 animals Alpk:APfSD rats per sex were administered 0, 300, 1000 and 2000 mg/kg bw glyphosate acid. Two weeks post administration all animals were observed daily for any changes in clinical condition. A detailed clinical observation was performed at weekly intervals. At scheduled termination 5 rats/sex/group were subjected to full *post mortem* examination. Selected nervous system tissues were examined microscopically.

Clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) occurred during Day 1 but were limited to 3 females in the highest dose group. One of these females was subsequently found dead on Day 2.
Slight reductions in food consumption, without any associated effects on body weight, were also observed during Week 1 for both sexes in the highest dose group.
Quantitative assessment of neurotoxic parameters and histopathological evaluation of the central and peripheral nervous confirmed no neurotoxic potential for glyphosate.

In conclusion, the NOAEL for neurotoxicity following single oral administration of glyphosate acid was 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	Y04707/034
Purity:	95.6% w/w
Stability of test compound:	The test substance was shown to be stable for the period of use.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rats
Strain:	Alpk:APfSD (Wistar-derived)

Source:	[redacted] (UK)
Age:	At least 28 days
Sex:	Males and females
Weight at dosing:	♂ 171.4 – 175.0 g; ♀ 144.6 – 148.7 g
Acclimation period:	Approx. 2 weeks
Diet/Food:	CT1 diet ([redacted] UK), *ad libitum,* except 24 h prior dosing
Water:	Tap water, *ad libitum*
Housing:	In groups of five, separated by sex, in multiple rats racks.
Environmental conditions:	Temperature: 19 – 23 °C Humidity: 40 – 70% Air changes: 25 – 30/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: Not reported. The study was conducted during May and June 1995.

Animal assignment and treatment
In an acute neurotoxicity study groups of ten male and ten female Alpk:APfSD (Wistar derived) rats were administered with a single oral dose of 0, 500, 1000 and 2000 mg/kg bw glyphosate acid by gavage.

Dosing Formulation Analysis
Verification of the achieved concentrations was done with samples of each preparation. Homogeneity was determined with samples from the low to high dose levels. The chemical stability of glyphosate acid in water was also determined for all dose formulations over a period of 10 days.

Clinical observations
Clinical observations were made prior to administration and daily thereafter. Any abnormalities together with the observation of no abnormality detected were recorded.

Body weight
The body weight of each rat was recorded on Days -7 and -1, immediately before dosing (Day 1), approximately 6 hours after dosing (Day 1) and on Days 8 and 15.

Food consumption
Food consumption for each cage of rats was recorded throughout the study and calculated on a weekly basis.

Functional Observational Battery
Prior to the start of treatment (Week -1) and on Day 1, 8 and 15, all animals were observed for signs of functional/behavioural toxicity. Detailed clinical assessments and functional performance tests were performed together with an assessment of sensory reactivity to different stimuli. Locomoter activity was also assessed at these time points.

Sacrifice and pathology
At scheduled termination, 5 rats/sex/group designated for neuropathology were sacrificed. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve. Neuropathological examination was performed on control and highest dose group animals only.

Statistics
Analyses of variance and covariance were carried out using the GLM procedure in SAS (1989). Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

II. RESULTS AND DISCUSSION

A. DOSING FORMULATION ANALYSIS
The achieved concentrations of glyphosate acid in water were within 3% of the nominal levels. The homogeneity was considered acceptable, with a deviation from the overall mean values of approximately ± 8%. The chemical stability was considered satisfactory.

B. MORTALITY AND CLINICAL OBSERVATIONS
Two females receiving 2000 mg/kg bw glyphosate acid showed subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia on the day of administration. One of these animals died on the subsequent day. The other one together with an additional female which showed diarrhoea on the day of administration regained full recovery the subsequent day.
One female receiving 500 mg/kg bw was found dead approximately 6 h after administration.
In the absence of any treatment-related clinical signs prior to death, and because no deaths were observed at the intermediate dose level of 1000 mg/kg bw, the death of this animal was considered not to be treatment related.
Distension of the abdomen was recorded for several males from all treated groups on the day of administration. However, in the absence of any dose relationship, this was not considered to be treatment-related.

C. BODY WEIGHT
No treatment-related effects were observed.

D. FOOD CONSUMPTION
During Week 1, mean food consumption was lower in animals receiving 2000 mg/kg bw glyphosate acid compared to controls, although the difference did attain statistical significance only in females (see Table 5.7-2). There was no evidence of treatment-related effects in animals receiving 500 or 1000 mg/kg bw.

Table 5.7-2: Intergroup comparison of food consumption (g/rat/day) during Week 1

Dose level of glyphosate (mg/kg bw)			
0 (control) Mean ± SD	500 Mean ± SD	1000 Mean ± SD	2000 Mean ± SD
Males			
29.9 ± 0.7	29.0 ± 0.1	30.1 ± 0.4	28.4 ± 0.2
Females			
22.4 ± 1.0	22.2 ± 0.2	22.8 ± 0.3	20.6* ± 0.3

* Statistically significant difference from the control group mean at the 5% level (Student's t-test, two-sided)

E. FUNCTIONAL OBSERVATIONAL BATTERY

Examinations of the functional observational battery did not identify any conclusive treatment- and dose-related effects

F. PATHOLOGY

Necropsy

No macroscopic findings were detected.

Histopathology

No microscopic findings were considered to be treatment-related.

III. CONCLUSION

Based on the study results the NOAEL for acute neurotoxicity, following single oral administration of glyphosate acid is 2000 mg/kg bw.

IIA 5.7.2 Delayed neurotoxicity following acute exposure

There were no indications for a neurotoxic potential of glyphosate observed in acute and subchronic neurotoxicity, acute, short-, and long-term toxicity toxicity studies. Therefore, a study for the assessment of delayed neurotoxicity following acute exposure is considered not necessary.

IIA 5.7.3 28-day delayed neurotoxicity

There were no indications for a neurotoxic potential of glyphosate observed in acute and subchronic neurotoxicity, acute, short-, and long-term toxicity, as well as in reproductive and developmental toxicity studies. Therefore, a 28-day delayed neurotoxicity studies is not required.

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IIA 5.7.4 Subchronic neurotoxicity – rat - 90 day

Annex point	Author(s)	Year	Study title
IIA, 5.7.4/01	[redacted]	1996b	Glyphosate Acid: Subchronic Neurotoxicity Study In Rats [redacted] Data owner: Syngenta Report No.: [redacted]4867 Date: 1996-03-11 GLP: yes unpublished

Guideline:	Study was pre-guideline, but satisfies in general the requirements of OECD 424 (1997)
Deviations:	None
Dates of experimental work:	1995-04-25 to August 1995

Executive Summary

In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks.

All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of

landing foot splay, sensory perception and muscle weakness, were performed at intervals. Locomotor activity was also monitored at intervals. At the end of the study, 6 rats/sex/group were killed and subjected to a full post mortem examination. Selected nervous system tissues were removed, processed and examined microscopically.

Administration of glyphosate acid produced treatment-related effects on growth and food utilisation in males receiving 20000 ppm, with no associated effects on food consumption. There were no treatment-related effects on bodyweight, food consumption or food utilisation for males receiving 2000 or 8000 ppm, or for females from all treated groups.

There were no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system showed no evidence of any changes which could be attributed to administration of glyphosate acid.

The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%
Stability of test compound:	Confirmed for the study period

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Alpk:APfSD
Source:	[redacted] UK
Age:	At least 6 weeks
Sex:	male and female
Weight at dosing:	♂ 215.0 – 218.6 g (mean); ♀ 173.5 – 178.8 g (mean)
Acclimation period:	Approximately 2 weeks
Diet/Food:	CT1 diet ([redacted] UK), *ad libitum* (except up to 24 hours prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	Four per cage per sex in stainless steel cages (26.5 x 50.0 x 20.7cm)
Environmental conditions:	Temperature: 19-23°C
	Humidity: 40-70%
	Air changes: 25-30/hour
	Photoperiod: 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-05-09 to August 1995

Animal assignment and treatment:
In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks. (equivalent to mean achieved dose levels of 0, 155.5, 617.1 and 1546.5 mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females) Glyphosate technical.
All diets were based on CT1 diet supplied by [redacted], UK. The experimental diets were prepared in 30 kg batches by direct addition of the test substance to 30 kg of CT1 diet and mixing thoroughly. The diets were stored at room temperature until required for use.

Samples from all dietary levels (including controls) were taken at intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet, under the conditions of storage used on this study, was determined for 2000 ppm and 20000 ppm diets prepared for use on a concurrent 1 year feeding study in the rat in the same laboratory.

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. A detailed physical examination was performed on each rat prior to start of treatment, and at weekly intervals thereafter.

Functional observational battery (FOB)
Prior to the start of treatment and during Weeks -1, 5, 9 and 14, all animals were observed for signs of functional/behavioural toxicity. The assessment involved observations in the home cage and/or while the rat was moving freely in a standard arena followed by manipulative/in hand tests Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind limb grip strength). The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration.

Locomotor activity:
Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks -1, 5, 9 and 14. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.

Body weight
Individual body weights were recorded in week -1, immediately prior to treatment), at weekly intervals thereafter, and at necropsy.

Food consumption and compound intake
Food consumption was recorded as required for each cage group throughout the study and calculated on a weekly basis. Food utilisation and compound intake were calculated.

Water consumption
Not reported.

Ophthalmoscopic examination
Not performed. However, ophthalmological data are available from other repeated dose studies.

Sacrifice and pathology
At the scheduled termination, all main study animals not required for neuropathology, were killed by overexposure to rising concentrations of carbon dioxide gas and were discarded without examination.

At termination, the six rats/sex/group designated for neuropathology were deeply anaesthetised with intraperitoneal sodium pentobarbitone and killed by whole body perfusion fixation with modified Karnovsky's solution. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.

Brain weight, brain length and brain width were determined.

Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either control diet or diet containing 20000 ppm glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.

The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse and the longitudinal plane.

Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.

Statistics
All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the GLM procedure in SAS (1989)[7].

The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).

II. RESULTS AND DISCUSSION

A. DOSING FORMULATION ANALYSIS
The achieved mean concentrations of Glyphosate acid in diet were within 4% of the nominal levels, with individual values being within 15% of nominal. There were considered acceptable. The homogeneity of the low- and high-dose diets was considered acceptable, with a deviation from the overall mean values of ± 4%. The chemical stability was considered satisfactory.

B. MORTALITY
No deaths occurred during the study.

C. CLINICAL OBSERVATIONS
There were no treatment-related clinical signs of toxicity.

[7] SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. Cary, NC: SAS Institute Inc., 1989

D. FUNCTIONAL OBSERVATIONS

Functional observational battery (FOB)

There were no clinical signs that could be attributed to administration of glyphosate acid.

There was an apparent increase in the incidence of miosis and decreased pupil response to light in males receiving 20000 ppm. However, as these signs were seen for several of these males pre-experimentally and were also present at a similar incidence in females with no obvious relationship to treatment, this was considered to be incidental and unrelated to administration of glyphosate acid.

Landing Foot Splay Measurements

There was no evidence of any treatment-related effect on landing foot splay.

Time to Tail-Flick

There was no evidence of any treatment-related effect on time to tail-flick.

Grip Strength Measurements

There was no evidence of any treatment-related effect on forelimb or hind limb grip strength.

Motor activity

There was no evidence of any treatment-related effect on locomotor activity.

During week 5, slightly reduced locomotor activity was recorded on occasions for females receiving 20000 ppm. However, in the absence of any treatment-related effects on motor activity for these animals at other time points during the study, this is considered to be incidental and unrelated to administration of glyphosate acid.

Table 5.7-3: Selected motor activity findings

		Dietary concentration (ppm)							
		Males				Females			
Week	Assessment period (min)	0	2000	8000	20000	0	2000	8000	20000
5	1-50	388.7	472.1	335.6	389.4	441.2	379.3	457.8	359.3
9	1-50	304.7	413.4*	298.4	527.3	512.3	488.9	555.1	557.0
14	1-50	299.4	395.0*	292.2	372.8	553.0	512.7	569.3	514.7

* Statistically significant difference from control group mean at the 5% level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1% level (Student's t-test, 2-sided)

E. BODY WEIGHT

Group mean bodyweight for males receiving 20000 ppm was statistically significantly lower than that of controls throughout the study. At week 14, group mean bodyweight for these animals was 92.8% that of controls, equating to a reduction in bodyweight gain of approximately 12%.

Group mean bodyweight for males receiving 8000 ppm was also marginally lower than that of controls from weeks 6 to 14. However, these differences did not attain statistical significance and were considered too small to be of biological importance.

For males receiving 2000 ppm, and for females at all dose levels, mean bodyweight was essentially similar to that of concurrent controls throughout the study.

Table 5.7-4: Intergroup comparison of bodyweights (g)

	Dietary concentration (ppm)							
	Males				Females			
Week	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
1	216.0	217.0	218.6	215.0	173.5	178.8	175.6	175.3
2	263.5	264.7	264.9	254.6**	192.7	200.6	196.1	194.3
4	338.2	340.7	339.6	323.7*	214.3	228.3**	224.9**	219.2
8	440.7	440.1	429.1	405.8**	253.6	262.1	260.4	255.4
14	534.7	532.8	526.5	496.1**	285.1	291.5	287.9	281.0

* Statistically significant difference from control group mean at the 5% level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1% level (Student's t-test, 2-sided)

F. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no effects on food consumption. The efficiency of food utilisation for males receiving 20000 ppm was statistically significantly lower than that of concurrent controls during weeks 1 to 8. There were no changes in the efficiency of food utilisation for males receiving 2000 or 8000 ppm or for females from all treated groups.

Table 5.7-5: Intergroup comparison of food utilisation (g growth/100 g food)

	Dietary concentration (ppm)							
	Males				Females			
Week	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
1-4	18.13	17.16	16.94	16.28*	9.47	9.73	9.36	9.61
5-8	11.52	10.69	10.35	9.93*	5.99	5.55	5.39	5.70
1-13	12.00	11.45	11.38*	10.82**	6.08	6.03	6.06	5.96

* Statistically significant difference from control group mean at the 5% level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1% level (Student's t-test, 2-sided)

The mean doses received for males and females respectively were 155.5, 617.1, 1546.5 and 166.3, 672.1, 1630.6 mg glyphosate acid/kg/day at dose levels of 2000, 8000 and 20000 ppm, respectively

G. PATHOLOGY

Brain measurements

There was no evidence of any effects on brain weight, length or width.

Necropsy

There were no macroscopic findings that were considered to be attributable to treatment.

Histopathology

There were no microscopic findings in the peripheral or central nervous system that were considered to be attributable to treatment.

III. CONCLUSION

Dietary administration of Glyphosate acid to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm produced evidence of toxicity in the form of reduced growth and reductions in food utilisation for males. Comprehensive histopathological evaluation of the nervous system showed no evidence of any changes in the peripheral or central nervous system which could be attributed to administration of glyphosate acid.

The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1546.5 / 1630.6 mg/kg bw/day for males and females, respectively.

IIA 5.7.5 Postnatal development neurotoxicity

There were no indications for a neurotoxic potential of glyphosate observed in acute and subchronic neurotoxicity, acute, short-, and long-term toxicity, as well as in reproductive and developmental toxicity studies. Therefore, it is not necessary to conduct a postnatal development neurotoxicity study.

IIA 5.8 Toxicity studies on metabolites

The metabolite aminomethyl phosphonic acid (AMPA) was investigated for acute and subchronic effects, mutagenicity and teratogenicity. These studies previously evaluated in the 2001 EU glyphosate evaluation have shown that AMPA has a lower toxicity than the parent compound and is devoid of any mutagenic or teratogenic potential.

Studies conducted after the 2001 EU evaluation, further investigated the potential dermal and oral toxicity, as well as skin sensitizing potential of AMPA. From these studies a dermal LD_{50} of > 2000 mg/kg bw/day for rat and an oral LD_{50} of > 5000 mg/kg bw/day for rat and mice was found ([redacted] 2002, IIA 5.8/03; [redacted] 1996, IIA 5.8/01; [redacted] 1988, IIA 5.8/02). In a Magnusson-Kligman Maximisation Test, no sensitizing effects of AMPA were observed ([redacted] 2002, IIA 5.8/04).

Table 5.8-1: Summary of toxicological studies with metabolites of glyphosate

	Reference (Data owner)	Type of study Species, Strain	Metabolite	Purity [%]	Exposure conditions / test method	Results
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1973 (MON)	Metabolism Rat	AMPA	not available	Metabolism after single oral dosing	Of 6.7 mg [14C]-AMPA, 74% appeared in faeces, 20% in urine, <0.1% in exhaled air; 0.06 of the total dose recovered from carcass; liver, kidney, and muscle exhibited residues of 6, 6 and 3 ppb
	Annex B.5.8.1.1 Glyphosate Monograph [redacted], 1993a (CHE)	Acute oral toxicity Rat	AMPA	99.2	Limit test	LD_{50} > 5000 mg/kg bw
Studies not reviewed in the 2001 evaluation	IIA 5.8/01 [redacted] 1996 (ALS)	Acute oral toxicity Mice	AMPA	99.33	Limit test	LD_{50} > 5000 mg/kg bw
	IIA 5.8/02 [redacted] 1988 (SYN)	Acute oral toxicity Rat, Wistar	AMPA	> 99	Limit test	LD_{50} > 5000 mg/kg bw
	IIA 5.8/03 [redacted] 2002 (FSG)	Acute dermal toxicity Rat, CD	AMPA	98	Limit test	LD_{50} > 2000 mg/kg bw

	Reference (Data owner)	Type of study Species, Strain	Metabolite	Purity [%]	Exposure conditions / test method	Results
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1993b (CHE)	Acute dermal toxicity Rat	AMPA	99.2	Limit test	LD_{50} > 2000 mg/kg bw
	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1993c (CHE)	Skin sensitisation Guinea pig	AMPA	99.2	MKT	Not sensitising
Studies not reviewed in the 2001 evaluation	IIA 5.8/04 [redacted] 2002 (FSG)	Skin sensitisation Guinea pig	AMPA	98	MKT	Not sensitising
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1993 (CHE)	4-week oral toxicity Rat	AMPA	99.2	gavage	NOAEL = 100 / 1000 mg/kg bw/day (♂/♀)
	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1993	13-week oral toxicity Rat	AMPA	99.2	gavage	NOAEL > 1000 mg/kg bw/day
	Annex B.5.8.1 Glyphosate Monograph [redacted] 1979 (MON)	90-day oral toxicity Rat	AMPA	99.96	diet	NOEL = 400 mg/kg bw/day
	Annex B.5.8.1. Glyphosate Monograph [redacted] 1991 (MON)	90-day oral toxicity Dog	AMPA	87.8	capsule	NOAEL = 300 mg/kg bw/day
	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1993a (CHE)	Genotoxicity in bacteria	AMPA	99.2	Reverse mutation in bacteria (Ames test)	negative

Reference (Data owner)		Type of study Species, Strain	Metabolite	Purity [%]	Exposure conditions / test method	Results
Studies not reviewed in the 2001 evaluation	IIA 5.8/05 [redacted]1988 (SYN)	Genotoxicity in bacteria	AMPA	99.2	Reverse mutation in bacteria (Ames test) in *S. typhimurium & E. coli*	negative
	IIA 5.8/06 [redacted]1996 (ALS)	Genotoxicity in Bacteria	AMPA	99.33	Reverse mutation test in *S. typhimurium & E. coli*	negative
	IIA 5.8/07 [redacted]2002 (ALS)	Genotoxicity *in vitro*	AMPA	99.9	UDS in rat hepatocytes	negative
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph [redacted]1991d (CHE)	Genotoxicity	AMPA	99.2	Micronucleus test	negative
	Annex B.5.8.1.1 Glyphosate Monograph [redacted]1992 (CHE)	Develop-mental toxicity Rat	AMPA	99.2	0, 100, 350, 1000 mg/kg bw/day; gavage gestation days 6-16	NOAEL > 1000 mg/kg bw/day (maternal and developmental)
	Annex B.5.8.1 Glyphosate Monograph [redacted]1991 (MON)	Develop-mental toxicity Rat	AMPA	94.68	0, 150, 400, 1000 mg/kg bw/day; gavage gestation days 6-15	NOAEL 150 / 400 mg/kg bw/day (maternal / developmental)

AMPA = Aminomethyl Phosphonic Acid
MKT = Magnusson Kligman Maximization Test

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, 5.8/01	[redacted]	1996	AMPA: Acute Oral Toxicity Study In Mice. [redacted] Data owner: Arysta Life Sciences Report No.: [redacted]96-0075 Date: 1996-11-11 GLP: yes not published

Guideline: OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)

Deviations: None

Dates of experimental work: 1996-09-24 to 1996-10-08

Executive Summary

The test substance, AMPA, was evaluated for its acute oral toxicity potential in ICR mice when administered as a gavage dose at a level of 5,000 mg/kg bw. No mortality occurred during the study. Neither clinical signs nor macroscopic lesions at necropsy were observed in any animals. All animals gained body weights 7 and 14 days after administration when compared with the body weights on the day of administration. The acute oral LD_{50} was calculated to be

LD_{50}, oral, mice > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance AMPA is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA
Description:	White powder
Lot/Batch #:	A-960719
Purity:	99.33%
Stability of test compound:	Stable for 1 year at RT.

2. Vehicle and/ or positive control: 1% carboxymethyl-cellulose (CMC)

3. Test animals:

Species:	Mice
Strain:	ICR (Crj:CD-1), SPF
Source:	[redacted] Japan, [redacted]
Age:	6 weeks
Sex:	Male and females
Weight at dosing:	♂ 30.5 – 34.6 g; ♀ 22.9 – 24.8 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet [redacted] ([redacted] Japan), *ad libitum* except for approx. 3 h before and after dosing
Water:	Tap water, *ad libitum*
Housing:	Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage.
Environmental conditions:	Temperature: 23 ± 3°C Humidity: 55 ± 15% Air changes: 12/hour 12-hour light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1996-09-17 to 1996-10-08

Animal assignment and treatment:
A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made 1, 3 and 6 h after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
No body weight losses were recorded in any animal 7 and 14 days after the administration.

D. NECROPSY
The gross necropsy conducted at termination of the study noted no observable abnormalities.

III. CONCLUSION

The oral LD_{50} of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.8/02	[redacted]	1988	Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat. [redacted] Data owner: Syngenta Report No.: [redacted]/2266 Date: 1988-08-10 GLP: yes not published
Guideline:			Not stated, but method is in accordance with OECD 401.
Deviations:			None
Dates of experimental work:			No date given in the report.

Executive Summary
The test substance, Aminomethyl Phosphonic Acid (AMPA), was evaluated for its acute oral toxicity potential in Wistar rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality due to the test substance occurred during the study. Signs of slight toxicity were observed following dosing, but all animals appeared normal by Day 4. Observed body weight losses were not regarded to be treatment-related since no associated clinical abnormalities or any abnormalities at necropsy were observed. The acute oral LD_{50} was calculated to be

LD_{50}, oral, rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance AMPA is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Aminomethyl Phosphonic Acid (AMPA)
Description:	White solid
Lot/Batch #:	Y06384/001/001 (CTL reference)
Purity:	100% (assumed)
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control: 0.5% (w/v) aqueous polysorbate 80

3. Test animals:

Species:	Rat
Strain:	Wistar (Alpk:APfSD), SPF
Source:	[redacted] UK
Age:	Approx. 8-9 weeks
Sex:	Male and females
Weight at dosing:	♂ 280 – 312 g; ♀ 204 – 214 g
Acclimation period:	At least 6 days
Diet/Food:	[redacted] Combined Diet ([redacted]), *ad libitum* except for approx. 24h before dosing
Water:	Tap water, *ad libitum*
Housing:	Suspended stainless steel/polycarbonate cages with stainless steel mesh floors in groups of max. 5 animals/sex/cage.
Environmental conditions:	Temperature: 15 – 24°C Humidity: 50 ± 10% Air changes: 20 – 30/hour 12-hour light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: No date given in the report.

Animal assignment and treatment:
A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 10 mL/kg bw. One animal was accidentally killed (by mis-dosing) on Day 1 and another animal was therefore substituted, but was dosed one day later. Observations for mortality and clinical/behavioural signs of toxicity were made once 30-90 minutes, 4 and 6 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded one day prior to dosing, the day of dosing (Day 1) and on Days 3, 5 or 6, 8 and 15. On Day 15 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Signs of slight toxicity (diarrhoea, chromodacryorrhea, piloerection, stains around nose, ungroomed appearance, signs of urinary incontinence) were seen in the animals, but these did not persist and all animals had recovered by Day 3 or 4.

C. BODY WEIGHT
All animals lost weight initially due to the pre-dose fast, but all then gained weight and had exceeded their initial bodyweight by Day 6. Moreover, one male lost weight between Day 6 and 8 and one further male and three females between Day 8 and 15. The reason was unclear as there were no associated clinical abnormalities, nor were there any abnormalities at necropsy.

D. NECROPSY
The gross necropsy conducted at termination of the study noted no observable abnormalities.

III. CONCLUSION

The oral LD_{50} of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.8/03	[redacted]	2002a	Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – LIMIT TEST [redacted] Data owner: Feinchemie Schwebda GmbH Report No.: 16168/02 Date: 2002-12-03 GLP: yes not published

Guideline:	OECD 402 (1987), EEC B.3 (1992)
Deviations:	None
Dates of experimental work:	2002-10-21 to 2002-11-01

Executive Summary
The test substance, Aminomethyl Phosphonic Acid (AMPA), was evaluated for its acute dermal toxicity potential in CD rats when administered at a level of 2000 mg/kg bw. No mortality occurred during the study. Neither clinical signs nor macroscopic lesions at necropsy were observed in any animals. All animals gained body weights 7 and 14 days after administration when compared with the body weights on the day of administration. The acute dermal LD_{50} was calculated to be

LD_{50}, dermal, rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance AMPA is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA (Aminomethyl Phosphonic Acid)
Description:	White solid powder
Lot/Batch #:	FA005563
Purity:	98.0%
Stability of test compound:	Stable until 2004-12-03 at RT.

2. Vehicle and/ or positive control: 0.5% aqueous hydroxypropylmethyl cellulose gel

3. Test animals:

Species:	Rat
Strain:	CD / Crl:CD
Source:	[redacted] Germany
Age:	20 - 22 days
Sex:	Male and female
Weight at dosing:	♂ 214 – 238 g ♀ 213 – 223 g
Acclimation period:	At least 5 days.
Diet/Food:	[redacted] Germany), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Individually in MAKROLON cages (type III) with granulated textured wood as bedding.
Environmental conditions:	Temperature: 22 ± 3° C Humidity: 55 ± 15% Air changes: not reported 12-hour light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 2002-10-21 to 2002-11-01

Animal assignment and treatment:
The acute dermal toxicity of AMPA was tested on five male and five female CD rats. One day before treatment the administration site was clipped free of hair. A single dose of 2000 mg/kg bw test substance prepared as suspension in 0.5% aqueous hydroxypropylmethyl cellulose gel was applied uniformly over an area of about 10% of the total body surface. The dosing volume was 10 mL/kg bw. The application site was covered with an occlusive dressing for 24 hours. After removal of the dressing, possible residual substance was removed. All animals were observed for overt signs of toxicity or behavioural changes before and immediately, 5, 15, 30 and 60 minutes, as well as 3, 6 and 24 h after administration and subsequently once daily for 14 days. Individual body weights were recorded before administration and on Days 7 and 14. All surviving animals were killed at the end of the 14-day observation period.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
No body weight losses were recorded in any animal 7 and 14 days after the administration.

D. NECROPSY
The gross necropsy conducted at termination of the study noted no observable abnormalities.

III. CONCLUSION

The dermal LD_{50} of the test material (AMPA) was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.8/04	[redacted]	2002b	Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitisation Test in Guinea Pigs according to Magnusson And Kligman (Maximisation Test) [redacted] Data owner: Feinchemie Schwebda GmbH Report No.: 16169/02 Date: 2002-12-03 GLP: yes unpublished

Guideline:	OECD 406 (1992); EEC B.6 (1996)
Deviations:	None
Dates of experimental work:	2002-10-12 to 2002-11-26

Executive Summary

AMPA (Aminomethyl Phosphonic Acid) was tested for its sensitizing effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 5% dilution of the test item in purified water and an emulsion of Freund's Complete Adjuvant (FCA)/purified water. The epidermal induction was conducted for 48 h under occlusion with the test item at 50% one week after the intradermal induction.

Two weeks after induction the animals were challenged by epidermal application of the test item at 50% under occlusive dressing. The study was performed using a control group consisting of five animals, one test group consisting of ten animals and a positive control group consisting of 20 animals.

None of the vehicle control or test animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment. Animals treated with the positive control benzocaine in 40% ethanolic 0.9% NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Based on the study results and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for skin sensitization.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA
Description:	White solid powder
Lot/Batch #:	FA005563
Purity:	98.0%
Stability of test compound:	At room temperature stable until December 31, 2004.

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	[redacted] Germany
Age:	22 days
Sex:	Male
Weight at dosing:	252 - 307 g; positive control group: 228 - 341 g
Acclimation period:	At least 5 days.
Diet/Food:	[redacted] Germany), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In pairs in Makrolon cages (type IV) with granulated textured wood bedding
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 55 ± 15% Air changes: no data 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 2002-10-12 to 2002-11-26

Animal assignment and treatment:
AMPA was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Male Dunkin Hartley guinea pigs, young adults with body weights ranging from 228 to 341 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals. The main study was performed in 10 test animals, 5 control animals and 20 positive control animals.
The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 5% either in purified water or in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and purified water.

On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10% in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50% to the clipped and shaved skin of the shoulder region and covered with an occlusive dressing, which was left in place for 48 hours.
The challenge was conducted on Day 21 by an occlusive patch at a concentration of 50% which was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle only. 24 and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.

The animals of the positive control group were treated with a 2% benzocaine solution intracutaneously in the induction phase and with a 5% solution topically in the induction phase and at challenge.

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.

Evaluation criteria for classification as a potential skin sensitizer:
At the 24-hour and/or 48-hour reading, 30% or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred.

B. CLINICAL OBSERVATIONS
No signs of systemic toxicity were observed.

C. BODY WEIGHT
All animals showed the expected gain in body weight.

D. SKIN REACTIONS
No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.
Animals treated with the positive control benzocaine in 40% ethanolic 0.9% NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

III. CONCLUSION

Based on the study results and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for skin sensitization.

Annex point	Author(s)	Year	Study title
IIA, 5.8/05	[redacted]	1988	Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E.Coli* [redacted] Data owner: Syngenta Report No.: [redacted]/2206 Date: 1988-09-21 GLP: yes not published

Guideline:	Comparable to OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC B.13/B.14 (2000)
Deviations:	None
Dates of experimental work:	1988-03-01 to 1988-09-21

Excecutive summary

In a reverse gene mutation, plate incorporation assay in bacteria (***Maron and Ames, 1983***), five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) and one strain of *Escherichia coli* (WP2 *uvrA* pKM101) were exposed to aminomethyl phosphonic acid.

In at least two separate experiments, the compound did not induce any significant, reproducible increase in the observed numbers of revertant colonies in any of the tester strains used, either in the presence or absence of an auxiliary metabolising system (S9). Although slight effects were observed in strain TA1537 in the first experiment, these were not reproducible in two further experiments with this strain. In each experiment, the positive controls responded as expected, indicating that the assay was working satisfactorily.

Under the conditions of this assay, aminomethyl phosphonic acid gave an unequivocal negative, ie non-mutagenic, response, when tested to a limit dose of 5000 µg/plate.

I. MATERIALS AND METHODS

A: MATERIALS:

Test Material:	Aminomethyl phosphonic acid
Description:	Crop metabolite of, and an impurity in, ICIA0224, white solid
Lot/Batch number:	48F-3893
Purity:	>99% a.i.
CAS#:	Not reported
Stability of test compound:	Confirmed by Sponsor

Control Materials:

Negative: Water

Solvent control (final concentration): Dimethylsulphoxide – DMSO (10 µL/plate)

Positive control: Nonactivation:

Acridine mutagen ICR191 TA1537
2-Aminoanthracene TA1537, WP2 uA
Daunomycin hydrochloride TA98
4-Nitro-o-phenylene diamine TA1538
N-Methyl-N´-nitro-N-nitrosoguanidine TA1535, TA100, WP2 uA

Activation:

2-Aminoanthracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA
Acridine mutagen ICR191 TA1537
2-Aminothracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA
N-Methyl-N´-nitro-N-nitrosoguanidine, WP2 uA

Mammalian metabolic system: S9 derived

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbitol		Mouse		Lung
			None		Hamster		Other
			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 (250:30:1 mM) and cofactor solution.

The cofactor solution was prepared in bulk as follows: 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).

Test organisms:

S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537	X	TA1538		list any others		

E. coli strains

	WP2 (pKM101)	X	WP2 *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, 1000, 200, 40, 8 and 1.6 µg/plate
Activated conditions: 5000, 1000, 200, 40, 8 and 1.6 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 date: Start: 10 May 1988 End: 23 May 1988

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 Co-factor Buffer mix) 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 Bonner 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 controls should show at least a two-fold increase in average reversion frequency rate and there should be a dose-response relationship.

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 effect(s) must be consistently reproducible.

REPORTED RESULTS

Mutagenicity assay: In two separate experiments, aminomethyl phosphonic acid did not induce any significant increases in the observed numbers of revertant colonies in *Salmonella typhimurium* strains TA1535,TA1538, TA98, TA100 and *Escherichia coli* WP2 *uvrA* pKM101 in either the presence or absence of an auxiliary metabolising system (S9).

In the first experiment, slight responses were observed in strain TA1537, reaching maxima of 1.9 x and 2.0 x background in the presence and absence of S9 respectively. These responses were only of limited dose-relationships, and were of limited statistical significance in both cases. In two further experiments, no significant increases in colony numbers were observed either with or without S9. This lack of reproducibility indicates that the observed effects in the first experiment are not due to compound-induced mutations.

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, aminomethyl phosphonic acid gave an unequivocal negative, ie non-mutagenic, response, when tested to a limit dose of 5000 µg/plate.

Annex point	Author(s)	Year	Study title
IIA, 5.8/06	[redacted]	1996	AMPA Reverse mutation test. [redacted] Data owner: Arysta LifeScience Study No.: [redacted] 96-0076 Date: 1996-12-09 GLP: yes not published
Guideline:			U.S. EPA FIFRA Guidelines, Subdivision F OECD guidelines 471, 472 (1983) Japan MAFF guidelines 59 NohSan N° 4200 (1985)
Deviations:			None
Dates of experimental work:			1996-09-09 to 1996-10-11

Executive Summary

Reverse mutation tests were performed on AMPA in *Escherichia coli* WP2 uvrA and four tester 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, AMPA is non-mutagenic to bacteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AMPA

Identification: AMPA
Description: White powder
Lot/Batch #: A-960719
Purity: 99.33%
Stability of test compound: Stable for 1 year at room temperature
Solvent used: Sterile water

2. control materials:

Negative: Sterile water
Solvent/final concentration: Water / 50 mg/mL
Positive: non-activation and activation

Strain	Positive controls	
	Without S9 (µg/plate)	With S9 (µg/plate)
TA100	AF – 2 (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 in DMSO; 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:

(c) 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.

(d) 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 5.8-2: preliminary dose range finding test

		Concentration (µg/plate)	Revertants (n° colonies/plate) TA100	TA1535	WP2 *uvr.A*	TA98	TA1537
- S9 mix		Solvent Control (H_2O)	150*	8*	20*	24*	7*
		200	167	11	21	12	8
		500	152	10	19	22	6
		1000	131	10	15	20	5
		2000	154	5	23	24	10
		5000	140	11	20	18	11
+ S9 mix		Solvent Control (H_2O)	106*	6*	28*	25*	10*
		200	92	7	19	16	16
		500	116	1	20	24	15
		1000	124	5	25	17	12
		2000	102	7	24	30	8
		5000	128	10	29	26	11
Positive Control	- S9 Mix	Compound	AD-2	NaN_3	AF-2	AF-2	9-AA
		µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	666*	673*	201*	658*	540*
	+ S9 Mix	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
		µg/plate	1	2	10	0.5	2
		Revertants/plate	450*	233*	415*	456*	84*

AMPA 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

AMPA 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 5.8-3: Reverse mutation tests without metabolic activation– Experiment 1

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix	Solvent Control (H_2O)	101±17	11±1	17±4	18±3	4±3
	313	84±7	10±5	17±6	14±2	5±3
	625	94±14	8±6	17±7	16±4	4±2
	1250	97±17	8±3	14±2	13±2	4±2
	2500	91±7	9±5	15±8	15±2	6±2
	5000	106±4	7±3	16±4	16±5	3±1
Positive Control (- S9)	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
	µg/plate	0.01	0.5	0.01	0.1	80
	Revertants/plate	619±57	619±45	160±22	667±60	710±73

* : Average ± SD

Table 5.8-4: Reverse mutation tests with metabolic activation– Experiment 1

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
+ S9 mix	Solvent Control (H_2O)	105±11	10±4	19±1	30±5	10±1
	313	105±5	12±2	16±4	28±5	9±3
	625	92±6	6±1	16±1	28±7	13±2
	1250	90±3	6±1	16±2	25±7	11±3
	2500	83±9	9±4	20±4	25±8	10±3
	5000	93±10	10±4	24±6	32±10	7±1
Positive Control (+S9)	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
	µg/plate	1	2	10	0.5	2
	Revertants/plate	529±33	184±5	384±20	407±11	94±2

* : Average ± SD

Table 5.8-5: Reverse mutation tests without metabolic activation– Experiment 2

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix	Solvent Control (H_2O)	120±3	9±3	15±3	18±4	3±2
	313	136±9	4±1	18±3	14±4	4±3
	625	124±16	5±2	16±3	13±3	3±2
	1250	107±11	6±4	12±4	15±2	3±3
	2500	96±6	9±4	12±3	16±6	4±0
	5000	117±2	7±3	20±5	13±2	3±2
Positive Control (- S9)	Compound	AF-2	NaN_3	AF-2	AF-2	9-AA
	µg/plate	0.01	0.5	0.01	0.1	80
	Revertants/plate	668±27	696±20	182±16	650±8	698±53

* : Average ± SD

Table 5.8-6: Reverse mutation tests with metabolic activation– Experiment 2

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
+ S9 mix	Solvent Control (H_2O)	95±3	8±2	17±3	28±5	7±2
	313	112±14	8±3	17±4	21±6	10±5
	625	84±5	11±4	16±5	21±5	7±3
	1250	106±8	7±2	17±4	28±9	7±1
	2500	97±4	11±3	16±2	21±1	6±1
	5000	115±12	9±3	22±2	22±3	6±5
Positive Control (+S9)	Compound	2-AA	2-AA	2-AA	2-AA	2-AA
	µg/plate	1	2	10	0.5	2
	Revertants/plate	584±56	169±28	461±8	334±14	82±4

* : Average ± SD

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 AMPA in the reverse mutation tests with or without metabolic activation. It is concluded that AMPA is non mutagenic for bacteria under the conditions used with this experiment.

Annex point	Author(s)	Year	Study title
IIA, 5.8/07	[redacted]	2002	Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro procedure with AMPA (Amino methyl phosphonic acid). [redacted] Data owner: Arysta LifeScience Study No.: [redacted]-R-02025 Date: 2002-09-10 GLP: yes not published

Guideline: OECD guideline n° 482

Deviations: None

Dates of experimental work: 2002-04-29 to 2002-07-02

Executive Summary

AMPA was examined for mutagenic potential by measuring its ability to induce Unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

Cytotoxicity of AMPA was estimated in a pre screen by exposing cells to 8 concentrations of AMPA for determination of survival. From these data a range of 5 test concentrations 0 (solvent control), 0.625, 1.25, 2.5, 5 and 10 µg/ml are selected for UDS analysis procedure.
2-acetamidofluorene prepared in DMSO was used as positive control.

Based on the results, AMPA did not have DNA-damaging activity in the bacteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AMPA

Identification: AMPA
Description: White crystalline powder
Lot/Batch #: 020404
Purity: 99.9%
Stability of test compound: Not mentioned in the report
Solvent used: William's E medium Gibco

2. control materials:

Negative: /
Solvent/final concentration: See above
Positive: 2-acetamidofluprene

3. activation: none

4. test organisms: Rats hepatocytes

5. test concentrations: 5 dose level were tested: 10, 5, 2.5, 1.25 and 0.625 mM with and without S9 metabolic activation

B: TEST PERFORMANCE

1. Test principle

Hepatocytes were isolated from livers of rats. The primary hepatocyte cultures were exposed to the test article in the presence of 3H thymidine which is incorporated into the DNA, if DNA damage is occuring. DNA repair systems then stimulated UDS and increased the incorporation of thymidine which was measured by grain counting after autoradiography of hepatocytes.

The following results are presented:
- The average NNG and standard deviation
- The percent of cells in repair and standard deviation (>=5)
- The average cytoplasmic and nuclear grain count
- The number of cells in S-phase

2. Statistics

Results were judged without statistical analysis.

3. Evaluation Criteria

Results are judged positive when:
- At any dose tested, group, mean NNG value greater than 0 NNG and 20% or more of cells responding (NNG value >=5)
- An increase is seen in both NNG and the percentage of the cells in repair
- A dose related increase is seen in both NNG and the percentage of the cells in repair
- Any induction of UDS can be reproduced in an independent experiment.

4. Validity Criteria

The assay is considered valid if:
- Negative control slides have a group mean NNG value within the historical range
- The positive control have group mean NNG values of less than 5 NNG counts with 50% or more cells having NNG counts of 5 or more and statistically significant relative to the solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

None

B. MUTATION ASSAYS

Negative control gave a group mean NNG value of less than zero with a percentage of cells in repair comparable with historical control data. In positive controls, group mean net nuclear grain count (NNG) values as well as percentage of cells in repair obtained were within the range of historical control. The sensitivity of the cell type used to a DNA damaging agent requiring metabolism for its action, 2-acetamidofluorene was demonstrated. Thus, the validity criteria of the test were fulfilled.
The findings of both experiments are summarized in the tables below.

Table 5.8-7: UDS data of the first experiment

Concentration (µg/ml)	Net Nuclear Grain Count (NNG)		Net Nuclear Grain Count of cells in repair (NNG >5)		% cells in repair (NNG >5)	
	Mean	+/- sd	Mean	+/- sd	Mean	+/- sd
Solvent control	-2.38	5.20	5.63	0.51	4.21	1.53
0.625	-3.81	5.29	6.61	1.56	3.89	1.53
1.25	-3.61	5.23	6.99	0.56	4.02	1.53
2.5	-3.93	5.24	6.83	0.91	5.18	1.53
5	-3.35	5.10	6.28	1.66	3.45	1.00
10	-2.04	4.82	6.91	0.74	5.43	1.53
Positive control: 2-acetamidofluorene 6.25 µM	30.81	18.22	31.38	4.84	96.61	2.00

Table 5.8-8: UDS data of the second experiment

Concentration (µg/ml)	Net Nuclear Grain Count NNG		Net Nuclear Grain Count of cells in repair NNG >5		% cells in repair NNG >5	
	Mean	+/- sd	Mean	+/- sd	Mean	+/- sd
	-4.62	5.81	5.37	0.21	3.74	2.08
	-4.77	5.46	6.90	0.50	1.78	0.00
	-4.04	5.35	6.87	1.00	4.49	1.53
Solvent control	-3.81	5.82	7.07	1.61	6.18	2.08
0.625	-3.47	5.81	7.83	0.29	7.73	1.73
1.25	-5.58	6.06	6.97	1.29	3.76	1.53
Positive control: 2-acetamidofluorene 6.25 µM	17.57	11.18	17.73	3.29	94.94	9.45

Over the two experiments (Tables 5.8.1.1-1 and 5.8.1.1-2), group mean net nuclear grain count (NNG) values at the dose range tested from 10 to 6.25 mM were less than zero (-2.04 to –3.81 vs –2.38 in solvent control in the first UDS assay and –5.58 to –4.77 vs –4.62 in solvent control in the second UDS assay), that is to say below the threshold value of 0 NNG for a positive response.
Furthermore, no significant increase in the percentage of cells in repair at any dose of AMPA tested when compared with the respective controls (5.43 to 3.89% vs. 4.21% in solvent control in the first assay and 3.76 to 1.78 % vs. 3.74% in solvent control in the second assay). In addition, in cells in repair, group mean net nuclear grain count (NNG≥5) values were comparable with the solvent controls (6.91 at 10 mM to 6.61 at 0.625 mM vs. 5.63 in control in the first assay and 6.97 ta 10 mM to 6.90 at 0.625 mM vs. 5.37 in control in the second assay).

III. CONCLUSIONS

Under the conditions of this experiment, **AMPA did not reveal any genotoxicity activity** in the Unscheduled DNA synthesis assay.

IIA 5.9 Medical data

IIA 5.9.1 Report on medical surveillance on manufacturing plant personnel

Monsanto Glyphosate Manufacturing Industrial Hygiene Monitoring Data, [redacted] USA

Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto [redacted] manufacturing facility are available for the years 1981-1998 and are presented below. No such data are available from a Monsanto European manufacturing facility. Based on the measured low exposures to glyphosate in the manufacturing setting (well below the ADI) and low toxicological concern, glyphosate specific medical monitoring is not considered necessary. These data are air concentration measurements which are conservatively applied as 100% bioavailable to calculations of mean and maximum daily exposures.

Table 5.9-1: Particulate exposures from glyphosate technical acid operations involving wetcake; e.g., supersack or container filling operations. Values are time weighted averages.

Glyphosate Technical Dust (mg/m^3)					Mean Daily Exposure* (mg/kg/day)	Maximum Daily Exposure* (mg/kg/day)
Sample Type	# Samples	Range	Mean	SD		
All	179	0.0003-0.2594	0.00647	0.0218	0.00108	0.04323
Personal	176	0.0003-0.2549	0.00658	0.022	0.00109	0.04248
Area	3	0.0008-0.024	0.00153	0.0008 1	0.00026	0.00400
Operator	158	0.0008-0.2594	0.00727	0.0236	0.00121	0.00393
Maintenance	16	0.0005-0.0053	0.00296	0.0014 4	0.00034	0.00088
Lab	2	0.0003-0.0004	0.00035	N/A	0.00006	0.00007

* based on breathing 10 m^3 air/shift and 60 kg worker

Table 5.9-2: glyphosate isopropylamine salt liquid formulation bottling, drumming and tote filling operations. Values are time weighted averages.

Glyphosate IPA Salt- Liquid Formulations (mg/m^3)					Mean Daily Exposure** (mg/kg/day)	Maximum Daily Exposure** (mg/kg/day)
Sample Type	# Samples	Range	Mean	SD		
All	72	0.0001-0.47	0.085	0.105	0.01050	0.05804
Personal	58	0.0001-0.47	0.0251	0.106	0.00310	0.05804
Area	14	0.004-0.28	0.0932	0.105	0.01151	0.03458
Operator	54	0.0001-0.47	0.0966	0.11	0.01193	0.05804
Maintenance	4	0.0041-0.0088	0.00792	0.00187	0.00098	0.00099

** based on breathing 10 m^3 air/shift and 60 kg worker and divided by 1.3496 to convert IPA salt to technical acid

Improvements in manufacturing facility containment and ventilation systems over recent years further reduce the likelihood of operator exposures within glyphosate manufacturing facilities.

IIA 5.9.2 Report on clinical cases and poisoning incidents

See IIA 5.9.4. Clinical cases and poisoning incidents are referenced in order to address the clinical signs and symptoms of poisoning.

IIA 5.9.3 Observations on exposure of the general population and epidemiological studies

Please refer to the literature review, captured in section IIA 5.10 in regards to epidemiologic studies.