The magnitude of the effect does not appear to increase with age plus the lack of a consistent effect for females does suggest this to be of limited toxicological importance.

At the 18 month evaluation there was an increase in plasma electrolytes for both sexes. Sodium and chloride values for males and females and potassium values for males only were increased compared with controls. Female calcium levels were lower than controls. These elevations/decrements were also observed at lower dose levels but were not seen in a dose related trend. In addition at the 12 month evaluation for satellite females a lower sodium value was seen for females. Values for all calcium and chloride values are presented as follows:

Table 5.5-41: Calcium and chloride values (mmol/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Calcium								
Month 6 (Satellite)	2.587	3.693	2.701	3.752	2.617	3.637	2.508	3.604*
Month 12 (Satellite)	2.530	2.602	2.543	2.587	2.458	2.475	2.514	2.483
Month 18 (Main)	2.231	2.775	2.523	2.645*	2.656	2.554**	2.598	2.468**
Month 24 (Main)	2.431	2.293	2.487	2.396	2.511	2.288	2.297	2.347
Chloride								
Month 6 (Satellite)	107.7	105.8	107.1	106.1	107.0	106.1	108.5	106.7
Month 12 (Satellite)	105.6	103.9	105.1	104.8	104.3	104.7	105.9	104.2
Month 18 (Main)	103.3	101.8	105.8**	104.2**	105.8**	106.4**	107.6**	107.8**
Month 24 (Main)	104.5	103.4	104.4	103.1	104.3	102.2	105.4	102.8

* p < 0.05; ** p < 0.01

At intermediate level similar findings to the highest dose level were seen for plasma electrolytes at the 18 month evaluation. A slight increase in alkaline phosphatase activity was seen for satellite group males at 12 months. At the low dose level there was a similar effect on the plasma electrolytes for both sexes at the 18 month evaluation of main group animals. Whilst these observations were seen at the highest dose level, the lack of dose response or the effect being limited to one sex does make the toxicological significance questionable.

All other differences were isolated in their finding and are therefore not toxicologically relevant.

I. URINALYSIS

There were no treatment-related effects observed.

J. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed during the study period.

Organ weights

No effects on organ weight values were observed.

Histopathology

Adipose infiltration of the bone marrow was seen for the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, greater effects were seen among male rats dosed at the highest level and this attained statistical significance for terminal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the influence of other pathological conditions upon marrow cellularity in ageing rats, the effect was not altogether convincing but cannot be dismissed. A similar effect was not seen among male rats in the remaining treatment groups but among premature deaths for animals of both sexes at the intermediate level and only low-dosed females. However, the

variable duration of exposure and significant background pathology for premature death animals further negates this as an effect of treatment upon marrow cellularity for female rats.

Moreover, at the highest dose level there was a significant difference in the site of mineral deposition within the kidneys compared with controls. Pelvic mineralisation was commonly seen in both sexes and was more prevalent among female rats; however corticomedullary mineralisation was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia in both sexes; which is considered to be a consequence of the decreased mineral deposition.
The effects on pelvic and corticomedullary mineralisation, and hyperplasia of the pelvic/papillary epithelium were confined to high dose animals with no indication of a similar effect at any other treatment level for either sex.

No other treatment-related changes were observed.

Neoplastic changes
No significant effects associated with tumour development were observed.

III. CONCLUSION

Based on the study results the NOAEL in rats after chronic exposure to Glyphosate technical for 24 month is 24000 ppm (corresponding to 1229.7 mg/kg bw/day for combined sexes). It is concluded that Glyphosate technical is not carcinogenic in rats.

IIA 5.5.3 Carcinogenicity study in the mouse

Carcinogenicity studies that were not assessed during the 2001 glyphosate evaluation are summarised below.
A combined toxicity and carcinogenicity study in mice ([redacted] 2001, 5.5.3/01) demonstrated a slightly higher mortality in the high dose group. Mortality was within the upper end of the the historical control range. However, treatment with glyphosate might slightly have affected the mortality at the highest dose of 10000 ppm, and because a relationship to treatment was unclear a conservative NOAEL for toxicity at the mid dose of 1000 ppm (150.5 mg/kg bw/day for combined sexes) was set for this study. The number of malignant lymphoma, the most common tumour in the mouse, was slightly elevated in the high dose group compared to control, but this was considered as incidental background variation based on historical control data and was not considered to be related to treatment. However it should be noted that the high dose group received a daily achieved dose of 1460 mg/kg bw/day which is in excess of the limit dose recommended by most current international guidelines.
In the study by [redacted] 1997 the low effect level was 8000 ppm (equivalent to 787 mg/kg bw/day) in females only based on a reduction in body weight gain. At the top dose of 40000 ppm (equivalent to 4348/4116 mg/kg bw/day in males and females respectively) signs of toxicity included loose stools, reduced body weight gain, food consumption and food utilisation, caecum distention and increased absolute and relative caecum weight (without corollary histopathological findings), increased incidence of anal prolapsed consitent with histopathological erosion/ulceration of the anus.
The most recent 80-Week dietary mouse study was conducted by [redacted] 2009. There were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 810/1081 mg/kg bw/day in males and females respectively.
Overall the lowest effect level observed in the long-term mouse studies was 787 mg/kg bw/day in females in the [redacted] (1997) study and the wide range of NOEL/NOAELs of 151 – 1081 mg/kg bw/day is an artifact of dose selection (see Table 5.5-42).

Table 5.5-42: Summary of long-term toxicity and carcinogenicity studies in mice

	Reference (Owner**)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.5.2.2 Glyphosate Monograph [redacted] 1993b (CHE 2)	2-year, oral diet Mouse, CD-1	0, 100, 300, 1000	1000 (1000)	> 1000 Not clearly identified
	Annex B.5.5.2.1 Glyphosate Monograph [redacted] 1983 (MON 3)	2-year, oral diet Mouse, CD-1	♂ 0, 157, 814, 4841 ♀ 0, 190, 955, 5874	157/190 (4841/5874)	841/955 Decreased body weight, histological changes in liver and urinary bladder (epithelial hyperplasia)
Studies not reviewed in the 2001 evaluation	IIA 5.5.3/01 [redacted] 2001 (FSG 2)	18-month, oral diet Mouse, Swiss albino	0, 15, 151, 1460 (0, 100, 1000, 10000 ppm)	151 (1460)	1460 Increased mortality
	IIA 5.5.3/02 [redacted] 2009b (NUF 2)	18-month, oral diet Mouse, CD-1	0, 200, 1500, 5000 ppm	810/1087 (♂/♀) 940 (♂+♀) 810/1087 (♂/♀)	No treatment-related effects
	IIA 5.5.3/03 [redacted] 1997 (ALS 2)	18-month, oral diet Mouse, CR	0, 1600, 8000, 40000 ppm	8000 ppm 1600 ppm (= 838/153 mg/kg bw/day (♂/♀) (4348/4116 (♂/♀))	8000 ppm (≅ 787 mg/kg bw/day) (♀): retarded growth 40000 ppm: pale-coloured skin ♂, loose stool, retarded growth, reduced food consumption and food efficiency, caecum distension and increased absolute and relative caecum weight without histopathological findings increased incidence of anal prolapse, in consistent with histopathological erosion/ulcer of the anus

* NOAEL for carcinogenicity
** Number refers to the data presented in Figure 5.11-1.

Tier II summaries are presented for the 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, 5.5.3/01	[redacted]	2001	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice [redacted] Data owner: Feinchemie Schwebda GmbH Study No.: [redacted]1559.CARCI-M Date: 2001-10-10 GLP: yes not published

Guideline: OECD 451 (1981)

Deviations: None

Dates of experimental work: 1997-12-18 - 1999-06-29

Executive Summary

The carcinogenic potential of glyphosate technical was assessed in an 18-month feeding study in male and female Swiss albino mice. Groups of 50 mice per sex received daily dietary doses of 0, 100, 1000, and 10000 ppm glyphosate technical (equivalent to an average intake of 14.7, 150.5 and 1460.3 mg/kg bw/day). Observations covered survival, clinical signs, neurological changes, body weight, food- and water consumption, ophthalmological examinations, masses formation, blood smears with differential count analysis, organ weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues and lesions for all control and high dosage group animals died, sacrificed moribund or killed at termination.

The survival after 18-month of treatment was 56, 60, 56 and 46% in males and 68, 68, 60 and 60% in females in the control through high dosage groups, respectively. The mortality (combined for both sexes) was slightly increased at the high dose level with 38, 36, 42 and 47% for the control, low, mid- and high-dose group, respectively. Despite being in the upper range of the historical control data for mortality, the mortality in the high dose is considered to represent a LOAEL for safety reasons. There were no treatment-related effects on clinical signs, behaviour, eyes, body weight, body weight gain, food consumption, and differential white blood cell counts in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects. The number of malignant lymphoma, the most common tumour in the mouse, was slightly elevated in the high dose group compared to control, but this was considered as incidental background variation based on historical control data and in agreement with the study director.

Based on the slightly higher mortality and lower survival rates in the high dose groups, the NOAEL was considered 1000 ppm.

In conclusion, glyphosate technical was not carcinogenic in Swiss albino mice following continuous dietary exposure of up to 1460.3 mg/kg bw/day (average for both sexes) for 18 months. The NOAEL for toxicity was 149.7 mg/kg bw/day for male mice and 151.2 mg/kg bw/day for female mice (150.5 mg/kg bw/day for combined sexes).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification: Glyphosate

Description: Solid white, odourless crystals

Lot/Batch #: 01/06/97

Purity:	> 95.14 % (w/w)
Stability of test compound:	Expiry: December 1999

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	Swiss albino, HsdOla: MF1
Source:	[redacted] UK
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 25 – 47 g, females: 21 – 26 g
Acclimation period:	5 days
Diet/Food:	[redacted] rat/mouse powder food maintenance meal – low in germs ([redacted] Germany), *ad libitum*
Water:	Well water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	In groups of five per sex in polypropylene mouse cages with stainless steel top grill and sterilized clean paddy husk bedding.
Environmental conditions:	Temperature: 19 - 25°C Humidity: 30- 70% Air changes: 12- 15/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1997-12-23 to 1999-06-29

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 50 Swiss albino mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 14.5, 149.7 and 1453 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466 mg/kg bw/day for females) glyphosate technical in diet for 18 month. The dose levels were chosen based on results of a 50-day pre-study in mice. Test diets were prepared prior to start of treatment and then in intervals ranging from 10 to 23 days. Diets were prepared in quantities of 10, 12 or 15 kg. For preparation of 12 kg diet mixtures 1.2 g, 12 g and 120 g for the low-, mid- and high-dose group, respectively, of the test substance was mixed with approximately with 0.5 kg basal diet and blended for 3 minutes. This pre-mix was then mixed manually with approximately 0.5 kg food and then added in portions to the remaining bulk amount of food (approximately 11.0 kg) and blended in a stainless steel ribbon mixer for 20 minutes.
The homogeneity of the test material in diet was determined at beginning of treatment, and at 12 and 18 month. Analyses for achieved concentration were done at three and six month of the study. The stability of glyphosate technical in the diet was determined prior to start of the study for the 100 and 10000 ppm dose levels.

Clinical observations
A detailed veterinary examination of all mice was done before and after grouping and monthly thereafter. A check for clinical signs of toxicity, appearance, behaviour, and neurological changes and mortality was made once daily on all mice. For mice with observed tumours a separate record was maintained with details of the tumour development.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from week 1 to week 13 and subsequently at weeks 26, 39, 52, 65 and 68. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology
Blood smear samples were collected at 9 month and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Ophthalmological examination
Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mydriasis was induced before examination by adding 1% Tropicamide solution into the eyes. All other grossly visible eye findings were recorded also at the daily observations.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately or preserved in 10% buffered neutral formalin until necropsy.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: adrenals, kidneys, liver and gall bladder, ovaries, and testes.
Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: adrenals, bone & bone marrow (sternum and femur (incl. joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), heart, , jejunum, kidneys, larynx, liver and gall bladder, lungs, lymph nodes (mandibular, mesenteric, and superficial inguinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and all lesions and tumours/masses.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically.

Statistics
Body weight, body weight gain, food consumption and differential leukocyte counts of different groups were compared by Bartlett´s test for homogeneity of intra group variances. Heterogeneous data were transformed using log transformation. Data with homogeneous intra group variances were subjected to one-way analysis of variance using ANOVA. When “F” values were significant, Dunnetts pair wise comparison of means of treated groups with control means was done individually.
Incidence of gross lesions and non-neoplastic histopathological changes and incidences of benign and malignant neoplasms in the test substance groups were statistically compared with control group by Z-test where necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto´s incidental tumour analysis. When a significant difference over the control group was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t-test. All analyses and comparisons were evaluated at the 5% level and statistically significant differences ($p \leq 0.05$) were indicated

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 8.37% at the 100 ppm level and 6.99% at the 10000 ppm level, when stored at room temperature in PE bags inside stainless steel drums.

Analyses for homogeneity at the start and at 12 and 18 month of treatment indicated that the dose preparations were homogeneous. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 10% of the nominal concentration for all diet samples. The overall mean achieved concentrations were 94.0 ± 1.66, 949.5 ± 15.84 and 950.7 ± 142.28 as compared to the nominal concentrations of 100, 1000 and 10000 ppm, respectively.

B. MORTALITY

The cumulated pre-terminal deaths (including moribund sacrifice) are summarised in Table 5.5-43 below.

Table 5.5-43: Cumulated mortalities after 78-week dietary exposure to glyphosate technical

Sex	Historical control#		Dose group (ppm)**			
	min- max*	Mean ± SD	0	100	1000	10000
Male	11/50 – 27/50	18 ± 5	27 (6)	20 (6)	22 (8)	27 (8)
Female	12/50 – 20/50	16 ± 3	16 (7)	16 (7)	20 (2)	20 (3)
Combined sex	12/100 – 47/100	17 ± 4	38 (18)	36 (13)	42 (10)	47 (11)

Derived from the control groups of 9 studies performed in the timeframe embracing the study summarised here
* Number of dead animals / total number of animals
** Total number of animals per group = 50
() number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table 5.5-44: Percentage survival at termination after 18-month dietary exposure to glyphosate technical

Sex	Dose group (ppm)			
	0	100	1000	10000
Male	56	60	56	46
Female	68	68	60	60
Combined	62	64	58	53

The survival percentage was slightly decreased at the high dose level, but the decrease did not attain statistical significance.

As can be seen from the historical control data, the mortality in the high-dose group is, even though at the upper end, but within the historical control range. Although the treatment with glyphosate technical might slightly have affected the mortality at the highest dose of 10000 ppm, and a relationship to treatment is unclear, the worst-case NOAEL is set at 1000 ppm, corresponding to 150.5 mg/kg bw/day (combined sexes, see Table 5.5-45).

C. CLINICAL OBSERVATIONS

There were no significant treatment-related clinical signs of toxicity observed.

D. BODY WEIGHT

There were no significant treatment-related effects on male and female body weight and overall body weight gain during the conduct of study.

In males incidences of slightly decreased body weights in week 10 at 100 ppm and in months 7 and 8 at 1000 ppm were considered incidental, since no effects on body weights were observed in the high-dose group.

In females decreased net body weight gain was observed in month 18 at 100 ppm only. Therefore, this finding was also considered as incidental.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.
The observed slightly lower food consumption observed in males in week 1 at 100 ppm and in weeks 1 and 7 at 10000 ppm was considered incidental, since the changes were minimal and the effects was not consistent during the remaining parts of the study period.
In females lower food consumptions were observed in week 2 for all dose levels, in week 26 at 10000 ppm. Higher food consumption occurred in week 11 at 100 ppm and in weeks 3 and 4 at 10000 ppm. These findings were also considered incidental, since the changes were minimal and food consumption during the remaining parts of the study was comparable with the control group.

The calculated mean daily test substance intake is summarised in Table 5.5-45 below.

Table 5.5-45: Group mean compound intake levels

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	100	14.5	15.0	14.7
3 (mid)	1000	149.7	151.2	150.5
4 (high)	10000	1453.8	1466.8	1460.3

* based on actual food intake and body weight data

F. HAEMATOLOGY

Differential leukocyte counts at 9 and 18 month
There were no significance treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophile counts and slightly lower lymphocyte counts in high dose males at 9 month were within the historical control ranges. The slightly higher eosinophil counts, higher neutrophil and monocyte counts, as well as slightly lower lymphocyte counts observed in high dose females at 18 month were comparable with historical control values and therefore considered incidental.

Differential leukocyte counts of moribund sacrificed mice
Although the differential leukocyte count data were not statistically analysed, they appeared to be within the range of biological variation.

G. OPHTHALMOLOGICAL EXAMINATION

There were no treatment-related findings observed at the ophthalmological examinations performed at 6, 12 and 18 month of treatment.

H. NECROPSY

Gross pathology
There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.
In animals found dead or sacrificed moribund across control and all dose levels the incidence of enlargement of superficial inguinal lymph nodes and thymus in mid dose females and in the high dose for combined sexes was statistically significant increased. These enlargements were associated with neoplasms of the hemolymphoreticular system. Other changes included enlargement of various lymph nodes, and thymus, both associated with neoplasms of the hemolymphoreticular system, enlargement of the spleen, associated with neoplasia and amyloidosis and increased extramedullary haematopoiesis. The low incidence of observed liver enlargements was associated with neoplasia and amyloidosis. However, none of these findings were dose-dependent.
In mice sacrificed at termination the following changes were observed: Kidney surface rough/uneven in high dose males, discoloration / enlargement of mesenteric lymph nodes in high dose females and discoloration in high dose combined sex, and enlargement of spleens in both sexes combined at the high dose were significantly higher than in control mice. Since none of these changes showed a dose-

dependency, and the corresponding histopathological changes were not significantly higher in these groups, the findings were considered incidental.

Organ weights
There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology
There were no treatment-related histopathological findings observed in any dose group of either sex.

In mice found dead or sacrificed moribund during the study period the following significant histopathological changes were seen. Cystic glands of the stomach were significantly increased in high dose mice of both sexes combined. However, the incidence of these findings was similar to historical control data and did not show a dose dependency. Therefore, these finding was considered incidental. Increased haematopoiesis was seen in the bone (femur) of high dose males, mid- and high-dose animals combined sex. Cell debris in tubules of epididymides was increased in mid dose males and the incidence of sub-capsular cell hyperplasia was increased in adrenals of low dose males. In addition, the incidence of kidney nephropathy in mid-dose females, as well as the incidence of lymphocytic infiltration of epididymides in mid dose males was significant decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental.

In mice sacrificed at termination the following more frequent observed changes were observed: Cystic glands of the stomach were significantly increased in low-, mid- and high-dose males. However, this finding was not dose dependent.
Degenerative heart changes were higher in high dose males and females, and significant higher in combined sex. Since the incidences were similar or slightly higher than historical controls, and no dose-dependency was observed this finding is considered incidental. The number of malignant lymphoma was slightly elevated in the high dose group compared to control. This tumour of the hemolymphoreticular system is one of the most common tumours of mice accounting for the highest percentage of spontaneous tumours in this species. Therefore, the observed tumour incident is considered incidental and not treatment-related. In addition, there was no increase with dose and the incidences of this tumour varied with sex and fate (i.e. pre-terminal and terminal deaths). In mandibular lymph nodes lymphoid hyperplasia was significantly increased in low and mid-dose males and combined sex, whereas the incidence was significantly lower in high dose females. In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level in combined sex. In spleen extramedullary haematopoiesis was significantly increased in females and combined sex at the low dose level. In the absence of any dose-relation these findings, as well as several not statistically significant changes considered incidental (see tables below).

Table 5.5-46: Summary of non-neoplastic histopathological findings for dead and moribund animals

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Number examined	22	20	22	27	16	16	20	20	38	36	42	47
Stomach Cystic glands (n)	8	8	9	16	1	4	5	6	9	12	14	22+
Kidney Nephropathy (n)	9	7	10	12	5	1	1	3	14	8	11	15
Bone (femur) Increased haematopoises (n)	1	1	8+	5	0	1	2	3	1	2	10+	8+
Epididymes Cell debris in tubules (n)	0	1	4	0	--	--	--	--	--	--	--	--
Epididymes Lymphocyte infiltration (n)	4	1	0	3	--	--	--	--	--	--	--	--
Heart Degenerative changes (n)	11	14	13	16	4	2	4	9	15	16	17	17
Adrenals sub-capsular cell hyperplasia (n)	3	8+	7	10	12	11	13	15	15	19	20	25
Mandibular LN extramedullary haematopoiesis (n)	3	2	5	3	1	1	1	2	4	3	6	5

n = number of animals affected; LN = lymph node
+ significantly increased; -- not examined/determined

Table 5.5-47: Summary of non-neoplastic and neoplastic histopathological findings at termination

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Stomach(N)	28	30	28	23	34	-	-	30	62	64	58	53
Cystic glands (n)	9	19+	20+	17	22	-	-	19	31	-	-	36
Kidney(N)	28	6	4	23	34	7	1	30	62	64	58	53
Nephropathy (n)	7	4	3	6	5	1	0	2	12	6	3	8
Bone (femur) (N) Increased haematopoises (n)	28 1	-	-	23 0	34 4	2 0	1 0	30 2	62	--	--	53
Epididymes (N) Lymphocyte infiltration (n)	28 0	1 0	- --	23 1	2 --	-- --	-- --	-- --	-- --	-- --	-- --	-- --
Mandibular LN (N) extramedullary haematopoiesis (n)	28 5	30 7	28 9	23 9	34 3	33 9	28 7	30 4	62 8	64 16	58 16+	53 13
Heart (N) Degenerative changes (n)	28 14	2 1	-- --	23 17	34 2	-- --	- -	30 6	62 16	-- --	-- --	53 23+
Adrenals (N) sub-capsular cell hyperplasia (n)	28 15	-- --	-- --	23 13	34 27	- --	- --	29 22	62 42	-- --	-- --	53 35
Hemolymphoreticular system (N) malignant lymphoma (n)	28 1	30 3	28 3	23 6	34 9	34 10	30 6	30 13	62 10	64 13	58 9	53 19

N = number examined; n = number of animals affected; LN = lymph node
+ significantly increased; -- not examined/determined

Table 5.5-48: Incidences of lamlignant lymphoma and comparison with historical control

			Dietary concentration of glyphosate (ppm)							
			Males				Females			
	♂	♀	0	100	1000	10000	0	100	1000	10000
Dead & moribund										
Number examined	75	77	22	20	22	27	16	16	20	20
Number affected	20	49	9	12	13	13	9	10	13	12
Percentage affected	26.7	63.6	41.0	60.0+	59.0+	48.0	56.0	63.0	65.0	60.0
Mean %	26	61.8	--	--	--	--	--	--	--	--
Range %	0-44	0-100	--	--	--	--	--	--	--	--
			--	--	--	--	--	--	--	--
Terminal sacrifice										
Number examined	175	175	28	3028	23	34	34	30	30	28
Number affected	26	50	1	3	3	6+	9	10	6	13
Percentage affected	14.9	28.9	3.6	10.0	10.7	26.1+	26.5	29.4	20.0	43.3+
Mean %	14.8	28.8	--	--	--	--	--	--	--	--
Range %	8-24	20-43	--	--						--
			--	--						--
All fates										
Number examined	250	250	50	50	50	50	50	50	50	50
Number affected	46	99	10	15	16	19+	18	20	19	25
Percentage affected	18.4	39.6	20.0	30.0	32.0	38.0+	36.0	40.0	38.0	50.0+
Mean %	18.4	41.6	--	--	--	--	--	--	--	--
Range %	6-30	14.58	--	--	--	--	--	--	--	--

+ significantly increased; -- not examined/determined

III. CONCLUSION

Based on mortality at the upper limit of the historical control range, the NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is conservatively set at 1000 ppm, corresponding to 149.7 mg/kg bw/day for males, 191.2 mg/kg bw/day for females, and 150.5 mg/kg bw/day for both sexes combined. It is concluded that Glyphosate is not carcinogenic in mice.

Annex point	Author(s)	Year	Study title
IIA, 5.5.3/02	[redacted]	2009b	Glyphosate technical: Dietary Carcinogenicity Study in the Mouse [redacted] [redacted] Project No.: 2060-0011 Date: 2009-04-22 GLP: yes not published

Guideline:	OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA OPPTS 870.4200 (1996)
Deviations:	None
Dates of experimental work:	2005-10-10 - 2007-11-19

Executive Summary

The carcinogenic potential of Glyphosate technical was assessed in an 18-month feeding study in male and female CD-1 mice. Groups of 51 mice per sex received daily dietary doses of 0, 500, 1,500, and 5,000 ppm Glyphosate technical (equivalent to an average intake of 84.7, 266.8 and 945.6 mg/kg bw/day). Observations covered clinical signs, body weight, food and water consumption, palpation of masses, organ

weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues for all control and high dosage group animals killed at termination. In addition, differential white blood cell counts were performed for animals that were killed or died in extremis and for selected animals at twelve and eighteen month of treatment. The dose-levels were chosen based on available toxicity data.
There were no treatment-related deaths or clinical signs in any of the dose-groups. In the carcinogenicity study, survival after 78 weeks of treatment was 76, 80, 76 and 69% in males and 73, 75, 75 and 78% in females in the control through high dosage groups, respectively.
There were no treatment-related effects on body weight gain or food and water consumption noted. No significant treatment-related effects were noted on differential white blood cell counts in both sexes. There were no treatment-related trends in the proportion of masses observed, number of mice affected or time to appearance of palpable masses. Gross pathology, organ weight data and histopathological examination revealed no treatment-related effects.
In conclusion, Glyphosate technical was not carcinogenic in the CD-1 mouse following continuous dietary exposure of up to 945.6 mg/kg bw/day (average for both sexes) for 18 months. The NO(A)EL for toxicity was 810 mg/kg bw/day for male mice and 1081 mg/kg bw/day for female mice, the highest dosage tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification:	Glyphosate
Description:	White crystalline solid
Lot/Batch #:	H05H016A0
Purity:	95.7%
Stability of test compound:	Expiry: 2008-03-25

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	CD-1, Crl:CD-1 (ICR) BR
Source:	[redacted] UK
Age:	Approx. 5 – 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 22 – 32 g, females: 18 – 28 g
Acclimation period:	At least ten days
Diet/Food:	Rat and Mouse [redacted] Ground diet No. 1, [redacted]), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Initially in groups of three per sex in polypropylene solid-floor cages.
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 55 ± 15% Air changes: at least 15/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 2005-10-10 to 2007-11-19

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm (equivalent to mean achieved dose levels of 0, 84.7, 266.8 and 945.6 mg/kg bw/day) Glyphosate technical in diet. Additional 12 mice per sex, designated for veterinary controls, were housed and maintained alongside treated animals. Ten animals per sex from each group were set aside for an interim kill (toxicity assessment), which was carried out on the survivors after 39 weeks of dosing. The remaining 50 mice per sex and dose-level were dosed for a maximum of 79 weeks (carcinogenicity assessment).

Test diets were prepared prior to start of treatment and then weekly by mixing a known amount of the test substance with a small amount of basal diet and blending for 10 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for achieved concentration monthly for the first six months and then every three months thereafter.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all mice and recorded weekly. Observations for morbidity and mortality were made twice daily. Additional unscheduled examinations were performed on animals that showed ill-health.
All surviving animals were palped weekly for size, position and appearance of new or existing masses.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Body weights were also determined before sacrifice. Bodyweight data were reported only until Week 77.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination. Food consumption data were reported only until Week 77. Food efficiency and compound intake was calculated from the recorded food consumption data.

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

Haematology
Blood smear samples were collected after 12 months and at termination from all animals, and from mice that were killed in extremis. Differential white cell counts were performed on all control and high-dose animals and on the animals killed in extremis.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, and all animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen, and testes.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aortic (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions incl. palpable

masses, head (incl. pharynx, nasopharynx and paranasal sinuses), heart, Harderian and lacrimal glands, ileum, jejunum, kidneys, larynx, liver and gall bladder, lungs (with bronchi), mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of the liver, lungs and kidneys, as well as gross macroscopic lesions and palpable masses from low and intermediate dose groups at termination were examined microscopically.

Statistics
All data were summarised in tabular form and analysed by computerised analysis using ProvantisTM Tables and Statistics Module. For each variable the of variance incorporating Student´s t-test and F-test. For each variable the most suitable transformation of data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANOVA and Bartlett´s test. The lowest treatment-related significant effects were determined using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no response is found but the data showed non-homogeneity of means, data were further analysed by a stepwise Dunnet (parametric) or Steel (non-parametric) test to determine significant differences from control. If required, pair-wise tests are performed using Students t-test (parametric) or the Mann-Whitney U test (non-parametric)
The levels of probability chosen as significant were $p < 0.01$** and $p < 0.05$*.
Histopathology data were analysed using Chi squared analysis (differences in the incidence of lesions occurring with an overall frequency of 1 or greater) and the Kruskal-Wallis one-way non-parametric analysis of variance (comparison of severity grades).
The levels of probability chosen as significant were $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.1$.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Analyses for homogeneity and stability indicated that the dose preparations were homogeneous and stable for at least six weeks. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 5% of the nominal concentration for all but 1 sample (500 ppm – level), which was + 10% of the nominal concentration.
The group mean achieved doses are summarised below.

Table 5.5-49: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Achieved dose level (mg/kg bw/day)*				Overall mean
		Males		Females		
		Mean	Range	Mean	Range	
1 (control)	0					
2 (low)	500	71.4	33 – 104	97.9	55 – 155	84.7
3 (mid)	1500	234.2	101 – 365	299.5	176 – 466	266.8
4 (high)	5000	810	461 - 1143	1081.2	610 - 1728	945.6

* based on actual food intake and body weight data

The results show a higher test material intake for males when compared to males for each dose level. Highest intakes were achieved within the first few treatment weeks, with subsequent decline thereafter. The mean intake for each dose group is therefore 84.7, 266.8 and 945.6 mg/kg bw/day for 500, 1500, and 5000 ppm, respectively.

B. MORTALITY

No treatment-related effects on the deaths occurred during the study, as well as no treatment-related effects on the time of death. From three male mice that were killed in extremis, examination results suggest that the morbidity of these animals was due to fighting between cage mates.

Table 5.5-50: Cumulated mortalities after 78-week dietary exposure to Glyphosate technical

	Dose group (ppm)			
Sex	**0**	**500**	**1500**	**5000**
Male	12 (6)	10 (8)	12 (6)	16 (6)
Female	14 (10)	13 (7)	13 (10)	11 (8)

( ): number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table 5.5-51: Percentage survival at termination after 78-week dietary exposure to Glyphosate technical

	Dose group (ppm)			
Sex	**0**	**500**	**1500**	**5000**
Male	76	80	76	69
Female	73	75	75	78

C. CLINICAL OBSERVATIONS

There were no significant treatment-related clinical signs of toxicity observed.

There were no trends in the proportion of palpable masses observed during the study period. A significant proportion observed showed evidence for regression before the animal reached the point of death or termination. Based on the results (see Table 5.5-52) no treatment-related effect on the development of palpable masses is seen for either sex. The slight increase in the mean number of masses per animal for high-dose females and mid-dose males was considered a coincidence. The median time to appearance of palpable masses was comparable for all dose groups of either sex.

Table 5.5-52: Group summary of palpable masses

Dose	Total number of animals in group		Number of animals with palpable masses		Total number of masses per group		Mean number of masses per animal		Median time (weeks) to appearance of masses	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0	51	51	28	23	45	38	0.88	0.75	42.00	45.75
500	51	51	32	28	49	49	0.96	0.96	42.00	46.08
1500	51	51	39	23	60	38	1.20	0.75	42.43	44.83
5000	51	51	25	33	49	51	0.96	1.00	41.67	42.50

D. BODY WEIGHT

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

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

F. WATER CONSUMPTION

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

G. HAEMATOLOGY

There were no significance differences in the proportions of white blood cell counts for either sex at both 12 and 18 month.

H. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

There were no treatment-related histopathological findings observed in any dose group of either sex.

III. CONCLUSION

Based on the study results the NOEL and NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is 810 mg/kg bw/day for males, and 1081 mg/kg bw/day for females. It is concluded that Glyphosate technical is not carcinogenic in mice.

Annex point	Author(s)	Year	Study title
IIA, 5.5.3/03	[redacted]	1997	HR-001: 18-Month Oral Oncogenicity Study in Mice [redacted] Data owner: Arysta LifeScience Study No.: [redacted]94-0151 Date: 1997-06-18 GLP: yes not published
Guideline:			Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 451 (1981).
Deviations:			None
Dates of experimental work:			1995-02-21 to 1996-09-06

Executive Summary

In order to evaluate the oncogenic potential of HR-001 in mice, the test substance was administered to SPF ICR mice –Crj:CD-1) by incorporating it into a basal diet at a concentration of 0, 1600, 8000 or 40000 ppm for a period of 18 months (78 weeks). During the treatment period, all animals were observed for clinical signs and measured body weights as well as food consumption. At week 21, urinalysis was carried out on 20 males from all groups. Differential leukocytes counts were determined on the blood smears from 10 males and 10 females of all groups at week 52 and after 78 weeks of treatment, organ weight analysis was conducted on 10 males and 10 females which were served to the determination of differential leukocytes counts. All animals of both sexes were subjected to necropsy and histopathological examinations.

- 40,000 ppm groups In clinical observations, the incidence of pale-coloured skin was increased in males. In addition, loose stool was observed in all cages beginning at week 21 in males and at week 20 in females. Retarded growth was persistently observed during treatment period showing

statistically significant differences in weight from week 16 to 36 in males and from week 6 to end of treatment in females. These changes were associated with depressed food consumption and food efficiency. At necropsy, the increased incidences of distention of caecum were noted in males and females at terminal kill and in all animals examined, which were consistent to increase in absolute and relative weights of the caecum. However, no abnormalities were recorded in the caecum histopathologically. In males a significant increase was noted for the overall incidence of anal prolapsed which was correspondent to erosion/ulcer of the anus in histopathology.

- 8,000 ppm group: Retarded growth was observed in females with statistically significant decreases in weight at week 6 and weeks 9-24. No treatment related changes were seen in males.

- 1,600 ppm: There were no treatment related changes in either sex in any parameters.

Histopathological examinations failed to show increases of any types of neoplastic lesions in all treatment groups of both sexes.

Based on the results, the No-Observable Adverse Effect level (NOAEL) was set at 8,000 ppm (equivalent ot 838.1 mg/kg/da y) for males and 1,600 ppm (equivalent to 153.2 mg/kg/day) for females.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification:	HR-001	
Description:	Solid crystals	
Lot/Batch #:	J-941209	J-950308
Purity:	97.56%	94.6%
Stability of test compound:	Not mentioned in the report	

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	SPF ICR (Crj:CD-1)
Source:	[redacted] Japan, [redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Males: 15 – 25 g, females: 14 – 23 g
Acclimation period:	9 days in males; 7 days in females
Diet/Food:	Certified diet [redacted], *ad libitum*
Water:	Filtered and sterilized water, *ad libitum*
Housing:	In groups of four per sex in aluminium cages with wiremesh floors
Environmental conditions:	Temperature: 24 ± 2°C Humidity: 55 ± 15% Air changes: 15/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-02-21 to 1996-09-06

Animal assignment and treatment:
Groups of 50 males and 50 females Specific –Pathogen-Free (SPF) ICR (Crj : CD-1) mice received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 40 000 ppm for a period of 18 months.

Clinical observations
All animals were conducted a cage-side observation daily for clinical signs and their deaths during the study. In addition, a detailed examination including palpation of the body was performed at least once a week. Moribund animals showing marked delibility were euthanized by exsanguinations under deep ether anesthesia and necropsied when an unfavourable prognosis was predicted. Dead animals were taken from the cage as soon as possible after discovery o minimize the loss of tissues by cannibalism and necropsied. Mortality was expressed as ratios of cumulative number of animals found dead or killed in extremis to effective number of animal group.

Body weight
Individual body weights were recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 76. Body weights were also measured at week 78, at the end of treatment, and used for calculation of relative organ weights. Group mean body weights were calculated at each measurements.

Food consumption and compound intake
Food consumption by each cage was recorded for a period of 3 or 4 consecutive days weekly during the first 13 weeks and every 4 weeks from week 16 to 76. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology
Blood smear samples were collected at week 52 and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: brain, adrenals, kidneys, spleen, liver and gall bladder, ovaries, and testes.
Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, pharynx, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically. The following tissues were examined: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions

Statistics

Body weight, food consumption and organ weights were evaluated by Bartlett´s test for homogeneity of intra group variances. When group variances were homogenous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences exist among groups. When the analysis of variance was significant, Dunnett's or Scheffe's multiple comparison test was applied. When the group variance were heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant Dunnett type mean rank test or Scheffe's type mean rank test was applied.
Mortality was assessed by a life table analysis.
Urinalysis were analyzed by Mann-Whitney's U test to compare data between the treatment groups and the controls.
Mann-Whitney's U test was used to analyze difference of the differential leukocyte counts between the high dose groups and the controls. For comparison of the data from all groups, Dunnett's and Scheffe's multiple comparison test was applied. The data from males killed in extremis during the treatment were examined by Mann-Whitney's U test.
Fisher's exact probability test was used to analyze the data of clinical signs and incidences of gross lesions at necropsy and histopathological lesions.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 8.37%
Homogeneity of the test substance in diet was analysed on the samples taken from the top, middle, and bottom portion of the mixer. The coefficient of variation for each test diet was within 5.2% or less. The results indicated that homogeneity of the test substance in diet was satisfactory in each test diet.
In order to verify concentration of the test substance in test diets, every batch of test diet was analysed during the treatment period. Mean concentration of the test substance in test diet at a nominal level of 1 600, 8 000 or 40 000 ppm was 1 561± 86.7, 7 790±394.4 or 38 783±1 655.0 (mean + standard deviation) ppm, respectively. The values were within 97-98% of the target concentrations and satisfied the acceptable limit of concentration for test substance.

B. MORTALITY

No significant differences were noted for mortality between the treated groups and the respective control of either sex. Cumulative mortality of each group of either sex is shown in the following table:

Table 5.5-53: Final mortality at termination of treatment (%)

Dose group (ppm)	Male	Female
0	24/50 (48)	18/50 (36)
1 600	16/50 (32)	14/50 (28)
8 000	23/50 (46)	10/50 (20)
40 000	21/50 (42)	15/50 (30)

C. CLINICAL OBSERVATIONS

Statistically significant changes in clinical signs observed in the treated groups of either sex are shown in the following table:

Table 5.5-54: Statistically significant changes in clinical signs:

	Male				Female			
	Dose group (ppm)							
	0	**1 600**	**8 000**	**40 000**	**0**	**1 600**	**8 000**	**40 000**
Number of animals examined	50	50	50	50	50	50	50	50
Perinasal region : tactile hair loss	0	3	3	6*	5	13*	9	8
Anus : mass(es)	0	0	0	8**	0	0	0	0
Integument :								
wound	22	16	20	6*	3	0	0	0
Erosion/Ulcer	9	5	12	8	16	4**	1**	2**
Swelling	16	6*	13	9	6	2	0	1
Mass(es)	15	13	13	10	13	11	9	4*
Pale-colored skin	2	3	6	10*	4	2	6	6
Hair loss	11	12	21*	12	22	23	18	14
Wetted fur	11	9	7	4*	1	1	1	1

* : p<0.05 ; **, p<0.01 (Fisher's exact probability test).

In the 40 000 ppm group, males showed increased incidences of tactile hair loss, pale-colored skin, and mass(es) of anus and decreases of wound and wetted fur. In females of this group, decreased incidences were observed in ulcer/erosion and mass(es) of skin. Although, in addition to these signs, loose stool was observed in the cages of both sexes beginning at week 21 in males and 20 in females, the group housing failed to identify which animal excreted the loose stool.
In the 8 000 ppm group, males showed an increased incidence in hair loss of the skin and females represented decreases in ulcer/erosion and swelling of the skin.
In the 1 600 ppm group, males showed a decrease in swelling of the skin and females represented an increase in tactile hair loss as well as a decrease in ulcer/erosion of the skin.
None of the observed effects seems to be dose-related. Whatever the dose tested, females were more sensitive to erosion/ulcer of the integument than males.

D. BODY WEIGHT

In the 40 000 ppm group, males and females showed retarded growth during the treatment manifesting significantly lowered weights at weeks 16 to 36 in males and at weeks 6 and thereafter in females compared to the respective control. At the end of treatment, mean average weights were 93% and 86% of the respective control in males and females respectively.
In the 8 000 ppm group, females showed significantly decreased weights at week 6 and weeks 9 to 24 compared to the control and the final mean average weight was 92% of the control at the end of the treatment, while growth rate in males was comparable to the control.
In the 1 600 ppm group, males and females showed similar growth curves to the controls during the treatment period.
Effects on the body weight were more important in females than in males. These effects were durable in the 40 000 ppm female group whereas they were stopped at week 36 in the male group of the same treatment dose. Sporadic effects were observed in the 8000 ppm female group. No significant effects were seen in the 1600 ppm male and female groups.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

In the 40 000 ppm group, males showed significant depressions in food consumption at weeks 1 and 68, revealing an overall group mean food consumption at 94% of the control during the treatment period. Females in this group also showed significantly decreased food consumption at weeks 1, 4, 8, 12, 20, 28, 40, 48 and 68, revealing an overall group mean food consumption at 93% of the control during the treatment period.
In the 8 000 ppm group, females showed significantly lowered food consumption at weeks 28, 40, and 68 compared to the control manifesting an overall group mean food consumption at 96% of the control. Whereas, food consumption in males was comparable to the control during the treatment period.

No statistically significant effects was observed in the 1600 ppm group either in males or females.
The food consumption depressions were more important in female than in males. They were not time-related
Overall average chemical intake in each treated group of either sex was calculated from food consumption and nominal concentration as shown in the following table:

Table 5.5-55: Calculated test substance intake in mg/kg bw/day:

Dose level (ppm)	Dose level (mg/kg bw/day) Male	Female
1 600	165.0	153.2
8 000	838.1	786.8
40 000	4348	4116

F. HAEMATOLOGY

Statistically significant changes in differential leucocyte counts observed in the treated group of either sex are shown in the following table.

Table 5.5-56: Statistically significant changes in haematology parameters:

Parameter	Sex	Fate of animals[a]	Dose group (ppm) 1600	8000	40000
Lymphocytes	Males	ke	ND[b]		↑ 172
	Females	tk			↑[c] 163
Neutrophil (segmented)	Males	ke	ND		↓ 81

Numbers in the above table show values in the treated groups when the corresponding value in the control group is 100.
a: ke, killed in extremis; tk, terminal kill
b: ND, not determined
c: Dunnett's or Scheffe's multiple comparison test
↓↑: Mann-Whitney's U test

In the 40000 ppm group, males killed *in extremis* during the treatment period showed an increase of lymphocytes in differential leukocyte counts and a decrease of neutrophil (segmented form). In females of this group, differential count of lymphocytes was significantly increased at week 78.

There were no significant differences in differential leukocyte counts at other intervals of examination in the 40000 ppm group of both sexes, males killed *in extremis* in the 8000 ppm group, and females at week 78 in the 8000 and 1600 ppm groups compared to the controls. No significant treatment-related effects were conceived in morphology of the leukocytes.

G. NECROPSY

Gross pathology

Statistically significant changes in incidence of macroscopic lesions observed in the treated groups of either sex are shown in the following table.

Table 5.5-57: Statistically significant changes in macroscopic lesions:

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
External appearance: Loss of tactile hair	0	0	1	5*	4	8	8	0*
Soiled fur on external genital region	9	7	2*	6	0	0	0	0
Spleen: Swelling	5	1*	4	2	7	2	3	3
Lung: Mass(es)	4	12	11*	9	8	6	18	8
Cecum: Distention	0	0	0	11**	0	0	0	16**
Kidney: Cyst(s)	4	4	2	0*	2	0	4	1
Uterus: Cyst(s)	-	-	-	-	6	2	2	0*

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
Skin: Loss of hair	1	4	7*	6	8	11	16	5
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Lymph nodes (mesenteric): Swelling	0	2	0	5*	1	2	1	4
Lymph nodes (others): Swelling	5	2	4	9	0	3	4*	4*
Kidney: Coarse surface	4	2	1	1	6	3	0*	4
Skin: Loss of hair	5	4	7	4	11	5	2*	4
Wound	6	2	3	0*	0	0	0	0
Ulcer/Erosion	6	3	4	6	5	3	0	0
All (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
External appearance: Loss of tactile hair	0	0	1	6*	5	11	9	3
Lymph nodes (cervical): Swelling	5	3	6	9	12	9	4*	7
Lymph nodes (mesenteric): Swelling	0	2	0	6*	3	2	1	5
Spleen: Swelling	16	4**	12	14	17	8*	8*	10
Lung: Mass(es)	9	14	17	15	10	7	20*	11
Cecum: Distention	0	0	0	14**	0	0	0	18**
Anus: Anal prolapse	0	0	0	5*	0	0	0	0
Kidney: Pale in color	6	2	4	2	7	4	1*	4
Coarse surface	6	2	2	1	4	4	0**	5
Testis: Atrophy	-	2	5	0*	-	-	-	-
Uterus: Cyst(s)	-	-	-	-	6	2	2	0*
Eye: Opacity	1	1	3	2	2	1	0*	0*
Auricle: Partial amputation	6	2	1	0*	4	2	0	1
Skin: Loss of hair	6	8	14*	10	19	16	18	9*
Wound	9	3	1	1*	0	0	0	0
Ulcer/Erosion	7	4	9	6	8	3	1*	0**
Swelling	7	1*	3	1*	3	0	0	0

Tk: Terminal kill
Ke/fd: Killed in extremis or found dead
All: All animals examined
(N=): Number of animals examined
*, p<0.05 (Fisher's exact probability test); **, p<0.01

In the 40000 ppm group, males and females showed significant increases in icidence of distention of the cecum at terminal kill after 78 weeks of treatment. Significant increases in incidence of the lesion were also noted in all animals examined recording 28% (14/50) in males and 36% (18/50) in females. Distended cecum was filled with loose stool-like materials. In addition, males showed an increase in loss of tactile hair and a decrease of cyct(s) in the kidney in thosenecropsied at terminal kill, and an increase of swelling in the lymph nodes (mesenteric) and a decrease of wound in the skin in those killed in extremis or foud dead during the treatment period when compared to the controls. Among these, significant differences in incidence were also noted in all animals examined for increases in loss of tactile hair and swelling of the lymph nodes (mesenteric) and a decrease in wound in the skin. Moreover, significant differences in incidence were also noted in all animals examined for an increase in anal prolapse of the anus and decreases in atrophy of the testis, partial amputation of the auricle, and swelling of the skin. Females showed decreases in loss of tactile hair and cyst(s) of the uterus in those necropsied at terminal kill, and an increase in swelling of the lymph nodes (others) and a decrease in ulcer/erosion of the skin in those killed in extremis or found dead during the treatment period. Among these, significant differences in incidence were noted in all animals examined for decreases in cyst(s) of the uterus and ulcer/erosion of the skin. Moreover, significant differences in incidence were also noted in all animals examined for decreases in opacity of the eye and loss of hair of the skin.

In the 8000 ppm group, males showed increases in mass(es) of the lung and loss of hair of skin and a decrease in soiled fur on external genital region in those necropsied at terminal kill when compared to the control. An increased incidence was also noted in all animals examined for loss of hair of the skin. Females killed in extremis or found dead during the treatment period in this group showed an increase in swelling of the lymph nodes (others) and decreases in coarse surface of the kidney and loss of hair of the skin. Moreover, significant differences in incidence were noted in all animals for an increase in mass(es) of the lung and decreases in swelling of the lymph nodes (cervical) and spleen, pale in color and coarse surface of the kidney, opacity of the eye, and ulcer/erosion of the skin.

In the 1600 ppm group, males showed decreased incidences in swelling of the spleen in those necropsied at terminal kill and in all animals examined and in swelling of the skin in all animals examined, while females disclosed a decreased incidence in swelling of the spleen in all animals examined.

Organ weights
In the 40000 ppm group, males and females showed significant increases in absolute and relative weights of the cecum. The percentages of the values to those of the respective control were 173% in males and 187% in females for absolute weight, respectively, and 174% and 212% for relative weight, respectively. In females, relative weight of the kidney was also increased significantly at a level of 111% of the control.

Histopathology
Neoplastic lesions
The table below shows neoplastic lesions in the treated groups of either sex with statistically significant differences in incidence from those of the controls.

Table 5.5-58: Statistically significant changes in histopathology findings:

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Hematopoietic & Lymphatic system: General: Malignant lymphoma	0	0	1	5*	4	8	8	0*

Tk: Terminal kill
(N=): Number of animals examined
*, p<0.05 (Fisher's exact probability test)

As to neoplatic lesions, the incidence of malignant lymphoma was significantly decreased in females of the 1600 ppm group necropsied at terminal kill compared to the control. Neither increases in incidence nor nearly occurrences compared to the controls were noted for neoplatic lesions in the treated groups of both sexes.

Non-neoplatic lesions
Statistically significant changes in incidence of non-neoplastic lesions observed in the treated groups of either sex are shown in the following table.

Statistically significant changes in incidence of non-neoplastic lesions:

Table 5.5-59: Statistically significant changes in non-neoplastic lesions:

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Spleen: Increased extramedullary hematopoiesis	5	2	4	3	6	5	1*	4
Liver: Micro-granuloma	1	5	5	4	15	16	14	7*
Kidney: Cortical cyct(s)	9	6	9	0*	2	1	5	0
Tibio-femoral joint: Proliferation of cartilageous tissue	14	17	11	15	18	14	11*	15

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Bone marrow (femur): Increased hematopoiesis	6	3	7	6	7	1*	1	2
Lymph nodes (cervical): Plasma cell hyperplasia	6	1	5	4	5	3	0	0*
Spleen: Amyloid deposition	2	3	2	0	8	3	0*	1*
Small intestine: Amyloid deposition	1	1	1	0	5	0*	0	2
Liver: Amyloid deposition	3	3	2	0	10	3	0**	1**
Thyroid: Amyloid deposition	2	2	2	0	8	1*	0*	2
Parathyroid: Amyloid deposition	1	1	2	0	7	1	0*	2
Skin: Wound	9	5	9	4	9	5	1*	3
All (N=)	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*	*(50)*
Bone marrow (femur): Increased hematopoiesis	9	6	10	10	9	2*	2*	2*
Bone marrow (sternum): Increased hematopoiesis	9	3	9	10	9	3	2*	2*
Bone marrow (Vertebra): Increased hematopoiesis	9	6	10	10	9	3	2*	2*
Lymph nodes (cervical): Plasma cell hyperplasia	6	2	8	9	5	3	1*	0*
Lymph nodes (mesenteric): Myeloid cell aggregation	3	0*	3	2	4	1	1	1
Spleen: Increased extramedullary hematopoiesis	20	7*	14	14	13	10	5*	9
Amyloid deposition	3	3	4	0	10	3*	0**	1**
Lung: Alveolar epithelial cell hyperplasia	0	5*	1	3	4	5	5	
Small intestine: Amyloid deposition	2	1	1	0	8	0**	0**	3
Liver: Micro-granuloma	1	6	5	5	16	16	14	7*
Amyloid deposition	5	3	4	0*	12	3*	0**	1**
Kidney: Cortical cyct(s)	10	8	13	2*	5	1	5	0*
Glomerular amyloidosis	1	1	2	0	7	2	0**	2
Uterus: Amyloid deposition		-	-	-	6	0*	0*	1
Thyroid: Amyloid deposition	3	2	4	0	11	1**	0**	2**
Parathyroid[c]: Amyloid deposition	2	1	4	0	10	1**	0**	2*
Eye: Cataract	4	5	5	5	5	2	0*	2
Skin: Skin subacutaneous abscess	3	1	2	5	5	1	0*	1

Tk: Terminal kill
Ke/fd: Killed in extremis or found dead
All: All animals examined
(N=): Number of animals examined
*, p<0.05; **, p<0.01 (Fisher's exact probability test)
c: The number animals examined in the control, 1600, 8000 or 40000 ppm groups were 46, 48, 48 or 46 in males and 48, 48, 50 or 49 in females, repscetively.

In the 40000 ppm group, males showed significant decreases in incidence of amyloid deposition in the liver in all animals examined and cyst(s) in the kidney in those necropsied at terminal kill and in all animals examined, when compared to the control. In these males, erosion/ulcer in the anus was observed in a total of 8 animals including 6 cases killed in extremis or found dead during the treatment period and 2 cases necropsied at terminal kill. There was even a large abscess in one case. Among these, regressive hyperplasia of mucous eithelium of the large intestine was seen in 2 cases with severe lesions in the anus. However, as the histopathological examinations were carried out only on the anus which were observed macroscopic lesions, the incidence of erosion/ulcer in the anus was not assessed by a statistical method. In

females of this group, statistical significant decreases in incidence were noted in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), cyst(s) in the kidney, micro-granuloma in the liver, and amyloid deposition in the spleen, liver, thyroid, and parathyroid. Among these, significant decreases in incidence were also noted for micro-granuloma in the liver in those necropsied at terminal kill and plasma cell hyperplasia in the lymph nodes (cervical) and amyloid deposition in the spleen and liver in those killed in extremis or found dead during the treatment period.

In the 8000 ppm group, although males did not show any non-neoplastic lesions with statistically significant differences in incidence from the control, females disclosed significant decreases in incidence of proliferation of cartilageous tissue in the tibio-femoral joint in those necropsied at terminal kill, wound in the skin in those killed in extremis or found dead during the treatment period, and subcutaneous abscess in the skin in all animals examined. In addition, significant decreases in incidence, when compared to the control, were observed in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), extramedullary hematopoiesis in the spleen, amyloid deposition in the spleen, small intestine, liver, kidney (glomerular amyloidosis), uterus, thyroid, and parathyroid, and cataract in the eye. Among these, the incidences of extramedullary hematopoiesis in the spleen in those necropsied at terminal kill and amyloid deposition in the spleen, liver, thyroid, and parathyroid in those killed in extremis or found dead during the treatment period were also decreased significantly.

In the 1600 ppm group, males in all animals examined showed a significant increase in incidence of alveolar epithelial cell hyperplasia in the lung and decreases in incidence of myeloid cell aggregation in the lymph nodes (mesentery) and extramedullary hematopoiesis in the spleen. In females of this group, the incidences in all animals examined were decreased significantly in increased hematopoiesis in bone marrow (femur) and amyloid deposition in the spleen, small intestine, liver, uterus, thyroid, and parathyroid. Among these, significantly decreased incidences were also noted for increased hematopoiesis in bone marrow (femur) and amyloid deposition in the small intestine and thyroid in those killed in extremis or found dead during the treatment period.

III. CONCLUSION

Based on the results, no oncogenic potential was observed in glyphosate after treated to mice at a dietary level of as high as 40 000 ppm for a period of 18 months (78 weeks).
No observable effect level and sure toxic level in the present study were established as follows.

	No-observable effect level	Based on
Males	8 000 ppm (838.1 mg/kg/day)	- Increased incidences of tactile hair loss, pale-colored skin and mass(es) of anus at 40 000 ppm - Decrease in food efficiency at 40 000 ppm - Decrease in urinary pH at 8000 and 40000 ppm - Increase of lymphocytes and decrease in neutrophil (segmented form) at 40 000 ppm - Increase in mass(es) of the lung and loss of hair of skin at 1600 ppm but not observed at 40 000 ppm. - Increase in distension and absolute and relative weight of the cecum at 40 000 ppm - Increase in incidence of alveolar epithelial cell hyperplasia in the lung at 1600 ppm but not observed at higher doses.
Females	1 600 ppm (153.2 mg/kg/day)	- Increase in tactile hair loss at 1600 ppm but not observed in higher dose groups - Decrease in food efficiency at 8000 ppm - Increase in swelling of the lymph nodes and in mass(es) of the lung

IIA 5.5.4 Mechanism of action and supporting data

Considered not necessary. Glyphosate showed no carcinogenic potential in the longterm toxicity studies.

IIA 5.6 Reproductive toxicity

The potential of glyphosate to cause toxic effects on **reproduction** (reproductive performance, fertility, development) was examined in several multi-generation studies in rats. In the previous 2001 EU glyphosate evaluation no specific reproductive toxicity potential was shown for the active substance. Weak effects on the offspring consisting of a reduced pup weight were seen only at high dose levels and was associated with signs of paternal toxicity. Treatment-related effects in parent animals were similar to those seen in sub-chronic and chronic toxicity studies and occurred at comparable dose levels. Since the last review three new studies have been performed. The results of all studies are summarised in Table 5.6-1 and described below.

Developmental toxicity studies were performed in rats and rabbits.
Glyphosate does not cause teratogenicity. Adverse effects on the number of viable foetuses and the foetal weight were noted in rats and rabbits at higher dose levels also causing maternal toxicity. A reduced ossification and a higher incidence of skeletal and/or visceral anomalies at these dosages were also indicative of foetotoxicity. Overall, there is an inconsistent pattern of the most commonly occurring cardiac defects at maternally toxic doses without a clear dose effect. The lowest NOAEL for developmental effects was 300 mg/kg bw/day in rats ([redacted] 1991; IIA 5.6.11/05) and 175 mg/kg bw/day in rabbits ([redacted] 1996; IIA 5.6.11/03).
An endocrine disruption potential of glyphosate can be excluded based on the relevant endpoints evaluated in the reproductive and developmental toxicity studies. This conclusion is confirmed by the absence of any treatment-related findings in reproductive tissues and organs related to the endocrine system in repeated dose toxicity studies conducted with glyphosate (see IIA 5.3 and IIA 5.5).
In addition, dominant lethal assays performed in rats and mice (see IIA 5.4, Table 5.4-21) were negative.

IIA 5.6.1 Two generation reproductive toxicity in the rat

In the 2001 EU glyphosate evaluation a number of multigeneration studies were reviewed. It was concluded that glyphosate acid did not indicate a specific hazard for reproduction. It concluded that weak effects on the offspring as evidenced by reduced pup weight were confined to high dose levels where compound related effects were observed in the parent animals. Since the last review three additional studies have been conducted. Study summaries are available for these new studies below.
In the first additional study by [redacted] (1997) parental toxicity was evident at doses of 30000 ppm and consisted of reduced body weight, soft stool and distension of the caecum (see Table 5.6-1) which was consistent with findings in the sub-chronic and chronic rats studies conducted at this laboratory. In this study, effects in offspring consisted mainly of reduced body weight and distension of the caecum at 30000 ppm only.
In the [redacted] (2000) study the only effect of treatment was a reduction in the bodyweight of the F1A pups in the 10000 ppm group (1063/1634 mg/kg bw/day in males and females respectively) with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the mating period. The fertility and reproductive performance of each generation of parental animals and the clinical condition and survival of their offspring were not adversely affected by treatment.
In the most modern study by [redacted] (2007) there was no treatment-related effects on reproductive performance, parents or offspring.
In the previously reviewed study ([redacted] 1992) there were minimal histopathological changes on the salivary glands in parental and offspring animals noted at the highest dose (i.e., 10000 ppm) and to a lower extent at the mid-dose (i.e., 3000 ppm). This observation was also noted in other repeated dose studies with glyphosate but is considered an adaptive response to high dietary doses of glyphosate, which is a strong organic acid, and can therefore cause irritation of the oral cavity leading to increased salivary excretion (see chapter IIA 5.10). Overall the lowest effect level for parental toxicity was 668-771 & 752-841 mg/kg bw/day in males and females respectively based on slightly reduced body weight in F1 males,

increased food and water consumption F1 females in the [redacted] (1992) study The relevant parental NOEL/NOAELs ranged from 197-1063 mg/kg bw/day for males and 226-1634 mg/kg bw/day for females. There were no effects on reproduction (reproductive performance, fertility, parturition, lactation, sperm parameters and oestrus cycle) noted in any of the dose groups in any of the studies.
The lowest effect level for the offspring was 1063/1634 mg/kg bw/day in males and females respectively based on reduced body weight of first generation pups during lactation. The relevant NOEL/NOAELs for reproductive toxicity ranged from 197-1063 mg/kg bw/day for males and 226-1634 mg/kg bw/day for females. The range of NOEL/NOAELs is large as a consequence of variation in dose level selection between studies.

Table 5.6-1: Summary of reproductive toxicity

	Reference (Owner)	Type of study / Species	Dose levels (ppm)*	NOAEL (mg/kg bw/day (ppm))		LOAEL Targets / Main effects
				Parental	Offspring / reproductive	
Studies not reviewed in the 2001 evaluation	IIA 5.6.1/01 [redacted] 2007 (NUF)	2-generation, diet, rat, Sprague-Dawley	0, 1500, 5000, 15000	1063/1634 ♂/♀ (15000)	1063/1634 ♂/♀ (15000)	No treatment-related effects on parents, offspring and reproduction
	IIA 5.6.1/02 [redacted] 2000 (SYN/MON)	2-generation, diet, rat, Alpk:AP$_f$SD	0, 1000, 3000, 10000	NOAEL: 322 / 459 ♂/♀ (3000)	NOAEL: 322 / 459 ♂/♀ (3000)	1063/1634 ♂/♀ (10000 ppm): Parental: body weight of F1 males ↓ during pre-mating Offspring: reduced body weight of F1A pups during lactation No effects on reproduction
	IIA 5.6.1/03 [redacted] 1997 (ALS)	2-generation, diet, rat, Sprague-Dawley	0, 1200, 6000, 30000	417-458 and 485-530 ♂/♀ (6000)	417-458 and 485-530 ♂/♀ (6000)	2150-2411 & 2532-2760 ♂/♀ mg/kg bw/day (30000 ppm): Parental: loose stool, slight decrease in mean body weight in F1 ♂ at 2nd generation selection, caecum distension Offspring: reduced body weight (F0 ♂ and F1 ♀ during lactation), caecum distension No effects on reproduction
Studies from the 2001 evaluation	Annex B.5.6.1.2 Glyphosate Monograph IIA 5.6.1/04 [redacted] 1993a (FSG)	2-generation, diet, rat, Wistar	0, 100, 1000; 10000	ca. 700-800 (10000)	ca. 700-800 (10000)	No treatment-related effects

	Reference (Owner)	Type of study / Species	Dose levels (ppm)*	NOAEL (mg/kg bw/day (ppm)) Parental	NOAEL (mg/kg bw/day (ppm)) Offspring / reproductive	LOAEL Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.6.1.2 Glyphosate Monograph IIA 5.6.1/05 [redacted] 1981 (MON)**	3-generation, diet, rat, CD	0, 3, 10, 30 mg/kg bw/day	30	30	No treatment-related effects
	Annex B.5.6.1.2 Glyphosate Monograph IIA 5.6.1/06 [redacted] al., 1992 (CHE)	2-generation, diet, rat, Sprague-Dawley	0, 1000, 3000; 10000	197/226 (♂/♀) (3000)	197/226 (♂/♀) (3000)	668-771 & 752-841♂/♀ mg/kg bw/day (10000 ppm): Parental: slightly reduced body weight in F1 males, increased food and water consumption F1 females Offspring: no effects No effects on reproduction
	Annex B.5.6.1.2 Glyphosate Monograph [redacted] al., 1991a (CHE)	1-generation dose range finder, diet, rat, Sprague-Dawley	0, 3000, 10000, 30000	Not established	Not established	Parental: soft faeces, increased urination, reduced body weight gain and food consumption, increased water intake, GI disturbances, salivary gland changes Offspring: soft faeces, and reduced food consumption and pup weight, GI disturbances, salivary gland changes No effects on reproduction
	Annex B.5.6.1.1 Glyphosate Monograph IIA 5.6.1/07 [redacted], 1990 (MON)	2-generation, diet, rat, Sprague-Dawley	0, 2000, 10000, 30000	ca. 772/757 ♂/♀ (10000)	ca. 772/757 ♂/♀ (10000)	1983-2322 & 2320-2536 mg/kg bw/day (30000 ppm): Parental: reduced body weight, soft faeces, equivocal effect on litters size Offspring: reduced body weight No effects on reproduction
	Annex B.5.6.1.2 Glyphosate Monograph [redacted] 1985 (Alkaloida)	3-generation, diet, rat, Wistar	0, 200, 1000, 5000	ca. 460/500 ♂/♀ (5000)	ca. 462/502 ♂/♀ (5000)	No effects

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

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

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/01	[redacted]	2007	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat [redacted] Data owner: Nufarm [redacted] project no.: 2060/0013 Date:2007-10-31 (amended 2008-04-08 and 2008-08-08) GLP: yes not published

Guideline: OECD 416 (2001), JMAFF 2-1-14 (2001), US-EPA OPPTS 870.3800 (1998)

Deviations: None

Dates of experimental work: 2005-11-18 to 2006-11-06

Executive Summary

Glyphosate Technical was administered by dietary admixture to three groups of 28 male and female F0 generation Sprague-Dawley rats each, at dietary concentrations of 1500, 5000 and 15000 ppm (equivalent to a mean achieved dosage of 104, 351 and 1063 mg/kg bw/day for males and 162, 530 and 1634 mg/kg bw/day for females respectively). A further group of 28 male and 28 female F0 animals was exposed to basal laboratory diet to serve as a control.

Clinical signs, bodyweight development, food and water consumption were monitored during the study. After 10 weeks of treatment, pairing of animals within each dose group was undertaken on a 1:1 basis. At weaning of offspring from the F0 mating phase, groups of 24 male and 24 female offspring from each dose group were selected to form the F1 generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*, followed by the termination of all F0 male dose groups. The offspring selected for the F1 generation were reared for at least 10 weeks and then paired within each dose group to produce the F2 litters. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups.

Oestrous cycle assessment was performed daily for three weeks prior to mating for both the F0 and F1 generations. Observations for positive evidence of mating were recorded together with the start and completion of parturition. During the maturation phase of the F1 generation offspring, males and females were evaluated for sexual maturation. The ano-genital distance was recorded for all F2 generation offspring on Day 1 *post partum*. During the lactation phases daily clinical observations were performed on all surviving offspring, together with litter size. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days *post partum*.

All animals at termination were subjected to a gross necropsy examination and histopathological evaluation of selected tissues was performed.

The following treatment-related effects were observed:

During the end of the lactation phases, females showed less bodyweight loss when compared to controls for the F0 and F1 generations. There was no adverse effect on bodyweight change for males throughout the treatment period, or for females during the pre-pairing and gestation phases of the study.

An increase in liver weights was noted for females treated with 15000 ppm from both generations. No such effect was noted for males treated with 15000 ppm or for animals of either sex treated with 5000 or 1500 ppm. However, this finding was considered as an adaptive response to treatment and not as an adverse health effect.

There were no treatment-related histopathological changes for F0 generation animals. Treatment-related changes in the F1 generation were confined to the presence of lower incidences and severities of cortical vacuolation of the adrenal glands for treated males when compared to controls.

Conclusion:
The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 15000 ppm for two successive generations resulted in possible treatment-related changes at 15000 ppm. Therefore the NOAEL was considered to be 15000 ppm for adult toxicity for both the F0 and F1 generations.
The NOAEL for reproductive and developmental toxicity for both generations and offspring was considered to be 15000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7% (w/w)
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) IGS BR
Source:	[redacted] UK
Age:	Approximately 8 weeks
Sex:	Males and females
Weight at dosing:	Males: 138 – 257 g; females: 140 – 195 g
Acclimation period:	At least 14 days
Diet/Food:	Rodent [redacted] (certified) diet ([redacted] UK), *ad libitum*
Water:	Tap water *ad libitum*
Housing:	Initially in groups of up to four in polypropylene cages with stainless steel grid floors and tops, suspended over polypropylene trays lined with absorbent paper. During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation in polypropylene cages with solid floors and stainless steel lids, furnished with softwood flakes.
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 55 ± 15% Air changes: at least 15/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 2005-11-18 to 2006-11-06

Animal assignment and treatment:
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females) glyphosate technical in diet. The dose levels were chosen based on results of a previously conducted study. After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. At weaning of offspring from the F0 mating phase, groups of twenty-four male and twenty-four female offspring from each dose group were selected to form the F1 generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*, followed by the termination of all F0 male dose groups. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups.

Diet preparation and analyses
For preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet at a constant speed for 19 minutes in a Hobart QE200 mixer. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes in a Hobart H800 mixer.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration weekly for the first four weeks of the study and monthly thereafter.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight
Individual body weights were recorded for F0 males on Day 1 (prior to treatment) and at weekly intervals for F0 and F1 males until termination. F0 and F1 females were weighed daily until mating was evident. Bodyweights for females showing evidence of mating were recorded on Days 0, 7, 14 and 21 *post coitum*. Females with live litters were weighed on Days 1, 4, 7, 14 and 21 *post partum*.

Food consumption and compound intake
During the maturation period, weekly food consumption was recorded for each cage of adults. For females showing evidence of mating, food consumption was recorded for the periods covering Days 0 - 7, 7 - 14 and 14 – 21 *post coitum*. For females with live litters, food consumption was recorded for the period covering Days 1 - 4, 4 - 7, 7 - 14, 14 - 21 *post partum*.
Food conversion efficiency (the ratio of bodyweight change / dietary intake) was calculated retrospectively for males for both the pre-mating and post-mating phases of the study. For females, food conversion efficiency was only calculated for the pre-mating phases of the study. Due to offspring growth, milk production and weaning, food efficiency could not be accurately calculated for the gestation and lactation phases of the study.

Water consumption
Water intake was observed daily by visual inspection of water bottles for any overt change.

Reproduction parameters
Oestrus cycle
Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous.

Pregnancy and parturition
Pregnant females were observed at approximately 0830, 1230 and 1630 hours daily, and at approximately 0830 and 1230 hours on weekends and public holidays. In addition, the females were observed around the period of expected parturition. The date of mating, date and time of start and end of parturition and duration of gestation was recorded.

Litter data
The following litter data were recorded:
The number of offspring born, the number of offspring alive recorded daily and reported on Day 1, 4, 7, 14, 21 post partum. On Days 1, 4 and 21, the sex of individual offspring was recorded. The clinical condition of offspring during lactation, as well as individual offspring and total litter weights were recorded after birth on Day 1, 4, 7, 14.

Physical and sexual development
All live offspring were observed for the detachment and unfolding of pinna, incisor eruption and eyelid separation and assessed for reflexological response to stimuli by assessing surface righting reflex on Day 1 *post partum* and air righting reflex on Day 17 *post partum*. Pupillary reflex and auditory startle response were performed on Day 21 *post partum*.

All selected F1 offspring were observed for sexual development and the body weight for each individual animal at the time of sexual maturation was recorded. In addition, the ano-genital distance was recorded for all F2 generation offspring on day 1 post partum

Sacrifice and pathology
All surviving adult females and surviving offsprings except offsprings selected to form the F1 generation, as well as surviving males were sacrificed on Day 21 post partum.
All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For females the uterine implantation sites were counted. In addition, the corpora lutea of all ovaries from pregnant females were counted at necropsy.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study sampled, weighed and preserved, except for the thyroids, which were weight after fixation:
adrenals, brain, left cauda epididymis, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, thymus, thyroid glands, and uterus (with cervix and oviducts).
The following organs from one male and one female offspring from the F0 and F1 pairings were weighed: brain, spleen, thymus, and uterus.

The following tissues were preserved from all F0 males and females from each dose group in 10% buffered formalin, except for the right epididymis, right testis, which were fixed in Bouins fluid and 70% IMS: adrenals, coagulating gland, right epididymis, ovaries, right testis, pituitary, prostrate, seminal vesicles, Uterus (with oviducts) and cervix, vagina and all gross lesions.

A detailed histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on animals that died or were killed in extremis.
During the histopathological examination there were indications of treatment-related changes in the adrenal glands for the F1 animals. Thus, the microscopic examination was subsequently extended to include similarly prepared sections of adrenals from the F1 animals from the 5000 and 1500 ppm dose groups.

Semen assessment
At necropsy of adult F0 and F1 males at least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the testis and cauda epididymis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number
From ten control and ten high dose females of the F1 generation slides of the ovaries were prepared and analysed for visible oocytes. The identified oocytes were classified as small, medium or large follicles.

Statistics

Organ weight (absolute and relative to terminal bodyweight), weekly bodyweight gain, litter weights and offspring bodyweights were assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogenous, pair wise comparisons were conducted using Dennett's test. Where Levene's test showed unequal variances the data were analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney 'U' test.

The non-parametric methods were also used to analyse implantation loss, offspring sex ratio and developmental landmarks and reflexological responses.

Probability values (p) are presented as follows:

$p < 0.001$ ***
$p < 0.01$ **
$p < 0.05$ *
$p \geq 0.05$ (not significant)

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:

1. Chi-squared analysis for differences in the incidence of lesions occurring with an overall frequency of one or greater.
2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

Probability values (p) were calculated as follows:

$p < 0.001$ +++ --- ***
$p < 0.01$ ++ -- **
$p < 0.05$ + - *
$p < 0.1$ (+) (-) (*)
$p \geq 0.1$ N.S. (not significant)

(+)-signs indicate positive differences from the control group and (-)-signs indicate negative differences.
* refer to overall differences between group variation which is non-directional.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations at nominal concentrations of 1500, 5000 and 15000 ppm were stable for at least six weeks at ambient temperature.

Analyses for homogeneity at the start of treatment indicated that the dose preparations were homogeneous.

Analyses for achieved concentration performed on ten separate occasions demonstrated that the prepared dietary admixture concentrations given to the animals were in the range of 83 to 102% of the nominal concentration.

B. TEST COMPOUND INTAKE

The group mean achieved dosages are summarised in Table 5.6-2 below.

Table 5.6-2: Group mean achieved dose levels

Group	Dietary concentration (ppm)	Estimated dose level (mg/kg bw/day)	Mean achieved dose level (mg/kg bw/day)			
			Males	Females		
				Maturation	Gestation	Lactation
Control	0	0	0	0	0	0
Low	1500	75	104	126	108	252
Intermediate	5000	250	351	423	358	808
High	15000	750	1063	1273	1109	2520

C. MORTALITY

There were no test substance related mortalities.

Four unscheduled deaths occurred during the study. In the F0 generation one male of the low dose group and one female of the mid dose group was killed on humane reasons on Days 87 and 103, respectively. The male exhibited a mass of about 3 x 4 cm on the lower jar. The female was in extremis following a suspected prolonged parturition. One high dose female was found dead on Day 97 possibly due to complications during parturition.
In the F1 generation one control female was killed on Day 99 following severe clinical signs (pallor of the extremities, lethargy, pilo-erection, hunched posture and staining around the ano-genital region); however the aetiology of the signs was not established.

D. CLINICAL OBSERVATIONS

No treatment-related clinical signs of toxicity were noted. Clinical signs observed in control and treated animals of the F0 and F1 generation are summarised in Table 5.6-3 and Table 5.6-4 below. These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

Table 5.6-3: Observed clinical signs in F0 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Abrasion to dorsal region	2/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Generalised fur loss	5/28	5/28	3/28	5/28	2/28	6/28	2/28	3/28
Red/brown staining around snout	4/28	0/28	4/28	0/28	3/28	3/28	5/28	0/28
Red/brown staining of fur	1/28	0/28	0/28	0/28	2/28	2/28	1/28	0/28
Red/brown staining around eyes	0/28	0/28	1/28	1/28	0/28	0/28	3/28	0/28
Swollen face (due to overgrowth tooth)	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Cranial abrasion	0/28	0/28	0/28	0/28	0/28	0/28	2/28	0/28
Red stained urine	0/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Facial scab formation	1/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation	1/28	0/28	1/28	0/28	1/28	0/28	0/28	0/28
Large mass under lower jar	0/28	0/28	1/28	0/28	0/28	0/28	0/28	0/28
Mass on dorsal region	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation around right eye	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Physical injury to tail apex	0/28	0/28	0/28	1/28	0/28	0/28	0/28	0/28
Stained fur on head	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Red swollen ears	0/28	/28	0/28	0/28	0/28	1/28	0/28	1/28
Blood seen without evidence of offspring born	0/28	1/28	0/28	0/28	028	0/28	0/28	0/28
Blood around vagina (suspected prolonged parturition, killed in extremis)	0/28	0/28	0/28	0/28	0/28	1/28	0/28	0/28
Pilo-erection	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Exophthalmia	0/28	/28	0/28	0/28	0/28	0/28	0/28	1/28

* x/y: number affected / total number of animals in group

Table 5.6-4: Observed clinical signs in F1 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Generalised fur loss	3/28	4/28	0/28	2/28	0/28	6/28	0/28	4/28
Red/brown staining around eyes	2/28	1/28	0/28	0/28	1/28	0/28	0/28	0/28
Red/brown staining of fur	0/28	1/28	2/28	0/28	0/28	2/28	1/28	1/28
Red/brown staining around snout	1/28	7/28	1/28	0/28	4/28	7/28	1/28	4/28
Scabbing and fur loss around eye	0/28	/28	0/28	/28	0/28	/28	1/28	/28
Protruding sternum	0/28	2/28	0/28	3/28	0/28	3/28	0/28	0/28
Lethargy	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Hunched posture	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Staining around ano-genital region	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Pallor of extremities	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28

* x/y: number affected / total number of animals in group

E. BODY WEIGHT

No adverse effect of bodyweight change was evident for treated animals in comparison to controls throughout the treatment period for both the F0 and F1 generations except for *post-partum* females treated with 15000 ppm (see Table 5.6-5). During the final week of lactation, both the F0 and F1 generations showed statistically significant less bodyweight loss in comparison to controls ($p < 0.001$ and $p < 0.01$ respectively).

Table 5.6-5: Body weight changes during lactation (Group mean values)

Dietary concentration (ppm)	No. of animals		Body weight Change (g) at Day 4	7	14	21
			F0 Generation			
0 (Control)	26	mean	15	22	0	-23
		sd	14	9	15	10
1500	27	mean	18	16	3	-26
		sd	9	9	13	13
5000	26	mean	16	18	1	-23
		sd	14	13	11	11
15000	26	mean	18	18	1	-8***
		sd	11	12	14	14
			F1 Generation			
0 (Control)	26	mean	14	9	9	-16
		sd	11	13	14	13
1500	27	mean	14	16	3	-21
		sd	7	11	9	17
5000	26	mean	17	10	5	-17
		sd	12	10	13	13
15000	26	mean	16	11	10	-4**
		sd	9	9	12	13

sd - standard deviation
** - significantly different from control group $p < 0.01$
*** - significantly different from control group $p < 0.001$

F. WATER CONSUMPTION

Daily visual inspection of water bottles showed no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations, when compared to their concurrent controls.

G. REPRODUCTIVE PARAMETERS

Oestrus cycle

There were no toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

H. LITTER DATA

Size and Viability

No overt differences in litter size and viability were detected. The mean numbers of corpora lutea and subsequent number of implantations did not indicate any adverse effect of dietary exposure and pre and post implantation loss for treated animals were essentially similar to controls. There were no toxicologically significant differences in sex ratio for both F0 - F1 and F1 - F2 litters.

Growth and Development

No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.

Clinical signs

No clinically observable signs of toxicity were observed for offspring from treated animals.

I. PATHOLOGY

Necropsy

There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, nor in the offspring.

Organ weights

F0 females treated with 15000 ppm displayed statistically significant increases in liver weights, both absolute and relative to terminal bodyweight ($p < 0.001$). An increase in liver weights was also noted for F1 females treated with 15000 ppm (absolute: $p < 0.05$, relative: $p < 0.01$). In the absence of any histopathological changes in the liver, and as increased liver weights without histopathological changes were also noted in another repeated dose toxicity study this finding is considered as an adaptive response rather than an adverse effect. Furthermore, F0 females treated with 15000 ppm displayed an increase in kidney weights, both absolute ($p < 0.001$) and relative to terminal bodyweight ($p < 0.01$) (see Table 5.6-6). No such observations were detected for males treated with 15000 ppm from either generation.

Table 5.6-6: Liver and kidney weights (relative and absolute) of females (Group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)			
			Liver		Kidney	
			Absolute	Relative	Absolute	Relative
			F0 Generation			
0 (Control)	26	mean	15.0328	4.3103	2.4315	0.6977
		sd	1.0493	0.2864	0.1706	0.0548
1500	27	mean	15.1465	4.3027	2.5395	0.7233
		sd	1.4948	0.3435	0.1602	0.0560
5000	27	mean	15.8791	4.3570	2.5654*	0.7062
		sd	1.7649	0.2810	0.2361	0.0592
15000	26	mean	16.9704***	4.6806***	2.7096***	0.7490**
		sd	1.7620	0.2977	0.2203	0.0521

Dietary concentration (ppm)	No. of animals		Organ weight (g)			
			Liver		Kidney	
			Absolute	Relative	Absolute	Relative
			F1 Generation			
0 (Control)	22	mean	16.4887	4.5970	2.6792	0.7483
		sd	2.0275	0.4038	0.4137	0.1070
1500	23	mean	16.3848	4.6047	2.5777	0.7257
		sd	1.7744	0.2858	0.2776	0.0647
5000	24	mean	17.2591	4.6543	2.8124	0.7585
		sd	2.0969	0.3628	0.5326	0.1229
15000	23	mean	18.0724*	4.9591**	2.7660	0.7578
		sd	1.2434	0.3130	0.2616	0.0517

sd - standard deviation
* - significantly different from control group $p < 0.05$
** - significantly different from control group $p < 0.01$
*** - significantly different from control group $p < 0.001$

There were no toxicologically significant intergroup differences detected for the brain, spleen or thymus for offspring of either sex from either generation. Furthermore, there were no differences in uterus weights for treated females from either generation when compared to controls.

Sperm assessment
There were no toxicologically significant effects on the concentration, motility or morphology of samples of sperm from treated F0 and F1 generation males when compared to their controls. Furthermore, no abnormal sperm were detected in the control and treated males from either generation.

Oocyte assessment
There were no toxicologically significant differences in follicle numbers for F1 females treated with 15000 ppm when compared to controls.

Histopathology
No treatment-related changes were detected in the F0 generation animals.

In the F1 generation cortical vacuolation of the adrenal glands was observed with a lower incidence and with generally lower grades of severity among males treated with 15000 ppm ($p < 0.05$), 5000 ppm ($p < 0.05$ - 0.01), and 1500 ppm ($p < 0.1$ - 0.05) when compared to controls. The group distribution of incidence and of severity grades may also suggest a consequence of treatment. However, the absence of a dose-related response, may suggest that a higher than normal background incidence of the condition among control male rats may have contributed to the effect on this occasion.

Table 5.6-7: Incidence of adrenal cortical vacuolation in males at terminal kill

	Historical control data	Dietary concentration (ppm)							
		0		1500		5000		15000	
Generation	--	F0	F1	F0	F1	F0	F1	F0	F1
Animals examined	234	28	24	27	24	28	24	28	24
adrenal cortical vacuolation									
Absent	153	20	7	--	14	--	16	16	14
Present	81	8	17	--	10*	--	8**	12	10***
Minimal	57	6	10	--	6	--	6	8	7
Slight	23	2	7	--	4	--	2	4	2
moderate	1	0	0	--	0	--	0	0	1
% present	34.6	28.6	71%	--	24	--	33	42.9	42

* - significantly different from control group $p < 0.1 – p < 0.05$
** - significantly different from control group $p < 0.01 – p < 0.05$
*** - significantly different from control group $p < 0.05$

All remaining morphological changes were those commonly observed in laboratory maintained rats of the age and strain employed and, since there were no differences in incidence or severity between control and treatment groups, all were considered to be without toxicological significance.

III. CONCLUSION

The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 15000 ppm for two successive generations resulted in possible treatment-related changes at 15000 ppm. The effects however were considered not to represent an adverse health effect, therefore the NOAEL was considered to be 15000 ppm (equivalent to 1063 and 1634 mg/kg bw/day, for males and females, respectively) for adult toxicity for both the F0 and F1 generations.
The NOAEL for reproductive and developmental toxicity, for both generations and offspring was considered to be 15000 ppm.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/02	[redacted]	2000	Glyphosate acid: Multigeneration reproduction toxicity study in rats [redacted] Data owner: Syngenta, Monsanto Report No.: [redacted]6332 Date: 2000-06-16 GLP: yes not published

Guideline: OECD 416 (2001), Annex V 67/548/EEC, 9.ATP 87/302/EEC, OJEC, L133, 47-50 (1988), US-EPA OPPTS 870.3800 (1998)

Deviations: None

Dates of experimental work: 1998-09-01 to 1999-12-10

Executive Summary

Glyphosate acid was administered by dietary admixture to three groups of 26 male and female F0 generation Alpk:AP$_f$SD rats each, at dietary concentrations of 1000, 3000 and 10000 ppm. A further group of 26 male and 26 female F0 animals was exposed to basal laboratory diet to serve as a control.

After 10 weeks, the animals were mated and allowed to rear the F1A litters to weaning. The regime was repeated with the F1 parents (26 per sex and dose) selected from the F1A litters to produce the F2A litters after a 10 week pre-mating period. The remaining surviving F0 females and unselected offspring were terminated at Day 29 *post partum*, followed by the termination of all F0 male dose groups. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups. Diets containing glyphosate acid were fed continuously throughout the study. Clinical signs, bodyweight development, food and water consumption were monitored during the study.
Oestrous cycle assessment was performed daily for three weeks prior to mating for both the F0 and F1 generations. Observations for positive evidence of mating were recorded together with the start and completion of parturition. During the maturation phase of the F1 generation offspring, males and females were evaluated for sexual maturation. During the lactation phases daily clinical observations were performed on all surviving offspring, together with litter size. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days *post partum*.
All animals at termination were subjected to a gross necropsy examination and histopathological evaluation of selective tissues was performed if necessary.

The bodyweights of the F1A pups in the 10000 ppm group were lower in comparison with the control group from Day 2 to Day 29 *post partum* although a similar effect was not observed for the F2A pups. In line with this, compound-related reductions in bodyweight and food consumption were evident only in F1 males given 10000 ppm. No further treatment related effects were observed.

Conclusion:
The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of the Alpk:APfSD rat resulted in possible treatment-related changes at 10000 ppm, where a reduction in the bodyweight of the F1A pups in the 10000 ppm group with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the pre-mating period was observed. The effects however were considered not to represent an adverse health effect, therefore the NOAEL was considered to be 3000 ppm, for adult toxicity for both the F0 and F1 generations (equivalent to 322 mg/kg bw/day for males and 459 mg/kg bw/day for females).
The NOAEL for reproductive and developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid, technical
Description:	White solid
Lot/Batch #:	Y04707/082
Purity:	97.6% (w/w)
Stability of test compound:	At least 10 years at ambient temperature

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Alpk:APfSD (Wistar-derived)
Source:	[redacted], UK
Age:	At least 5 weeks old
Sex:	Males and females
Weight at dosing:	Males: approx. 160 g; females: approx. 140 g
Acclimation period:	At least 14 days
Diet/Food:	CT1 diet ([redacted] UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Rats were house in pairs (same sex) in multiple rat racks (with rats of the same group in adjacent cages). During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until termination. Males were housed up to four per cage after being used for mating.

Environmental conditions: Temperature: 22 ± 3°C
Humidity: 50 ± 20%
Air changes: at least 15/hour
12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: not reported

Animal assignment and treatment:
In a two-generation reproduction study groups of 26 Alpk:AP$_f$SD rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate acid in diet. The dose levels were chosen based on results of a previously conducted chronic toxicity study.
After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. On Day 29 *post partum*, groups of twenty-six male and twenty-six female offspring from each dose group of the F0 generation were selected to form the F1 generation. F0 males were terminated after the completion of littering and females were terminated on or soon after Day 29 of lactation. Unselected offspring were terminated at Day 29 *post partum*. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned off on Day 29 *post partum* and terminated thereafter.

Diet preparation and analyses
For preparation of diet mixtures (60 kg) a known amount of the test substance was mixed with a small amount of basal diet in a mortar using a pestle. Further milled diet was added to give a pre-mix of 1000 g. Each pre-mix was grounded at a constant speed for 15 min with an automatic pestle and mortar. This pre-mix was then added to a larger amount of basal diet and blended for further 6 minutes in a Pharma Matrix Blender Model PMA 150S ([redacted]). Control diet was treated in the same way but without addition of the test substance. The stability and homogeneity of the test material in diet were determined in the lowest and the highest dose. Dietary admixtures were analysed for achieved concentration at a 2 month interval.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight
Individual body weights were recorded for F0 adults immediately prior to treatment and weekly thereafter throughout the pre-mating period. F0 males were weighed weekly thereafter until termination. Successfully mated F0 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 *post partum*. Initial body weights for the F1 adults were recorded at selection on Day 29 *post partum* and weekly thereafter throughout the pre-mating period. F1 males were weighed weekly thereafter until termination. Successfully mated F1 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 *post partum*. All rats were weighed at termination.

Food consumption and compound intake
Food consumption for each cage was recorded throughout the pre-mating period and calculated on a weekly basis. Food utilisation was calculated as the bodyweight gained by the rats in the cage per 100 g of food eaten. Food consumption was also recorded for females during gestation and lactation and calculated on a weekly basis.

Reproduction parameters
Oestrus cycle
Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous. A vaginal smear was also taken and examined from all F0 and F1 females at termination.

Reproductive performance
The success of mating (production of viable litter) was established. Length of gestation was measured in days from the date of the positive smear to the date of birth. Pre-coital interval was measured as the number of days from the date of pairing to the date of the positive smear.

Litter data
The following litter data were recorded:
The number of offspring born and the number of offspring alive were counted within 24 h after parturition and thereafter on Day 5, 8, 15, 22 and 29 *post partum.* The sex and the litter weight was also recorded at these times. Any clinical findings were recorded. Litters were examined for dead or moribund pups at least once daily.

Physical and sexual development
All selected F1 offspring were observed for sexual development and the body weight for each individual animal at the time of sexual maturation was recorded.

Sacrifice and pathology
All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, were sacrificed on Day 29 post partum. Males were sacrificed at completion of the littering. All adult animals and offspring, including those dying during the study were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For F0 and F1 females the uterine implantation sites were counted.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study were sampled, weighed and preserved:
adrenal gland, brain, left and right epididymides and caudae, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, uterus (with cervix and oviducts).
The following organs from one male and one female offspring from the F1 pairings were weighed: brain, spleen and thymus.
The following tissues were preserved from all F0 males and females from each dose group in 10% buffered formalin, except for the left epididymis, left testis which were fixed in Bouins fixative: adrenals, brain, coagulating gland, left epididymis, ovary, left testis, pituitary, prostrate, seminal vesicle, uterus (with oviducts) and cervix, vagina and all gross lesions.

Beside all pups killed in extremis (age 1 to 29 days) 3 male and 3 female per F2-litter were given a macroscopic examination at termination on Day 29 *post partum.* One of the 3 pups/sex/litter was used for organ weight determination as described above. Following tissues were stored from these pups: brain, spleen, thymus, salivary gland. Abnormal tissue from all these pups were taken and fixed as described earlier.

The reproductive organs from animals suspected of reduced fertility were processed for histopathological examination.

Semen assessment
At necropsy of adult F0 and F1 males sperm were taken from the right distal cauda epididymis. At least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the right testis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number
Primordial and small growing follicles were quantified in the left ovary of all F1 females from the control and high dose groups. Quantification was done using five 5 µm thick sections cut from the central third of each ovary and taken at least 100 µm apart and as evenly spaced as possible.

Statistics

One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: analyses of variance (ANOVA), analyses of covariance, ANOVA followed by analyses of covariance, as well as ANOVA following the double arcsine transformation of Freeman and Tukey (1950), or ANOVA following a square root formation, or Fisher's Exact Test.

All analyses were carried out in SAS (1996). For Fisher's Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance, with the exception of pup organ weights, allowed for the replicate structure of the study design.

Least-squares means for each group were calculated using the LSMEAN Option in SAS PROC MIXED. 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.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The chemical stability of glyphosate acid in the diet at nominal concentrations of 1,000 and 10,000 ppm was consistent for at least 6 weeks (at room temperature). Homogeneity of the test substance in the dietary mixture was satisfactory, percentage deviations from the overall mean were within 4%. The mean achieved concentrations of glyphosate acid in the preparations were within 9% of the nominal concentrations and the overall mean concentrations were within 3% of the nominal concentrations.

B. TEST COMPOUND INTAKE

The group mean achieved dosages are summarised in Table 5.6-8 below.

Table 5.6-8: Group mean achieved dose levels F0 and F1-generation

Group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)			
		Males	Females		
			Maturation	Gestation	Lactation
Control	0	0	0	0	0
Low	1000	108.0	113.9	90.4	227.9
Intermediate	3000	322.2	346.8	277.9	752.4
High	10000	1072.9	1136.2	910.9	2424.8

C. MORTALITY

There were no test substance related mortalities.

Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively. In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

D. CLINICAL OBSERVATIONS

No treatment-related clinical signs of toxicity were noted.

During the pre-mating period, annular constrictions were visible on the tails of the F0 and F1 male and female rats. Almost all males and approximately half of the females, in all groups, were affected. Scaly

tail was also observed in some of the animals. These findings were considered incidental to the administration of glyphosate acid in the diet. Other recorded changes in clinical condition were either isolated occurrences or of an incidence comparable with that of the control group
These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

E. BODY WEIGHT

There was no effect of glyphosate acid on bodyweight adjusted for initial weight for the F0 rats, males and females, during the pre-mating period. For the F1 males given 10000 ppm, bodyweight was slightly lower at week 1, in comparison with the control group. Thereafter, bodyweights adjusted for initial weight remained lower than the controls for the duration of the pre-mating period and were statistically significant different from week 2 through to week 8 (see Table 5.6-9). There was no effect of 10000 ppm on the bodyweight of the Fl females and no effect of 3000 or 1000 ppm on the bodyweight of the F1 males or the F1 females (see Table 5.6-9). There was no effect of glyphosate acid on bodyweight adjusted for initial weight for either the F0 or F1 rats during gestation or lactation.

Table 5.6-9: Body weight during the pre-mating period-F1 generation (Group mean values)

F1 generation	body weight (g)							
	Control (0 ppm)		Low (1,000 ppm)		Mid (3,000 ppm)		High (10,000 ppm)	
week	♂ (n=25)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)
1	80.2	74	81.1	75.2	78.1	74.2	75.3	73.4
2	130.1	115.4	132.0	115.7	128.6	114.7	127.6*	115.2
3	188.5	152.6	190.7	154.7	186.5	151.2	183.3*	152.3
4	246.2	178.3	247.6	180.2	242.8	176.5	237.3**	179.4
5	300.2	201	304.1	202.7	296.5	199.7	289.5**	202.1
6	345	219.8	347.5	224.1	334.5	217.2	328.7**	218.4
7	377.2	231.7	382.4	237.1	369	228.3	360.5**	234.4
8	403.6	241.9	410.1	245.1	395.3	237.2	387.0*	245.6
9	425	250.3	433.3	253.6	416.3	245.1	411.8	252.5
10	443.4	259.7	453.1	263.8	435.1	251.7	431.6	258.1
11	461	265.7	471.3	271.2	455.5	258.8	449.7	266.9

* - significantly different from control group $p < 0.05$
** - significantly different from control group $p < 0.01$

F. FOOD CONSUMPTION

There was no effect of glyphosate acid on food consumption for the F0 generation, all F1 females and F1 males of the low and intermediate level dose group during the pre-mating period. Only F1 males of the high-level dose group showed significantly lower food consumption throughout the pre-mating period. There was no effect of glyphosate acid on food utilisation for the F0 generation, all F1 females and F1 males of the low and intermediate dose group during the pre-mating period. Food utilisation was slightly higher for F1 males given 10000ppm glyphosate acid, the difference from control being statistically significant for weeks 5-8 only. There was no effect of glyphosate acid on food consumption for either the F0 or F1 rats during gestation or lactation.

G. REPRODUCTIVE PARAMETERS

Oestrus cycle

There were no consistent toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation
There were no treatment-related effects on pre-coital interval, mating performance, and gestation length for both F0 and F1 generation animals.

H. LITTER DATA

Size and Viability
No overt effects of glyphosate acid on pup survival or on litter size during lactation were detected.

In both generations the incidence of whole litter losses was low and similar across all groups. Glyphosate acid treatment did not affect the percentage of post-implantation loss. The proportion of F1A and F2A pups born live was slightly higher in the glyphosate acid groups than in the control group. There was no effect of glyphosate acid on litter size at birth or during the time of lactation for either the F1A or F2A pups. The proportion of litters with all pups surviving and the proportion of pups surviving during lactation were also unaffected by the treatment. An increased proportion of litters with all pups surviving noted for the F1A litters in the 10000 ppm group in comparison with the control group were not present for the F2A litters since the F2A controls showed an improvement over the F1A controls. Sex distribution within the litters was not altered by the administration of glyphosate acid.

Growth and Development
There was no effect of glyphosate acid on pup weight at birth for the F1A or F2A pups. Thereafter, the bodyweights of the F1A pups in the 10000 ppm group were lower in comparison with the control group The differences from control were statistically significant for males from day 8 through to day 29 and for females, from day 5 through to day 29. A similar effect was neither observed for the F2A pups in the 10000 ppm group nor for the F1A pups of the low and intermediate dose level groups. There was no effect of glyphosate acid on total litter weight of either generation. Also the day of age when preputial separation or vaginal opening occurred in the F1 parents was unaffected by treatment.

Clinical signs
No clinically observable signs of toxicity were noted for offspring from treated animals.

I. PATHOLOGY

Necropsy
No macroscopic findings that could be attributed to the treatment with glyphosate acid were observed in any animal of the F0 and F1 generations.

The incidence of unilateral pelvic dilatation was slightly higher (9/69) in F2A females in the 10,000 ppm group compared with the other groups. Unilateral pelvic dilatation is a very common spontaneous change in the Alpk:APfSD strain of rat. There was no increase in incidence in the F0 or F1 adults or in the F1A pups and, as an isolated observation, it is considered incidental to treatment with glyphosate acid.

Organ weights
The treatment of rats with glyphosate acid did not affect the weight of the adrenal glands, brain, right cauda epididymis, epididymides, kidney, liver, ovary, pituitary gland, prostate gland, spleen, seminal vesicles, testes or uterus. For the F0 males given 10000 ppm glyphosate acid, liver and kidney weights adjusted for bodyweight were statistically significantly greater than in the control group. Similar changes were not observed in the F1 males given 10000 ppm glyphosate acid. Absolute and relative values were comparable with the control group (see Table 5.6-10). The weight changes seen in the liver and kidney of the F0 males were therefore considered not to be treatment related. For the F0 males given 3000 or 10000 ppm glyphosate acid, brain weight adjusted for bodyweight was statistically significantly greater than in the control group. Absolute values were comparable with the control group (see Table 5.6-10). Similar changes were not observed in the F1 animals. The weight changes seen in the brain of the F0 males were therefore considered to be incidental to treatment.

Table 5.6-10: Liver, kidney and brain weights (relative and absolute) of males (Group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)					
			Liver		Kidney		Brain	
			Absolute	Relative	Absolute	Relative	Absolute	Relative
			F0 Generation					
0 (Control)	25	mean	19.3	3.4	3.20	0.57	2.11	0.38
		sd	2.6	0.2	0.38	0.04	0.09	0.03
1000	26	mean	19.1	3.5	3.17	0.58	2.12	0.39
		sd	2.3	0.2	0.36	0.04	0.08	0.03
3000	26	mean	18.7	3.5	3.11	0.58	2.12	0.40
		sd	1.9	0.2	0.27	0.03	0.07	0.03
10000	25	mean	19.7	3.6	3.23	0.59	2.13	0.40
		sd	2.7	0.2	0.38	0.04	0.07	0.03
			F1 Generation					
0 (Control)	25	mean	21.4	3.7	3.42	0.6	2.11	0.37
		sd	2	0.3	0.31	0.05	0.07	0.02
1000	26	mean	21.4	3.7	3.45	0.59	2.12	0.37
		sd	3.3	0.4	0.37	0.04	0.07	0.02
3000	26	mean	20.1	3.6	3.32	0.6	2.1	0.38
		sd	2.6	0.3	0.31	0.04	0.07	0.03
10000	26	mean	19.1*	3.6	3.36	0.62*	2.1	0.39
		sd	2.3	0.3	0.28	0.04	0.07	0.03

sd - standard deviation
* - significantly different from control group $p < 0.05$

There was no effect of glyphosate acid on brain, spleen or thymus weight.

For the F1A female pups in the 10000 ppm group absolute thymus weight was statistically significantly lower than in the control group. There was no effect of glyphosate acid on the thymus weight of the F2A pups. The observation in the F1A females is therefore considered incidental to treatment with glyphosate acid.

Sperm assessment
In F0 and F1 males no effect of glyphosate acid on the number of sperm, sperm motility parameters or sperm morphology was observed.

Oocyte assessment
There was no effect of 10000 ppm glyphosate acid on the number of primordial and small growing follicles in the left ovary of the F1 parent animals.

Histopathology
No treatment-related changes were detected in the F0 and F1 generations.

III. CONCLUSION

The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10,000 ppm for two successive generations of the Alpk:APfSD rat resulted in possible treatment-related changes at 10,000 ppm, where a reduction in the bodyweight of the F1A pups in the 10000 ppm group with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the pre-mating period was observed. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 3000 ppm (equivalent to 322 and 459 mg/kg bw/day for males and females, respectively) for maternal and offspring for both the F0 and F1 generations.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/03	[redacted]	1997	HR-001: A two-generation reproduction study in rats [redacted] Data owner: Vysta Life Sciences Study No.: [redacted]96-0031 Date: 1997-06-19 GLP: yes not published

Guideline: OECD 416 (1981), US-EPA FIFREA Guidelines Subdivision F (1984), Japan MAFF Guideline 59 Nohsan No. 4200 (1985)

Deviations: None

Dates of experimental work: 1996-04-16 to 1997-03-31

Executive Summary

To evaluate the potential effects of HR-001 on reproduction groups of 24 Sprague-Dawley rats per sex were fed diets containing test substance concentrations of 0, 1200, 6000 and 30000 ppm for two consecutive generations. Clinical signs, bodyweight development, food consumption were monitored during the study. Reproductive parameters (oestrus cycle, mating, fertility and gestation indices, sperm assessment) were also evaluated. Gross pathological examinations were performed on all animals. Organ weight determinations and histopathological examinations were also performed on designated animals.

Litter data determined covered the total number of live and dead pups, the number of males and females, viability indices, body weights and clinical signs.

There were no treatment-related signs of toxicity noted in parental animals of the low- and mid-dose groups. At 30000 ppm treatment-related adverse effects consisted of defecation of loose stool in F0 and F1 males and females, and decreased body weights in F0 and F1 males. Also in the high-dose group distension of the caecum and increased liver and kidney weights in F0 and F1 males and a decreased prostrate weight in F1 males were observed at necropsy.

Reproductive performance was not affected by the treatment in any dose group. The slightly lower gestation indices observed in F1 females of the mid- and high-dose group were considered unrelated to treatment, as shown by the results of the reciprocal crosses of F1 animals with untreated rats.

No treatment-related alterations were observed in offspring of the low- and mid-dose groups. In the high-dose group pups of both sexes of the F1 and F2 generation showed significant decreased body weights and a significant increase in the incidence of distension of the caecum.

Oral dietary administration of 0, 1200, 6000 and 30000 ppm HR-001 to Sprague-Dawley rats for two successive generations resulted in treatment-related signs of toxicity in parental rats at 30,000 ppm. Therefore, the NOAEL for maternal toxicity is considered to be 6000 ppm, equivalent to 417-458 mg/kg bw/day and 485-530 mg/kg bw/day for males and females, respectively.
The NOAEL for reproduction is 30000 ppm, since the reproductive performance was not affected in any dose group. Based on the body weight effects and increased incidences of caecum distension the NOAEL for offspring is considered to be 6000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-950308
Purity:	94.61% (w/w)
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley: Crj:CD(SD)
Source:	[redacted] Japan, [redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Males: 132 - 148 g; females: 112 - 126 g
Acclimation period:	7 days
Diet/Food:	Certified pulverized feed ([redacted]), *ad libitum*
Water:	Filtered, sterilized well water, *ad libitum*
Housing:	During acclimatisation in groups of five per sex in suspended wire-mesh stainless steel cages). During pre-mating, and mating periods animals were housed in groups o 3/sex/cage. During mating one male and one female were housed in aluminium cages with wire-mesh floors and fronts. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until termination. Males were housed up to four per cage after being used for mating.
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: 1996-04-16 - 1997-03-31

Animal assignment and treatment:
In a two-generation reproduction study groups of 24 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1200, 6000 and 30000 ppm HR-001 in diet. The dose levels were chosen based on results of a preliminary reproductive study in Crj:CD (SD) rats..
After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation was designated Day 0 of gestation. Copulated females were placed individually into breeding boxes with nestle material. The day of completed parturition was designated Day 0 of lactation. On Day 4 *post partum*, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were sacrificed. Groups of 24 male and 24 female offspring from each dose group of the F0 generation were selected to form the F1 parents. Unselected offspring were sacrificed and subjected to a gross necropsy.

The offspring selected for the F1 generation were dosed for 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals. F1 parental rats which failed to produce F2 offspring (10 males and 10 females with normal external genitalia and oestrus cycle) were mated with untreated rats of the same strain and sacrificed thereafter for fertility assessment (reproductive performance).

Diet preparation and analyses
Diets were prepared monthly during the pre-mating period, and biweekly during the breeding period. For each dose level a specified amount of the test substance was mixed with a small amount of basal diet in a mortar. This pre-mix was stirred into the remaining part of the diet. The diets were stored at about 4 °C in the dark. Analyses for homogeneity were done for each dose level of the first diet preparation. Analyses for achieved concentration were done for all prepared diets.

Clinical observations
A check for clinical signs of toxicity and mortality was made once daily on all F0 and F1 parental animals. A detailed physical examination was performed on males prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. Females were examined prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Body weight
Individual body weights F0 and F1 males adults were determined prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. F0 and F1 females were weighed prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Food consumption and compound intake
Food consumption for each cage was recorded and daily food consumption was calculated. Determination of food consumption was made on a weekly basis during the pre-mating period for males and females and during the breeding period for males. In addition, for females total food consumption was determined at the following intervals: Day 0-7, 7-14, 14-20 of gestation and of days 0-7, 7-14 and 14-21 of lactation.
Compound intakes in parental animals were calculated during the pre-mating periods for each sex on a weekly basis.

Reproduction parameters
Oestrus cycle
The oestrus cycle was checked daily by microscopically examination of vaginal smears. Examinations were done for each female for one week prior to mating until copulation was confirmed.

Reproductive performance
Mating indices for males and females were calculated separately after copulation was confirmed. In addition, fertility and gestation indices, the length of gestation, as well as the number of implantation sites were determined.

Sperm assessment
An assessment of motility and morphology of epididymal sperm was done at necropsy for 10 males per group, which were selected for the organ weight measurement, as well as for males that failed to impregnate females.

Litter data
Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group. Viability indices, were determined for each litter on lactation days 0, 4 and 21. Body weights were determined on lactation days 0, 4, 7, 14 and 21.
A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. A detailed physical examination was done on lactation days 0, 4, 7, 14 and 21.

Sacrifice and pathology
All surviving parental F0 and F1 males and females were sacrificed on Day 21 post partum and subjected to a gross pathological examination. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. The following organs and tissues were preserved: adrenals, aorta, brain, caecum, colon, duodenum, epididymis, eyes, gross lesions, head (incl. nasal cavity, paranasal sinuses, buccal mucosa and ears), heart, ileum, jejunum, kidneys, larynx, liver, lung, mammary gland, oesophagus, ovaries, pancreas, pharynx, pituitary, prostrate, rectum, seminal vesicles, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus (cornua and cervix) and vagina.

F1 and F2 pups that were not selected on Day 4 of lactation were also killed and necropsied on that day. In addition, F1 weanlings that were not selected for parental animals of the F1 generation and all F2 weanlings were necropsied at 22-26 and 21-26 days of their age, respectively. The same organs, as described above, were preserved from one animal per sex per litter of the F1 and F2 weanlings necropsied.

The following organs weights of 10 F0 and F1 males and females from each dose group that were sacrificed at the end of the study as well as from pairs of parental animals that failed to mate: adrenal gland, brain, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), testes, uterus.
A histopathological examination was performed on the reproductive organs and pituitary of the control and high dose F0 and F1 parental animals that survived until scheduled termination. A histopathological examination of the reproductive organs and pituitary in the low and mid-dose group was only performed on pairs of animals that had failed to produce offspring.
In addition, a histopathological examination was performed on organs with significant weight change, and on all organs with gross pathological changes.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Bartlett´s test for equality of variance (p=0.05) followed by parametric analyses of variance in one-way classification (p=0.05) or Dunnett´s t-test or Scheffé ´s multiple comparison test (p=0.05, 0.01 or 0.001); or Bartlett´s test followed by Kruskal-Wallis test (P=0.05) and Dunnett-type mean rank test or Scheffé-type mean rank test ((p=0.05, 0.01 or 0.001). Fisher´s exact probability test ((p=0.05, 0.01 or 0.001) and Mann-Whitney´s U-test (p=0.05 or 0.01) were also used.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Based on the results of the dose-finding study the chemical stability of the test substance in the was given for 5 weeks (at room temperature) in sealed plastic bags in the dark, and for at least 2 weeks after being released from the plastic bags.
Homogeneity of the test substance in the dietary mixtures was satisfactory, percentage deviations from the overall mean were within 4%. The mean achieved concentrations of HR-001 in the diet preparations were in the range of 90 – 105% of the nominal and therefore acceptable.

B. MORTALITY

F0 and F1 males

Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 12 and 18, respectively. In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

F0 and F1 females

There were no mortalities observed during the study period.

C. CLINICAL OBSERVATIONS

F0 and F1 males

There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.
At 30000 ppm F0 and F1 parental males exhibited loose stool with incidences during the pre-mating growth and breeding periods of 3/24 and 2/24 for the F0 generation, and of 13/24 and 0/24 for the F1 generation, respectively, with a significant difference in the value for the pre-mating growth period of the F1 generation. Since this finding was not observed in other groups including control, defecation of loose stool was considered to be treatment-related.
Statistically significant differences were also observed in the incidence of hair loss during the breeding period for F0 males in all test substance groups. However, the occurrence of this change in the treated groups was rather lower than controls, and was considered to be incidental.
During the study period, one F0 male and one F1 male in the control group and one F1 male in the 6000 ppm group showed malocclusion of the incisors, respiratory wheezing, and red sebum. The aforementioned one F1 male of the 6000 ppm group also showed distension of the abdomen. These animals were euthanatised within several days after discovery due to unfavourable prognosis. Necropsy noted a fracture of the facial bones in all cases, suggesting that the alterations were caused by an accident in the cage. Accident malocclusion of incisors was also observed in one F1 male in the 1200 ppm group. However, test substance treatment of this animal was continued until termination of the study because its condition was improved.

F0 and F1 females

There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.
In F0 and F1 parental females, loose stool was also observed at 30000 ppm. The incidences during the pre-mating growth period and the lactation and post-weaning period were 1/24 and 6/24 for the F0 generation, and 4/24 and 2/24 for the F1 generation, respectively, with a significant difference in the value for the lactation and post-weaning period of the F0 generation.

D. BODY WEIGHT

F0 and F1 males

Mean body weights of F0 and F1 males in the 30000 ppm group were consistently lower than those in the control group from treatment week 1 to the day of necropsy, and the differences from controls at treatment weeks 1-12 and 14 for the F0 generation, and treatment weeks 1-6 for the F1 generation were statistically

significant. In the 1200 and 6000 ppm groups, mean body weights of F0 and F1 parental males were comparable to the controls throughout the study.

F0 and F1 females

There were no significant differences in mean body weights of F0 females in any treatment group when compared to control. In F1 females in the 30000 ppm group, mean body weight on lactation day 0 was significantly higher than that in the control group. In the 1200 and 6000 ppm groups, mean body weights of F1 parental females were comparable to the controls throughout the study.

E. FOOD CONSUMPTION AND TEST COMPOUND INTAKE

F0 and F1 males

In F0 males, mean food consumption at treatment week 13 in the 1200 ppm group was significantly higher than that in the control group. Since there was no such increase observed in the mid- and high-dose groups throughout the study, this change was not thought to be treatment-related.

In F1 males in the 30000 ppm group, mean food consumption at treatment week 4 was significantly lower than that in the control group, but the values on the other treatment weeks in this dose group were comparable to the controls. In the 1200 and 6000 ppm groups, mean food consumption of F1 males was comparable to the controls throughout the study.

F0 and F1 females

In F0 females, the values on treatment weeks 2-4 in the 30000 ppm group were significantly higher than the controls. Inversely, the value on lactation days 7-14 in this dose group was significantly lower than those in the control group. So it was unclear these changes were treatment-related or not. In the 1200 and 6000 ppm groups, mean food consumption of F0 females was comparable to the controls throughout the study.

In F1 females in the 1200 and 6000 groups, mean food consumption on lactation days 14-21 were significantly higher than those in the control group. However, these changes were thought to be incidental because no such increase was observed in the highest dose group. In the 30000 ppm group, mean food consumption of F1 females was comparable to the controls throughout the study.

The group mean achieved dosages are summarised in Table 5.6-11 below.

Table 5.6-11: Group mean achieved dose levels F0 and F1-generation

Group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)			
		Males		Females	
		F0	F1	F0	F1
Control	0	0	0	0	0
Low	1200	83.6	91.7	96.9	104.8
Intermediate	6000	417	458	485	530
High	30000	2150	2411	2532	2760

F. REPRODUCTIVE PARAMETERS

F0 males and females

Reproductive performance of F0 parental animals was not adversely affected by test substance treatment, and no significant differences were observed in such parameters as percentage of females having normal oestrous cycle, mating index, fertility index, gestation index, duration of gestation, number of implantation sites , and number, motility and morphology of epididymal sperm between the control group and the treated groups.

F1 males and females
In F1 parental animals, reproductive parameters in the treated groups were also comparable to the controls with the exception of gestation index and number of implantation sites, on which some biases were occasionally observed.
The significant higher number of implantation sites at 1200 ppm when compared to control was considered to be unrelated to treatment, since there was no increase noted at 6000 and 30000 ppm.
A similar bias was also found in the fertility index. The fertility indices in the control, 1200, 6000 and 30000 ppm groups were 95.8 (23/24), 95.8 (23/24), 87.5 (21/24) and 79.2% (23/24), respectively, with somewhat low values in the 2 higher dose groups. However, these decreases were considered to be incidental because the differences between the control and treated groups were not statistically significant, and because, as described below, normal reproduction results were obtained in the F1 parental animals, which had failed to produce offspring in this study, after remating with untreated animals.

Among the total of ten F1 females mated with untreated males, only one female in the 30000 ppm group did not undergo pregnancy. Histopathological of this female showed no abnormalities in the reproductive organs and pituitary. So the cause of infertility of this female was not known. The other nine F1 females were proved to have normal reproductive performance. One F1 male in each of the 1200, 6000 and 30000 ppm groups could not successfully impregnate untreated females mated. These 3 males had histopathological abnormalities in the testes and epididymides, and abnormalities in the sperm parameters, as a cause of infertility. However, the other 7 males were proved to have normal reproductive performance. Thus, the majority of F1 males and females which had failed to produce offspring were proved to have normal reproductive performance.

G. LITTER DATA

Number of pups delivered
Mean number of F1 and F2 pups delivered in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Sex ratio
Sex ratios of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Viability index
The viability indices of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Body weights
F1 pups
There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls. F1 pups of both sexes in the 30000 ppm group, showed significantly higher mean body weights on lactation day 0 than the controls. However, mean body weights on days 14 and 21 were significantly decreased when compared controls.

F2 pups
There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls during the lactation period. In F2 pups in the 30000 ppm group, mean body weights of both sexes on day 21 of lactation were significantly lower than those in the control group.

Clinical signs
There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.

During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

I. PATHOLOGY

Necropsy

F0 and F1 generation

Necropsy of parental animals of both sexes noted several findings in all groups including the control group. Among these alterations, the incidences of distension of the caecum in F0 and F1 males and females of the 30000 ppm group were significantly higher than those of the controls, and were considered treatment-related. Statistically significant differences from controls were also found in the incidences of hair loss in F0 males of the 1200, 6000 and 30000 ppm groups. However, the values were rather lower than controls and were considered to be incidental. Other findings were low in their incidences and considered not treatment-related.

F1 and F2 pups

Necropsy of stillbirths found on lactation days 0, pups found dead during lactation days 1-4, and pups killed to reduce the litter size on lactation day 4 demonstrated no treatment-related abnormalities in any of the F1 and F2 pups.

During days 5-21 of lactation, only 2 F1 pups in the 1200 ppm group were found dead. Necropsy of these dead pups were not performed due to advanced autolysis.

Necropsy of F1 and F2 weanlings in the 30000 ppm group noted distension of the caecum, suggesting a treatment-related occurrence. In the 1200 and 6000 ppm groups, no treatment-related abnormalities were observed in any of the F1 and F2 weanlings.

Organ weights

F0 and F1 males:

There were no effects in the absolute and relative organ weights in F0 and F1 males of the low- and mid-dose groups. At 30000 ppm relative weights of the liver and kidneys of F0 and F1 males were significantly higher than the control values. These increases were considered treatment-related. In F1 males in the high-dose group, there was also a significant decrease noted in the absolute and relative weights of the prostate. Besides these changes, the relative brain weight of F0 males in the 30000 ppm group was significantly higher than the control value. However, this finding was considered to be the change associated with the low body weights in this group.

F0 and F1 females

In F0 females, the absolute and relative weights of all organs were comparable between the control and treated groups. In F1 females in the 30000 ppm group, the absolute and relative weights of the liver and kidneys were significantly higher than the controls, and these increases were considered treatment-related. Significantly higher-than-control value was also observed in the absolute kidney weight in the 6000 ppm group. However, this increase was not considered treatment-related because statistical significance in the difference between the control and 6000 ppm groups disappeared when all F1 females were subjected to the weighing of the kidneys fixed in 10% neutral buffered formalin. The significant lower relative ovarian weight observed in F1 females in the 1200 ppm group was considered to be an incidental finding because no such decrease was observed in the mid- and high-dose groups.

Histopathology

F0 and F1 generations

In all F0 and F1 males and females in the 30000 ppm group, histopathological examinations of the reproductive organs and pituitaries did not indicate any treatment-related alterations.

No treatment-related histopathological alterations were also evident in the following organs in which significant weight changes were detected: kidneys of F1 females in the 6000 ppm group; kidneys of F0 males and F1 males and females in the 30000 ppm group; and liver of F1 males and females in the 30000 ppm group.

III. CONCLUSION

The oral administration of HR-001 to rats by dietary admixture at a maximum dose level of 30000 ppm for two successive generations of Sprague-Dawley rat resulted in maternal toxicity at 30000 ppm. Thus, the NOAEL for maternal toxicity is 6000 ppm, equivalent to 417-458 mg/kg bw/day and 485-530 mg/kg bw/day for males and females, respectively.
The NOAEL for reproduction is 30000 ppm, since the reproductive performance was not affected in any dose group. Based on the body weight effects and increased incidences of caecum distension the NOAEL for offspring is considered to be 6000 ppm.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/04	[redacted]	1993	Two Generation Reproduction Study in Wistar Rats. [redacted] Data owner: Feinchemie Schwebda GmbH Study No.: [redacted]885-[redacted] Date: 1993-08-27 GLP: yes not published

Guideline: OECD 416 (1983)

Deviations: None

Dates of experimental work: May 1991 - April 1992 (not further specified)

Executive Summary

To evaluate the potential effects of glyphosate technical on reproduction groups of 30 Wistar rats per sex were fed diets containing test substance concentrations of 0, 100, 1000, and 10000 ppm (equivalent to 0, 7.7, 77 and 770 mg/kg bw/day) for two consecutive generations up to weaning of third (F2) generation.

First parental (F0) animals were treated for at least 8 weeks before mating. The first pairing produced the F1 litter from which the second parental generation was selected. All F2 litters were sacrificed at weaning. All groups were observed for clinical signs, body weight development, food consumption, mating behaviour, vaginal smear and pups observation (number, sex, survival and body weight). During gestation and lactation periods the dams were observed for body weight and food consumption. The data were statistically analysed. From all groups of parental (F0 and F1) rats, major visceral organs and reproductive organs were subjected to detailed gross necropsy. Lesions if any found in reproductive organs/tissues in all groups were subjected to histopathological examination. All pups were subjected to detailed gross pathology.

Dietary administration of glyphosate at up to 10000 ppm for two successive generations in Wistar rats showed no treatment and dose related significant and consistent changes in the incidence of clinical signs, mortality rate, body weight change and food consumption during treatment, gestation and lactation periods. No treatment and dose related consistent effects on number of pups born (combined and individual sex) and their growth were observed; however the total litter weight and female pup weight tended to be higher in treatment groups occasionally.

The reproductive performance parameters like (a) number of dams littered (b) number of dead pups at first observation (c) mean litter size; pup survival parameters like live birth index and survival index for 24 hours, day 4, 7, 14 and 21 and fertility index parameters for sires and dams have though shown some incidental significant changes compared to the control group; however, these changes were not consistent

in both the generations and there was no relationship with treatment or the dose of the test compound in the diet.

In the two generation reproduction study conducted on glyphosate at dietary dose levels of 0, 100, 1000 and 10000 ppm no major effects on general health, growth of parents, gestation/lactation period, body weight and food consumption, gross necropsy findings of pups and parents were observed. The test compound did not cause any treatment or dose related consistent changes in parental mortality, parturition performance, mean litter size, pup weight and male and female fertility index. The NOAEL for maternal toxicity was therefore considered to be 10000 ppm. The NOAEL for offspring was found to be 10000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Chemical name	N-(Phosphonomethyl) glycine
Description:	Odourless, white crystal
Batch #:	60
Purity:	96.8%
Date of receipt	11/9/1990
Stability of test compound:	More than two years at ambient temperature

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Wistar rats (Random bred)
Source:	[redacted] India
Age at start of treatment (F0):	6 weeks
Sex:	Males and females
Mean body weight at initiation of dosing:	Males: 160 - 190 g; females: 141 - 160 g
Acclimation period:	7 days
Diet/Food:	Standard [redacted] brand powdered rat feed manufactured by [redacted] India
Water:	Deep bore well water passed through activated charcoal filter and exposed to UV rays ([redacted] on-line water filter cum-purifier manufactured by [redacted], India) was provided in glass bottles *ad libitum*
Housing:	Groups of five/three rats of same sex per cage depending on the size of the animals were accommodated in standard polypropylene rat cages (size: L 430 x W 270x H 150 mm) with stainless steel top grill; bedding material (paddy husk) was changed three times per week.

Environmental conditions: Temperature: 22 ± 3°C
Humidity: 40-70%
Air changes: 10-15/hour
12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: May 1991 to April 1992 (not further specified)

Animal assignment and treatment:
In a two-generation reproduction study groups of 30 Wistar rats per sex of the F0 generation received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical in diet.
After at least 8 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation (vaginal smear) was designated Day 0 of gestation. On Day 4 *post partum*, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were sacrificed. Groups of 30 male and 30 female offspring from each dose group of the F0 generation were selected to form the F1 parents.
The offspring selected for the F1 generation were paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals.

Diet preparation and analyses
The required quantities of test compound were weighed and mixed manually with 1.0 kg of powdered rat feed to prepare the premix. The premixes were added to the bulk of remaining quantities of feed and mixed in ribbon mixer. Prepared feed bulks were sampled at different intervals for assaying test compound concentration in experimental diet.

Clinical observations
All animals were observed daily throughout the study and any visible clinical signs were recorded with details on type, severity, time of onset and duration. Any animal found dead or sacrificed *in extremis* was necropsied and macroscopically abnormal tissues were retained.

Body weight
Males were weighed weekly until termination. Females were weighed weekly during pre-mating, on Gestational Days 0, 6, 13, and 20 and on Days 1, 4, 7, 14 and 21 of lactation.
Offspring were weighed sex-wise as litters on Days 1, 4, 7, 14 and 21 *post partum*.

Food consumption and compound intake
Food consumption for each cage of males was recorded weekly until termination. Food consumption of females was recorded weekly during pre-mating and at the following intervals: Days 0-6, 6-13, 13-20 of gestation and Days 1-4, 4-7, 7-14 and 14-21 of lactation.

Reproduction parameters
Reproductive performance
The following reproductive indices were recorded: Male and female fertility index, fecundity index, mean number of implantations, parturition percentage, percentage mortality of pregnant dams, percentage of live pups born, in females the pre-coital interval (time elapsed between initial pairing and detection of mating) and duration of gestation.

Litter data
Total number of live and dead pups, viability indices (mean viable litter size on day 0, live birth index), litter weight, individual sex and observations on individual pups (if any) were determined within 24 hours after birth. Survival indices were determined on Days 2, 4, 7, 14 and 21 of lactation. Body weights were

determined on Lactation Days 0, 4, 7, 14 and 21. A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. On Day 4 *post partum*, offsprings were culled to reduce litter size to eight.

Sacrifice and pathology
All surviving parental F0 and F1 males and females and the non-selected weanlings from F1 and all F2 weanlings were sacrificed and subjected to a gross pathological examination. Tissue collection was done for parent generation only. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible.
The following organs and tissues were preserved from all F0 and F1 parents of all groups: Ovaries, uterus, vagina, testes, epididymides, seminal vesicles, prostate, coagulation glands, pituitary, adrenals, liver and kidneys. The organs were examined for gross pathological changes and those found abnormal were examined histopathologically.
Females failing to get mated within 21 days and females failing to produce a viable litter by Day 25 *post coitum* were necropsied and any macroscopically abnormal tissue was retained for histopathological examination. The presence of corpora lutea, implantations and resorptions was examined in females which had failed to produce a viable litter.
On Day 4 *post partum*, offsprings were culled to reduce litter size to eight, where possible; culled offspring or found dead were necropsied. All F2 pups were sacrificed at weaning.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Dunnett´s t-test (for body weight, food consumption, litter number, litter weight, gestation and lactation period), Z Test (for mating performance, fertility index, gestation index, live birth index, viability index, lactation index, pups survival data, number of dead pups at birth, survival indices, number littered) and t/r test (for dose-response relationship).

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
In-house stability study for glyphosate technical was carried out at 0, 2000 and 20000 ppm. Chemical stability was given for 30 days at room temperature with a loss of less than 7% at 0, 2000 and 20000 ppm levels in experimental diet when stored in polyethylene lined stainless steel drums.
The mean achieved concentrations of glyphosate in the diet preparations were analysed; the achieved concentrations were in the range of 96-100% of the nominal and therefore acceptable.

B. MORTALITY
F0 and F1 males
There were no deaths in male animals.

F0 females
In the females there were three deaths, two in the low dose group, (one dystokia and one suppurative pneumonia) and one in the high dose group (cause of death not ascertained).

F1 females
One dam in low dose group died of dystokia and no other mortalities were seen.

C. CLINICAL OBSERVATIONS
F0 generation
Nasal discharge and snuffling and cannibalism were seen in all groups. No other treatment related changes in clinical signs were observed.

F1 generation
The incidence of clinical signs was low and not treatment or dose related.

D. BODY WEIGHT

F0 males

Initial body weight of treatment groups was higher compared to the control group and this trend continued during the entire treatment period. The absolute weight gain (difference between initial and terminal) during entire treatment period was similar to control group in low and high dose while in mid dose it was slightly higher.

F0 females

No significant treatment related differences were noted between treated and control groups.

F1 males

Mid dose group body weight (both initial and subsequent weeks) was more than control. In high dose group initial body weight (Week 0) was higher than control but at Week 2 and 3 it was less. However in this group the body weight tended to be higher (not significant) during last seven weeks.

F1 females

The body weight of all treatment groups at selection (Week 0) was higher than in the control group and continued to be significantly higher than in the control group for up to Week 10 in mid and high dose groups. Body weights of the high dose group dams on Days 0, 6 and 13 of gestation period were significantly higher compared to controls but the body weight gain was statistically not significantly different. Another incidental significant finding was higher body weight (Gestational Day 0-20) of mid dose group dams compared to controls. Absolute body weight of mid dose group on Lactation Days 1 and 4 and that of high dose group during all periods of lactation was significantly higher than in control group. The mid dose group had lost body weight during Days 7-14, 14-21 and 1-21 of lactation period as compared to control.

E. FOOD CONSUMPTION AND TEST COMPOUND INTAKE

F0 parents

Mean food consumption of males was comparable to the controls throughout the study. High dose female animals tended to consume significantly more food than controls during gestation. During lactation low and mid-dose females consumed significantly less than controls, especially for the Periods 7-14 and 14-21. High dose females consumed significantly more food for Lactation Days 4-7 as compared to controls.

F1 males

Treatment groups did not show consistent and dose related changes as compared to control group. However initially (Weeks 0-2) mid and high dose groups consumed significantly less feed and later on a few occasions mid dose group showed increased consumption.

F1 females

Treatment group dams did not show treatment and dose related consistent difference from control group; on a few occasions the treatment groups showed both increased/decreased food consumption over control. During gestation there was no statistically significant inter group difference in feed consumption between control and treatments during gestation period. Low dose dams consumed significantly less food than controls during different lactation periods (except for Day 7 and Period 7-14). Mid and high dose group dams did not show any treatment and dose related changes over control except for an incidental finding of increased and decreased feed consumption on Day 7, 14 and Period 7-14 and 14-21 respectively in mid dose group.

F. REPRODUCTIVE PARAMETERS

Reproductive performance parameters of F0 parental animals such as female fertility index, number of implantations, gestation index, duration of gestation, live birth index, and duration of gestation were not significantly different between treated and control groups. Male fertility index was significantly higher in low and high dose groups over control.

F0 generation
On Day 1 of lactation, mean litter size was significantly less than control in low and mid dose groups and the mean viable litter size at birth was significantly less in low dose group; the number of live pups on Day 1 was significantly lower in the mid-dose group.

F1 generation
Reproductive performance parameters of F1 parental animals such as male and female fertility index, fecundity index, parturition percentage and mortality of pregnant dams was not different between treatment and control groups. The incidence of dams not littered tended to be higher in the mid-dose group compared to controls. A significantly decreased number of implantations was observed in low and mid dose groups; the percentage of live pups born was significantly reduced in the in mid dose group and significantly increased in the high dose group.

Table 5.6-12: Reproductive parameters of F0- and F1-generation

	Group 1 - control 0 ppm		Group 2 100 ppm		Group 3 1000 ppm		Group 4 10000 ppm	
	F0	F1	F0	F1	F0	F1	F0	F1
Number of dams in group	30	30	30	30	30	30	30	30
Number of dams littered	29	26	26	27	28	22	30	28
Mean litter size	11.3	11.7	9.8*	10.4	9.9*	10.9	10.4	11.9
Mean viable litter size at birth	11.0	11.7	9.7*	10.4	9.9	10.9	9.9	11.9
Number of pups alive on day 1	320	305	253	281	276*	239	296	334
Mean number of implantations	12.1	13.4	11.2	11.6*	11.0	12.0*	12.3	12.9
Percentage of live pups born [%]	87.9	87.6	83.5	86.5	86.5	79.7*	80.0*	92.8**

*significantly decreased; **significantly increased

G. LITTER DATA

Number of pups delivered
Mean number of F1 and F2 pups delivered and mean litter sizes in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Sex ratio
Sex ratios of F1 and F2 pups in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Viability index
F1 pups
In the low dose group the pup survival index for Days 4, 14 and 21 was significantly lower than in controls. In the mid dose group the live birth index and Day 14 survival index were higher and Day 4 survival index was lower compared to controls. In the high dose group on Day 14 and 21 survival index was higher than in controls. Dose response relationship was not seen in these parameters.

F2 pups
There were no statistically significant inter group differences between control and treatment groups in parameters of F2 litters at first observation including incidence of external abnormalities in pups. The mean number of pups (combined and individual sex) during different periods of lactation did not show statistically significant differences compared to control group.

The group mean values of pup survival data parameters like: live birth index, 24 hours survival index and survival index for Days 4, 7, 14 and 21 did not show any significant inter group difference between control and treatment groups.

Body weights

F1 pups

Mean litter weight of combined sex and female pups in treatment groups were significantly more than control group on Day 1 and 4, respectively. On Day 7 combined sex litter weight and male pup weight was significantly less than control in low dose group while in high dose group it was more than control group. On Day 21 the mean body weight of complete litter and individual sex pups of mid dose group were more than control group. None of these showed any apparent dose response relationship.

F2 pups

Combined sex litter weight on day one and that of female pups of all treatment groups was higher than in controls; in addition combined sex litter weight in low and mid dose groups and that of male and female in mid dose group was higher than control on Day 4. In high dose group the male pup body weight on Day 14 and 21 was lower than control. None of these parameters showed any dose response relations.

Clinical signs

There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.

During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

I. PATHOLOGY

Necropsy

F0 generation

The gross pathological lesions seen were consolidated lungs with ecchymoses, chronic liver changes, kidneys with cysts and dilated pelvis, and hypoplastic testes (1 in the control group, 2 in the mid-dose and 1 in the high-dose group). The incidence was low and did not appear to be compound or dose related.

F1 generation

The gross pathological lesions seen were consolidated and collapsed lungs with emphysema, hydronephrotic kidneys, and unilateral hypoplastic testes. The lesions observed were few and appeared to be incidentally. A single incidence of unilateral testicular hypoplasia was observed in each of the three treatment groups, hydronephrosis was seen in two animals in the high dose group.

F1 pups

A higher incidence of emaciated pups was recorded for the mid and high dose groups compared to controls. A low incidence of minor developmental abnormalities like Kinky tail, rudimentary tail, kidney hydro-nephrosis and dilated pelvis occurred without dose-response relation.

F2 pups

A higher incidence of emaciation has been observed in pups of high dose group. Occasional not treatment and dose related incidence of hydronephrosis and dilated pelvis in kidney have been recorded.

Histopathology

F0 generation

Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one control animal, two mid dose and one high dose animal. The control and high dose animals showed degenerative changes in the seminiferous tubules while the mid dose group were normal. These changes appeared to be incidental and not compound related.

F1 generation
Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one animal in each of the three treatment groups; the testes in the low and mid dose groups showed unilateral degenerative changes and giant cell formation in the seminiferous tubules and focal chronic inflammation. The testes in the high dose were normal though unequal in size. The changes appeared to be incidental and not compound related.

III. CONCLUSION

The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of Wistar-Dawley rats resulted in no maternal toxicity. The NOAEL for reproduction is considered to be 10000 ppm, since the reproductive performance was not affected in a dose-related manner. The NOAEL for offspring is 10000 ppm, since no treatment-related effects on offspring could be observed.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/05	[redacted]	1981	A three generation reproduction study in rats with glyphosate [redacted] Data owner: Monsanto Study No.: 77-2063; [redacted]77-417 Date: 1981-09-31 GLP: no pre-GLP Not published
Guideline:			None (pre-guideline)
Deviations:			Not applicable
Dates of experimental work:			1978-06-13 to 1980-04-09

Executive Summary
Rats were administered glyphosate oral via diet continuously for three successive generations. Each generation (F0, F1 and F2) consisted of 12 male and 24 female [redacted] CD® rats. Dietary concentrations were adjusted weekly during growth, and between mating rest periods, to achieve dose levels of 3, 10, and 30 mg/kg bw/day. A concurrent control group (plain diet) was included in the study.
Each parent generation was mated to produce two litters. Offspring from the second litters of the F0 and F1 parents (F1b and F2b litters, respectively) were selected to be parents for subsequent generations. Offspring not included in the selection procedure and offspring from the first litter intervals of each generation (F1a, F2a, and F3a) were sacrificed at weaning and given a gross post-mortem examination. Pathological and histopathological examinations were conducted from control and high-dose parent generations (F0, F1 and F2) and from control and high dose F3b offspring at weaning (10/sex/group).
Parameters evaluated for each generation included: mortality, body weight, food consumption, clinical observation, maternal body weights (gestation/lactation), reproduction-fertility indices (mating, pregnancy and fertility indices), litter data at parturition and organ weight data. Offspring from each litter interval were evaluated during a 21-day lactation period for growth, survival, sex distribution data and gross post-mortem observations including organ weight data (F3b offspring only).

No treatment-related effect was evident in adult mortality data, body weight and food consumption data (growth and rest periods), and clinical observation data throughout the study (F0, F1 and F2 generation). Considerable variability was seen in the mating-fertility indices during this study in both, the control and treated groups, in particular during the F1 and F2 generations. In the control group, mating indices were

low for both mating intervals of the F1 generation and in the F2 generation these indices were higher than normally encountered. Similar fluctuations were seen in mating indices for some of the treated groups during these same intervals. Throughout the study no consistent effect was seen in mating indices, fertility indices (male) or pregnancy rate data to suggest an adverse effect of treatment.

No adverse effects of treatment were evident in maternal weight data, gestation length, parturition data (number of live/dead pups at birth) or litter survival indices throughout the study. Concerning the offspring, no treatment-related effects were indicated in sex distribution data, body weights, survival or gross post-mortem findings. Likewise, no effect of treatment was evident in mean organ weight data (absolute and relative to body or brain weight) for randomly selected Day 21 F3b offspring.
No adverse effect of treatment was evident in organ weight data for the F0, F1 generations and F2 adult males. In the F2 treated females, a non-dose related, albeit statistically significant, decrease in mean liver/body weight ratio was evident; liver/brain weight ratios for these females showed a similar reduction; however, these differences from control were not statistically significant.
Gross post-mortem evaluations of the adult generations and histological evaluations of tissues from randomly selected F0, F1 and F2 high-dose animals and F3b high-dose offspring did not indicate a treatment-related effect.

Conclusion:
Oral dietary administration of 0, 3, 10 and 30 mg/kg bw/day glyphosate to CD-rats for three successive generations resulted in no treatment-related signs of toxicity in parental rats. Therefore, the NOAEL for parental toxicity is considered to be 30 mg/kg bw/day a for males and females, respectively.
The NOAEL for reproduction and the NOAEL for offspring was found to be 30 mg/kg bw/day, since the reproductive performance was not affected in any dose group and no adverse effects on offspring were observed.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	Fine white powder
Lot #:	XHJ-64
Purity:	considered 100% active ingredient for dosing preparations;
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	CD® (Sprague-Dawley derived)
Source:	[redacted]
Age at treatment initiation:	43 days
Sex:	Males and females
Mean weight at initiation of dosing:	Males: 139.9 - 144.3 g; females: 118.0 - 119.2 g
Acclimation period:	7 days
Diet/Food:	Standard laboratory diet ([redacted]), *ad libitum*

Water:	Automated watering system ([redacted]), *ad libitum*
Housing:	Individually (except during mating and lactation), in elevated stainless steel wire mesh cages; nesting material: [redacted]® hardwood shavings added to cages on Day 19 of gestation and changed when wet or soiled through Day 14 of lactation
Environmental conditions:	12 hours light/dark cycle No details on temperature and humidity reported

B: STUDY DESIGN AND METHODS

In life dates: 1978-06-14 - 1980-04-09

Study design

Table 5.6-13: Study design

Group	Dose level (mg/kg bw/day)	No. of adults initially assigned to mate F0, F1, F2		No. of matings per generation F0, F1, F2	Gross post-mortem examination	Histopathology of F0, F1 and F2 parents, F3b weanlings	
		Males	Females			Male	Female
1 Control (plain diet)	0	12	24	2	All	10	10
2	3	12	24	2		none	none
3	10	12	24	2		none	none
4	30	12	24	2		10	10

Animal assignment and treatment:
In a three generation reproduction study groups of 12 male and 24 female CD rats received beginning 63 days prior to mating of the F0 generation daily dietary doses of 0, 3, 10 and 30 mg glyphosate/kg bw in diet. Diet samples were taken at four week intervals for analysis of achieved test substance concentrations.

Mating: One male and two females of equivalent dose levels were caged together nightly until a sign of mating (sperm and/or copulation plug in the vaginal was observed or until 15 days had elapsed with no evidence of mating. The day on which evidence of mating was observed was defined as Day 0 of gestation.

In this study, the first litters (F1a, F2a and F3a) from each mating were raised to weaning and discarded. Rats produced by the second matings (F1b and F2b) were selected to become parents of succeeding generations or to be subjected to complete gross necropsy (F3b).

Diet preparation and analyses
Diets were prepared weekly during the study and were adjusted on the basis of body weight and food consumption.

Clinical observations
A check for clinical signs of toxicity and mortality was made twice daily. A detailed physical examination was performed on adult generations at weekly intervals throughout the study.

Body weight
Body weights of all animals were determined weekly during growth and rest periods of all generations. Pregnant females were weighed on Days 0, 6, 15 and 20 of gestation and lactating females were weighed on Days 0, 4, 14 and 21 of lactation.

Food consumption and compound intake
Food consumption was recorded weekly during growth and rest periods of all generations. Test substance intake was calculated from individual body weight and food consumption data and reported as a group mean value for weekly intervals during the growth and rest periods of all generations.

Reproduction parameters
The day on which evidence of mating was observed was designated as Day 0 of gestation; the day of delivery was designated as Day 0 of lactation.
Mating indices, pregnancy rates, length of gestation and male fertility indices were recorded.

Litter data
Pups of all generations were examined daily for general appearance and mortality. On Days 0, 4, 14, and 21 they were counted to record the number of live and dead pups. Body weights were determined on Days 0, 4, 14, and 21 as a litter and on Day 21 individually.
Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group on Days 0 and 21 of lactation. Viability indices, were determined for each litter on Lactation Days 0, 4 and 21.

Sacrifice and pathology
Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. All adult males and females were sacrificed after pup selection of the last Fb litter (F0, F1) and after last F3b litter weaned (F2) by lethal exposure to ether. Pups that were found dead or stillborn pups were weighed and given a gross post-mortem examination including internal sex determination, presence of milk in stomach. F1a, F2a, F3a and F3b animals were sacrificed at weaning, given a gross post-mortem examination and abnormal tissues were saved. F1b and F2b animals which were not selected as future parents were sacrificed after ensuing selection of parental animals, given a gross post-mortem examination and abnormal tissues were saved.

The following organs and tissues were preserved from all parents (F0, F1, F2) and from 10/sex/group of the F3b weanlings: adrenals, aorta, bone and bone marrow (sternal), brain, colon, duodenum, eyes with optic nerve and Harderian gland, gonads (ovaries and testes), heart, ileum, kidney (2), liver (2 sections), lung with main stem bronchi, lymph nodes (mesenteric), mammary gland (right inguinal), pancreas, pituitary, salivary gland, skeletal muscle (biceps femoris with right sciatic nerve), skin, spinal cord, spleen, stomach, thyroid/parathyroid, urinary bladder, uterus/prostate, gross lesions, tissue masses, thymus.
Microscopic examination of histological sections of these tissues were done for 10 male and 10 female animals from control and high-dose groups of F0, F1 and F2 parents and of F3b offsprings.

The following organs were weighed from all parents sacrificed after weaning of the second litters and from eighty F3b weanlings (10 males and 10 females per group): adrenals, gonads, kidneys, brain, spleen, liver, heart and pituitary.

All pups of the second litter of the F2 parents (F3b) were necropsied at weaning and specified tissues were preserved for selected animals in each group.

Statistics
Body weights, body weight gain, maternal body weights, food consumption, number of offspring, offspring body weights, terminal body weights and organ weight data (absolute and relative), offspring survival, litter survival, pup viability index at birth, mating indices, pregnancy rates and male fertility indices data were compared to the control. Statistically significant differences were evaluated using several methods including Dunnett's test, ANOVA, Barlett's test, Kruskal-Wallis test and Fisher Exact Test.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Not reported.

B. MORTALITY
F0 adults (2 dead females in mid-dose group)
In the F0 generation, no unscheduled mortality occurred in the control, low- or high-dose groups. One female of the mid-dose group died during on Lactation Day 20 of first litter having 13 live pups at time of death. A second female of the mid-dose group died on Lactation Day 7 of second litter; this female delivered eight pups - seven live and one dead - and all pups were dead at time of death. No mid-dose F0 male died.

F1 adults (1 dead female in mid-dose group, 1 dead female in high-dose group)
In the F1 generation, no unscheduled mortality occurred in the control or low-dose groups. In the mid dose group one female was killed in a moribund condition during the post-mating period for the second litter. This female had mated during the first mating but did not deliver a litter; during the second mating this female had not mated. No other mortality occurred in the mid dose group. In the high dose group one female died due to an accident (animal was caught in the feeder jar). A second high-dose female died on Day 21 of gestation for the second litter; the uterus of this female contained 15 term foetuses. No other mortality occurred in the high-dose group.

F2 adults (1 dead female in low-dose group, 1 dead male in mid-dose group)
In the F2 generation, no unscheduled mortality occurred in the control or high-dose groups. In the low-dose group one female died during the F3a lactation period. This female delivered a litter containing only dead pups (13 pups) and died the day after parturition. No other mortality occurred in the low-dose group. In the mid-dose group one male was killed in a moribund condition during the period between mating of the first and second litters. This male had mated and impregnated both females during the first mating period. No other mortality occurred in the mid dose group.

C. CLINICAL OBSERVATIONS
Clinical observation data were similar between the control and treated groups for each generation interval throughout the study. No adverse treatment effects were indicated.

D. BODY WEIGHT
Mean body weight data during the growth and rest periods were comparable between the control and treated groups for each generation, throughout the study. Likewise, mean weight gain during the growth periods were comparable between these same groups for both sexes throughout all generations. No treatment effect on body weight data during the growth and rest periods was evident.

E. FOOD CONSUMPTION AND TEST COMPOUND INTAKE
Mean food consumption data were considered comparable between the control and treated groups (both sexes) during the growth and rest periods for each generation, throughout the study. No adverse effect of treatment on food consumption was evident throughout the study. Mean weekly test substance intake values ranged from 2.8 to 3.3 mg/kg bw/day for the low-dose group, from 9.5 to 11.2 mg/kg bw/day for the mid-dose group and from 27.7 to 33.1 mg/kg bw/day for the high dose group for all generations including both genders.

F. REPRODUCTIVE PARAMETERS
Male and female mating indices and male fertility indices during both mating intervals of the F0 generation were considered comparable between the control and treated groups. During the second mating interval of the F0, pregnancy rates were lower than control in each of the treated groups; however, no indication of a dose-relationship was evident as the lowest pregnancy rate was seen in the mid-dose group. This reduction in pregnancy rate for the mid-dose group was not statistically significant. In the absence of

a dose-response relationship the reduction in pregnancy rate during this mating interval (F1b) in the treated groups was not considered treatment-related.

In the F1 generation, mating indices (males and females) for both litter intervals were comparable between the control and treated groups. It is note-worthy that for both mating intervals of this generation, mating indices for control and some treated groups were lower than normally encountered in multi-generation studies. The reason for the poorer mating performance in this generation was unclear but no treatment effect was indicated since mating indices were lowest in the control group. Pregnancy and male fertility indices for the first mating interval of the F1 were comparable between the control and treated groups. During the second litter interval, pregnancy rates were lower than those seen for the first interval in control and treated groups. The lowest pregnancy rate was seen in the high-dose group; however, this difference from the control value was not statistically significant. Pregnancy rates for the low- and mid-dose groups, during the second mating interval, were considered comparable to control. Male fertility indices for this same mating interval were considered comparable between the control and treated groups.

In the F2 generation mating indices for the treated groups were lower than control for each mating interval. During the first mating interval of the F2 generation, the female mating indices were lower than control in each of the treated groups; however, only in the high-dose group was this difference from control statistically significant. The female mating index for the control group at this interval was 100% which is higher than normally encountered. The female mating indices observed for the control group in this study have shown considerable variability ranging from 70.9 to 100%. The poor mating performance for the treated groups during the first mating interval is attributed to two males in each treatment group that did not mate either female in their mating unit (each mating unit was comprised of one male and two females).

During the second mating interval of the F2 generation, male mating performance improved in the mid- and high-dose groups as both mid-dose males and one of two high-dose males that did not mate during the first mating interval, mated and impregnated at least one female. Male mating indices for the low-dose group remained unchanged as the same two males that did not mate during the first interval, failed to mate during the second interval. Pregnancy and fertility indices for the treated groups were comparable to control for both litter intervals of the F2 generation.

Mean gestation length was comparable between the control and treated groups for each pregnancy interval in each generation. Over the entire study, no consistent dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment.

G. LITTER DATA

Litter size

Mean litter size data on Day 21 of lactation (weaning) was comparable between the control and treated groups for each litter interval throughout the study.

Sex ratio

Pup sex distributions ratios at Day 0 and 21 were generally comparable between the control and treated groups for each litter interval for each generation. No adverse treatment effect on sex distribution data was evident.

Viability index

The mean numbers of live, dead and total pups at birth and pup viability at birth for each pregnancy interval, were comparable between the control and treated groups for each generation. The litter survival indices were comparable between the control and treated groups for each lactation interval in the F0, F1 and F2 generation. In the F0 generation, postnatal survival indices for Days 0-4 and 4-21 were comparable between the control and treated groups for the first lactation interval (F1a). For the second litter interval of the F0, postnatal survival indices for the Day 0-4 interval were comparable between the control and treated groups. During the Day 4-21 interval, survival indices were significantly lower than control in each treatment group. The increase in pup mortality during this interval (i.e. Days 4-21) was attributed to high

pup mortality within one or more litters at each treatment level. In the low-dose group the lower pup survival was attributed to one female that experienced complete litter mortality (litter contained 14 live pups at Day 4). In the mid-dose group, one female died on Day 7 of lactation and all seven pups in her litter died during the Day 4-7 lactation interval. Additionally, three mid-dose litters lost five or more pups from their litters during the Day 4-21 lactation interval. In the high-dose group, one female lost nine of l2 pups during the Day 4-21 lactation interval.
In the F1 and F2 generations postnatal survival indices for Days 0-4 and 4-21 during both litter intervals were considered comparable between the control and treated groups. Some statistically significant differences in these indices were observed between the control and treated groups; however, no trend was evident through successive generations to indicate an adverse effect of treatment.

Body weights
Maternal body weights
Mean body weight data during the gestation and lactation intervals and mean weight change during these same periods were comparable between the control and treated group for each pregnancy interval from each generation throughout the study. No treatment effect was indicated in gestation / lactation body weight data throughout the study.

Offspring body weights
Mean pup body weight data during each litter interval for each generation were comparable between the control and treated groups. No adverse effects of treatment on pup weight data was evident.

Adult animals (F0, F1 and F2)
Mean terminal body weight data were comparable between the control and treated groups for both males and females throughout the study.

I. PATHOLOGY

Necropsy
F0, F1 and F2 generations
Gross necropsy of parental animals of both sexes did not indicate any adverse effect of treatment.

F1, F2 and F3 offspring
Gross post-mortem observations of offspring at weaning (F1a, F2a, F3a, F3b) or post-weaning (F1b, F2b) did not demonstrate an adverse effect of treatment. Likewise, evaluation of dead pups recovered at birth and during the 21-day lactation period did not note a treatment-related effect.

Organ weights
F0, F1 and F2 generations
Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females from the F0 and F1 generations. Some statistically significant differences were noted between control and treated groups both in mean organ weight data and in the relative weight data; however, no trends were evident within dose levels or through these generations.
In the F2 generation, mean organ weight data (absolute and relative) for the males were comparable between the control and treated groups. In the F2 female group, mean liver/body weight ratios were significantly lower than control in each of the treated groups; however, no clear dose-relationship was apparent. Mean liver/brain weight ratios for the treated F2 females were lower than control; however, these differences from control values were not statistically significant. Mean spleen weights (absolute and relative to brain and body weights) were significantly higher than the control value in the F2 mid-dose female group; however, mean spleen weight data for the low- and high-dose F2 females were comparable to control values. In the absence of an effect on spleen weight in the high-dose Fg female group, the change seen in spleen weight data for the mid-dose females was considered spurious and not biologically meaningful. Other mean organ weight data (absolute and relative to body weight or brain weight) for the treated F2 female groups were considered comparable to control data.

F3b offspring
Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females. No treatment-related effect was evident in organ weight data for the F3b offspring.

Histopathology
In total 160 male and female rats (40 adults of each generation F0, F1 and F2 and 40 weanlings of F3b) were examined microscopically. No microscopic findings were considered treatment related. Proliferative tissue changes diagnosed as neoplasms were few. The microscopic tissue alterations, neoplastic and non-neoplastic, were indicative of common incidental histological findings.

III. CONCLUSION

The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 30 mg/kg bw/day for three successive generations of CD rats resulted in no treatment-related signs of toxicity in parental animals. The NOAEL for reproduction is 30 mg/kg bw/day, since the reproductive performance was not affected in any dose group. The NOAEL for offspring is 30 mg/kg bw/day, since no adverse effects on offspring were observed.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/06	[redacted]	1992	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat. [redacted] Data owner: Cheminova Project no.: [redacted]47/911129 Date: 1992-05-14 GLP: yes not published
Guideline:			OECD 416 (1983), US-EPA FIFRA 83-4 (1982)
Deviations:			None
Dates of experimental work:			1990-03-29 - 1991-03-22

Executive Summary
Glyphosate technical was administered by dietary admixture to three groups of 28 male and female F0 generation Sprague-Dawley rats each at dietary concentrations of 1000, 3000 and 10000 ppm (equivalent to a mean achieved dosages of 66.4 – 76.1, 196.8 – 230.2 and 668.1 – 771.3 mg/kg bw/day for males and 75.3 – 82.1, 226 – 244.9 and 752.3 – 841.1 mg/kg bw/day for females, respectively). An additional group of 28 male and 28 female F0 animals was exposed to basal laboratory diet to serve as a control.
Each parent generation was mated to produce two litters. Offspring from the first litters of the F0 parents (F1A litters) were selected to be parents for the F1 generation (24/sex/group). Offspring not included in the selection procedure and offspring from the second litter of each generation (F1B and F2B) were sacrificed at Day 21 *post partum* and given a gross post-mortem examination. Parent animals were sacrificed shortly after termination of the second litter.
Clinical signs, bodyweight development, food and water consumption, mating performance, pregnancy rate, and length of gestation of adults were monitored during the study. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days *post partum*. All animals at termination were subjected to a gross necropsy examination and weighing of selected organs and histopathological evaluation of selected tissues for the F0 and F1 adults was performed.

Treatment at 10000 ppm produced marginal signs of toxicity in parent animals and minimal histological changes in the target organ (salivary gland). Histological changes in the salivary gland were also noted at 3000 ppm. This finding is considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse. In the offspring no treatment-related effects were apparent at dietary administration up to 10000 ppm.

Conclusion:
Oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in minimal effects consisting of increased food and water consumption of F1 females, possibly reduced bodyweights of F1 males and minimal histological changes in the salivary glands in F0 and F1 adults at 10000 ppm. The only finding associated with treatment at 3000 ppm were minimal histopathological changes in the salivary glands of F0 and F1 adults. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and females, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White solid
Lot/Batch #:	206-Jak-119-1
Purity:	99.2%
Stability of test compound:	Stable during the treatment period

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) BR VAF/Plus
Source:	[redacted] UK
Age:	Approximately 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 143 – 201 g; females: 106 – 175 g
Acclimation period:	At least 15 days
Diet/Food:	[redacted] Diet No.2, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During pre-mating periods, animals were housed in groups of four in metal cages with wire mesh front, floor and top. During the first week of F1A and contingency animals of F2B animals were housed in plastic cages. During mating animals were housed on an 1:1 basis in plastic cages where females stayed after mating for breeding. Males were re-housed in former metal cages.
Environmental conditions:	Temperature: 23 ± 4°C Humidity: 45 ± 24% Air changes: not reported 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1990-03-14 to 1991-03-22

Animal assignment and treatment:
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate technical. The dose levels were chosen based on results of a previously conducted study. After at least 70 days of treatment pairing of animals within each dose group was undertaken on a 1:1 basis to produce the F1 litters. At Day 21 *post partum* of offspring from the F0 mating phase, groups of 24 male and 24 female offspring from each dose group were selected to form the F1A generation. The remaining pups were sacrificed. Approximately 10 days following the weaning of all F1A pups, F0 males and females were re-mated. At Day 21 *post partum* all F1B pups were sacrificed. F0 males and females were terminated shortly after weaning of F1B pups.
The selected F1A animals were dosed from approximately Week 4 of age for at least 84 days and then mated on a 1:1 basis (sibling pairings were avoided). On Day 4 *post partum* F2A litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 *post partum* all F2A pups were sacrificed. Approximately 10 days following the weaning of all F2A pups, F1 males and females were re-mated. On Day 4 *post partum* F2B litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 *post partum* all F2B pups were sacrificed. F1 males and females were terminated shortly after weaning of F2B pups.

Diet preparation and analyses
For the weekly preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 7 minutes in a rotary double-cone-blender.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration throughout the study.

Clinical observations
A check for clinical signs or ill health was made once daily and recorded daily for the first week of treatment and on a weekly basis thereafter. Rats showing marked signs of ill health or reaction to treatment were killed and subjected to necropsy.

Body weight
Individual body weights were recorded at the start of each generation (F0: Week 6 of age; F1A: Week 4 of age) and subsequent at weekly intervals. Females were weighed daily during mating and continued until parturition. Weights were reported for Days 0, 7, 14, 17 and 20 of pregnancy. Females with live litters were weighed on Days 0, 7, 14 and 21 *post partum*.

Food consumption and compound intake
Food consumption was recorded on a weekly basis from allocation throughout the first pre-mating phase of each generation. During this period food conversion ratios and achieved intake (mg/kg bw/day) were calculated.

Water consumption
Water intake was observed daily during the initial and final two weeks of the first pre-mating period for each generation and from allocation for the F0 generation.

Reproduction parameters
Vaginal smears were taken daily during the 20-day mating period to examine the oestrus cycle and median *pre-coital* time. Additionally, date of mating and duration of gestation was recorded.

Litter data
The number of offspring born and the number of offspring alive were recorded daily. Pups were weighed on Days 0 and 4 and all litters containing more than eight pups were culled to eight retaining, where possible, ideally 4 pups per sex. The remaining pups were also weighed on Days 8, 12, 16 and 21. Dead and culled young were subjected to necropsy.

Sacrifice and pathology
All adult animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

The following organs were weighed of adults: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostrate (with seminal vesicles and coagulating gland), testes (with epididymides), thymus.

The following tissues were preserved from all adults: adrenals, aorta, bone (femur and joint), bone marrow (sternum), brain, cranial vault (for lachrymal glands, teeth, nasal turbinates, inner ear), caecum, colon, duodenum, eyes, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (cervical/mesenteric), mammary gland, macroscopically abnormal tissues, oesophagus, ovaries, pancreas, pituitary*, prostate with seminal vesicles (with coagulating gland)*, rectum, salivary gland, sciatic nerve, skeletal muscle, skin, spinal column (vertebral column), spleen, stomach, testes (with epididymides)*, thymus, thyroids (with parathyroids), tongue, trachea (with larynx and pharynx), urinary bladder, uterus (with cervix)* and vagina*.

Histology of the reproductive tract was restricted to adults of the control and high-dose group and any apparently infertile animals at the lower dietary concentrations and confined to tissues marked with an asterisk (*).

Statistics
Two tailed significance tests were performed on adult parameters (water consumption, food consumption, bodyweight, organ weights) and litter data. Evaluation of other parameters were found not to be useful. Significances at 1% and 5% were reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Stability analyses indicated that the dose preparations at nominal concentrations of 500 and 30000 ppm were stable for up to 18 days during storage under animal room conditions.
Analyses for homogeneity at nominal concentrations of 500 and 30000 ppm indicated that the dose preparations were homogeneous.
Analyses for achieved concentration performed at 4-5 weekly intervals demonstrated that the prepared dietary admixture concentrations given to the animals were within ± 15% of the nominal concentration in all groups.

B. TEST COMPOUND INTAKE
The group mean intakes of glyphosate are summarised in Table 5.6-14 and Table 5.6-15 below.

Table 5.6-14: Group mean achieved intakes of glyphosate - F0 generation

Group	Dietary concentration (ppm)	Mean intakes (Week 1 - 10) (mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	66.4	75.3
Intermediate	3000	196.8	226.0
High	10000	668.1	752.3

Table 5.6-15: Group mean achieved intakes of glyphosate - F1 generation

Group	Dietary concentration (ppm)	Mean intakes (Week 5 - 16) (mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	76.1	82.1
Intermediate	3000	230.2	244.9
High	10000	771.3	841.1

C. MORTALITY

There were no test substance related mortalities.

Four unscheduled deaths occurred during each generation.
In the F0 generation one female of the low-dose group and one male of the high dose group were killed for humane reasons during Week 15 and 23, respectively. The female exhibited pilo-erection and thin appearance and the necropsy noted thickened forestomach, invaginated stomach and abnormal contents in the gastro-intestinal tract. The male was unable to use hind limbs, exhibiting aberrations of brain and spinal cord at necropsy. Another male of the high-dose group died during Week 3 with effects on pancreas and liver noted at necropsy. One control male was sacrificed during Week 16 following poor condition, however, the aetiology of the signs was not established.

In the F1 generation one female of the low-dose group was killed following a procedural error. In the mid-dose group one male died during Week 34 but autolytic changes precluded a valid necropsy. Moreover, one male and one female died and were sacrificed respectively, during Week 23. Necropsies failed to identify a specific cause of death.

D. CLINICAL OBSERVATIONS

No treatment-related clinical signs of toxicity were noted. General signs were observed in occasional animals from both generations and were not related to treatment.

E. BODY WEIGHT

No adverse effect of bodyweight change was evident for treated animals in comparison to controls for both generations.
However, absolute mean body weights in high-dose F1 males were slightly lower as compared to control. In addition it was noted that during the first mate of each generation, bodyweight gains during the initial stages of pregnancy tended to be slightly lower than controls at all dietary levels. Since no consistent dose-response was apparent these effects cannot conclusively be attributed to treatment.

F. FOOD AND WATER CONSUMPTION

Apart from a slightly higher but not statistically significant food consumption of high-dose F1 females during the second half of the pre-mating period, there were no marked intergroup differences in food consumption of males or females.
Apart from a slight increase among high-dose F1 females (attaining statistical significance in Week 16), no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations when compared to their concurrent controls.

G. REPRODUCTIVE PARAMETERS

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

H. LITTER DATA

Size and Viability

No overt differences in litter viability were detected.
In the high-dose group total litter size at birth was consistently, but not significantly, lower than controls across all four matings and remained lower than controls at Day 4 in three of the four matings. Since the

mean litter size at birth within each mating, was not always the lowest litter size recorded, this finding could not be clearly attributed to treatment.

Growth and Development
No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.

Clinical signs
No clinically observable signs of toxicity were observed for offspring from treated animals.

I. PATHOLOGY

Necropsy
There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, or offspring.

Organ weights
There were no overt or statistically significant treatment-related changes in any organ weights analysed in either generation.

Histopathology
No treatment-related changes in tissues associated with the reproductive tract were detected in the F0 or F1 generation animals.

Examination of two previously identified target organs, the parotid and submaxillary salivary glands, was initially performed only in the control and high dose groups. Due to effects seen in the parotid gland, examination was extended to the remaining treatment groups. For the submaxillary gland, examination was extended to only the F0 and F1 females in the low- and mid dose group. The findings are summarised in Table 5.6-16.

Table 5.6-16: Incidence of salivary gland findings

Observation	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
F0 Generation								
Animals examined	27	28	28	26	28	27	28	28
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	2	2	3	12	0	2	5	17
submaxillary	0	-	-	0	0	1	4	14
F1 Generation								
Animals examined	24	24	23	23	24	23	24	23
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	1	0	4	11	0	0	4	9
submaxillary	0	-	-	0	0	0	0	3

- = not examined

Treatment-related minimal changes were apparent in the parotid salivary gland of both F0 and F1 males and females in the mid- and high-dose groups and the submaxillary salivary gland of the F0 females in the mid- and high-dose groups and F1 females in the high-dose group. This finding is similar to those seen occasionally in other subchronic and long-term dietary studies and is considered to be an adaptive

response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse. There were no effects on the salivary glands noted in the low-dose group.

III. CONCLUSION

The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in minimal effects consisting of increased food and water consumption of F1 females, possibly reduced bodyweights of F1 males and minimal histological changes in the target organ (salivary glands) in F0 and F1 adults at 10000 ppm. The only findings associated with treatment at 3000 ppm were minimal histopathological changes of the salivary glands in F0 and F1 adults. No effects were apparent at 1000 ppm. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and females, respectively.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/07	[redacted]	1990	Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats [redacted] Data owner: Monsanto Report No.: [redacted]-1038[redacted] Project No.: [redacted]88-106[redacted]38038 Date: 1990-08-27 GLP: yes not published
Guideline:			Not stated but in general accordance with OECD 416 (1983)
Deviations:			Yes: no data on food efficiency; no details on fertility indices, number of live births and post-implantation loss, number of pups with grossly visible abnormalities,
Dates of experimental work:			1988-10-24 to 1989-10-13

Executive Summary

30 Sprague Dawley rats sex/dose group (F0 and F1a generation) were fed daily with glyphosate at concentrations of 2000, 10000 and 30000 ppm (corresponding to 132-140, 666-711, 1983-2230 mg/kg bw for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated from F0 and F1a adults)) through two generations for approximately 11 (F0-generation) and 14 weeks (F1a-generation), respectively. Animals of the F1a-generation were mated twice to produce the F2a and F2b-generations. Pairing of animals within each dose group was undertaken on a 1:1 basis. At weaning of offspring from the F0 mating phase, groups of 30 male and 30 female offspring from each dose group were selected to form the F1a generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*. Males were sacrificed after completion of mating phase. The offspring selected for the F1a generation were dosed for approximately 14 weeks and then paired within each dose group to produce the F2a and F2b litters. At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.

Clinical observations for mortality and moribundity were performed twice daily, and detailed observations for signs of toxicity once weekly, when diets were prepared. Body weights were weekly determined for

adults and on day 0, 4 (pre- and post-culling), 14 and 21 of lactation for offspring. Food consumption was determined weekly for adults and on days 0-7, 7-4 and 14-21 of gestation and lactation.
All animals at termination and which died, or were sacrificed moribund, were subjected to a gross necropsy. Histopathological evaluation of selected tissues was performed for control and highest dose level animals.

No significant changes concerning mortality, mating, fertility, organ weights, gross pathology and histopathology were observed. In the high dose group at 30000 ppm, soft stool in adults were observed in both sexes, being accompanied by reduced body weights of adult animals (about 8%) and pups (about 10%) when compared to controls. This effect was assumed to be treatment-related. Furthermore, decreased pup weights were observed when pups began supplementing their milk with the glyphosate-containing food. In the 10000 ppm-dose group, decreased pup body weights were observed, too, but the effect was less pronounced and occurred not in both sexes of all generations.
At the highest dose level of 30000 ppm a slightly, and statistically not significant, reduced average litter size was observed in the F0 and to a lesser degree in the F1 dams. A reduction was not noted when F1 animals were re-mated and treatment-relation was considered to be equivocal.

Conclusion:
Daily oral administration of glyphosate to Sprague-Dawley rats via the diet at concentrations of 2000, 10000 and 30000 ppm to two generations identified treatment-related effects at 30000 ppm, which became manifest in soft stools in adults and consequently reduced body weights in the parent and offspring animals. This effect was less pronounced in the pups of the 10000 ppm dose group and not in both sexes of all generations.
Therefore, the NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations.
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate (Identification code: T880068)
Description:	White powder
Lot/Batch #:	XI-203
Purity:	97.67%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Albino Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approximately 7 weeks (F0 adults)
Sex:	Males and females
Weight at study start (F0):	Males: 165 – 207.6 g; Females: 135.6 – 162.7 g
Acclimation period:	No data