2.3 Long-term studies of toxicity and carcinogenicity

Mice

Study 1: Knezevich, A.; Hogan, G. (1983) A Chronic Feeding Study of Glyphosate (Roundup Technical) in Mice: Project No. 77-2061: Monsanto Report BDN-77- 420.

In a carcinogenicity study, glyphosate (purity 99.7%) was administered to groups of 50 male and 50 female CD-1 mice/sex/dose in the diet at dose levels of 0, 1000, 5000, or 30,000 ppm (equal to 0, 157, 814, 4841 mg/kg bw per day for males and 0, 190, 955, and 5874 mg/kg bw per day for females) for 24 months. Cage-side and detailed clinical observations were done. Body weight and food intake were monitored throughout the study. Water consumption was measured during months 12 and 24. Erythrocyte, as well as total white cell counts and differentials, were done at months 12, 18, and 24. Tissues and organs were collected from all mice whether dying during the study or at terminal sacrifice. Microscopic analyses were done on all collected tissues. This study was conducted prior to the establishment of GLP requirements.

Analysis of treated diets demonstrated that glyphosate could be homogeneously mixed with rodent diet and that it remained stable in the diet for the one week feeding period used in this study. Glyphosate test concentrations averaged approximately 95% of the target concentrations throughout the study. There were no physical or behavioural signs of toxicity, which were considered to be related to glyphosate administration. The incidences of yellow staining of the anogenital area, scabbing on the ears, alopecia, excessive lacrimation, displacement of the pupils, and ocular opacities were observed in all groups of male and female mice, none were dose-related and all occurred at low incidences. Mortality was not affected by the treatment. Body weights for both males and females of the high-dose group were consistently less than controls throughout the study. Although the decreases were slight (1% to 11%), several were statistically significant. Other statistically significant decreases were also noted in the mid- and low-dose animals; however, these changes were sporadic and did not reflect a recognizable dose-response relationship. Although sporadic statistically significant effects were noted for food consumption in treated male and female mice, none were dose- or treatment-related. Also no treatment-related effects were observed for water consumption. No biologically or toxicologically relevant effects were noted on total RBC or WBC counts, HGB, HCT, or platelet counts. There were no changes observed in the absolute or relative organ weights that were considered to be due to glyphosate administration. Several statistically significant changes in organ/body weight ratios were observed, but these were attributed to the statistically significant decreases in terminal (fasted) body weights rather than a specific organ effect. There were no dose-response relationships or any correlated gross or microscopic observations in any of the organs.

No remarkable treatment-related effects were noted at necropsy. Statistically significant positive trends were observed for central lobular hepatocyte hypertrophy, centrilobular hepatocyte necrosis (Table XX) and chronic interstitial nephritis in males, and for proximal tubule epithelial basophilia and hypertrophy in females. Statistically significant increases in the incidence of lesions in treatment groups vs. control were observed for centrilobular hepatocyte necrosis in high-dose males and proximal tubule epithelial basophilia and hypertrophy in high-dose females. Regarding the kidney findings, while the incidences and/or dose response trends of these individual microscopic kidney lesions were found to be statistically significant, they are considered to be part of a spectrum of lesions, which, as a whole, constitute spontaneous renal disease.

Table XX. Incidences of hepatocellular lesions in all mice

	Sex	Concentration in the diet (ppm)			
		0	1000	5000	30000
Centrilobular hypertrophy	M	9/49[a]	5/50	3/50	17/50
	F	0/49	5/50	1/49	1/49
Centrilobular necrosis	M	0/49[a]	2/50	2/50	10/50[a,b]

[a] Statistically significant linear trend ($p \leq 0.01$) using the Cochran-Armitage test

[b] Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-Square test

Neoplastic outcomes were of the type commonly encountered in mice of this age and strain. Of the tumour types observed, bronchiolar-alveoli tumours of the lungs, hepatocellular neoplasms, and tumours of the lymphoreticular system, none were dose-related and were seen in all treatment groups (Table XX). Lymphoreticular tumours were more frequently observed in female mice, but the incidences were low and did not approach statistical significance. With the possible exception of kidney tumours (renal tubular adenomas) in males, all tumour types were considered spurious and unrelated to treatment.. The renal tumour incidence as provided in the study report is shown in Table XX. At the request of the US Environmental Protection Agency (USEPA), the Pathology Work Group (PWG) examined all sections of the kidneys from this study as well as additional renal sections. The PWG noted that because differentiation between tubular-cell adenoma and tubular-cell carcinoma is not always clearly apparent and because both lesions are derived from the same cell type, it appropriate to combine the incidences for purposes of evaluation of statistical analysis. Statistical analyses performed by PWG are presented in Table XX. The PWG concluded that these lesions are not compound-related based on the following considerations: 1) renal tubular cell tumours are spontaneous lesions for which there is a paucity of historical control data for this mouse stock; 2) there was no statistical significance in a pairwise comparison of treated groups with the controls and there was no evidence of a significant linear trend; 3) multiple renal tumours were not found in any animal; and 4) compound-related nephrotoxic lesions, including pre-neoplastic changes, were not present in male mice in this study. Additionally, there was no increase in non-neoplastic renal tubular lesions in male mice (e.g. tubular necrosis/regeneration, hyperplasia or hypertrophy). Although the incidence of tubular adenomas exceeded the historical control range (0-3.3%) for the testing laboratory, the increase at the high dose was not statistically significant compared to the concurrent controls.

Table XX. Incidence of neoplasia in male and female mice treated with glyphosate for 24 months

Organ / Effect	Dose (ppm)			
	0	1000	5000	30,000
	Males			
Lung				
Bronchiolar alveolar adenoma	5/48	9/50	9/50	9/50

Bronchiolar alveolar adenocarcinoma	4/48	3/50	2/50	1/50
Lymphoblastic lymphosarcoma with leukemic manifestations	1/48	4/50	3/50	1/50
Liver				
Hepatocellular adenocarcinoma	5/49	4/50	6/50	4/50
Hepatocellular carcinoma	0/49	0/50	0/50	2/50
Lymph node (mediastinal)				
Lymphoblastic lymphosarcoma with leukemic manifestations	1/45	2/49	1/41	2/49
Kidney				
Renal tubular adenoma	0/49	0/49	1/50	3/50
Lymphoblastic lymphosarcoma with leukemic manifestations	1/49	3/49	2/50	2/50
Females				
Lung				
Bronchiolar alveolar adenoma	10/49	9/50	10/49	1/50
Bronchiolar alveolar adenocarcinoma	1/49	3/50	4/49	4/50
Liver				
Hepatocellular adenocarcinoma	1/49	2/50	1/49	0/49
Composite lymphosarcoma	2/49	1/50	0/49	4/49

Table XX. Incidence of renal tumours in male mice as reported by the PWG using Cochran-Armitage Trend & Fisher's Exact Test

	Dose (ppm in diet)			
Tumor Type	0	1000	5000	30000
Adenomas (%) P =	1/49 (2) 0.4422	0/49 (0) 1.0000	0/50 (0) 1.00000	1/45 (2) 0.7576
Carcinomas (%) P =	0/49 (0) 0.0635	0/49 (0) 1.0000	1/50 (2) 0.5051	2/50 (4) 0.2525

Combined	1/49	0/49	1/50	3/50
(%)	(2)	(0)	(2)	(6)
P =	0.0648	1.0000	0.7576	0.3163

In conclusion, the NOAEL for the systemic toxicity in the carcinogenicity study in mice was 5000 ppm (equal to 814 mg/kg bw/day for males and 955 mg/kg bw/day for females) based on slightly reduced body weights, increased centrilobular hepatocellular necrosis in high-dose males and proximal tubular epithelial basophilia in high-dose females seen at the systemic LOAEL of 30,000 ppm; equal to 4841 mg/kg bw/day for males and 5874 mg/kg bw/day for females (Knezevich and Hogan, 1983).

Study 2: Bhide, M.B. (1988): Carcinogenicity and chronic toxicity study of glyphosate (technical) in mice. Excel Industries Ltd.

Bhide, M.B. (1988): Carcinogenicity and chronic toxicity study of glyphosate (technical) of Excel Industries Ltd. From Referenced in: Draft Assessment Report on Glyphosate, Annex B B-5: Toxicology and metabolism, 1998. Verbatim from Draft Assessment Report on Glyphosate, Annex B B-5: Toxicology and metabolism, 1998: The DAR assessment concluded that the study is unacceptable for a reliable assessment of carcinogenicity since the number of animals used was too small. In addition, the highest dose level of 300 ppm is considered too low. However, the study can be considered to provide supplementary information with regard to chronic toxicity. **Summarized here for JMPR DISCUSSION-**

Groups of 25 male and 25 female Balb/c inbred albino mice (source not specified; 5 to 8 weeks old at the start of treatment) per dose were administered glyphosate technical (batch and purity not given; manufacturer: Excel Industries Ltd., Bombay, India) for 80 weeks at dietary levels of 0, 75, 150 and 300 ppm. The actual mean daily compound intake was not calculated.

Survival was not affected by treatment and overt clinical signs of toxicity did not occur. There was a trend of decreased body weight in high dose male animals towards the end of the treatment period. In females, a similar trend was obvious from the beginning of the study up to week 21 at the highest and the mid dose level. During the last 20 weeks of the administration period, mean body weight was reduced again but only in the female group receiving 300 ppm. Food consumption was markedly diminished in high dose males from week 9 onwards and in high dose females from week 6. Haematology and clinical chemistry did not reveal treatment-related changes neither after 9 nor after 18 months. Mean organ weights were not affected. Gross and histopathological examination did not provide evidence of lesions that could be attributed to glyphosate administration. The incidence of neoplasia was not increased. The total number of tumours was considerably low in all groups.

The NOAEL for chronic toxicity was 150 ppm based on the impact of treatment on body weight and food consumption. When the usual conversion factor of 10 is applied, this value would correspond to a daily intake of 15 mg/kg bw. A NOEL could not be established since a weak effect on body weight also in mid dose females cannot be completely excluded. In contrast, the study author concluded that toxicological effects did not occur up to the highest dietary level of 300 ppm although the reduction in body weight and food consumption was mentioned in the study report. It should be noticed that body weight and food intake were not affected at much higher doses in the other available long-term studies in mice. Thus, it is not likely that these effects were actually related to treatment (Bhide, 1988).

Study 3 :Vereczkey, L. and Csanyi, E. (1992/ revised version): 18-month carcinogenicity study of

glyphosate in mice.

Vereczkey, L. and Csanyi, E. (1992/ revised version): 18-month carcinogenicity study of glyphosate in mice. Referenced in: Draft Assessment Report on Glyphosate, Annex B B-5: Toxicology and metabolism, 1998. The DAR assessment concluded that no conclusion available due to low quality of the study report. The study is unacceptable for a reliable assessment of carcinogenicity since the number of animals surviving up to scheduled termination and subjected to pathological examination was too small. In addition, the highest dose of 300 ppm is apparently not sufficient for evaluation of carcinogenicity since no evidence of toxicity was obtained at that dose level. However, the study can be considered to provide supplementary information with regard to chronic toxicity. The study was not conducted in accordance with GLP. **Summarized here for JMPR DISCUSSION-**

Glyphosate (purity not indicated since the respective supplement was not submitted to the Rapporteur; manufacturer not given) was administered to groups of 50 male and female CFLP/LATI mice (bred in a facility in Godollo, Hungary; 26 - 30 days old at study initiation) per dose at dietary levels of 0, 100 and 300 ppm. The actual daily intake was not calculated. The administration period was 18 months.

There was a considerably high mortality rate in all study groups. Thus, only 11, 14, and 23 male animals and 14, 16 and 14 females survived up to scheduled termination in the control, low and high dose groups and were available for pathological examination. Because clinical signs of toxicity were lacking and since mortality did not increase with dose, a treatment-related impact on survival is not likely. Body weight and food consumption were not affected. Gross and histopathological examination did not reveal treatment-related changes. The overall tumour rate was rather high in all study groups including the controls. However, no significant difference in tumour incidence was observed between the groups.

There was no clear evidence of adverse effects of glyphosate administration up to the highest tested dose of 300 ppm (about 30 mg/kg bw/day) which is considered the NOEL in this study. However, the scientific value of this experiment is rather limited (Vereczkey and Csanyi 1982, revised 1992).

Study 4: Atkinson C, Martin T, Hudson P, Robb D (1993) Glyphosate: 104 Week Dietary Carcinogenicity Study in Mice. Inveresk Research International report No.: 7793. Cheminova report No.: 154 GLY.

In a carcinogenicity study, glyphosate (purity 97.5 – 100.2%) was administered to groups of 50 CD-1 mice/sex/dose in the diet at doses of 0, 100, 300, or 1000 mg/kg bw/day for 104 weeks. The dietary concentrations were adjusted weekly for the first 13 weeks and every 4 weeks thereafter. No interim sacrifices were done. Mortality, body weight, body weight gain, and food consumption were monitored throughout the study. WBC differential counts were done during Weeks 52, 77, and 102 of the study. Following premature deaths or at scheduled sacrifice, organ weights were measured and tissues collected for microscopic analyses.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. There were no unscheduled deaths during the course of the study that were attributable to the administration of glyphosate. No treatment-related clinical signs of toxicity were observed. There were no biologically relevant or toxicologically significant effects over the 104-week study on body weight or body weight gain of male and female CD-1 mice. Although statistically significant effects were noted, none were due to treatment with the test material and were typically higher than in corresponding control mice. No treatment-related effects were noted on food or water consumption. Ophthalmoscopic examinations, urinalysis and clinical chemistry parameters were

not evaluated. There were no remarkable intergroup differences in differential blood counts in either sex at any of the time-points tested. The absolute and relative to body thymus weights of male mice in the 300 and 1000 mg/kg bw/day groups were statistically significantly increased. The increase in thymus weights were slight in magnitude and lack of a dose response. No histologically correlates were found microscopically. No increase in absolute or relative to body thymus weights were found in female mice. The incidence of lung masses was slightly increased in high-dose male mice (control 10/50, low-dose 13/50, mid-dose 12/50 and high-dose 18/50), however, histopathology failed to reveal adverse lung findings. No increase in lung masses was found in female mice. The occurrence of mineral deposits in the brain was significantly increased in males at the highest dose when compared with the control group (13/50 compared with 4/49). It should be noted that this is a common finding in mice of this age and strain.

There were no statistically significant increases in the incidence of any tumours, both benign and malignant in either sex when compared to the control. However, the number of animals with multiple tumour types was slightly increased in the high-dose group of both sexes (males 16/50 and females 11/50) compared to the control (males 11/50 and females 6/50). This led to a slight increase in the total number of tumours in the high-dose group of both sexes (males 60 and females 43) compared to the control (males 49 and females 36). Haemangiosarcoma was evident in 4/50 high-dose males, 2/50 low-dose females and 1/50 high-dose females compared to 0/50 in the controls. Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue was evident in 2/50 low and high-dose males and 3 low- and intermediate- and 1/50 high-dose females when compared to the respective controls (0/50). Due to a lack of dose relationship, and the lack of statistical significance and the incidences in this study falling within the background ranges, these changes are not considered to be due to administration of glyphosate. Other tumours seen were considered to be typical for mice of this age and strain.

In conclusion, administration of glyphosate to CD-1 mice for 104 weeks produced no signs of carcinogenic potential at any dose. The NOAEL for carcinogenicity and systemic toxicity was 1000 mg/kg bw per day, the highest dose tested (Atkinson et al., 1993a).

Study 5: Sugimoto, K. (1997), HR-001: 18-Month Oral Oncogenicity Study in Mice, The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan, Laboratory Project I IET 94-0151, Sankyo Co., Ltd., 7-12, Ginza 2-chome, Chou-ku, Tokyo 104, Japan, not published. Also referred to as Arysta Life Sciences, 1997.

In a carcinogenicity study, glyphosate (HR-001, purity 97.56 and 94.61%; two lots) was administered to groups of 50 male and 50 female Specific-Pathogen-Free (SPF) ICR (Crj: CD-1) mice/dose in the diet at dose levels of 0, 1600, 8000 or 40000 ppm (equal to 0, 165, 838.1, or 4348 mg/kg bw per day for males and 0, 153.2, 786.8, or 4116 mg/kg bw per day for females) for 18 months. During treatment, all animals were observed for clinical signs and changes in body weight, and food consumption was measured. At week 21, urinalysis was carried out on 20 males from all groups. Differential leukocyte 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 and also animals killed in extremis during the treatment as possible. At final necropsy after 78 weeks of treatment, organ weight analysis was conducted on 10 males and 10 females which were served to the determination of differential leukocyte counts. All animals of both sexes were subjected to necropsy and histopathological examinations.

At 1600 ppm, there were no treatment-related changes in either sex in any parameters. At 8000 ppm,

retarded growth was observed in females with statistically significant decreases in weight at 6 and weeks 9-24. No treatment-related changes were seen in males. At 40000 ppm, the incidence of pale-colored skin 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 the treatment period showing statistically significant differences in weight from week 16 to 36 in males and from week 6 to the end of the treatment in females. These changes were associated with depressed food consumption and food efficiency. At necropsy, the increased incidences of distention of the cecum were noted in males and females at terminal kill and in all animals examined, which were consistent to increases in absolute and relative weights of the cecum. However, no abnormalities were recorded in the cecum histopathologically. In males, a significant increase was noted for the overall incidence of anal prolapse which was correspondent to erosion/ulcer of the anus histopathologically.

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

Based on these results, the no observable adverse effect level NOAEL is 1600 ppm (153.2 mg/kg/day) and the lowest observable adverse effect level (LOAEL) is 8000 ppm (838.1 mg/kg/day) for females based upon retarded growth with statistically significant decreases in weight at 6 and weeks 9-24. For males the NOAEL was 8000 (838.1 mg/kg bw per day) and the LOAEL was 40000 ppm (4116 mg/kg/day) based on a significant increase was noted for the overall incidence of anal prolapse which was correspondent to erosion/ulcer of the anus histopathologically (Sugimoto, 1997).

Study 6: Kumar, D.P.S. (2001) Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice Toxicology Department Rallis Research Centre, Rallis India Limited, Peenya II Phase, Bangalore, 560 058 India. Data owner: Feinchemie Schwebda GmbH, Study No.: Toxi: 1559.CARCI-M, Date: 2001-10-10 not published, ASB2012-11491 (EU data to JMPR)

In a carcinogenicity study, glyphosate (purity >95%) was administered to groups of 50 HsdOla:MF1 Swiss Albino mice/sex/dose in the diet at doses of 0, 100, 1000, or 10000 ppm (equal to 0, 14.5, 149.7 or 1453 mg/kg bw/day for males and 0, 15.0, 151.2, or 1466.8 mg/kg bw per day for females) for 18 months. The stability, homogeneity and dietary concentrations were measured periodically. 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. Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mortality, body weight, body weight gain, and food consumption were monitored throughout the study. WBC differential counts were done at 9 months and at terminal sacrificed from all surviving animals and from mice killed in extremis. 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. Adrenals, kidneys, liver and gall bladder, ovaries and testes from 10 mice/sex/dose were weighed and selected tissues were examined histopathologically from control and high dose animals and animals that died or were killed in extremis.

All prepared diets were stable for 30 days, homogenously distributed and achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 10% of the nominal concentration for all diet samples. There was no of effect of treatment on mortality, clinical signs, body weights, body weight gains, food consumption, ophthalmoscopic examination, and organ weights (absolute and relative to body). There were no significant treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophil 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.

In mice found dead or sacrificed moribund, cystic glands of the stomach were significantly increased in high dose males and for 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 lymphocyte infiltration of epididymides in mid dose males was significantly decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental. Furthermore, it is necessary to consider the frequency of this finding in animals surviving to scheduled termination. At terminal sacrificed, cystic glands of the stomach were significantly increased in low-, mid- and high-dose males but without a dose-response. Degenerative heart changes were higher in high-dose males and females, and significantly higher when sexes were combined. However, the percentage incidences were similar or slightly higher than in the historical controls and severity did not increased dose hence considered incidental. In mandibular lymph nodes lymphoid hyperplasia was significantly increase 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.

The number of malignant lymphoma (Table XX) 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 tumours incidence is considered incidental and not treatment-related.

An increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose. The incidence was statistically significantly elevated as compared to the actual control groups in this study, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Even though malignant lymphoma is a common tumour in mice (accounting for 54.6% of all tumours in this study), it cannot be completely excluded that the higher incidence in the top dose groups were somehow related to treatment. However, as noted in Greim et al. 2015 there is concern that there was a viral infection within the colony of mice used in this study, which confounds the interpretation of the lymphoma findings.

Table XX Incidence of malignant lymphoma in glyphosate treated mice and comparison with the 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	30	28	23	34	34	30	30
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

In conclusion, the systemic toxicity NOAEL in this 18 months carcinogenicity study in mice is 1000 ppm; equal to 149.7 mg/kg bw per day based on increased incidence of malignant lymphomas compared to controls seen at the high dose of 10,000 ppm; equal to 1453 mg/kg bw per day. Glyphosate was not carcinogenic in mice at doses up to 10,000 ppm; the highest dose tested (Kumar, 2001).

Study 7: Wood, E., Dunster, J., Watson, P., Brooks, P. (2009b), Glyphosate technical: Dietary Carcinogenicity Study in the Mouse, Harlan Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK SPL Project No.: 2060-0011 Date: 2009-04-22 - 503 - Glyphosate – Annex B.6: Toxicology and metabolism 18 December 2013 not published, ASB2012-11492

In a carcinogenicity study, glyphosate (purity 95.7%) was administered to groups of 51 male and 51 female CD-1 mice/dose in the diet at dose levels of 0, 500, 1500 and 5000 ppm (equal to 0, 71.4, 234.2, and 810 mg/kg bw per day for males and 0, 97.9, 299.5, and 1081.2 mg/kg bw per day for females) for 79 weeks. 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. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were done. Body weight and food intake were monitored throughout the study. Water consumption was observed daily. 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. Gross pathological examinations was conducted at sacrifice and on mice died pre-maturely and moribund. Selected organs were weighed from 10 mice/sex/dose. Histopathological examination was performed on all sampled tissues from control and high dosed animals and on animals that died or killed in extremis.

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

exception of one low dose sample, which was + 10 % of the nominal concentration. There were no treatment related effects on the number of mortalities observed and no significant differences in the rate of death during the course of the study were observed. There were no significant treatment related clinical observations reported during the course of the study. There were no treatment related effects on body weights, body weight gains, food consumption and water consumption during the study. There were no significant differences in proportion of white cell populations of either sex when observed at both 12 and 18 months. There were no trends in the proportion of palpable masses observed during the study period. 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. There were no treatment-related findings observed in organ weights or relative organ weights. There were no treatment-related histopathological findings observed in any dose group of either sex.

In conclusion, the NOAEL for carcinogenicity and systemic toxicity in mice is 5000 ppm (equal to 810 and 1081.2 mg/kg bw per day in male and female mice, respectively); the highest dose tested (Wood et al. 2009).

Study 8: Michiko Takahashi (1999). Oral Feeding Carcinogenicity Study in Mice with AK-01, Nippon Experimental Medical Research Institute Co., Ltd., Technical PROJECT No. H-95056, 3303-58 Ohdo, Agatsuma-cho, Agatsuma-gun, Gunma, Japan

In a carcinogenicity study, glyphosate (purity 97.5%) was administered to groups of 50 male and 50 female Crj:CD-1 mice/dose in the diet at dose levels of 0, 500, 5000 and 50000 ppm (equal to 0, 67.6, 685, and 7470 mg/kg bw per day for males and 0, 93.2, 909, and 8690 mg/kg bw per day for females) for 78 weeks. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were done. Body weight and food intake were monitored throughout the study. Differential white cell counts were performed at week 52 and full haematological parameters evaluation at the end of the treatment. Gross pathological examinations was conducted at sacrifice and on mice died pre-maturely and moribund. Selected organs (Brain, liver, (right and left) kidneys, (right and left) adrenal glands, and (right and left) testes) were weighed. Histopathological examination was performed on all sampled tissues from control and high dosed animals and on animals that died or killed in extremis.

Prepared diets were stable at room temperature for 4 months and the test compound was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations were ranged from 80 to 110% of the nominal concentrations. In the 50,000-ppm group, loose stool was found throughout the treatment period in all males and females, of which some animals showed improvement as treatment was continued. In the same group, 9 males and 8 females had treatment-related anus prolapse at Week 10 or later. Other clinical signs and their incidences were similar in the control and treated groups. A statistically significant difference in mortality rate in males was noted between the 50,000 ppm group and the control group at Week 26 or later. Mortality in the mid and low dose males and females of all dose group was not affected by the treatment. In the 50,000 ppm group, compared with the control group, body weight gain significantly decreased or appeared to decrease throughout the treatment period in males and at Week 24 or later in females. No effects of treatment was observed in treated males and females in the mid and low dose at any time interval compared to controls. In the males and females from the 50,000-ppm group, compared with the control group, food

consumption decreased and the change was considered to be related to the test substance. No treatment related changes were observed in haematological parameters. In the females from the 50,000-ppm group, compared with the control group, the relative weights of right and left kidneys significantly increased. These changes were considered to be related to the test substance, though no corresponding histopathological findings were observed. In addition, decreases in the absolute weights of liver and right and left kidneys and significant increases in the relative weights of brain, left kidney, left adrenal gland, and right and left testes in males, and a decrease in the absolute weight of brain in females were noticed in the 50,000-ppm group. All these changes were considered to be unrelated to the test substance, because they were accompanied by decreased body weight. Macroscopic examination revealed luminal dilatation of the large intestine, which may be associated with loose stool, in most of the terminally sacrificed males and females from the 50,000-ppm group. In the 50,000-ppm group, treatment-related non-neoplastic lesions were found in the kidney in males and the rectum in males and females. The renal findings included significant increases in tubular epithelial cell hypertrophy, tubular dilatation, degeneration / necrosis and an increasing tendency in basophilic tubules (based on data from all animals). The rectal findings included significant increases in anus prolapse-associated erosion and luminal dilatation (Table XX).

Table XX Incidence of non-neoplstic lesions in mice treated with glyphosate for 78 weeks

	Male				Female			
Dose in ppm	0	500	5000	50000	0	500	5000	50000
Kidney								
No. of animals examined	50	50	50	50	50	50	50	50
Tubular Dilation	4	7	4	20**	8	12	5	8
Tubular epithelial cell hypertrophy	13	10	13	25*	13	17	14	13
Basophilic tubules	21	16	17	28	14	14	10	13
Tubular degeneration/necrosis	9	6	5	15	5	8	8	7
Rectum								
No. of animals examined	48	12	7	46	44	11	10	44
Luminal dilation	0	0	0	6*	0	0	0	6*
Erosion	0	0	0	3	0	0	0	6*

[a] The data represents in all animals at week 78

* $p<0.05$, ** $p<0.01$ (Fisher's exact probability test)

The observed neoplastic lesions of the kidney included renal cell adenoma in 3 males and renal cell carcinoma in 1 male in the 50,000-ppm group, and renal cell adenoma in 1 male in the 5,000-ppm group, with no renal tumor formation in females (based on data from all animals). The incidence of other tumour types in glyphosate treated groups and controls were similar. These tumours were re-examined by the original study pathologist in 2012 because Pesticide Expert Panel, Food Safety Commission of Japan requested additional information on historical control data and association with the non-neoplastic renal findings. The hematoxylin-eosin-stained kidney sections prepared in the original study were found to have faded and unevaluable; therefore, the paraffin-embedded blocks of 50 males in each group which had been stored for each observation period were sectioned and stained by hematoxylin and eosin for microscopic re-examination. The data from the re-examination and the original data are shown in Table XX

Table XX. Number and Incidence of Males with Renal Tumor by Dose

Dose (ppm)	Findings	Original study	Re-examination	Incidence
50,000	Renal cell adenoma	3	1	1/50 (2%)
50,000	Renal cell carcinoma	1	1	1/50 (2%)
5,000	Renal cell adenoma	1	1	1/50 (2%)
500	Renal cell adenoma	0	1	1/50 (2%)

The incidence of renal tumours in each treatment group did not statistically significantly differ from that in the control group, both in the original study report and re-examination (Fisher's exact probability test, P>0.05). The historical control data were not available. As described in the reexamination document, historical control values in the literature for renal cell carcinoma were 1/725 (0.13%) in males and 0/725 (0%) in females. The historical control values for renal cell adenoma were 3/564 (0.53%) in males and 0/564 (0%) in females (Baldrick and Reeve, 2007 and Chandra and Frith (1994). The re-examination report also provides reference data which were 0/55, 0/55, 1/55, 0/55, 0/55 (0%-1.8%) in males and 0/55, 0/55, 0/55, 0/55, 0/55 (0%) in females for renal cell carcinoma and 0/55, 1/55, 1/55, 1/55, 0/55 (0%-1.8%) in males and 0/55, 0/55, 0/55, 0/55, 1/55 (0%-1.8%) in females for renal cell adenoma. The results of the re-examination revealed that the incidence of tubular epithelial cell hypertrophy in each treatment group did not significantly differ from that in the control group. In addition, the tubular epithelial cell hypertrophy was localized. These findings indicate no association between the tubular epithelial cell hypertrophy and the development of renal tumors.

Baldrick P and Reeve L. Carcinogenicity Evaluation: Comparison of tumour data from dual control groups in CD-1 mouse. Toxicologic Pathology 2007; 35: 562-569.

Chandra M and Frith CH. Spontaneous renal lesions in CD-1 and B6C3F1 mice. Exp Toxic Pathol 1994; 46:189-198.

It is concluded that the renal cell tumours observed in this study are not considered to be treatment-related because (1) The incidence of renal tumors in the males from the 50,000-ppm group did not significantly differ from that in the control group; (2) It is uncertain that the non-neoplastic lesions found in males were associated with tumors, because these lesions developed even in the absence of tumors (see the EPA's evaluation mentioned above); (3) No females had a neoplastic lesion or non-neoplastic lesion, and (4) The highest dose (50,000 ppm) used in this study far exceeded the limit dose for mice (7,000 ppm) specified by the OECD and EPA.

In conclusion, the NOAEL was 5,000 ppm; equal to 685 mg/kg bw per day based on loose stool, decreased body weight gain, decreased food consumption, and the increased incidences of rectal and renal non-neoplastic lesions were observed in the males and females at the LOAEL 50,000 ppm; equal to 7470 mg/kg bw per day; the highest dose tested. Glyphosate was not carcinogenic in CD-1 mice (Takahashi, 1999).

Rats

Study 1: Lankas, G.R. (1981) A Lifetime Feeding Study of Glyphosate (ROUNDUP Technical) in Rats Bio/dynamics Inc., Division of Biology and Safety Evaluation, East Millstone, New Jersey, Data

owner: Monsanto Study/Project No.: 77-2062 (BDN 77-416 on the report with a hand written).

In a combined chronic toxicity and carcinogenicity study, groups of Sprague-Dawley rats (50/sex/dose) were fed diets containing glyphosate (purity 98.7%) at concentrations of 0, 30, 100 or 300 ppm for the first week. These concentrations were adjusted during the course of the study so that actual doses of 0, 3.05, 10.30, and 31.49 mg/kg/day in males and 0, 3.37, 11.22, and 34.02 mg/kg/day in females were maintained for approximately 26 months. Diets were analyzed for stability, homogeneity and dietary concentrations periodically. All rats were observed twice daily for mortality and toxic signs. Body weights and food consumption were determined at pre-test, weekly for 14 weeks and bi-weekly thereafter. Water consumption was determined for 10 rats/sex/group for two separate three-day periods at 18 and 24 months. Blood and urine samples were collected at months 4, 8, 12, 18 and 24 from 10 rats/sex/group. Selected haematological and clinical chemistry parameters were evaluated. Complete necropsies were performed on all rats that died or were sacrificed during or at the end of the study. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, testes/ovaries, pituitary, spleen and thyroid. The tissues were preserved for histopathology.

This study was conducted prior to the establishment of GLP requirements. The prepared diets were stable for one week and homogeneously distributed. There was no significant difference between the control and treated groups of both sexes with regard to the survival rate during the course of this study. Survival was approximately 80-90% through Month 20 of the study for all groups. No clinical observations attributable to substance administration were reported in any of the treated groups. Although statistically significant differences in mean food consumption were occasionally noted in the treated groups relative to the controls, these differences occurred sporadically and there was no dose-effect relationship. Water consumption of the treated groups was similar to that of the controls at the 18 and 24-month intervals. During the intermediate months of this study, the mean body weights of the treated animals were slightly lower than the controls. The maximum body weight reductions for males ranged from 6% in the high-dose group to 2-3% in the low-dose group. For females these differences were statistically significant only during months 20 and 21 and they were not dose-related. From month 24 until study termination the mean body weights of all treated groups were comparable to the controls. Haematology, blood biochemistry and urinalysis parameters deviated occasionally and some of them were statistically significantly different from controls. These differences were not dose-related and not consistent over time or between sexes. No statistically significant differences were noted in the terminal absolute and relative organ weights of the treated groups when compared to the controls. The few inter-group differences were neither dose-related nor consistent. Gross observations at necropsy were similar in incidence between treatment groups and controls of both sexes. These lesions consisted primarily of inflammatory and structural changes that are commonly found in rats of this strain in lifetime studies. The incidence and severity of the microscopic lesions were similar between the treatment groups and controls of both sexes. The most frequently observed changes occurred in the lungs and the kidneys, and were associated with chronic respiratory disease and chronic progressive nephropathy. Both lesions are a common age-related disease in this strain of rats.

A variety of neoplasms were found in both control and treated animals. The most common tumours were found in the pituitary of both sexes and in the mammary glands of the females. The incidence of all tumour-bearing animals in the treated groups and the controls were similar and did not exhibit any dose-effect relationship.

Table XX A summary of interstitial cell tumours finding in testes of rats after 26-months dietary

exposure to glyphosate and historical control data

Group	Controls (0)	3.05 mg/kg bw per day	10.3 mg/kg bw per day	31.49 mg/kg bw per day	
Interstitial Cell Tumours of the Testes					
Terminal sacrifice	0/15 (0%)	2/26 (7.7%)	1/16 (6.3%)	4/26 (15.4%)	
All animals	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)	
Recent Historical Control Data					
Study no.	1	2	3	4	5
Terminal Sacrifice	4/65 (6.2%)	3/11 (27.3%)	3/26 (11.5%)	3/24 (12.5%)	3/40 (7.5%)
All animals	4/116 (3.4%)	5/75 (6.6%)	4/113 (3.5%)	6/113 (5.3%)	5/118 (4.2%)

Combined historical control data for 5 studies are 16/166 (terminal sacrifice) and 24/535 (all animals).

*number of animals affected / total number of animals examined

( ): Percentage

The incidence of interstitial cell tumours in the testes was increased in the treated animals when compared to the controls (15% at the highest dose at terminal sacrifice). The increased incidence of interstitial tumours in males rats were not considered as treatment-related based on the following weight of evidence considerations: 1) lack of monotonic dose-response; 2) absence of pre-neoplastic lesions (i.e., interstitial cell hyperplasia); 3) the incidences were within the normal biological variation seen for this tumour type in this strain of rats; 4) the incidences in the concurrent controls (0%) was not representative of the normal background incidences noted in the historical control animals and 5) no interstitial cell tumours were seen when tested at much higher doses in the same strain of rats in an another study of glyphosate (Stout and Ruecker, 1990).

In conclusion, the NOAEL for systemic toxicity in rats after 26 months dietary exposure to glyphosate was 31.5 mg/kg bw per day, the highest dose tested. It was concluded that the glyphosate was not carcinogenic in rats (Lankas, 1981).

Study 2: Stout, L.D.& Ruecker, F.A. (1990) Chronic study of glyphosate administered in feed to Albino rats Monsanto Agricultural Company, St. Louis, Missouri, USA, Data owner: Monsanto, Project No.: ML-87-148, Date: 1990-09-26

In a combined chronic toxicity and carcinogenicity study, groups of Sprague-Dawley rats (60/sex/dose) were fed diets containing glyphosate (purity 96.5%) at dietary concentrations of 0, 2000, 8000 or 20,000 ppm 24 months. These levels were equal to 0, 89, 362 or 940 mg/kg/day for the males and 0, 113, 457 or 1183 mg/kg/day for the females. An interim sacrifice was conducted on 10 rats/sex/dose at 12 months. Prepared diets were analyzed for stability, homogeneity and dietary concentrations. All animals were observed twice daily for mortality and moribundity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week for the first 13 weeks and then every fourth week thereafter. Ophthalmic examinations were performed at pre-test and just prior to terminal sacrifice. Haematology, blood biochemistry and urinalysis determinations were conducted on 10 animals/sex/dose each at months 6, 12 (interim sacrifice), 18, and 24 (study termination). Ten animals/sex/dose were sacrificed at month 12, and all survivors were sacrificed at month 24. All animals were given a complete gross necropsy. Brain, kidneys, liver and testes with epididymides were weighed. Approximately 40 tissues were preserved and examined microscopically.

Stability analyses indicated that the neat test material was stable throughout the study. The stability and homogeneity of the diet mixtures were determined to be adequate. Analyses to verify dietary concentrations demonstrated that average glyphosate concentrations were 95% of target levels for all dose groups. There were no statistically significant differences in-group survival rates. At the end of the study, the percentages of animals surviving at 0, 2000, 8000, and 20000 ppm were 29, 38, 34, and 34 for males, respectively, and 44, 44, 34, and 36 for females. Various clinical signs were noted throughout the study. However, they were typical of those frequently observed in chronic studies and appeared to be randomly distributed in all groups. Therefore, none were considered to be related to administration of the test material. Statistically significant reductions in body weight were noted in high-dose females from week 7 through approximately the twentieth month. During this time, absolute body weights gradually decreased to 14% below the control value. Body weight gain in high dose females was also consistently reduced compared to control. At the point of maximum body weight depression (20 months), cumulative body weight gain was 23% less than control. Body weight gain in all treated male groups was comparable to controls. There were no statistically significant decreases in food consumption in either sex at any time in the study; significant increases were noted frequently in high dose males.

The ophthalmic examination prior to study termination revealed a statistically significant difference ($p<0.05$) between the incidences of control and high-level males displaying cataractous lens changes (0/15 vs. 5/20). This incidence (25%) was within the range (0 to 33%) observed in previously conducted studies at this laboratory with male CD rats. The incidences of cataractous lens changes in low- and mid-dose males, as well as all treated female groups, were comparable to their respective controls. An independent pathologist's examination also revealed a statistically significant increase ($p<0.05$) in cataractous lens changes in high-dose male animals (1/14 control vs. 8/19 high dose) and concluded that there appeared to be a treatment-related occurrence of lens changes affecting high-dose males. Histopathological examination of the eyes by the Monsanto histopathologist revealed the incidences of cataract and/or lens fibre degeneration as shown in Table XX. Due to the small number of rats examined ophthalmologically and affected at termination, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes in high-dose males appears to be exacerbated by treatment.

Table XX: Incidences of cataract and lens fibre degeneration in male rats

	Dose (ppm in diet)*			
	0	**2,000**	**8,000**	**20,000**
Terminal sacrifice	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

* Number of rats affected / number of rats examined

There were various changes in haematology and serum chemistry parameters. However, the changes were not consistently noted at more than one time point, were within historical control ranges, small in magnitude, and/or did not occur in a dose-related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically insignificant. There was a statistically significant increase in urine specific gravity in high-dose males at the 6 months. Statistically significant reductions in urine pH were also noted in high-dose males at months 6, 18, and 24. This may have been related to the renal excretion of glyphosate, which is an acid. Statistically significant increases in liver weight were noted in high-dose males: liver-to-body weight ratio at 12 months, absolute liver weight and liver-to-brain weight ratio at 24 months. There were no other statistically significant changes in organ weights, which occurred

in a dose-related manner. Gross abnormalities observed at necropsy were not considered related to glyphosate administration. Histopathological examination revealed an increase in the number of mid-dose females displaying inflammation of the stomach squamous mucosa. This was the only statistically significant occurrence of non-neoplastic lesions. The incidences of this lesion in all groups of animals are shown in Table XX. Although the incidence (15%) of this lesion in mid-dose females was slightly outside the historical control range (0 to 13.3%) for the laboratory, there was no dose-related trend across all groups of treated females and there was no significant difference in any male group. Therefore, the finding was not considered treatment-related.

Table XX: Incidence of inflammation of the stomach squamous mucosa in the rat

	Concentration in the diet (ppm)			
	0	**2,000**	**8,000**	**20,000**
Males	2/58	3/58	5/59	7/59
Females	0/59	3/60	9/60**	6/59

** $p \leq 0.01$; Fisher Exact Test with Bonferroni inequality

The only statistically significant difference in neoplastic lesions between control and treated animals was an increase in the number of low-dose males (14%) with pancreatic islet cell adenomas, shown in Table XX. The historical control range for this tumour at the testing laboratory is 1.8 to 8.5%, but a partial review of studies reported in the literature revealed a prevalence of 0 to 17% in control males with several values greater than or equal to 8%. The incidences of islet cell adenomas did not follow a clear dose-related trend in the treated male groups as indicated by the lack of statistical significance in the Peto trend test. This indicates that the distribution of incidences in the four groups was most likely random. The authors also noted that there was considerable inter-group variability in the numbers of females with this tumour (5/60, 1/60, 4/60 and 0/59 in the control, low-, mid- and high –dose groups, respectively). There was no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a control male, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support a conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to glyphosate administration.

Table XX: Incidence of pancreatic islet cell findings

Finding	Sex	Dose Group in ppm*			
		0	2000	8000	20000
Hyperplasia	Males	2/58 (3%)	0/57 (0%)	4/60 (7%)	2/59 (3%)
	Females	4/60 (7%)	1/60 (2%)	1/60 (2%)	0/59 (0%)
Adenoma	Males	1/58 (2%)	8/57** (14%)	5/60 (8%)	7/59 (12%)
	Females	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)
Carcinoma	Males	1/58 (2%)	0/57 (0%)	0/60 (0%)	0/59 (0%)
	Females	0/60 (0%)[a]	0/60 (0%)	0/60 (0%)	0/59 (0%)

Adenoma	Males	2/58 (3%)	8/57 (14%)	5/60 (8%)	7/59 (12%)
	Females	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)

* Number of rats affected / number of rats examined

** Statistically significant at p≤ 0.01 (Fisher exact test with Bonferroni inequality)

There was a statistically significant trend for hepatocellular adenomas in males only, but a significant trend was not seen for adenomas and carcinomas combined (p > 0.05) (Table XX). These tumours were not considered to be treatment related since 1) the incidences for these tumours were within the historical control range (1-18%) for the testing facility; 2) absence of pre-neoplastic lesions (i.e., cell hyperplasia or preneoplastic foci); and 3) there was no evidence of progression to malignancy (adenoma to carcinoma).

Table XX: Incidence of hepatocellular tumours in males

Finding	Concentration in the diet (ppm)			
	0	2000	8000	20000
Adenoma	2/44#* (5%)	2/45 (4%)	3/49 (6%)	7/48 (15%)
Carcinoma	3/44 (7%)	2/45 (4%)	1/49 (2%)	2/48 (4%)
Adenoma Carcinoma Combined	5/44 (11%)	4/45 (9%)	4/49 (8%)	9/48 (19%)

Number of rats affected / number of animals examined, excluding those that died or were sacrificed prior to study week 55.

* Statistically significant at p<0.05 (Cochran-Armitage Trend Test)

An increased incidence of thyroid C-cell adenomas was observed in the 8000 and 20000 ppm dose group in both sexes but did not reach statistical significance compared to the control animals. There was a statistically significant dose trend for C-cell adenomas and adenomas/carcinomas combined in females as shown in Table XX. The historical control range in the testing laboratory for C-cell adenomas was 1.8 – 10.6% for males and 3.3 – 10% for females. The range for C-cell carcinomas was 0- 5.2% and 0 – 2.9% in males and females, respectively. These tumours are not considered to be related to treatment because: 1) the increased incidences in males were not statistically significant; 2) there was no evidence of a progression from adenoma to carcinoma; 3) and there were no dose-related increases in the incidence or severity of preneoplastic lesions (hyperplasia).

Table XX: Incidence of thyroid C-cell tumours in male and females.

Finding	Sex	Concentration in the diet (ppm)			
		0	2000	8000	20000
Adenoma	Males	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)
	Females	2/57* (4%)	2/60 (7%)	6/59 (10%)	6/55 (11%)
Carcinoma	Males	0/54 (0%)	2/55[c] (4%)	0/58 (0%)	1/58 (4%)

	Females	0/57 (0%)	0/60 (0%)	1/59[c] (2%)	0/55 (0%)
Adenoma Carcinoma Combined	Males	2/54 (11%)	6/55 (11%)	8/58 (14%)	8/58 (14%)
	Females	2/57* (4%)	2/60 (3%)	7/59 (12%)	6/55 (11%)

Number of rats affected / number of animals examined, excluding those that died or were sacrificed prior to study week 55.

* Statistically significant at $p<0.05$ (Cochran-Armitage Trend Test)

In conclusion, the NOAEL in this study in rats is 8000 ppm; equal to 362 mg/kg bw per day based on decreased in body weight gains in females and cataractous lens changes in males seen at the LOAEL of 20000 ppm; equal to 940 mg/kg bw per day. It is concluded that glyphosate is not carcinogenic in rats (Strout and Ruecker, 1990).

Study 3: Atkinson, C., Strutt, A., Henderson, W., et al. (1993). 104-Week chronic feeding/ oncogenicity study in rats with 52-week interim kill. Inveresk Research International (IRI), Tranent, Scotland. Study No. 438623; IRI Report No. 7867. April 7, 1993. Unpublished.

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 98.9 and 98.7% , two batches) was administered to 85 Sprague-Dawley rats/sex/dose in the diet for 104 weeks in amounts that varied in concentration to deliver 0, 10, 100, 300, and 1000 mg/kg/day to both sexes over the course of the study. Designated for the toxicity portion of the study were 35 rats/sex/dose with the remainder designated for the oncogenicity portion of the study. An interim sacrifice was conducted on 15 rats/sex/dose after 52 weeks of glyphosate administration. Animals were inspected twice daily for signs of toxicity and mortality. Clinical examinations were conducted on all animals prior to initiation of the study, and weekly during the study. Palpitation for tissue masses was included. Animals were weighed weekly during weeks 1-13 and once monthly thereafter. Food consumption was measured by cage weekly during weeks 1-13 and once monthly thereafter. Water consumption was monitored by visual inspection throughout the treatment period. An ophthalmoscopic examination was carried out on 20 males and 20 females from each group designated as the oncogenicity study before treatment commenced and on 20 males and 20 females from the Control and High dose designated oncogenicity study groups at Weeks 25 and 51. In addition all Control and High dose oncogenicity and toxicity study rats were examined at Week 102. Blood was collected from the retro-orbital sinus of fasted animals for hematology and clinical chemistry while under light ether anesthesia. Samples were obtained from 10 animals/sex/group in the toxicity study at 14, 25, 51, 78, and 102 weeks. Urine samples were obtained from 10 animals/sex/group at 14, 26, and 53 weeks, and from 10 animals/sex/group in the toxicity study at 14, 25, 51, 78, and 102 weeks. Fifteen males and 15 females from each toxicity study group were killed and necropsied after 52 weeks. All remaining toxicity study and surviving oncogenicity study animals were killed and necropsied after 104 weeks. All premature decedents were also necropsied. Selected organs were weighed from all interim kill animals and 10 males and 10 females from the oncogenicity study. All collected tissues were examined microscopically on all decedents prior to Week 52, those sacrificed at 52 weeks, and the control and high-dose animals sacrificed at the end of the study. Only the salivary glands were examined on the decedents after 52 weeks and the rats from the other dose groups at terminal sacrifice.

Pale feces were observed during weeks 16-104 in both sexes at the high dose and in females from the low-mid and high-mid dose levels. This sign was not considered to be toxicologically significant. There were no statistically significant differences between each group receiving glyphosate and the control group, in either sex in survival rates. No treatment-related effect was observed in food consumption, water consumption and haematology, ophthalmoscopic examinations and gross pathology data. Males from the high-dose group had statistically lower mean body weight ($p \leq 0.01$) by 5% to 11% beginning Week 2 of the study until Week 104, and at termination was 10% lower (-14% weight gain). Females at the high dose had statistically lower body weight ($p \leq 0.05$) by 5% to 12% beginning Week 20 through Week 80 (with several exceptions), and at termination was 8% lower (-11% weight gain). Statistically significantly increased alkaline phosphatase activity (ALP) activities (+46% to +72%) were observed in males at the high dose throughout the study except for the 51 week interval when the mean value was 31% higher than control. Elevated ALP activities were observed in females at the high dose (+34% to +53%) throughout the study, and through most of the study at the high-mid dose by +20% to +67%, though not always statistically significant. These changes in the ALP activity are considered as doubtful toxicological significance. Urinalysis data showed reduced pH (5.5-6) in males at the high dose throughout the study.

The absolute liver weight was decreased statistically significantly in females at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight the difference was statistically significant at all three doses. While in males, the absolute liver weight was decreased significantly at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight the difference was not statistically significant. The parotid salivary gland weight was increased significantly in males at 100, 300 and 1000 mg/kg bw per day (56-111%) after 52 weeks, but not after 104 weeks. The combined weight of the sublingual and submaxillary salivary glands was significantly increased by 13% (22% after correcting for body weight) at 1000 mg/kg bw per day after 52 weeks. In females, the parotid gland was not affected but the sublingual and submaxillary combined weight was significantly higher by about 15%. The changes in salivary gland weights were accompanied by increased incidence of mild to severe parotid salivary gland cell alterations and slight to moderate mandibular salivary gland cell alterations were observed in both sexes at the 52-week and 104-week intervals. The lesions were described as cells and/or acini that appeared larger and stained in a weakly basophilic manner without showing a tendency toward proliferative or degenerative changes over time. In males, the increased incidence and severity of lesions in the parotid gland were significant ($p \leq 0.01$) at 100, 300, and 1000 mg/kg bw/day at 52 weeks, and significant at 300 and 1000 mg/kg bw/day at 104 weeks. The increased incidence of lesions in the mandibular gland were significant at 300 and 1000 mg/kg bw/day at 52 weeks and significant ($p \leq 0.001$) at 100, 300, and 1000 mg/kg bw/day at 104 weeks. In females, the increased incidence of parotid lesions was significant at 300 and 1000 mg/kg bw/day at 52 weeks, and significant at 100, 300, and 1000 mg/kg bw/day at 104 weeks. The increased incidence in the mandibular gland lesions was significant at the high dose at both 52 and 104 weeks. The incidence and/or severity of kidney nephropathy decreased in males at 100, 300, and 1000 mg/kg bw/day at 52 weeks and at the high dose at 104 weeks. Urothelial hyperplasia significantly decreased in females from the high dose group at both the 52-week and 104-week intervals.

All groups had neoplastic lesions, however, No treatment-related neoplastic lesions were observed in male or female rats when histopathology data from treated groups were compared to that of controls at terminal sacrifice (104 weeks).

In conclusion, the LOAEL in male and female Sprague-Dawley rats administered glyphosate for 104 weeks in the diet was 100 mg/kg bw/day based on microscopic lesions in the parotid and mandibular

salivary glands. The NOAEL was 10 mg/kg bw/day. There was no treatment-related increase in tumour incidence at doses up to 1000 mg/kg bw per day (Atkinson et al. 1993).

Study 4: Milburn GM (1996) Glyphosate Acid: One Year Dietary Toxicity Study in Rats, Central Toxicology Laboratory, Zeneca unpublished report no. CTL/P/5143, study dates 3 April 1995 to 3 June 1996.

In a chronic study in rats, groups of 24 male and 24 female Alpk:APfSD (Wistar-derived) rats were given diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000 or 20000 ppm (equal to 0, 141, 560, and 1409 mg/kg bw per day for males and 0, 167, 671, and 1664 mg/kg bw per day for females) for 1 year. Analysis of diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study. The animals were monitored daily for mortality and clinical observations. Body weights and food consumption were measured and at the end of the scheduled treatment period, the rats were killed and subjected to a full examination post mortem. Blood and urine samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no unscheduled deaths during the course of the study that could be attributed to the administration of glyphosate. Apart from a small increase in the number of male and female animals in the group receiving glyphosate at 20000 ppm that showed wet or dry urinary staining, there were no other treatment-related clinical observations and no treatment-related ophthalmological findings. Bodyweights of top dose animals were lower than concurrent controls throughout the study (Table XX). Bodyweights of animals receiving 8000 ppm glyphosate were slightly reduced (not significantly in males and significantly only from week 46 in females). There was no effect on bodyweight in animals receiving 2000 ppm glyphosate. The changes in body weights in males and females were not considered adverse since the magnitude of change small (less than 10%). (PREVIOUS JMPR CONSIDERED ADVERSE AT 8000 and 20,000 PPM)

Table XX: Inter-group comparison of mean body weight (selected time points)

Weeks	Dietary concentration of glyphosate (ppm)							
	Males				Females			
	0 (control)	2000	8000	20000	0 (control)	2000	8000	20000
2	203.3	202.6	203.1	198.1**	158.0	156.5	155.6	151.7**
10	443.2	445.9	431.7	412.8**	256.2	252.5	251.1	248.3*
30	599.2	593.1	577.3	568.2*	305.6	306.0	298.3	291.8**
46	643.1	640.1	629.4	620.9	338.6	333.5	321.9*	322.1*
52	649.1	642.7	639.4	633.0	349.7	338.6	330.4*	327.3**

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

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

Food consumption was lower and food utilization was slightly less efficient at 20000 ppm, the reductions being most marked at the start of the study. There was a trend for reduced food intake for

females at 8000 ppm, which correlates with the reduction in body-weight gain at this dose in the latter stages of the study.

Table XX: Selected clinical chemistry findings in rats given diets containing glyphosate for 1 year

Parameter/week	Dietary Concentration of Glyphosate Acid (ppm)							
	Males				Females			
	0 Control	2000	8000	20000	0 Control	2000	8000	20000
Cholesterol/14	2.46	2.53	2.31	2.28*	2.13	2.28	2.26	2.21
27	3.09	3.05	2.75*	2.70**	2.62	2.67	2.76	2.78
Triglycerides/14	1.56	1.63	1.28**	1.28**	0.94	0.92	0.89	0.95
27	1.51	1.43	1.15**	0.97**	1.07	1.10	1.13	1.10
Alkaline Phosphatase/14	248	281	342**	429**	161	201*	227**	292**
27	221	250	306**	412**	135	171	200**	254**
53	232	258	291**	379**	87	100	114	160**
Aspartate Aminotransferase/ 27	120	105.3	108.4	113.3	157.1	130.4	116.9*	132.8
53	122.1	123.0	118.3	132.0	133.9	178.7*	198.3**	138.3
Alanine Aminotransferase/14	84.3	92.8	110.9**	109.6**	66.2	79.3	88.2**	90.5**
Creatine Kinase/14	118.2	123.5	127.3	143.7**	96.7	107.5	107.3	124.1**

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

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

From Milburn (1996)

Some statistically significant differences from control in haematological parameters were seen but there was no evidence of a relationship to dose, the differences were small and not seen consistently at all time points, therefore they were considered to be unrelated to the administration of glyphosate. Deviations in some clinical chemistry parameters, such as reductions in plasma concentration of cholesterol and triglycerides or a dose-related increase in plasma ALP activity throughout the study as well as occasional increases in the activities of plasma AST, ALT and creatine kinase, were mostly confined to groups receiving the high and intermediate doses (Table XX). In the absence of any histopathological findings these marginal changes are not considered to be of toxicological significance. There was no evidence of any effect of glyphosate on urine parameters. There were no findings at examination post mortem related to treatment. There were no treatment-related effects on organ weights.

At necropsy, there were no gross pathological findings that could be attributed to treatment and no consistent organ weight changes. An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland were seen in both sexes receiving 20000 ppm (Table XX). At 8000 ppm, examples of focal parotid basophilia were of minimal severity and the incidence was slightly above to that in the control animals. No other microscopic findings could be ascribed to administration of glyphosate.

Table XX: Incidence of focal basophilia of parotid acinar cells in rats given diets containing glyphosate for 1 year

Severity	Dietary concentration of glyphosate (ppm)							
	Males				Females			
	0 (control)	2000	8000	20000	0 (control)	2000	8000	20000
Minimal	2	0	3	10	2	0	6	8
Slight	0	0	0	3	0	0	0	5
Moderate	0	0	0	0	0	0	0	2

Similar numbers and types of neoplasms were diagnosed in the control group and in the group receiving glyphosate at 20000ppm, but the duration of the study was not sufficiently long to enable final conclusions to be made with regard to carcinogenicity.

In conclusion, the NOAEL in the 1-year toxicity study in rats was 8000 ppm; equal to 560 mg/kg bw per day based on the increased incidence of basophilia of parotid acinar cells seen at 20000 ppm; equal to 1409 mg/kg bw per day (Milburn, 1996).

Study 5: Suresh, T.P. (1996) Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats, Rallis Research Centre, Rallis India Ltd., Bangalore, India, Data owner: Feinchemie Schwebda GmbH, Study No.: 886.C.C-R, Date: 1996-07-18, GLP: not published, TOX9651587

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 96.8 and 90.0%, two batches) was administered to 50 Wistar rats/sex/dose in the diet for up to 2 years at a concentration of 0, 100, 1000, or 10,000 ppm (equal to 0, 6.3, 59.4 or 595.2 mg/kg bw per day for males and 0, 8.6, 88.5, or 886 mg/kg bw per day for females). In additional one vehicle control (acetone) with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. Veterinary examination was made before and after grouping and at the end of each month of experimental schedule. Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. 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. Individual blood samples were collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. At the scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex/group was subjected to clinical chemistry analysis. Individual urine samples

were collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months. Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups. Selected organs were weighed from 10 rats/sex/dose. 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, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

The stability of glyphosate was determined at 2000 and 2000 ppm which demonstrate that prepared diets were fairly stable for 30 days at room temperature with a loss of less than 7%. The analysis of diets indicated that the achieved concentrations were within acceptable range. There were no treatment related effects on mortality, clinical observations, body weights, body weight gains, food consumption, urinalysis and haematology. The following significant dose related changes of the blood chemistry parameters were seen at the high dose: a decrease in GGT level at 12 months in male rats, a decrease in albumin level at 6 months in female rats and increase in AP (alkaline phosphatase) level at 6, 12 and 18 months in female rats. The increase AP in high dose females were 235, 231, 194, and 249 (U/L) at 6, 12, 18 and 24 months, respectively, compared to corresponding control value of 133, 141, 101, 254 for females at 6, 12, 18 and 24 months, respectively.

There were no treatment-related macroscopic findings observed during the study period. There were no treatment-related findings observed in organ weights or relative organ weights. None of the significant microscopic changes, both increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed showed dose relationships, hence appeared to be incidental and not related to the treatment with the test compound. At terminal sacrifice, the incidence of cataracts in males were 3/20, 3/20, 1/18 and 6/29 at 0, 100, 1000 and 10,000 ppm, respectively. At terminal sacrifice, the incidence of cataracts in females were 1/24, 1/26, 5/33 and 4/21 at 0, 100, 1000 and 10,000 ppm, respectively. The historical data on neoplasm incidence for the test species indicates that the incidences of various tumours observed in the present study are within the range. The types of tumours seen were also comparable to the historical records. No statistically significant inter group difference between the control and low, mid and high dose treatment groups has been recorded in respect of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either sex-wise or for combined sex.

In conclusion, the NOAEL in this carcinogenicity study in rats is 10,000 ppm; equal to 595.2 mg/kg bw per day; the highest dose tested. There was no evidence of carcinogenicity in rats at glyphosate doses up to 10,000 ppm (Suresh, 1996).

Study 6: Bhide, R.M. (1997), Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat, Indian Institute of Toxicology, Pune India, Study Nol.: 1231, Sankyo Co., Ltd., Japan, not published.

In a combined chronic toxicity and carcinogenicity study, groups of 50 Sprague Dawley rats per sex received daily dietary doses of 0, 3000, 15000 and 25000 ppm (0, 0.15/0.21, 0.78/1.06 and 1.29/1.74 g/kg/day [M/F]) Glyphosate technical for two years. In addition, for the control and each dose group 20 rats per sex included for interim sacrifice in Week 52 to study non-neoplastic histopathological changes (chronic toxicity study). Selected dose levels were the same except for the highest dose which was 30000 ppm. Here the dietary doses correspond to 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively. Test diets were prepared

weekly by mixing appropriate amounts of the test substance with the basal diet. The stability and homogeneity of the test substance in food was determined in-house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were performed monthly during the study period.

No treatment-related clinical signs or deaths were observed in the satellite groups, e.g. the chronic toxicity study. In the carcinogenicity study, e.g. after 104 weeks, male animals of the high dose group exhibited slight but statistically insignificant higher mortalities. No significant toxic signs were observed in treated or control groups. Significantly reduced body weight gain that lasted throughout study until Week 104 was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination. There were no treatment-related effects on food consumption for either sex or group noted during the study. The results show a higher test material intake for females when compared to males for each dose level. The mean intake in the chronic toxicity study for each dose group is 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively. The mean intake in the carcinogenicity study for each dose group is 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0.21, 1.06 and 1.74 g/kg bw/day (females) for 3000, 15000 and 25000 ppm, respectively.

Ophthalmological examinations revealed no abnormalities. Haematological examination did not reveal any abnormalities attributable to the treatment. Regarding the clinical chemical investigations, a significant increase in the alkaline phosphatase level was only seen in the high dose of the carcinogenicity study at study termination. Other significant changes observed in haematological, and biochemical parameters were within the range of the historical control data and hence appear to be of no biological significance. Urinalysis did not reveal any abnormalities attributable to the treatment. There were no treatment-related macroscopic findings observed during the study period.

Significant and dose-dependent effects in the chronic toxicity study were found in both sexes of the high-dose group. In males, weights of kidneys, brain and testes were increased. In females, in addition to kidneys and brain, the liver weight was increased as well.

Histopathological changes were found at all dose levels including control, hence it is concluded that these are no treatment-related effects. There were no treatment-related neoplasms observed.

Based on mild toxic effects on body weight gain and the increased organ weights without histopathological changes, the NOAEL in rats after chronic exposure to Glyphosate technical for 24 months is 25,000 ppm (1290/1740 mg/kg bw/day [M/F]) (Bhinde, 1997).

Study 7: Enomoto, K. (1997), HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, The Institute of Environmental Toxicology, Kodaira-shi, Tokyo, Japan, Arysta Life Sciences, Study No.:IET 94-0150, 1997-07-01, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-11487, unpublished. Also referred to as Arysta Life Sciences, 1997.

In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats/sex/group received daily dietary doses of 0, 3000, 10000, and 30000 ppm (0, 104/115, 354/393 and 1127/1247 mg/kg bw/day [M/F]) HR-001. In addition, 30 rats/sex/group were included for interim

sacrifices at 26, 52, and 78 weeks.

In the 3000 dose ppm group, significant increases in incidence of decreased spontaneous motor activity, bradypnea, and soiled fur and a significant decrease in incidence of tactile hair loss were observed in males. Analysis of location of the soiled fur demonstrated predominant occurrences of the sign in the external genital region and foreleg. Females in this group showed significant increases in incidence of ptosis and tactile hair loss. In the 10000 ppm dose group, the incidence of tactile hair loss was significantly decreased in males and significantly increased in females when compared to the respective control.

In the 30000 dose group neither sex showed an increase in mortality, although mortality in males was lower than the control during the last half of the treatment period with statistical significance in most of the weeks. In all other groups mortality was comparable to control. Significant increases in incidence of bradypnea, palpable masses, and soiled fur were observed in males when compared to the control. Analysis of location of each mass showed that the ones in the tail were present in 27 males, which was apparently high in incidence compared to 11 of the control. The incidences of masses in other locations were comparable to the control. With respect to soiled fur, the sign was located at the external genital or perianal region. Males in this group also showed significant decreases in incidence of tactile hair loss, wound, and hair loss. In females, a significant increase in incidence of wetted fur was observed; the sign was mainly seen in the external genital region. Besides the signs mentioned above, loose stool was observed in all cages of this group from Week 24 in males and Week 23 in females until the end of the treatment.

The test compound at the doses tested did not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

Based on the effects in this study, the no observable adverse effect level (NOAEL) is 3000 ppm (104/115 mg/kg bw/day) and the LOAEL is 10000 ppm (354/393 mg/kg bw/day) based upon clinical signs (an increase in tactile hair loss in females). There was an increased incidence of multiple clinical signs at 30000 ppm (Enemoto, 1997).

Study 8: Brammer A (2001) Glyphosate Acid: Two Year Dietary Toxicity And Oncogenicity Study In Rats. Central Toxicology Laboratory, UK. Syngenta unpublished report no: CTL/PR1111/REGULATORY REPORT. Study dates: 1 April 1998 to 16 October 2000

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 97.6%) was administered to 64 Alpk:APfSD Wistar rats/sex/dose in the diet for up to 2 years at a concentration of 0, 2000, 6000, or 20,000 ppm (equal to 0, 121, 361, and 1214 mg/kg bw per day for males and 0, 145, 437, and 1498 mg/kg bw per day for females). An interim sacrifice was conducted on 12 rats/sex/dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in diet were determined. Clinical observations (including ophthalmoscopy), bodyweights, food consumption, haematology and clinical biochemistry (blood and urine), were measured throughout the study. A functional observational battery, including motor activity, was conducted in week 52 in animals allocated to the chronic toxicity assessment of the study. At the end of the scheduled period the animals were killed and subjected to a full examination post mortem. Cardiac blood samples were taken for

clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

The mean achieved concentrations of glyphosate in each dietary preparation were within 10% of the nominal concentration and the overall mean concentrations were within 1% of nominal. The diets were homogenously distributed and prepared diets were stable at room temperature for 45 days. Survival in the control, low and mid-dose group males approached 25% by week 104 of the study (criteria for termination of the study) although survival in the high-dose group was significantly better. Survival in the females was similar across all groups and better than in the lower dose group males. There was a treatment-related increase in the incidence of red-brown staining of tray papers (particularly in males), and isolated observations of red/brown coloured urine noted in 3 males and 1 female fed 20000 ppm glyphosate. The body weights of the males and females fed 20000 ppm glyphosate were statistically significantly lower than controls throughout the study, however, they were not considered as toxicologically relevant since maximum decreased in body weights were approximately 5 and 8% for males and females, respectively. Food consumption and food utilization was statistically significantly lower in high dose males and females. Ophthalmoscopic examination did not reveal any treatment related effects. There were no treatment-related observations noted in the functional observational battery, grip strength measurements, motor activity, landing foot splay measurements and time to tail flick. Haematological parameters were not affected by the treatment. There was a statistically significantly increases in alkaline phosphatase activity at all doses in both sexes up to week 79. There was evidence at one or more time points of increases in the activities of plasma alanine aminotransferase, aspartate aminotransferase and total bilirubin but statistically significant only at 6000 and/or 20000 ppm. In the absence of any histopathological findings these marginal changes are not considered to be of toxicological significance. Plasma triglycerides and cholesterol were consistently decreased for all or part of the study in males at 20000 ppm. Plasma creatinine values were lower in all treated female groups at week 27 and in females receiving 6000 and 20000 ppm at week 14, but in the absence of any effects later in the study, this is considered to be of no toxicological significance. Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate compared to controls. An increase in the incidence and severity of blood/red blood cells was present in males and, to a lesser extent, in females fed 20000 ppm glyphosate. There were no consistent, dose-related effects on organ weights that were considered to be indicative of a toxicologically significant effect of glyphosate.

Macroscopic findings were seen in males fed 6000 ppm and/or 20000 ppm and consisted of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes. A minor increase in the incidence but not severity of proliferative cholangitis in the liver was present in males fed 20000 ppm glyphosate at interim and terminal kill. Moreover, in males fed 20000 ppm glyphosate acid an increased incidence of hepatitis and periodontal inflammation was observed. There were a number of changes in the kidneys of both sexes fed 20000 ppm glyphosate, notably renal papillary necrosis, with or without papillary mineralisation, and transitional cell hyperplasia. The incidence was greater in males than females. These findings are considered to be related to treatment but are consistent with the feeding of high doses of an acidic material, which may also have caused the microscopically observed prostatitis and periodontal inflammation. The decrease in the incidence of tubular degeneration of the testis in males fed 20000 ppm is considered to be without adverse consequence (Table XX). The incidence of prostatitis was higher than the control groups in all treated males but it was within historical background levels in all treated groups but, as the control value in this study was low, the relationship to treatment at the high- dose level cannot be entirely dismissed.

Table XX- Inter-group comparison of selected microscopic findings

Finding	Dietary concentration of glyphosate (ppm)							
	Males (N=64)				Females (n=64)			
	0	2000	6000	20000	0	2000	6000	20000
Liver: Proliferative cholangitis	56	57	55	64	55	58	59	61
Liver: Hepatitis	8	6	9	13	6	7	4	6
Kidney: Papillary necrosis	0	1	0	14	0	1	2	5
Kidney: Transitional cell hyperplasia	2	3	0	5	3	1	0	1
Prostate: Prostatitis	13	22	23	37	-	-	-	-
Testis: Unilateral tubular degeneration	18	13	18	5				
Periodontal inflammation	25	27	23	42	18	24	32	28

In contrast to the previous one-year feeding study in rats by Milburn (1996) microscopic changes were seen in the liver and kidneys but not the salivary glands of rats fed 20000 ppm glyphosate even though the study was conducted on the same strain of the rats and in the same laboratory.

There was an increase in the incidence of hepatocellular adenomas in male rats at the high dose when compared to controls (Table XX). This increase was not considered to be treatment-related due to: 1) absence of dose-response relationship; 2) lack of progression to malignancy; 3) no evidence of pre-neoplastic lesions; 4) the incidences were within the range (0–11.5%) of historical controls for this strain (Wistar) of rats in 26 studies conducted during the relevant time period (1984–2003) at the testing laboratory; and 5) the 0% incidence in concurrent controls is lower than the average background incidence for liver adenomas in male Wistar rats.

Table XX. Incidence of hepatocellular adenomas in males rats.

Finding	Concentration in the diet (ppm)			
	0	2000	6000	20000
Adenoma	0/52#** (0%)	2/52 (4%)	0/52 (0%)	5/52* (10%)

Number of tumour-bearing animals/Number of animals examined.

In conclusion, the NOAEL is 6000 ppm; equal to 361 mg/kg bw per day based on indication for kidney, prostate and liver toxicity seen at 20,000 ppm; equal to 1214 mg/kg bw per day. There was no evidence of carcinogenicity in rats at glyphosate doses up to 20,000 ppm (Brammer, 2001).

Study 9: Wood, E., Dunster, J., Watson, P., Brooks, P (2009) Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat, Harlan Laboratories Ltd., Shardlow, Derbyshire, UK Study No.: 2060-0012, Date: 2009-04-23, amended 2009-05-08 not published, ASB2012-11490.

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 95.7%) was administered to 51 Han Crl:WI (GLx/BRL/HAN) IGS BR Wistar rats/sex/dose in the diet for up to 104

weeks at a concentration of 0, 1500, 5000, or 15000 ppm (equivalent to mean achieved dose levels of, 0, 95.0, 316.9 or 1229.7 mg/kg bw per day). To ensure that a received dose of 1000 mg/kg bw/day overall was achieved, the highest dose level was progressively increased to 24000 ppm. In addition, three satellite groups with 15 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. The satellite control group with 12 rats per sex served as veterinary control. The animals were to be used for investigations should any health problems have developed with study animals. No such problems occurred and therefore the observations of these animals have not been included in the report. Clinical signs, functional observations, body weight development and food and water consumption were monitored during the study. Clinical chemistry and haematological examinations were performed on ten animals per sex from the satellite and main groups at 3, 6 and 12 months. Further haematological and clinical chemistry investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. Urinalytical investigations were performed on ten animals per sex from satellite groups at 3, 6 and 12 months and from main groups at 18 and 24 months. Necropsy was conducted for all animals surviving until study termination (main groups: 104 weeks; satellite groups: 52 weeks) as well for all animals found dead or killed in extremis. Selected organs were weighed from 10 animals/sex/group that were killed at the end of the study and for all animals from satellite groups at termination. Histopathological examination was initially carried out on all tissues collected from control and high dose groups; all pre-terminally dead and moribund sacrificed rats and on all lesions and palpable masses of the terminally sacrificed rats from the low and mid dose groups. Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

Stability analysis demonstrated that the prepared diets were stable for at least six weeks. Analysis of the diet indicated that the achieved dietary concentrations were within acceptable range. No significant treatment related effects were observed on mortality, clinical signs, behavioural assessments, functional performance tests (motor activity, grip strength values), sensory reactivity, body weights, body weight gains, food consumption, water consumption, palpable masses, ophthalmoscopic examinations, haematology, clinical chemistry, urinalysis, organ weights, and macroscopic findings.

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, a generally greater effects were seen among male rats dosed at15000 ppm and this attained statistical significance for terminal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment for male rats at 15000 ppm. 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. There was a higher incidence of higher grades of severity of adipose infiltration seen among premature deaths but among premature deaths for animals of both sexes at 5000 ppm and females only at 1500 ppm. 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.

There was no influence of treatment upon the development of neoplasia in any organ or tissue, and no effect of treatment upon the overall frequency of benign or malignant tumours.

In conclusion, based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 15000 ppm (equal to mean achieved dose level of 1229.7 mg/kg bw/day); the highest dose tested. Glyphosate was not carcinogenic in rats at doses up to and including 15,000 ppm; the highest dose tested (Wood et al. 2009).

Study 10 A COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDY OF AK-01 BULK SUBSTANCE BY DIETARY ADMINISTRATION IN RATS

Nippon Experimental Medical Research Institute Co., Ltd, PROJECT No. H-95053, February 17, 1999
3303-58, Oaza Odo, Agatsuma-machi, Agatsuma-gun, Gunma

Study director Michiko Takahasi

Hardisty J. F. 2013. PATHOLOGY WORKING GROUP REVIEW OF THE HISTOPATHOLOGIC CHANGES IN THE KIDNEY A COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDY OF AK-01 BULK SUBSTANCE [GLYPHOSATE] BY DIETARY ADMINISTRATION IN RATS. NIPPON EXPERIMENTAL MEDICAL RESEARCH INSTITUTE CO., LTD, STUDY NO: H-95053, EPL PROJECT NUMBER 911-004

In a combined chronic toxicity and carcinogenicity study, groups of Fischer F344/DuCrlCrlj rats (50/sex/dose) were fed diets containing glyphosate (purity 97.5%) at concentrations of 0, 500, 4000 or 32000 ppm (equal to 0, 25, 201, and 1750 mg/kg bw per day for males and 0, 29.7, 239 and 2000 mg/kg bw per day for females) for 104 weeks. An interim sacrifice was conducted on 14 rats/sex/dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in diet were determined. Clinical observations (including ophthalmoscopy), bodyweights, food consumption, haematology and clinical biochemistry (blood and urine), were measured throughout the study. A functional observational battery, including motor activity, was conducted in week 52 in animals allocated to the chronic toxicity assessment of the study. At the end of the scheduled period the animals were killed and subjected to a full examination post mortem. Blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

Prepared diets were stable at room temperature for 4 months and the test compound was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations were ranged from 80 to 110% of the nominal concentrations. All males and females in the 32000 ppm group showed diarrhea or soft stool from immediately after the start of administration almost throughout the administration period. Mortality was not affected by the treatment. Statistically significantly reduced body weights were observed throughout the study in high dose males (beginning

week 1) and females (beginning week 2). Food consumption in all dosed group decreased or increased (no statistical significance) at various intervals. The only treatment related effects observed in urinalysis are increased urinary proteins in 3 females of the high dose group at 104 week. These changes were thought to be related to the histological changes in the kidney. There were no remarkable changes in males in any dose group, in females in any other dose group or at any other examination time. Males and females in the 32000 ppm group showed statistically significant decreases or tendencies toward decreases in erythrocyte count, hematocrit and hemoglobin concentration in Weeks 26, 52 and 78, and males in this group also showed significant increases in platelet count and leukocyte count in Week 52 and a significant increase in platelet count in Week 78. In the 4000 ppm group, females showed a significant decrease in erythrocyte count in Week 26 (94% of the control value) and males showed significant decreases in erythrocyte count (96% of the control value) and hematocrit (95% of the control value) in Week 52. In males and females in the 500 ppm group, there were no significant differences from those of the 0 ppm group in any examination item. The historical control values for haematological parameters from the performing laboratory were not available, however, the historical control data for Fisher Inbred Strain F344/ DuCrlCrlj were compared with study results. Through the study, except for at week 104, control group erythrocyte counts and hematocrit values are higher than the literature reported range for this strain of rats. This suggests that erythrocyte and hematology values for the control groups of the TAC study were unusually high, and that statistically significant decreases in test groups may not be toxicologically significant or relevant. Males and females in the 32000 ppm group showed tendencies toward decrease in albumin at each examination time, and the values were statistically significant in males and females in Week 26 and in males in Week 78 compared to those of the 0 ppm group. In addition, males in this group showed significant increases in γ-GTP, alkaline phosphatase and total bilirubin in Week 52. Otherwise the following changes were observed, but they were thought to be unrelated to administration of the test article since they were not observed continuously or they were not observed in the 32000 ppm group: significant decreases in creatinine, GPT and total bilirubin in males or females in Week 26 in the 32000 ppm group; and significant increases in creatinine, total protein and albumin in females in the 500 ppm group. Ophthalmoscopic examination of the eyes did indicated treatment related effects opacity in 1 female at Week 104 at high dose, which was considered as incidental change. In the 32000 ppm group, a statistically significant increase in the weight of the kidney weight (relative to body weight) was observed in males in the Week 79 scheduled sacrifice group and in males and females in the Week 105-106 scheduled sacrifice group. Otherwise the following changes were recorded, but they were thought to be changes due to suppressed body weight gain since there were no corresponding abnormalities in histopathological examination: significant increases in the relative weights of the brain and liver in males in the Week 79 and Week 105-106 scheduled sacrifice groups and females in the Week 105-106 scheduled sacrifice group and a significant decrease in the absolute weight of the adrenal in males in the Week 105-106 scheduled sacrifice group in the 32000 ppm group; and a significant decrease in the absolute weight of the brain in males in the Week 79 scheduled sacrifice group in the 4000 ppm group. At necropsy, males and females in the 32000 ppm group showed an increase in luminal dilatation of large intestine in the animals that were necropsied on schedule in Week 79 of administration, but there were no histological changes. Thymic involution was increased in the 32000 and 500 ppm groups in the data of all females and pituitary hypertrophy not recorded in the 32000 ppm group in the data of all males, but they were thought to be incidental changes since they are age-related changes and the incidence of the occurrence of each lesion was comparable.

In histopathological examination, an increase in glomerulosclerosis was observed in females in Week 105-106 scheduled necropsy group in the 4000 ppm group, and increases in eosinophilic granule/hyaline droplet in tubular epithelium in the kidney in females in Week 79 scheduled necropsy

group and in males and females in Week 105-106 scheduled necropsy group, and an increase in glomerulosclerosis in females in Week 105-106 scheduled necropsy group in the 32000 ppm group. Monsanto and TAC, co-sponsored the Pathology Work Group (PWG) to re-evaluate the microscopic kidney findings, specifically glomerulosclerosis, chronic nephropathy and hyaline droplet renal tubule degeneration in female rats. The PWG concluded (Hardisty 2013) that the kidneys of male and female rats from a 2-year oral (dietary) carcinogenicity study with glyphosate did not confirm the study pathologist's reported conclusions that the incidence of glomerulosclerosis and the presence of eosinophilic granules/hyaline droplets of renal tubule epithelium were related to test article administration. The PWG did not observe any histologic evidence of renal toxicity in the kidney sections examined. The only frequently observed finding in the kidneys of male and female rats was chronic progressive nephropathy which was similar in incidence and severity in control and treated groups. No treatment related tumours were observed in this study.

In conclusion, the NOAEL is 4000 ppm; equal to 201 mg/kg bw per day based on the decreased in body weights, transient hematological effects, diarrhea, urine parameters, clinical chemistry effects, increased kidney weight relative to body weight seen at 32000 ppm; equal to 1750 mg/kg bw per day; the highest dose tested. It was not carcinogenic in rats at doses upto 32,000 ppm (Takahasi, 1999 Japan TAC study).