B.5.3.2.3 DOG

At all, six subchronic dog studies are available. Among these, there were feeding studies as well as experiments performed by capsule administration of glyphosate. The duration of the dosing period varied between three months and approximately one year. In one of the experiments, an IPA salt based formulation was tested instead of the active ingredient. The studies are summarized in table B.5.3.2.3-1. It was decided to report the study by Gobordhun (1991) in detail since this is a completely valid and GLP-like study covering the longest administration period of one year.

Table B.5.3.2.3-1: Summary of subchronic oral studies with glyphosate in dogs

Study type	Dose levels	NOEL/NOAEL	Target organs/ Main effects	Reference	Submitted by (notifier)
Beagle dogs, 12-month study; capsule administration	0-30-300-1000 mg/kg bw/d	NOAEL: 300 mg/kg bw/d	Clinical signs; equivocal impact on body weight gain.	Gobordhun, 1991	Monsanto/Cheminova
Beagle dogs, 12-month study; capsule administration	0-20-100-500 mg/kg bw/d	NOEL: 500 mg/kg bw/d	No treatment-related effects.	Reyna, 1985	Original study not submitted but referred to by Monsanto/ Chemi-nova, Barclay, Sinon and Sanachem; evaluated by WHO/FAO (JMPR) in 1986
Mongrel dogs, 90-day feeding study with 30-d recovery period	0-100-250-500 mg/kg bw/d	NOEL: 250 mg/kg bw/d	Body weight gain and food consumption↓.	Bhide, 1985*	Luxan; Barclay
Beagle dogs; 6-month study; capsule administration of an IPA salt based formulation (MON 0139)	0-10-60-300 mg/kg bw/d	NOAEL: 300 mg/kg bw/d	AP↑; equivocal impact on body weight gain.	Reyna, 1983	Monsanto/Chemi-nova
Beagle dogs, 12-month feeding study	0-30-100-300 ppm	NOAEL: 300 ppm (ca 8 mg/kg bw/d)	Histology: equivocal liver findings.	Verecskey and Csanyi, 1982*	Alkaloida
Beagle dogs, 3-month feeding study	0-200-600-2000 ppm	NOAEL: 600 ppm (15 - 29 mg/kg bw/d)	Liver: organ weight↓, congestion, equivocal histological findings.	Verecskey and Csanyi, 1981*	Alkaloida

* study of limited scientific value providing supplementary information only

B.5.3.2.3.1 DOG, 12-MONTH STUDY

Gobordhun, R. (1991): Glyphosate: 52 week oral toxicity study in dogs. Inveresk Research International Ltd., Tranent, Scotland; IRI Report no. 7502, Project no. 642675. Dates of experimental work: 29 August 1989 - 30 August 1990. The study was submitted as part of the joint dossier of Monsanto and Cheminova.

Material and methods:
Test method: The study was run according to a guideline of the U.S.EPA (FIFRA, 83-1) and was in compliance with OECD guideline 452 ("Chronic Toxicity Studies", adopted 12 May 1981).
Deviations: Some examinations were carried out in excess of guideline requirements (see below). Neither summary incidences nor individual data on the occurrence of clinical signs were given in the study report.
GLP: Yes.
Acceptance: The study is considered acceptable.

Test system: Four male and four female purpose bred Beagle dogs (supplied by Consort Ltd., Hereford, England) per dose received glyphosate technical (source: Cheminova A/S, Lemvig, Denmark) orally, by capsule administration, once daily for 52 consecutive weeks. Three batches of the test material (206-Jak-25-1, purity: 98.6%; 206-Jak-59-5, purity: 99.5% and 229-Jak-5-1, purity: 98.9%) were used during the course of the study. The bulk powder was encapsulated in hard, clear gelatin capsules. The individual test substance amount to be given was calculated weekly on the basis of each dog's most recently recorded body weight. Multiple capsule administration was necessary in the high dose groups and, to ensure equal conditions, in the controls. The dose levels of 0 (vehicle control group receiving empty capsules), 30, 300 and 1000 mg/kg bw/day were selected on the basis of the results from a previous maximum tolerated dose study (Gobordhun and Oshodi, 1989; see B.5.3.1.3.1). Regular analyses of the capsule preparations revealed deviations of the actual from the nominal compound concentration varying within tolerable limits (<5%). Encapsulated glyphosate was shown to be stable for at least 7 days.
The animals were kept under standard conditions and had free access to domestic mains quality drinking water. Each dog was offered 400 g of a complete dry diet per day, approximately one hour following daily capsule administration.
In-life and *post mortem* examinations were performed in accordance with or even in excess of the OECD guideline requirements. Only these additional investigations are mentioned here. Ophthalmoscopy was performed at pre-test and again during weeks 13, 29, 39 and 51 of treatment. Blood was drawn for laboratory investigations for the first time before treatment commenced already. In addition, plasma level studies and faecal analysis for the presence of occult blood were performed. Activity of red blood cell, plasma and, at termination, of brain cholinesterase activity was determined. At necropsy, adrenals, brain, gonads, heart, kidneys, liver, lungs, pancreas, pituitary, prostate, spleen, thymus, thyroid and uterus were weighed and then examined microscopically, together with the other organs mentioned in the guideline. Tissues and organs from all animals were subjected to histopathology. With regard to effects observed in a long-term study in rats (see section B.5.5.1.1), it should be noticed that also the salivary glands (submaxillary, sublingual and parotid glands) were weighed and examined histologically.
Statistics: Statistical methodes applied included the "F-max" test, a parametric ANOVA, Student's t-test using Fischer's F-protected leAST significance difference test, the Kruskall-Wallis ANOVA test and Dunn's z-test.

Results:
All animals survived throughout the study period. It was stated in the study report that the only clinical sign attributed to treatment was the more frequent occurrence of changes in faecal consistency (soft/loose/liquid) in the high dose animals 4 - 6 hours after dosing. This finding was also recorded on isolated occasions for a few animals of the intermediate dose groups. Unfortunately, the numeric values for the number of affected individuals and the frequency of such episodes cannot be given since these data were not contained in the original report.
The body weight tended to be lower in all treated male groups and in high dose females. This was due to a decrease in body weight gain, however, statistical significance was not reached. Food consumption was not affected.

Table B.5.3.2.3.1-1: Total body weight gain (kg) in Beagle dogs administered glyphosate for 52 weeks

Dose level (mg/kg bw/d)	0	30	300	1000
Male animals	4.8	4.0	3.6	3.6
Female animals	3.6	3.9	4.1	2.9

Haematology, blood clinical chemistry, urinalysis and faecal analysis did not reveal treatment-related effects. Plasma level studies indicated good proof of absorption and dose-related levels of glyphosate. Cholinesterase activity was

not impaired. Organ weights were not affected. There were no remarkable gross or histopathological findings that could be attributed to compound administration.

Conclusion:
The NOAEL in this study was 300 mg/kg bw/day based on the changes in faecal consistency suggesting a rather common unspecific effect of treatment. A NOEL could not be established since a minor impact on body weight gain at least in male dogs cannot be excluded.
The study author, in contrast, had argued that this decrease was due to random variation since statistical significance was not reached and assumed the NOEL at this mid dose level, therefore.

B.5.3.2.3.2 FURTHER STUDIES IN DOGS (90 DAYS, 6 OR 12 MONTHS)

Bhide, M.B. (1985): Subacute oral toxicity in dogs (for 90 days) of glyphosate (technical) of Excel Industries Limited Bombay. Indian Institute of Toxicology, Bombay, India; Report identification and dates of experimental work not given. The study report was submitted to the Rapporteur by the notifier Luxan and, independently, by the notifier Barclay. The report contains also the description and the results of a preliminary 14-day dose finding study. The study is considered supplementary due to serious reporting deficiencies, e.g. the year when the study was performed is not indicated in the original report. A respective information is given only in the notifiers Tier I documents. Statistical analysis of the results was not reported.
Groups of three Mongrel dogs per sex and dose were administered glyphosate (purity not reported; for manufacturer see study title above) orally via their food at target dose levels of 0 (control group receiving 0.2% agar solution mixed in mutton soup), 100, 250 and 500 mg/kg bw/day for 90 days. In addition, a second (reversal) group receiving the mid dose of 250 mg/kg bw/day was sacrificed after a 30-day recovery period following treatment. Animals were observed daily for signs of toxicity. Body weight and food consumption were determined regularly. Blood samples for haematological (red and white cell parameters) and clinical chemistry (total serum protein, ALT, AP, blood urea nitrogen, glucose) investigations were taken at pretest, on day 46 and on days 91 and 121 just prior to sacrifice. Urinalysis was also performed. All animals were subjected to gross pathological examination and histopathology. Organ weights were determined.
There were no deaths during the study and no signs of toxicity were observed. Laboratory investigations and pathological examinations did not reveal indications of adverse effects. According to the study scientists, ALAT was increased in high dose animals, however, the respective mean values were exceptionally high at pretest already. The only findings which could be attributed to treatment were a reduction in body weight gain and, during the second part of the study, a decrease in food consumption. These effects were noted in both sexes but were confined to the highest dose level. Thus, the mid dose of 250 mg/kg bw/day is considered the NOEL in this study.

Reyna, M.S. (1983): Six month study of MON 0139 administered by gelatin capsule to Beagle dogs. Monsanto Environmental Health Laboratory, St. Louis, Missouri; Study no. 810166, Project no. ML-81-368. Dates of experimental work: 26 January 1982 - 13 August 1982. The report was submitted as part of the joint dossier of Monsanto and Cheminova. When the study was performed, GLP was not compulsory. The study is considered acceptable.
MON 0139 (62.49% isopropylamine salt of glyphosate, manufactured by Monsanto) was administered orally, via gelatin capsules, to groups of six male and six female Beagle dogs at daily dosages of 0, 10, 60 and 300 mg/kg bw for approximately 6 months. These dose levels were selected based upon the results of a six-week range-finding study in which moderate to severe emesis was

observed at doses of 300 mg/kg bw/day and above. The methods used for investigations generally complied with OECD guideline 409 (adopted 12 May 1981). All animals survived until termination of the study. There were no clinical signs of toxicity. In high-dose males, the body weight gain tended to be less than in the control group, however, statistical significance was not reached. No effect on body weight was noted in females. Food consumption was not affected. Ophthalmoscopy, haematology and urinalysis did not reveal evidence of treatment-related adverse effects. An apparent increase in alkaline phosphatase activity was observed in top dose males from the second measurement onwards reaching statistical significance in month five only. No other clinical chemistry parameters were affected. There were no consistent differences in organ weights and no pathological findings which could be attributed to treatment. Since the increase in AP activity was not accompanied by any other indication of liver damage and because the possible impact on body weight gain in one sex only at the highest dose level was of equivocal significance and could be well due to normal variation, the highest dose of 300 mg/kg bw/day is considered the NOAEL. The notifier even proposed to established the NOEL at this dose, however, the increase in enzyme activity should be taken into account since this can be assumed a treatment-related effect although, in the absence of overt toxicity, it is not considered an adverse one.

Vereczkey, L. and Csanyi, E. (1981): 3 month oral dietary toxicity study with glyphosate in dogs. Institute for Drug Research, Budapest, Hungary; Study no. 8012. Dates of experimental work: 24 November 1980 - 26 February 1981. The revised English version (issued in 1991) of the original Hungarian report was submitted to the Rapporteur by Alkaloida. Due to serious reporting deficiencies, this study is considered supplementary. When the study was performed, GLP was not compulsory.
Glyphosate (purity and manufacturer not stated since the respective supplement as mentioned in the study report was not available to the Rapporteur) was administered to groups of four male and four female Beagle dogs for three months via their food at target dietary concentrations of 0, 200, 600 and 2000 ppm. All animals survived the dosing period and there were no clinical signs of toxicity. Body weight and food consumption were not affected. Haematology, clinical chemistry (two sampling times during and near the end of the dosing period), urinalysis (prior to termination) and gross pathological examination did not reveal indications of adverse effects. It is stated in the report that liver weight was marginally lower at the highest dose level exhibiting weak statistical significance but numeric values were not given. In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" was described. In a smaller number of dogs (2 males, 1 female animal) and to a lesser degree, this was also seen at the mid dose level. This change was characterized by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three male and all female dogs in the highest dose group. The toxicological significance of these findings is equivocal. It should be taken into account that in the 12-month dog study in the same laboratory similar liver effects were noted but were not seen in any other dog study with glyphosate obtained from other manufacturers. It seems most appropriate to establish a NOAEL on the basis reduced liver weight at 600 ppm with an actual daily compound intake varying between 15 and 29 mg/kg bw.

Verecskey, L. and Csanyi, E. (1982): 12 month dietary toxicity study with glyphosate in dogs. Institute for Drug Research, Budapest, Hungary; Study no. 8012; dates of experimental work not given in the report. The revised English version (issued in 1992) of the original Hungarian report was submitted by Alkaloida. Due to reporting deficiencies, this study is considered supplementary only. When the study was performed, GLP was not compulsory.

Four Beagle dogs per sex and dose were fed diets containing 0, 30, 100 or 300 ppm of glyphosate (purity and source not specified). Body weight, food consumption and organ weights were not affected. There were no treatment-related clinical signs and no haematological, clinical chemistry, gross or histopathological changes with the possible exception of rounded hepatocytes and narrower sinusoids observed in the livers of some (2/4) high dose male dogs and mid (2/4) and high dose (3/4) females but not in the low dose and in the control groups. Since there was no further evidence of morphological or functional liver alterations the reported findings may be treatment-related, however, cannot be considered adverse effects. Thus, the NOAEL in this study was the highest dose of 300 ppm (about 8 mg/kg bw/day for the sexes combined).

A further study in Beagle dogs **(Reyna, 1985)** was performed at Monsanto Environmental Health Lab. (St.Louis, U.S.A.) and was submitted by Monsanto to the JMPR of WHO/FAO for its 1986 evaluation of glyphosate. It was not submitted to the Rapporteur for EU reregistration purposes although it is cited in the joint dossier of Monsanto and Cheminova. However, this study had been made available to the regulatory authorities in Germany for national registration purposes some years ago. The study is considered acceptable. A brief description and assessment of this study was published by FAO in 1987 (Pesticide residues in food, 1986 evaluations, Part II - Toxicology). The dossiers of the notifiers Barclay, Sinon [Shinung] and Sanachem refer to this JMPR evaluation.
Groups of 6 male and female dogs each were administered technical glyphosate (purity: 96.1%) in gelatin capsules at dose levels of 0, 20, 100 or 500 mg/kg bw/day for approximately one year. Body weight was not affected. There were no clinical signs of toxicity and no remarkable findings upon haematology, blood and urine clinical chemistry, gross and histopathological examination. As mentioned in the original study report, the pituitary weight was reduced in mid and high dose males. The respective mean organ weights were 0.084 g, 0.080 g, 0.062 g and 0.068 g in the control, low, mid and high dose groups. Because of the only small difference, the lack of concomitant histopathological findings and the absence of a consistent dose-response, this finding in one sex is not considered a toxicologically significant effect. In addition, one should notice that there were no remarkable findings in the pituitary or related organs neither in this nor in any other study with glyphosate. Thus, the highest dose of 500 mg/kg bw/day was the NOEL in this study.

B.5.3.3 OTHER ROUTES

B.5.3.3.1 DERMAL STUDIES

Three subacute (21 or 28 days) dermal studies with the active ingredient in rabbits and one in rats were submitted to the Rapporteur. These studies are summarized in Table B.5.3.3.1-1.

Table B.5.3.3.1-1: Subacute dermal studies with glyphosate in rabbits and rats

Species/strain/ duration of study	Dose levels	NOEL/NOAEL	Target organs/ Main effects	Reference	Submitted by (notifier)
NZW rabbit, 28 days (20 applications)	0-500-1000-2000 mg/kg bw/d	NOEL: 2000 mg/kg bw/d	No effects observed.	Tornai, 1994	Alkaloida
NZW rabbit, 21 days (15 applications) followed by 14-d recovery	0-500-1000-2000 mg/kg bw/d	NOEL: 2000 mg/kg bw/d	No effects observed.	Bhide, 1985*	Luxan; Barclay
NZW rabbit, 21 days (15 applications)	0-100-1000-5000 mg/kg bw/d	NOEL (systemic): 5000 mg/kg bw/d	Dermal irritation at the highest dose. No systemic effects.	Johnson, 1982	Monsanto/Cheminova
Sprague-Dawley rat, 21 days (daily administration)	0-1000 mg/kg bw/d (limit test)	NOEL (systemic): 1000 mg/kg bw/d	Evidence of weak dermal irritation. No systemic effects.	Heath, 1993	Monsanto/Cheminova

* study considered supplementary only

B.5.3.3.1.1 RABBIT

The study by Johnson (1982) is described in detail since a higher dose level than in the other studies was tested and since some local effects were observed.

Johnson, D.E. (1982): 21-day dermal toxicity study in rabbits. International Research and Development Corporation, Mattawan, Michigan; Study no. 401-168, Monsanto no. IR-81-195. Dates of experimental work: 28 July - 19 August 1981. The study was submitted as part of the joint dossier of Monsanto and Cheminova. This study had been evaluated by the JMPR of WHO/FAO in 1986. The notifiers Sinon [Shinung] and Sanachem refer in their dossiers to this evaluation as published by FAO in 1987 (Pesticide residues in food, 1986 evaluations, Part II - Toxicology).

Material and methods:
Test method: Not applicable. The study was run in accordance with standard operating procedures of the performing laboratory and with an internal protocol as approved by the sponsor.
Deviations: Not applicable.
GLP: No. When the study was performed, GLP was not compulsory.
Acceptance: The study is considered acceptable.
Test system: Glyphosate (purity not stated in the original report; manufacturer: Monsanto) was applied dermally at dosages of 100, 1000 or 5000 mg/kg bw/day to groups of 10 male and 10 female New Zealand White rabbits (source: Davidson´s Mill Farm, Jamesburg, New Jersey) per dose, five days a week for three consecutive weeks. One-half of the animals had intact skin whereas in the remaining half skin was abraded. A control group of the same size did not receive the test compound but was otherwise handled in the same manner. The test material (moistened with saline) was applied on each treatment day for six hours to the shorn back of the animals (approximately 30% of the body surface) and was covered by a gauze patch. Following removal, the test site was washed. The animals were daily observed for mortality, signs of systemic toxicity and irritation. Food consumption was estimated daily and body weights were recorded twice weekly. At termination, blood was drawn from five male and female rabbits per dose group for haematological and biochemical investigations. Following sacrifice, all animals were subjected to complete necropsy and selected organs were weighed. Histopathological examination was performed on a wide range of organs and tissues from all animals on study.
Statistics: Standard methods like analysis of variance, Bartlett´s test and an appropriate t-test using Dunnett´s multiple comparison tables were applied.

Results:
There were no premature deaths during the study. No clinical signs of systemic toxicity were noted. Body weight and food consumption were not affected. Haematological and clinical chemistry tests did not reveal evidence of adverse effects. Organ weights did not show notable intergroup differences and no treatment-related macroscopic or microscopic pathological lesions were observed. The only finding of toxicological significance was dermal irritation at the highest dose level characterized by doubtful or barely perceptible to very slight erythema and doubtful or barely perceptible edema. These changes were observed in rabbits with intact skin as well as in those with abraded skin. Dermal irritation did not cause lesions which were visible upon histopathological examination of the treated skin sites.

Conclusion:
No systemic toxicity was observed up to the highest dose level of 5000 mg/kg bw/day (`systemic NOEL´). In contrast, rather slight dermal irritation occurred at this top dose level.

Tornai, A. (1994): Repeated dose 28-day dermal toxicity study with glyphosate in rabbits. Institute of Toxicology, Keszthely, Hungary, on behalf of Alkaloida; Test code GLY-94-410/N, Sponsor´s Report no. MÜF 214/94. Dates of experimental work: 23 May 1994 - 20 June 1994. The report was submitted by Alkaloida. GLP: yes. The study is considered acceptable although dermal irritation scores were not tabulated.
Glyphosate (purity 99.6%; manufacturer: Alkaloida, Tiszavasvary, Hungary) was tested for dermal toxicity in New Zealand White rabbits over a period of 28 days. Groups of five male and five female rabbits each were exposed to dose levels of 0, 500, 1000 and 2000 mg/kg bw/day five times a week for 4 consecutive weeks. The test substance was applied to the clipped intact back of the animals under occlusion. Exposure time was six hours per treatment day. The study design and the investigations performed were in compliance with OECD guideline 410 (adopted 12 May 1981).
There was no mortality during the study and clinical signs of systemic toxicity did not occur. Signs of dermal irritation (very slight erythema) were seen in some animals in various dose groups but did not suggest a clear dose response. Considerable differences in body weight gain were noted among the groups, in particular in males, however, no obvious dose-related trend was to be seen. Food consumption was not affected. There were minor changes in a few clinical chemistry parameters like slightly higher blood urea concentrations in high dose males but according to the original report these values were still within the normal range observed for this kind of rabbits in the performing laboratory. Haematology, comparison of organ weights and gross and histopathological examinations did not reveal evidence of treatment-related adverse effects. Thus, the highest dose level of 2000 mg/kg bw/day can be considered the NOEL in this study.

Bhide, M.B. (1985): Subacute dermal toxicity (for 21 days in rabbits) of glyphosate (technical) of Excel Industries Ltd., Bombay. Indian Institute of Toxicology, Bombay, India; Report identification and dates of experimental work not given. The study report was submitted to the Rapporteur by the notifier Luxan and, independently, by the notifier Barclay. GLP: no. The study is considered supplementary only due to serious reporting deficiencies. Statistical analysis of the results was not reported. The report includes also the description of a range-finding study (dose levels up to 2000 mg/kg bw/day) with repeated dermal application of glyphosate to rabbits for 5 days. However, only the main study is briefly reported in this monograph.
Glyphosate (purity not stated; for manufacturer see study title above) was dermally applied for an exposure period of six hours per day to the intact skin of New Zealand White rabbits. The dose levels were 0, 500, 1000 and 2000 mg/kg bw/day and the groups consisted of three male and three female rabbits each.

Treatment was performed on five days a week for three consecutive weeks. The dosing period was followed by a 14-day reversal interval before sacrifice. The animals were daily observed for toxic signs and skin irritation. Food consumption was calculated daily and body weights were determined weekly. Laboratory investigations (haematology, clinical chemistry, urinalysis) were performed on days 0, 21 and 35. At termination, all animals were subjected to gross and histopathological examination.
There were no premature deaths and no signs neither of systemic toxicity nor of dermal irritation. Body weight and food consumption were not affected. Laboratory investigations and pathological examination did not reveal evidence of substance-related effects. The top dose level of 2000 mg/kg bw/day is considered the NOEL in this dermal rabbit study.
Remark:
According to the evaluation of glyphosate by the JMPR (1986, published in 1987), a further 21-day dermal study including a 28-day recovery period (Killeen, 1975) was performed in rabbits using an isopropylamine salt formulation. Effects were confined to dermal irritation. However, since this study was not made available to the Rapporteur, it could not be taken into consideration for this evaluation.

B.5.3.3.1.2 RAT

Heath, J. et al. (1993): Glyphosate: 3 week toxicity study in rats with dermal administration. Inveresk Research International Ltd., Tranent, Scotland; IRI Report no. 7839, Project no. 450881. Dates of experimental work: March/April 1992. The study was submitted as part of the joint dossier of Monsanto and Cheminova. The GLP-like study is considered acceptable.
A group of 5 male and 5 female Sprague-Dawley rats was dosed daily with 1000 mg glyphosate (98.7% pure, supplied by Cheminova A/S, Lemvig, Denmark)/kg bw via the dermal route over a period of 3 weeks. The rats were exposed for ca 6 hours per day. A second group of the same size received dermally the vehicle diethylphthalte only to act as a control. The study was performed as a limit test according to U.S.EPA recommendations. Blood samples were collected for haematology and clinical chemistry screens during week 3. At termination, rats were necropsied and selected organs weiged. All animals underwent limited histological examination.
No evidence of any systemic effects of treatment was elicited neither by in life observations, laboratory investigations nor *post mortem* examinations. Thus, the NOEL for systemic toxicity was 1000 mg/kg bw/day in this limit test. In contrast, mild transitory irritant effects (erythema and desquamation) were noted at the dosing site in the animals of the glyphosate-treated group.

B.5.3.3.2 INHALATIVE STUDIES

Only one study for inhalation toxicity of the active ingredient was submitted.

Bhide, M.B. (1985): Report on subacute inhalation toxicity in rats (14 days) of glyphosate (technical) of Excel Industries Ltd., Bombay. Indian Institute of Toxicology, Bombay, India; Report identification and dates of experimental work not given. The study report was independently submitted to the Rapporteur by the notifiers Barclay and Luxan.

Material and methods:
Test method: Not applicable.
Deviations: Not applicable.
GLP: No. When the study was performed, GLP was not compulsory.
Acceptance: The study is considered supplementary only since an effect dose was lacking. In addition, there were some reporting deficiencies. Furthermore,

statistical analysis of the results obtained was either not performed or not reported.
Test system: Four groups of 5 male and 5 female Wistar rats (bred at Indian Institute of Toxicology, Bombay, India) were exposed to an atmosphere containing glyphosate (purity not stated; for manufacturer see title above) for 6 hours a day, 5 days per week for two weeks. There were one low and one high dose group and two intermediate dose groups. One of the latter groups was sacrificed 14 days after the treatment period had been finished (reversal group). Two control groups of the same size were also included, one of them being exposed to filtered air only and the other to an atmosphere containing the vehicle propylene glycol. Glyphosate was mixed with the vehicle and nebulised by using compressed air. The animals were exposed in a dynamic inhalation chamber by mouth and nose route by restraining them in polypropylene tubes. The target, the nominal and the actually measured mean concentrations for the various groups are given in table B.5.3.3.2-1. With the exception of the exposure periods, animals were housed five per cage and had free access to food and drinking water.
The rats were examined individually before, during and after exposure for any signs of toxicity. Food consumption was recorded daily and body weight was determined at pretest and then on every 4th day during the study and on the day of termination. Blood samples for haematological (red and white blood cell parameters, thrombocyte count and prothrombin time) and clinical chemistry (total serum protein, blood urea nitrogen, glucose,ALAT and AP) investigations were taken before first exposure and after one week from some animals of each group and from all animals (except the recovery group) after the last exposure. From reversal group rats, blood was obtained at termination. Urinalysis was also performed. All rats were necropsied and the following organs were removed and weighed: adrenals, gonads, heart, kidneys, liver, and spleen. These organs as well as aorta, brain, eyes, intestines, larynx, lungs, lymph nodes (axillary and mesenteric), nose, oesophagus, pancreas, pituitary, seminal vesicle, stomach, thyroid, trachea, urinary bladder and uterus were examined microscopically.

Table B.5.3.3.2-1: Target, nominal and actually measured mean concentration (mg/l air) of the test material or the vehicle

Dose group	Target concentration	Nominal concentration	Measured concentration
Control (filtered air)	0	0	0
Vehicle control	5.0	16.5	5.6
Low dose	0.25	0.90	0.28
Intermediate dose	1.0	3.01	0.93
Intermediate dose (reversal)	1.0	3.10	0.90
High dose	4.0	11.7	3.8

Results
Atmosphere analysis: The actually achieved mean concentrations are given above. Mass median aerodynamic diameters of all atmospheres were within the respirable range of 0 - 7 µm on all occasions. The temperature, humidity, oxygen concentration and air flow rate were similar for all groups.
General observations: There was no mortality during the treatment or the recovery periods. Neither treatment-related clinical signs nor effects on body weight or food consumption were observed. In the vehicle control group and in all groups exposed to glyphosate, salivation, lacrimation, redness to nose and urination were observed during exposure. No change in the rate of respiration was seen.
Haematology, clinical chemistry, urinalysis: No adverse effects of treatment were noted in any of the groups.
Pathology: No significant changes in organ weights or upon gross examination were seen. Histopathology did not reveal evidence of adverse effects.

Conclusion:
Up to the highest concentration tested of approximately 3.8 mg/l air (mean actual concentration), repeated inhalative exposure of Wistar rats to glyphosate

did not exhibit any local (respiratory) or systemic toxicity. Only unspecific signs of reaction to treatment were observed during exposure. Using the conversion factor of 270 for rats, repeated inhalative exposure to a concentration of 3.8 mg/l air would correspond to a mean daily oral intake of 1026 mg/kg bw. This value would markedly exceed the dosages at which effects have been observed in subacute oral studies on rats.

Published literature

A further 4-week inhalation study was carried out in rats on behalf of Monsanto using a 1:3 dilution of a Roundup formulation. The original study was not submitted to the Rapporteur but has been evaluated by the WHO as part of the International Programme on Chemical Safety (IPCS) as published in 1994 (Environmental Health Criteria, 159, WHO, Geneva). The notifiers Sinon [Shinung] and Sanachem refer to this evaluation.

In this study (Monsanto Environmental Health Laboratory, St. Louis, Missouri; 1983), test concentrations representing 17, 53 and 120 mg Roundup/m^3 air were administered to rats as an aerosol spray for 6 hours/day on 5 days a week for four consecutive weeks. According to IPCS, the endpoints evaluated were survival, growth, (limited) haematology and blood biochemistry, organ weights and (limited) histopathology. In high dose females, an increase in the incidence of irritation of nasal turbinates (subacute inflammation), trachea (mononuclear cell infiltration) and lungs (perivascular lymphoid infiltrates/aggregates) was noted. No signs of systemic toxicity were reported. However, local respiratory tract irritation at a concentration of 0.120 mg/l air in female rats suggests a higher inhalation toxicity of the formulation tested as compared to the active ingredient. Using the conversion factor of 270 for rats, one could calculate a corresponding mean daily oral intake of 32.4 mg/kg for the formulation tested. This effect level would be below the lowest NOEL established in the subacute oral studies with glyphosate on rats.

B.5.4 MUTAGENICITY TESTING

Glyphosate was extensively tested for mutagenicity in a wide range of *in vitro* and *in vivo* systems. The extent and the quality of the database are considered sufficient for a reliable assessment. For evaluation of the mutagenic potential, one should additionally take into account that the compound is apparently not cancerogenic (see section B.5.5) and not teratogenic (see B.5.6.2). On the basis of the available data, the overall conclusion can be drawn that the active ingredient does not exhibit a genotoxic risk for humans.

Glyphosate does not cause gene (point) mutations neither in bacteria nor in mammalian cells. Likewise, the compound proved negative in the UDS assay *in vitro*. The potential of glyphosate to cause chromosome aberrations was investigated to a rather limited extent *in vitro* and by means of the bone marrow micronucleus test and cytogenetic analysis in a large number of studies *in vivo*. Most of these experiments did not reveal any indication of clastogenicity. There was only limited evidence of a possible compound-related effect in one study (Suresh, 1993) in which a higher frequency of micronuclei in bone marrow cells was seen in female mice receiving the extremely high dose of 5000 mg/kg bw/day by oral gavage on two consecutive days. In male animals, this finding was not confirmed. The high variation in micronucleus incidence among female dose groups and the possible occurrence of cytotoxicity at the highest concentration suggest that this isolated positive result could be due to chance. A cytogenetic study in the same laboratory (Suresh, 1994) was performed in the same mouse strain under similar conditions (i.e., dosages, administration route and frequency were identical) but failed to indicate structural chromosome aberrations suggesting that glyphosate was not clastogenic. The single oral administration of 5000 mg glyphosate kg/bw did not cause an increase in micronucleus incidence (Jensen, 1991). Glyphosate proved consistently negative in dominant lethal tests in rats and mice. However, when administered to male rats up to a dose as high as 5000 mg/kg bw, a temporary effect on reproduction was observed as a result of the apparent acute toxicity.

B.5.4.1 *IN VITRO* TESTING

B.5.4.1.1 TESTS FOR GENE (POINT) MUTATIONS

B.5.4.1.1.1 BACTERIA

Glyphosate was tested for the ability to induce gene mutations in a number of tests in bacterial systems. Usually, the method of Ames was followed. Most of these investigations were performed with glyphosate acid being the test material, however, occasionally salts or formulations (see B.5.4.3) were also tested. All the studies which were considered acceptable or at least supplementary as well as data obtained from the literature are summarized in table B.5.4.1.1-1. One experiment (Jensen, 1991) is described in detail since this GLP-like study is considered representative for the majority of Ames tests performed with glyphosate. The results of this study are in particular reliable since two different test methods were used both leading to the same conclusion. Beginning with the most recent one, all the further original studies which have been submitted to the Rapporteur for purposes of this evaluation together with published data are listed below.

Table B.5.4.1.1.1-1: Summary of tests for gene mutations in bacteria

Study type	Test material/ Purity	Test organism	Dose range/ Metabolic activation	Result	Reference	Submitted by (notifier)
Ames test	Glyphosate, 95%	S.typhimurium strains TA 98, 100, 1535, 1537, and 1538	8.0 - 5000 µg/plate; -/+ S9	Negative	Thompson, 1995	Herbex
Ames test	Glyphosate, purity not given	S.typhimurium strains TA 98, 100, 102, 1535 and 1537	50 - 5000 µg/plate; -/+ S9	Negative	Fassio, 1995	I.Pi.Ci.
Ames test	Glyphosate, 96%	S.typhimurium strains TA 98, 100, 1535, 1537, and 1538	1 - 1000 µg/plate; -/+ S9	Negative	Suresh, 1993*	Feinchemie
Ames test	Glyphosate IPA salt, 64%	S.typhimurium strains TA 98 and 100	0.01 - 100 µg/plate	Negative	Wang et al., 1993*	Sinon [Shinung]
Ames test	Glyphosate, 98.6%	S.typhimurium strains TA 98, 100, 1535, 1537	-S9: 160 - 2500 µg/ plate; +S9: 310 - 5000 µg/ plate	Negative	Jensen, 1991	Monsanto/ Cheminova
Ames test	Glyphosate, purity not given	S.typhimurium strains TA 98, 100, 1535, 1537, and 1538	8.0 - 5000 µg/plate; -/+ S9	Negative	Jenkinson, 1990*	Agrichem
Ames test	Glyphosate, purity not given	S.typhimurium strains TA 98, 100, 1535, 1537 and E.coli stain WP-2uvrA	10 - 1000 µg/plate -/+ S9	Negative	Bhide, 1986*	Barclay; Luxan
Ames test	Glyphosate, 98.4%	S.typhimurium strains TA 98, 100, 1535, 1537, 1538 and E.coli WP2 hcr strain	10 - 5000 µg/plate; -/+ S9	Negative	Shirasu et al., 1978; published by Li and Long, 1988	Monsanto/ Cheminova

* The study is considered to provide supplementary information only.

The studies with glyphosate active ingredient being the test material consistently revealed negative results in all tested strains of *Salmonella typhimurium* and *Escherichia coli*. In the experiment using the isopropylamine salt of glyphosate (Wang et al., 1993), no mutagenicity was observed but the dose levels tested were too low for definitive assessment.

Jensen, J.C. (1991): Mutagenicity test: Ames Salmonella assay with glyphosate, batch 206-JaK-25-1. Scantox A/S, Lille Skensved, Denmark on behalf of Cheminova; Laboratory no. 12323. Dates of experimental work: 5 February 1991 - 8 March 1991. This report was submitted as part of the joint dossier of Monsanto and Cheminova.

Materials and methods:
Test method: The study was performed in compliance with the requirements of OECD guideline 471 ("Salmonella typhimurium, Reverse mutation assay", adopted 1983).
Deviations: None.
GLP: A formal certificate of a national authority is lacking. However, it is stated in the original report that the investigations were carried out in compliance with the principles of GLP. A Quality Assurance Statement is included.
Acceptance: The study is considered acceptable.
Test system: Glyphosate (Batch 206-JaK-25-1, purity: 98.6%, supplied by Cheminova A/S, Lemvig, Denmark) was tested in the Ames test using the Salmonella typhimurium strains TA 100, TA 98, TA 1537 and TA 1535 for the ability to induce gene mutations. TA 100 and TA 1535 are specific testers for chemicals causing

base substitutions whereas TA 98 and TA 1537 are tester strains for frameshift mutations. Testing was done in the presence and absence of an external metabolic activation system (S-9 mix, prepared on the basis of rat liver microsomes). The concentrations tested were 160, 310, 630, 1300 and 2500 µg/plate without metabolic activation and 310, 630, 1300, 2500 and 5000 µg/plate in the trials with addition of an S-9 mix. These dosages had been selected on the basis of a preliminary toxicity test on strain TA 98. Mutagenicity testing was performed in two independent test series using two different methods: the plate incorporation assay and the preincubation assay. Three plates were used per dose and test. Positive and negative controls were included in both test series. The negative control substance was not specified. Positive control substances in the experiments without activation were sodium azide for the strains TA 100 and TA 1535 and 2-nitrofluorene for TA 98 and TA 1537. In the presence of S-9 mix, 2-aminoanthracene served as positive control material for all four strains. Evaluation criteria were not specified in the report.
Statistics: Analysis of variance and Student´s t-test were applied.

Results:
The test article did not induce statistically significant increases in the number of revertant colonies in any of the strains neither with nor without metabolic activation. In contrast, the positive control substances gave the expected responses. There were no clear signs of cytotoxicity observed. However, the generally lower number of revertants at the upper glyphosate concentrations might indicate that some toxicity occurred.

Conclusion:
Glyphosate was found non-mutagenic in this Ames test.

Further gene mutation tests in bacterial systems:
Thompson, P.W. (1995): Glyphosate: Reverse mutation assay "Ames test" using Salmonella typhimurium. Safepharm Laboratories Ltd., Derby, U.K.; Project no.: 710/20. Dates of experimental work: 25 August 1994 - 06 October 1994. This report was submitted by the notifier Herbex Produtos Quimicos. GLP: yes. The study is considered acceptable.
Remark: The purity of the test substance was not given in the original study report but a statement is included that this determination was the responsibility of the sponsor. Indeed, information on purity is included in the notifiers data package submitted to the Rapporteur.

Fassio, F. (1995): Study of the ability of the test article glyphosate to induce gene mutations in strains of Salmonella typhimurium. Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A., Colleretto Giacosa, Toskana, Italy; Experiment no. 940724. Dates of experimental work: 23 January 1995 - 06 February 1995. This report was submitted by the notifier Industria Prodotti Chimici (I.PI.CI.). GLP: yes (self-certification of the laboratory). The study is considered acceptable although the purity of the test substance was not given. However, it is stated that this information may be found in the notifiers "Confidential data package".

Suresh, T.P. (1993): Mutagenicity - Salmonella typhimurium reverse mutation assay (Ames test). Test compound: glyphosate technical (FSG 03090 H/05 March 90). Rallis Agrochemical Research Station, Bangalore, India; Study no. TOXI:887-MUT.AMES. Dates of experimental work: December 1992/January 1993. This report was submitted by the notifier Feinchemie. GLP: yes. The study is considered supplementary only since the highest dose tested (1000 µg/plate) was too low for definitive assessment of genotoxicity in the Ames test. It has been shown in other experiments that much higher dose levels of glyphosate can be tested.

Wang, S.-C.; Feng, H.-T. and You, B.-Y. (1993): Mutagenicity evaluation of glyphosate in Salmonella/microsomal reversion assay (Ames test). Taiwan

Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung, Taiwan; Report no. 87BMA012-E, original Chinese version issued in 1987. Dates of experimental work: 11 November 1986 - 30 November 1986. The report (English translation) was submitted by the notifier Sinon [Shinung]. GLP: no. When the study was performed, GLP was not compulsory. The study is at best considered supplementary only for the following reasons: The title is misleading because not glyphosate acid itself but the isopropylamine salt has been tested. It is not clear whether the given purity refers to the contents of glyphosate in the formulation or to the salt. Only two tester strains were included in the experiment. The dose levels tested were much too low. Thus, the scientific value of the study is rather limited, however, additional information is provided since it is one of the only few studies in which a salt and not the acid was tested for genotoxicity. Therefore, it was not excluded from evaluation despite the serious concerns.

Jenkinson, P.C. (1990): Agrichem glyphosate active: Reverse mutation assay "Ames test" using Salmonella typhimurium. Safepharm Laboratories Ltd., Derby, U.K.; Project no.: 300/1, Company file: R235. Dates of experimental work: 22 March 1990 - 06 April 1990. This report was submitted by the notifier Agrichem [Glyphosate Tulip Task Force]. GLP: yes (self-certification of the laboratory). The study is considered supplementary only since the purity of the test material was not reported.

Bhide, M.B. (1986): Report on mutagenicity tests with glyphosate (technical of Excel Industries Limited, Bombay. Ames bacterial test. Indian Institute of Toxicology; Report identification and data of experimental work not indicated. This report was submitted independently by the notifiers Barclay and Luxan. GLP: no. When the study was performed, GLP was not compulsory. The study is considered supplementary only since the highest dose tested (1000 µg/plate) was too low for definitive assessment of genotoxicity in the Ames test. Furthermore, there was no second experiment for confirmation of the results. In addition, serious reporting deficiencies were noted.

Shirasu, Y.; Moriya, M. and Ohta, T. (1978): Microbial mutagenicity testing on CP 67573 (glyphosate). Institute of Environmental Toxicology, Japan; Study no. ET 78-241. Dates of experimental work: not given. This report was submitted as part of the joint dossier of Monsanto and Cheminova. Results of further mutagenicity tests performed as part of this study are reported under section B.5.4.1.3 below. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable although there were some reporting deficiencies. It should be noticed that the main results of this study were published by Li and Long (1988, for complete reference see volume 2). Some notifiers refer to this publication.

The following study was considered unacceptable for evaluation purposes: **Antal, A. (1981)**: Mutagenic testing of glyphosate active principle and of glyphosate product: Ames and host mediated test. This report has been submitted by the notifier Alkaloida. The description of material and methods was so poor that an evaluation of the reliability of the results obtained (the test substance was considered non-mutagenic in both experiments) was not possible.

Published literature

Chan and Mahler (1992) reported an Ames test in four *Salmonella typhimurium* strains (TA 97, TA 98, TA 100 and TA 1535) as part of testing glyphosate in the U.S. National Toxicology Program. The test material was applied at concentrations ranging from 10 or 33 up to 10000 µg/plate with and without metabolic activation. The S9 mix was prepared on the basis of liver homogenates from rats and from Syrian hamsters and all activation trials were run with S9 mix from both sources and always used at two different concentrations (10 and 30%). In all experiments, glyphosate was not mutagenic confirming the results of

the original studies. The information on the testing procedure in this publication is rather limited. In particular, the purity of the test compound is not stated.

B.5.4.1.1.2 MAMMALIAN CELLS

In contrast to the extensive testing of glyphosate for gene mutations in bacteria, only two studies covering this endpoint in mammalian cells are available. A study in mouse lymphoma cells (Jensen, 1991) is described in detail since this experiment meets the current standards and since the method used is not only capable of detecting gene mutations but may also provide information on a clastogenic potential. All information available is summarized in table B.5.4.1.1-2. The studies are reported and/or briefly listed below the table. There was no evidence that glyphosate could cause gene mutations in mammalian cells. In addition, no indications of a clastogenic potential *in vitro* were obtained in an appropriate test system.

Table B.5.4.1.1-2: Summary of tests for gene mutations in mammalian cells

Study type	Test material/ Purity	Test system	Dose range/ Metabolic activation	Result	Reference	Submitted by (notifier)
Mouse lymphoma test	Glyphosate, 98.6%	Mouse lymphoma cells (L5178Y)	-S9: 0.61 - 5.0 mg/ml; +S9: 0.52 - 4.2 mg/ml	Negative	Jensen, 1991	Monsanto/ Cheminova
HGPRT assay	Glyphosate, 98.7%	Chinese hamster ovary (CHO) cells	-S9: 5 - 22.5 mg/ml; +S9: 5-25 mg/ml	Negative	Li, 1983; published by Li and Long, 1988	Monsanto/ Cheminova

Jensen, J.C. (1991): Mutagenicity test: In vitro mammalian cell gene mutation test with glyphosate, batch 206-JaK-25-1. Scantox A/S, Lille Skensved, Denmark on behalf of Cheminova; Laboratory no. 12325. Dates of experimental work: 4 April 1991 - 13 May 1991. This report was submitted as part of the joint dossier of Monsanto and Cheminova.

Materials and methods:
Test method: The study was performed in compliance with OECD guideline 476 ("In vitro mammalian cell gene mutation test", adopted 1983).
Deviations: None.
GLP: A formal certificate of a national authority is lacking. However, it is stated in the original report that the investigations were carried out in compliance with the principles of GLP. A Quality Assurance Statement is included.
Acceptance: The study is considered acceptable.
Test system: Glyphosate (Batch 206-JaK-25-1, purity: 98.6%, supplied by Cheminova A/S, Lemvig, Denmark) was added to cultures of mouse lymphoma (L5178Y) cells at dose levels of 0.61, 1.3, 2.5 and 5.0 mg/ml without metabolic activation and of 0.52, 1.0, 2.1 and 4.2 mg/ml in the activation trials. These dose ranges were determined on the basis of a preliminary toxicity test. The S9-mix for metabolic activation was produced by adding some chemicals to liver homogenates obtained from Wistar rats. For mutagenicity testing, cell cultures were exposed to the test material over a period of 4 (without activation) or 3 hours (in the presence of S9-mix). Thereafter, cells were sedimented by centrifugation and resuspended in fresh medium. The cell cultures were than seeded in microtiter wells in medium containing trifluorothymidine (TFT) which is selective for cells that have mutated from $TK^{+/-}$ to $TK^{-/-}$ as well as in normal medium. After an incubation period of 8 - 10 days, mutation frequency was calculated by counting and comparing the number of cell clones grown in both types of media.

Glyphosate was tested in two independent assays (with and without activation each). Duplicate cultures were used for each dose level. A solvent control (not specified) and positive controls were included in both assays. Positive control substances were 100 µg ethylnitrosourea/ml (without S9-mix, both test series) and dimethylbenzanthracene in the activation experiments (5 µg/ml in the first and 10 µg/ml in the second assay).
Evaluation criteria: According to the protocol of the study which was also submitted to the Rapporteur, at least a statistically significant and reproducible increase in the mutation frequency as compared to the negative control cultures and a dose-response were considered indicative of mutagenicity. An additional criterion was a mutation frequency at the dose level where the highest effect is found that was more than twice the concurrent spontaneous mutation frequency.
Statistics: Analysis of variance was performed. In the study protocol, the Chi-square test was also mentioned.

Results:
The mutation frequencies of the test cultures were generally similar to those seen in the negative control cultures in both test series with and without metabolic activation. There was no consistent and statistically significant increase neither in the occurrence of large nor of small colonies. Solvent control mutation frequency was in the expected range in both test series and, in contrast, the positive control substances gave a clearly positive response.

Conclusion:
Glyphosate was found non-mutagenic under the conditions of this mouse lymphoma cell gene mutation test. Since there was no increase in the number of small TFT-resistant colonies, there was also no indication of the induction of chromosome aberrations.

Further study for gene mutations in mammalian cells
Li, A.P. (1983): CHO/HGPRT gene mutation assay with glyphosate. Monsanto Environmental Health Laboratory, St. Louis, U.S.A.; Study no. ML-83-155, Project no. 830079. Dates of experimental work: 21 June 1983 - 09 September 1983. This report was submitted as part of the joint dossier of Monsanto and Cheminova. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable. It should be noticed that the main results of this study were published by Li and Long (1988). Some of the other notifiers refer to this publication.

B.5.4.1.2 TESTING FOR CHROMOSOME ABERRATIONS

Only two studies covering this endpoint *in vitro* were available (see table B.5.4.1.2-1). In both experiments, glyphosate proved negative. The clastogenic potential of glyphosate was mainly studied *in vivo* (see section B.5.4.2.1). In general, studies *in vivo* are considered much more relevant to predict a possible risk for humans. Accordingly, no further *in vitro* testing should be required. It is also not necessary to describe the available *in vitro* experiments in detail. Thus, they are only briefly listed here.

Table B.5.4.1.2-1: Summary of *in vitro* tests for chromosome aberrations

Study type	Test material/Purity	Test system	Dose range	Result	Reference	Submitted by (notifier)
Cytogenetic study (two independent assays)	Glyphosate, 96%	Peripheral human lymphocytes, exposure time: 24 and 48 h without metabolic activation; 3 h with activation (harvest after 24 or 48 h)	-S9 mix: 33 - 333 µg/ml; +S9 mix: 237 - 562 (both experiments taken together)	Negative	Van de Waart, 1995	Agrichem (Task Force)
Cytogenetic study	Glyphosate, purity not given	Chinese hamster ovary cells (CHO-K1), 3 h exposure (with/without metabolic activation)	-S9: 250 - 1000 µg/ml; +S9: 62.5 - 250 µg/ml	Negative	György, 1989*	Alkaloida

* The study is considered to provide supplementary information only.

Van de Waart, E.J. (1995): Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat). NOTOX B.V., 's-Hertogenbosch, The Netherlands; NOTOX Project 141918, Company file no. R480. Dates of experimental work: 15 March 1995 - 28 May 1995. This report was submitted by Agrichem [Glyphosate Tulip Task Force]. GLP: yes (self-certification of the laboratory). A formal GLP certificate of a national authority has not been submitted. The study is considered acceptable.

György, B. (1989): Report on the possible chromosome damaging effect of glyphosate in Chinese hamster ovary cells. National Institute of Hygiene, Department of Morphology, Budapest, Hungary. Report identification and dates of experimental work were not given. This report was submitted by the notifier Alkaloida. GLP: no. When the study was performed, GLP was not compulsory. The study is considered supplementary only due to reporting deficiencies, e.g. source and purity of the test material were not stated.

B.5.4.1.3 DNA DAMAGE AND REPAIR

The ability of glyphosate (acid) to react with cellular DNA and to cause DNA damage and repair was studied *in vitro* by means of assays for unscheduled DNA synthesis (UDS). One of these studies (Rossberger, 1994) is described in detail. In addition, one rec assay and one DNA repair test with glyphosate are available. Tests for the induction of sister chromatid exchanges (SCE) are also reported under this section since this method may elucidate changes on the DNA level although a positive SCE test can provide useful information concerning other endpoints of mutagenicity (e.g. chromosome aberration), too. All these studies are briefly listed below only. They are summarized in table B.5.4.1.3-1.

Both UDS assays with glyphosate acid being the test substance were clearly negative suggesting that the active ingredient does not react with cellular DNA.

Similarly, glyphosate proved negative in a Rec assay (Shirasu et al., 1978). In contrast, in a DNA repair assay using the isopropylamine salt (Wang et al., 1993a), cell growth of a DNA polymerase I-deficient *E.coli* strain was slightly inhibited at the highest dose level. This result was not confirmed in an independent experiment. Nontheless, the finding could indicate a weak effect on cellular DNA but such an effect was not observed in the other experiments measuring a possible impact on the DNA. Also, the IPA salt of the same glyphosate content proved negative in an SCE assay (Wang et al., 1993b), however, the concentrations tested were rather low. The same applies to the SCE test performed by Jenkinson (1990).

Table B.5.4.1.3-1: Summary of tests for DNA damage and repair *in vitro*

Study type	Test material/Purity	Test system	Dose range	Result	Reference	Submitted by (notifier)
UDS assay	Glyphosate, >98%	Rat primary hepatocytes (Sprague-Dawley)	0.20 - 111.69 mM	Negative	Rossberger, 1994	Feinchemie
UDS assay	Glyphosate, 98.7%	Rat primary hepatocytes (F344)	up to 125 µg/ml	Negative	Williams, 1983; published by Li and Long, 1988	Monsanto/ Cheminova
DNA repair ($pol A^+/A^-$) assay	Glyphosate IPA salt, 64%	E.coli strains W3110 ($pol A^{+}$) and P3478 ($pol A^-$)	0.0001 - 10 mg/ml; 20 µl/disk	Equivocal	Wang et al., 1993a*	Sinon [Shinung]
Rec assay	Glyphosate, 98.4%	Bac.subtilis strains H 17 and M 45	20 - 2000 µg/disk	Negative	Shirasu et al., 1978*	Monsanto/ Cheminova
SCE test	Glyphosate IPA salt, 64%	Chinese hamster ovary (CHO) cells (with and without metabolic activation)	0-1 - 100 µg/ml	Negative	Wang et al., 1993b*	Sinon [Shinung]
SCE test	Glyphosate, purity not given	Human lymphocytes (with and without metabolic activation)	78.125 - 625 µg/ml	Negative	Jenkinson, 1990*	Agrichem

* The study report is considered to provide supplementary information only.

Rossberger, S. (1994): DNA repair test with primary rat hepatocytes. ANAWA München AG, Planegg, Germany; Report of test 931564. Dates of experimental work: 01 February 1994 - 18 March 1994. This report was submitted by the notifier Feinchemie.

Material and methods:
Test method: It is stated in the report that OECD guideline 482 ("DNA damage and repair/Unscheduled DNA synthesis in mammalian cells in vitro", adopted 1986) was followed. However, for analysis of the results an essentially different method than recommended in the guideline was used.
Deviations: Instead of autoradiography or liquid scintillation counting procedures, incorporation of radioactivity into the DNA was determined on the basis of UV absorbance measurement and mathematical calculations.
GLP: Yes.
Acceptance: The study is considered acceptable.
Test system: Glyphosate technical grade (Lot F/93/032, purity: >98%, supplied by Feinchemie Schwebda GmbH, Köln, Germany) was tested in two separate experiments for the induction of UDS in primary hepatocytes obtained from male Sprague-Dawley rats by means of the bromodeoxyuridine density-shift method enabling the discrimination of DNA repair synthesis from normal DNA replication. The test compound was diluted in William´s medium E (also serving as negative control in this study) to give six concentrations per experiment ranging from 0.20 mM to 48.98 mM (test 1) and from 1.14 to 111.69 mM (test 2). These concentrations had been selected by pretesting the solubility and cytotoxicity of the test

material. Rat liver cells were exposed to glyphosate for 18 hours in the presence of 200 µM 5-bromodeoxyuridine (density label), 40 µm fluorodeoxyuridine and 10µCi ^{3}H-deoxycytidine per ml medium, the latter used for radioactive DNA labeling. Following incubation and preparation (isolation) of DNA, repaired (i.e. radiolabeled) DNA was separated from replicated (bromodeoxyuridine-substituted) DNA by ultracentrifugation in caesium salt density gradients. Incorporation of radioactivity into DNA was calculated by integrating the UV absorbance peak (exactly conincident with radioactivity binding) and dividing it by the amount of "light" (parental) DNA. Quantification of DNA (converting the integrated UV peak into µg DNA) was performed by means of a calibration curve. Dimethylnitrosamine and 2-acetamidofluorene served as positive control substances in this study.
Evaluation criteria: A test substance causing a reproducible and significant dose-related increase in radiolabel incorporation would be considered genotoxic.

Results:
Up to the highest concentrations tested, glyphosate did not significantly increase the frequency of DNA repair above negative control levels. In contrast, the positive control substances markedly enhanced DNA repair demonstrating that both cell viability and drug metabolizing enzyme activities were well preserved in the hepatocyte preparations.

Conclusion:
Under the conditions of this study, glyphosate did not cause DNA damage and repair.

Further tests for DNA damage and repair *in vitro*:
Wang, S.-C.; Feng, H.-T. and You, B.-Y. (1993): Mutagenicity evaluation of glyphosate in *Escherichia coli* DNA repair (polA^{+}/A^{-}) assay. Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung, Taiwan; Report no. 87BME014-E, original Chinese version issued in 1987. Dates of experimental work: 20 November 1986 - 05 December 1986. The report (English translation) was submitted by the notifier Sinon [Shinung]. GLP: no. When the study was performed, GLP was not compulsory. The title is misleading because not glyphosate acid but the isopropylamine salt has been tested. The study is considered supplementary only since it is not clear whether the given purity refers to the contents of glyphosate in the formulation or to the salt. A confirmatory experiment was not conducted even though there was some inhibition of cell growth at the top dose level.

Wang, S.-C.; Feng, H.-T. and You, B.-Y. (1993): Mutagenicity evaluation of glyphosate in sister chromatid exchange (SCE) assay. Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung, Taiwan; Report no. 87BMS013, original Chinese version issued in 1987. Dates of experimental work: 11 November 1986 - 30 November 1986. The report (English translation) was submitted by the notifier Sinon [Shinung]. GLP: no. When the study was performed, GLP was not compulsory. The title is misleading because not glyphosate acid but the isopropylamine salt has been tested. The study is considered supplementary only since it is not clear whether the given purity refers to the contents of glyphosate in the formulation or to the salt.

Jenkinson, P.C. (1990): Agrichem glyphosate active: OECD 479 sister Chromatid exchange in human lymphocytes in vitro. Safepharm Laboratories Ltd., Derby, U.K.; Project no.: 300/2, Company file: R237. Dates of experimental work: 30 May 1990 - 19 June 1990. This report was submitted by the notifier Agrichem [Glyphosate Tulip Task Force]. GLP: yes (self-certification of the laboratory). The study is considered supplementary only since the purity of the test material was not reported.
Williams, G.M. (1983): The hepatocyte primary culture/DNA repair assay on compound JJN-1020 (glyphosate) using rat hepatocytes in culture. American Health

Foundation, Naylor Dana Institute for Disease Prevention, Valhalla, New York on behalf of Monsanto; Report no. AH-83-181. Dates of experimental work: 31 August 1983 - 18 October 1983. This report was submitted as part of the joint dossier of Monsanto and Cheminova. GLP: No. When the study was performed, GLP was not compulsory. The study is considered acceptable although there were some reporting deficiencies, e.g. purity of the test material was not given (however, in the notifiers Tier II document, a purity of 98.7% is stated). It should be noticed that the main results of this study were published by Li and Long (1988, for complete reference see volume 2). Some notifiers refer to this publication.

Shirasu, Y.; Moriya, M. and Ohta, T. (1978): Microbial mutagenicity testing on CP 67573 (glyphosate). Institute of Environmental Toxicology, Japan; Study no. ET 78-241. Dates of experimental work: not given. This report was submitted as part of the joint dossier of Monsanto and Cheminova. Results of further mutagenicity tests performed as part of this study are reported under section B.5.4.1.1 above. GLP: no. When the study was performed, GLP was not compulsory. The study is considered supplementary only since the experiment was not conducted with metabolic activation. In addition, there were some reporting deficiencies. The results of this study have been reported by Li and Long (1988) and some notifiers refer to this publication.

B.5.4.2 *IN VIVO* TESTING

B.5.4.2.1 SOMATIC CELLS (STUDIES FOR CHROMOSOME ABERRATIONS)

A number of bone marrow micronucleus tests and cytogenetic studies in mice and rats have been performed to investigate the ability of glyphosate to cause chromosome aberrations *in vivo*. The results of these tests are reliable since it can be assumed from the kinetic studies (see chapter B.5.1.1) that the bone marrow is reached by the test substance. Beginning with the most recent, all available studies which were considered acceptable or at least supplementary are summarized in table B.5.4.2.1-1.

All but one of the original studies available to the Rapporteur unequivocally revealed that glyphosate does not cause chromosome aberrations *in vivo*. Limited evidence of a positive result is confined to the micronucleus test performed by Suresh (1993) suggesting that a very high daily dose of 5000 mg/kg bw/day administered on two consecutive days might increase the increase of micronuclei at least in female mice. Therefore, it was decided to report and discuss this study in detail whereas all the other experiments are only briefly listed below.

Table B.5.4.2.1-1: Summary of *in vivo* tests for chromosome aberrations

Study type	Test system	Test material/ purity	Dose levels/ Sampling time	Results	Reference	Submitted by (notifier)
Cytogenetic study	Swiss albino mice, bone marrow; daily oral administration on two successive days	Glyphosate, 96.8%	0-50-500-5000 mg/kg bw/d; sampling 24 h after the second dose	Negative for clastogenicity; mitotic index↓ at 5000 mg/kg bw	Suresh, 1994	Feinchemie
Micronucleus test	Swiss albino mice, bone marrow; daily oral administration on two successive days	Glyphosate, 96.8%	0-50-500-5000 mg/kg bw/d; sampling 24 h after the second dose	Male mice: negative; female mice: positive at 5000 mg/kg bw	Suresh, 1993	Feinchemie
Micronucleus test	NMRI mice, bone marrow; single oral administration	Glyphosate, 98.6%	0-5000 mg/kg bw; sampling after 24, 48 and 72 h	Negative	Jensen, 1991	Monsanto/ Cheminova
Micronucleus test	BKW mice, bone marrow; single oral administration	Glyphosate, purity not reported	0-4000 mg/kg bw; sampling after 24, 48 and 72 h	Negative for clastogenicity; signs of toxicity at 4000 mg/kg bw	Jenkinson, 1990*	Agrichem
Micronucleus test	NMRI mice, bone marrow; single oral administration	Glyphosate, purity not reported	0-2000 mg/kg bw; sampling after 24, 48 and 72 h	Negative	Antal et al., 1989*	Alkaloida
Cytogenetic study	Sprague-Dawley rats, bone marrow; single i.p. injection	Glyphosate, 98.7%	0-1000 mg/kg bw; sampling after 6, 12 and 24 h	Negative	Li, 1983; see also Li and Long, 1988	Monsanto/ Cheminova

* study providing supplementary information only

Suresh, T.P. (1993): Mutagenicity - Micronucleus test in Swiss Albino mice. Test compound: glyphosate technical (FSG 03090 H/05 March 90). Rallis Agrochemical Research Station, Bangalore, India; Study no. TOXI:889-MUT.MN. Dates of experimental work: 08 October 1991 - 12 October 1991. This report was submitted by the notifier Feinchemie.

Material and methods:
Test method: Testing was performed according to OECD guideline 474 ("Genetic toxicology - Micronucleus test", adopted 1984).
Deviations: The repeated (twofold) application of the test material is not sufficiently justified since glyphosate may cause cytotoxicity in the bone marrow as indicated by a reduction in mitotic index at the highest dose of 5000 mg/kg bw/day. However, this effect was only established when a cytogenetic study was performed one year later in the same laboratory using identical dose levels. Another deficiency is the lack of historical control data from the performing test facility.
GLP: Yes (according to self-certification of the laboratory). A formal certificate of a national authority for the time when the study was performed is lacking. However, it should be noticed that the test facility was inspected by representatives of the German GLP Federal Office in 1992 and GLP compliance was confirmed.
Acceptance: The study is considered acceptable.
Test system: Five male and five female Swiss albino mice (bred at Toxicology Department, Rallis Agrochemical Research Station, Bangalore, India) per group were administered glyphosate (purity: 96.8%; manufactured by M/s EPIC-Schwebda Chemicals Ltd., New Bombay, India) once daily for two consecutive days by oral

gavage at a constant dosing volume of 10 ml/kg bw. The dose levels of 50, 500 and 5000 mg/kg bw/day were selected on the basis of a preliminary dose range study. A negative control group (10 males/10 females) receiving the vehicle (i.e. refined groundnut oil) only and a positive control group of 5 animals per sex were also included in the study. Cyclophosphamide (100 mg/kg bw/day) served as positive control substance. The animals were sacrificed 24 hours after the second administration and bone marrow cell smears from femur were prepared on slides and stained by a combination of May-Gruenwald and Giemsa staining. A minimum of two thousand erythrocytes per animal (evaluated on four slides) was scored for polychromatic and normochromatic erythrocytes (PCE/NCE) and the incidence of micronuclei. Evaluation criteria were not given in the report.
Statistics: The methods applied were one way ANOVA and a t-test for the comparison of the percentages of micronucleated cells in the various groups and for assessment of dose-effect relationship.

Results:
General observations: There were no compound-related toxic signs and no mortalities in the treatment groups. At sacrifice, however, many animals in the mid and high dose groups as well as all mice receiving cyclophosphamide had lost body weight.
Micronucleus test: In female mice, a slight but statistically significant increase in the incidence of micronucleated red blood cells was noted at the top dose level. No increase was seen in the low and mid dose groups in females and at all dose levels in male animals. The ratio between polychromatic and normochromatic erythrocytes suggests that there was no significant cytotoxicity. Cyclophosphamide gave the expected increase in the occurrence of micronuclei in both sexes. This was accompanied by strong evidence of cytotoxicity as indicated by a markedly affected PCE:NCE ratio. The results are summarized in table B.5.4.2.1-2.

Table B.5.4.2.1-2: Erythrocyte differentiation and incidence of micronuclei in bone marrow smears of Swiss albino mice

Dose group	Sex	Ratio PCE:NCE	% PCE with micronuclei	% NCE with micronuclei	Total percentage of erythrocytes with micronuclei
Vehicle control	Males	1 : 1.1	0.69	0.62	0.65
Glyphosate, 50 mg/kg bw/d	Males	1 : 1.1	0.84	0.64	0.73
Glyphosate, 500 mg/kg bw/d	Males	1 : 1.2	0.73	0.22	0.45
Glyphosate, 5000 mg/kg bw/d	Males	1 : 1.3	0.89	0.47	0.65
Cyclophosphamide, 100 mg/kg bw/d	Males	1 : 1.9	2.33*	1.18*	1.58*
Vehicle control	Females	1 : 1.2	0.51	0.39	0.44
Glyphosate, 50 mg/kg bw/d	Females	1 : 1.3	0.28	0.15	0.21
Glyphosate, 500 mg/kg bw/d	Females	1 : 1.2	0.52	0.23	0.36
Glyphosate, 5000 mg/kg bw/d	Females	1 : 1.3	1.05*	0.46	0.72*
Cyclophosphamide, 100 mg/kg bw/d	Females	1 : 1.9	2.39*	1.65*	1.90*

* significantly higher as compared to control group

Conclusion:
There was a slight but statistically significant increase in the formation of micronuclei in bone marrow red blood cells of female mice in this study. However, this observation was confined to the highest dose level of 5000 mg glyphosate/kg bw and was not confirmed in the male animals at this extremely high dose which was even applied in duplicate, i.e. on two consecutive days. This is surprising since if there is a sex difference in a micronucleus test, males are usually more susceptible than female animals. The toxicological significance of the increase in micronucleus incidence in one sex is doubtful since the variation in the percentage of polychromatic erythrocytes with micronuclei is considerably high among female dose groups as compared to the control. The results obtained in male groups appear much more homogeneous.

Because of the twofold application of the test substance, it is difficult to compare this experiment with all the other micronucleus tests in which glyphosate proved negative since such a high overall dose (2 x 5000 mg/kg bw) was not adminstered in any other study. However, there is a cytogenetic study in the same mouse strain available (Suresh, 1994) which had been performed in the same laboratory under nearly identical conditions using the same dosages and test material of the same purity. This study did not provide any evidence of chromosome aberrations. However, a decrease in mitotic index at 24 hours following the second administration of the highest dose of 5000 mg/kg bw might was indicative of a certain degree of cytotoxicity to bone marrow cells. In the micronucleus test, there was no apparent cytotoxicity as measured by the PCE:NCE ratio but mitotic index is not determined in studies of this type. Mitotic index and PCE:NCE ratio may be independent indicators of cytotoxicity. In the cytogenetic study, the apparently mutagenic control substance cyclophosphamide markedly decreased the mitotic index in female mice but not in males whereas PCE:NCE ratio was equally reduced in both sexes in the micronucleus test. Thus, an effect of cytotoxicity at the highest dose level in the micronucleus test cannot be excluded since it has been shown in a comparable study that it may occur at such extreme dosages.

Further micronucleus tests and cytogenetic studies:

Suresh, T.P. (1994): Genetic toxicology - In vivo mammalian bone marrow cytogenetic test - Chromosomal analysis. Test compound: glyphosate technical (FSG 03090 H/05 March 90). Rallis Agrochemical Research Station, Bangalore, India; Study no. TOXI:890-MUT-CH.AB. Dates of experimental work: 11 January 1993 - 09 February 1993. This report was submitted by the notifier Feinchemie. GLP: yes. The study is considered acceptable.

Jensen, J.C. (1991): Mutagenicity test: Micronucleus test with glyphosate, batch 206-JaK-25-1. Scantox A/S, Lille Skensved, Denmark on behalf of Cheminova; Laboratory no. 12324. Dates of experimental work: 24 January 1991 - 01 February 1991. This report was submitted as part of the joint dossier of Monsanto and Cheminova. GLP: yes (self-certification of the laboratory). The study is considered acceptable.

Jenkinson, P.C. (1990): Agrichem glyphosate active: OECD 474 micronucleus test in the mouse. Safepharm Laboratories Ltd., Derby, U.K.; Project no.: 300/3, Company file: R236. Dates of experimental work: 10 May 1990 - 11 June 1990. This report was submitted by the notifier Agrichem [Glyphosate Tulip Task Force]. GLP: yes (self-certification of the laboratory). The study is considered supplementary only since the purity of the test material was not reported.

Antal, A.; Dömötör, C.B. and Kiss, M. (1989): Mutagenicity study of glyphosate in NMRI mice using the micronucleus test. Ministry of Agriculture, Centre of plant Protection and Agrochemistry, Toxicological Laboratory, Keszthely, Hungary; Report identification and dates of experimental work not given. This report was submitted by the notifier Alkaloida. GLP: no. Since the study must have been performed in 1989 or before, GLP was not compulsory. The study is considered supplementary only due to certain reporting deficiencies, e.g. purity of the test material as supplied by Alkaloida was not indicated.

Li, A.P. (1983): In vivo bone marrow cytogenetics study of glyphosate in Sprague-Dawley rats. Monsanto Environmental Health Laboratory, St. Louis, U.S.A.; EHL study 830083, Project no. ML-83-236. Dates of experimental work: 08 August 1983 - 12 August 1983. This report was submitted as part of the joint dossier of Monsanto and Cheminova. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable. It should be noticed that the main results of this study were published by Li and Long (1988, for complete reference see volume 2). Some notifiers (e.g. Barclay) refer to this publication.

The following study was considered unacceptable for evaluation purposes:
Bhide, M.B. (1986): Report on micronucleus test of glyphosate (technical) of Excel Industries Limited Bombay. Indian Institute of Toxicology, Bombay, India. This report was submitted to the Rapporteur independently by the notifiers Barclay and Luxan. GLP: no. The study is considered unacceptable due to serious deficiencies. The dose levels used (50 and 100 mg glyphosate per kg bw) are much too low for meaningful evaluation. Much higher doses have been applied in other studies using the same i.p. route of administration (see table B.5.4.2.1-1). The number of animals used (i.e. only four male mice) is too small, and sampling only at 6 hours after the second administration without appropriate justification is not sufficient for definitive assessment of clastogenicity. In addition, there are serious reporting deficiencies. Thus, a report identification (study number) and the dates of experimental work were not given. Purity of the test material was not indicated.

Published literature
Chan and Mahler (1992) reported investigations on the occurrence of micronuclei in peripheral (normochromatic) blood erythrocytes obtained at the end of a 13-week feeding study with glyphosate in B6C3F1 mice receiving dietary doses of 0, 3125, 6250, 12500, 25000 and 50000 ppm. There was no indication of a higher frequency of micronucleus formation up to the highest dose level tested. However, since this study does not comply with current guidelines for mutagenicity testing, and because the information submitted is rather limited, it is considered to provide additional information only. The purity of the test material was not stated in the report.

B.5.4.2.2 STUDIES IN GERM CELLS (DOMINANT LETHAL TEST)

Five reports on dominant lethal tests were submitted to the Rapporteur by different notifiers consistently revealing that glyphosate was not mutagenic in this test system neither in rats nor in mice. Only three studies were considered acceptable or at least supplementary for evaluation purposes and only those are summarized in table B.5.4.2.2-1, therefore. The most recent one using the by far highest doses of glyphosate (Suresh, 1992) is reported in detail. The other studies are listed below.

Table B.5.4.2.2-1: Summary of dominant lethal tests with glyphosate in rats and mice

Test system/Study design	Test material/ purity	Dose levels	Result (Mutagenicity)	Reference	Submitted by (notifier)
Wistar rats, single oral dose, 10 successive one-week mating periods (1:1 sex ratio)	Glyphosate, 96.8%	0-200-1000-5000 mg/kg bw	Negative	Suresh, 1992	Feinchemie
CFY rats; 8-week dietary administration followed by mating of each treated male with a total of 8 females over a period of four weeks	Glyphosate, purity not reported	0-6.8-20.5-70.4 mg/kg bw/d (calculated mean daily intake)	Negative	Antal, 1982*	Alkaloida
CD-1 mice, single oral dose, each treated male paired with a total of 16 females over a period of eight weeks	Glyphosate, 98.7%	0-200-800-2000 mg/kg bw	Negative	Wrenn et al., 1980	Monsanto/ Cheminova

* study providing supplementary information only

Suresh, T.P. (1992): Dominant lethal test in Wistar rats. Test compound: glyphosate technical (FSG 03090 H/05 March 90). Rallis Agrochemical Research Station, Bangalore, India; Study no. TOXI:888-DLT. Dates of experimental work:

03 September 1991 - 31 January 1992. This report was submitted by the notifier Feinchemie.

Material and methods:
Test method: The study was conducted in compliance with OECD guideline 478 ("Genetic toxicology: Rodent dominant lethal test", adopted 1984).
Deviations: None.
GLP: Yes (according to self-certification of the laboratory). A formal certificate of a national authority for the time when the study was performed is lacking. However, it should be noticed that the test facility was inspected by representatives of the German GLP Federal Office in 1992 and GLP compliance was confirmed.
Acceptance: The study is considered acceptable.
Test system: Groups of 30 male Wistar rats (random bred, source: Toxicology Department, Rallis Agrochemical Research Station, Bangalore, India) per group received a single dose of glyphosate (batch no.: 60, purity: 96.8%; manufactured by M/s EPIC-Schwebda Chemicals Ltd., New Bombay, India) by oral gavage. The dose levels of 200, 1000 and 5000 mg/kg bw were selected on the basis of a dose-range study and were suspended in refined groundnut oil to give a constant dosing volume of 10 ml/kg bw. A vehicle control group of 10 male rats and two positive control groups of 5 male animals each were also included in this study. The positive control substance was ethylmethanesulphonate (dissolved in distilled water) and was orally administered at daily doses of 100 mg/kg bw for five consecutive days (first control group) or as a single dose of 500 mg/kg bw (second group). Immediately after dosing, treated males were paired with untreated adult virgin females in a 1:1 mating ratio. Paired females were separated after 6 days and on day 8 following dosing, the treated males were again paired with new females. This procedure was repeated for a total of ten weeks. On day 16 after pairing, the dams were sacrificed and uteri along with the ovaries were removed and examined for corpora lutea, implantation sites, early and late resorptions and live fetuses. The treated sires were regularly observed for toxic symptoms. Dead animals were immediately necropsied. Evaluation criteria were not reported.
Statistics: The methods of statistical analysis included Bartlett´s test, Analysis of Variance, Dunnett´s multiple pairwise comparison, Mann-Whitney test and `z´-test.

Results:
General observations: There were no unscheduled deaths in the male groups receiving glyphosate. Some clinical signs like nasal discharge, snuffling, soft stools/diarrhea or rough hair coat were more frequently observed in the high dose group. An initial body weight loss was observed in high and mid dose males after dosing.
Mutagenicity testing: Following the first mating, the implantation and pregnancy rate were lower among the females mated with the high dose males. The incidence of early resorptions (pre-implantation losses) was markedly increased in this group and, accordingly, the number and percentage of live implants was lower. In addition, incidence and percentage of embryonic resorptions (described as "small moles") were higher in the mid and high dose group and appeared to increase with dose. Following the next and all the other matings, however, consistent and dose-related adverse effects on fertility did not occur any more although there was some variation between the groups occasionally reaching statistical significance for one or the other of the parameters examined. In contrast, the positive control substance caused a decrease in pregnancy rate down to zero percent in week 3 with a gradual recovery back to normal by 8 to 10 weeks. In addition, the number of embryonic resorptions was increased during week 1 already which gradually returned to normal level by week 9.
Pathology: At terminal sacrifice, no major gross lesion in visceral organs and in the reproductive system of the treated males were noted with the exception of unilateral testicular atrophy which was seen in three high dose sires. However,

individual data show that these lesions were not accompanied by an impaired reproductive performance of the affected males.

Conclusion:
Under the conditions of this study, glyphosate did not cause dominant lethal effects when once administered to male Wistar rats up to a dose as high as 5000 mg/kg bw. Acute toxicity of the compound was indicated by initial body weight loss in the high and mid dose group following treatment and by clinical signs in top dose males. Furthermore, it cannot be excluded that pathological testicular findings in few high dose males were related to treatment. Transient effects on reproduction at the highest and, to a much lesser degree, the mid dose level were confined to the first mating period and were considered to be due to the acute toxic effects of the test substance. The positive control substance caused the expected effects.

Further dominant lethal tests:
Antal, A. (1982): Mutagenic testing of glyphosate in rat by dominant lethal test. Ministry of Agriculture, Centre of plant Protection and Agrochemistry, Toxicological Laboratory, Keszthely, Hungary; Report identification and dates of experimental work not given. This report was submitted by the notifier Alkaloida. GLP: no. When the study was performed, GLP was not compulsory. The study can be considered at best supplementary. There are serious reporting deficiencies, e.g. purity of the test compound was not given and it is not unequivocally clear how many males were allocated to the individual test groups. It can be assumed that the group size (20 per group?) was smaller than required by the current guidelines. In addition, the dose levels appear to be too low for definitive assessment. It is known from other studies that much higher doses can be applied. However, it is the only dominant lethal test with repeated dietary administration and, therefore, the study may provide some additional information.

Wrenn, J.M.; Rodwell, D.E. and Jessup, D.C. (1980): Dominant lethal study in mice. International Research and Development Corp., Mattawan, Michigan, U.S.A. on behalf of Monsanto; Report no. IR-79-014, Study no. 401-064. Dates of experimental work: 04 June 1979 - 09 August 1979. This report was submitted as part of the joint dossier of Monsanto and Cheminova. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable although the purity of the test compound was indicated in the original report. However, this information is given in the notifiers Tier I document.

The following two studies were considered unacceptable for evaluation purposes:
Bhide, M.B. (1986): Report on dominant lethal study in rats with glyphosate (technical) of Excel Industries Limited Bombay. Indian Institute of Toxicology, Bombay, India. This report was submitted by the notifier Barclay. GLP: no. When the study was performed, GLP was not compulsory. The study is considered unacceptable due to serious deficiencies. The dose levels used (50 and 100 mg glyphosate/kg bw) are too low for meaningful evaluation, in particular when administered by oral gavage. Much higher doses have been applied in other studies (see table B.5.4.2.2-1). The number of animals used (i.e. only 10 male mice per group) is definitely too small. A concurrent positive control group was not included. In addition, there were serious reporting deficiencies. Thus, a report identification (study number) and the dates of experimental work were not given. Purity of the test material was not indicated.

Anonym (1987): Mutagenicity study (Dominant lethal assay). Sarabhai Research Centre (India?), Division of Biology. This report was submitted by the notifier Luxan. GLP: no. When the study was performed, GLP was not compulsory. The study is considered unacceptable since it does not comply with current standards. Although the dose levels for the single oral administration (1200 and 1800 mg/kg bw) could be considered high enough to provide information on mutagenicity in

the dominant lethal test, the number of treated male mice (only 8/group) is definitely too small. A positive control substance was not tested. In addition, there were serious reporting deficiencies. Thus, location and country of the performing laboratory, a report identification (study number) and the dates of experimental work were not given and the description of experimental procedure is very poor.

B.5.5 LONG-TERM TOXICITY AND CANCEROGENICITY

A number of long-term feeding studies was performed in rats and mice. A summary of the available studies is given in tables B.5.5-1 and B.5.5-2 below. In general, it was confirmed that glyphosate is a compound of relatively low toxicity. In particular, no evidence of cancerogenicity was obtained. Concerning non-neoplastic chronic effects, however, considerable differences among the studies were apparent. To a certain extent, this could be due to methodical reasons or to the more or less pronounced susceptibility of the strains used but one can not exclude that toxicological properties of glyphosate (or its impurities) when supplied from various manufactures could be somehow different.

Rat

Table B.5.5-1: Summary of long-term studies with glyphosate in rats

Strain/duration of the study	Dose levels	NOEL/NOAEL	Target organs/ Main effects	Reference	Submitted by (notifier)
Wistar, 2 years	0-100-1000-10000 ppm	NOAEL: 1000 ppm (ca. 60 mg/kg bw/d); NOEL: 100 ppm (ca 6.3 mg/kg bw/d)	Clinical chemistry findings indicating minor liver effects; weak evidence of cataracts.	Suresh, 1996	Feinchemie
Sprague-Dawley, 2 years	0-10-100-300-1000 mg/kg bw/d	NOEL: 10 mg/kg bw/d	Salivary glands (histologic lesions, organ weight↑); evidence of weak liver toxicity (clinical chemistry, organ weight↓); body weight↓.	Atkinson et al., 1993	Monsanto/ Cheminova
Sprague-Dawley, 2 years	0-2000-8000-20000 ppm	NOEL: 2000 ppm (ca 89 mg/kg bw/d)	Systemic effects: cataracts, body weight↓, liver weight↑. Local effects: inflammation of gastric mucosa.	Stout and Ruecker, 1990	Monsanto/ Cheminova
Sprague-Dawley, 26 months	0-3-10-31 mg/kg bw/d in male rats, 0-3.4-11-34 mg/kg bw/d in females (calculated intake; dietary levels regularly adjusted)	NOEL: 31 mg/kg bw/d (highest dose)	No treatment-related effects.	Lankas, 1981*	Monsanto, published by WHO/FAO (1986 JMPR evaluation)

* study providing supplementary information only

Three long-term studies in rats have been submitted to the Rapporteur for EU registration purposes. The study by Atkinson et al. (1993) was selected to be reported in detail because it is the most comprehensive revealing unique histological salivary gland findings and a rather low NOEL. The two other chronic studies (Stout and Ruecker, 1990; Suresh, 1996) were also considered acceptable for evaluation but are reported more briefly.
In addition, one more study (Lankas, 1981) was referred to by a number of applicants (i.e. Agrichem [Glyphosate Tulip Task Force], Alkaloida, Barclay, Sinon [Shinung], Monsanto/Cheminova and Sanachem). This study was performed on behalf of Monsanto and evaluated by the JMPR of WHO/FAO in 1986 and the current ADI value of the WHO is based on it. The original report was not submitted by any of the notifiers, however, had been made available to the Rapporteur for national authorization of glyphosate some years ago. The study is briefly

described in this monograph since it was taken into account for ADI determination (see section B.5.10.2).
There were no adverse effects on survival and no clinical signs of toxicity in any of the long-term rat studies. Body weight gain was compromized only in female Sprague-Dawley rats (Stout and Ruecker, 1990) at the upper dietary level of 20000 ppm, equivalent to a mean daily intake of 1183 mg/kg bw, i.e. the highest dose tested in chronic rat studies at all.
Some findings were assumed to reflect a weak effect on the liver. Atkinson et al. (1993) and Suresh (1996) reported an increase in alkaline phosphatase acitivity. Femle rats were apparently more affected than males. In Sprague-Dawley rats, liver weight was reduced with dose from 100 mg/kg bw/day onwards but only at interim sacrifice (Atkinson et al., 1993). In contrast, Stout and Ruecker (1990) described a slight but statistically significant liver weight increase in male Sprague-Dawley rats at the highest dose level of 20000 ppm after 24 months.
In addition, effects on the eyes and on salivary glands were reported. A higher incidence of cataracts was seen in male Wistar rats at 10000 ppm by Suresh (1996) and in male Sprague-Dawley rats at the top dose level of 20000 ppm (Stout and Ruecker, 1990).
Histological changes described as "cellular alteration" in the parotid and mandibular salivary glands and a higher organ weight of these glands were noted at 100 mg/kg bw/day and above (Atkinson et al., 1993). Similar changes have been observed in subchronic rat studies, too (see B.5.3). The toxicological significance of these findings is equivocal. A special study suggests an adrenergic mechanism behind (see B.5.8.2). In the other chronic rat studies, no effects on salivary glands were reported. However, at least in the study of Stout and Ruecker (1990), only the mandibular but not the parotid salivary glands were examined histologically.
Local effects on gastric mucosa were noted by Stout and Ruecker (1990) at the high and mid dose levels in both sexes. The more frequent occurrence of stomach mucosa inflammation indicating irritation might well correspond to epithelial hyperplasia in the urinary bladder as observed in a long-term mouse study (Knezevich and Hogan, 1983, see below) in which also glyphosate manufactured by Monsanto was administered.

Mouse

Table B.5.5-2: Summary of long-term feeding studies with glyphosate in mice

Strain/Duration of the study	Dose levelse	NOEL/NOAEL	Target organs/ Main effects	Reference	Submitted by (notifier)
CD-1; 2 years	0-100-300-1000 mg/kg bw/d	NOAEL: 1000 mg/kg bw/d	Not clearly identified.	Atkinson et al., 1993	Monsanto/Chemi-nova
Balb/c; 18 months	0-75-150-300 ppm	NOAEL: 150 ppm (ca. 15 mg/kg bw/d)	Body weight and food consump-tion↓.	Bhide, 1988*	Luxan; Barclay
CD-1; 2 years	0-1000-5000-30000 ppm	NOEL: 1000 ppm (157 mg/kg bw/d)	Body weight↓; histological changes in liver and urinary bladder.	Knezevich and Hogan, 1983	Monsanto/Chemi-nova
CFLP/LATI; 18 months	0-100-300 ppm	NOEL: 300 ppm (30 mg/kg bw/d)	No adverse ef-fects observed.	Vereczkey and Csanyi, 1982 (revised version issued in 1992)*	Alkaloida

* Study not acceptable for assessment of cancerogenicity. Supplementary information regarding chronic toxicity is provided.

Two comprehensive chronic studies in mice were available. These studies have shown that glyphosate is not oncogenic. Non-neoplastic effects assumed to be treatment-related were confined to high dose males in the study by Knezevich and Hogan (1983) and comprised hepatocyte hypertrophy and bladder epithelial hyperplasia. In addition, body weight was decreased in this male group receiving a daily dose as high as 30000 ppm. In contrast, the more recent study by

Atkinson et al. (1993) did not confirm these effects and did not reveal convincing evidence of other treatment-related changes although there were some findings of equivocal toxicological significance (increased thymus weight, mineral deposits in the brain) at least at the highest dose level of 1000 mg/kg bw/day.
In addition, two studies of rather limited scientific value have been submitted which were not considered sufficient for the assessment of cancerogenicity and cannot serve as basis for overall health evaluation. No adverse effects were noted by Vereczkey and Csanyi (1982) up to the highest dose of 300 ppm. In contrast, Bhide (1988) reported evidence of impaired food consumption and reduced body weight at least at 300 ppm. However, it is very unlikely that these findings were actually substance-related since the more comprehensive mouse studies mentioned above did not show adverse effects on body weight and/or food consumption at much higher dose levels using a much higher number of animals. The notifier Agrichem [Glyphosate Tulip Task Force] referred to a further 18-month mouse study revealing no evidence of a carcinogenic potential up to the highest dose of 300 ppm. Neither the original report nor the cited EPA assessment of this study were available to the Rapporteur.

B.5.5.1 RAT

B.5.5.1.1 LONG-TERM FEEDING STUDY IN SPRAGUE-DAWLEY RATS

Atkinson, C.; Everett, D.J.; Strutt, A.V.; Henderson, W. and Hudson, P. (1993): Glyphosate 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill. Inveresk Research International, Tranent, Scotland; IRI Report no. 7867, Project no. 438623. Dates of experimental work: 2 March 1990 - 9 March 1992. The study was submitted as part of the joint dossier of Monsanto and Cheminova.

Material and methods:
Test method: U.S.EPA Pesticide Assessment Guidelines Subdivision F, 83-5. The cancerogenicity part of the study was performed in compliance with OECD guideline 451 ("Carcinogenicity Studies") and the separate chronic toxicity study included was run according to OECD guideline 452 ("Chronic Toxicity Studies"), both adopted in 1981.
Deviations: None. Some investigations were carried out in excess of guideline requirements. Thus, ophthalmological examinations were conducted. At necropsy after 52 and 104 weeks, brain cholinesterase was determined in 10 animals per sex and dose. In addition, water consumption was monitored.
GLP: Yes.
Acceptance: The study is considered acceptable.
Test system: Glyphosate technical (Batch nos. 229-JaK-5-1, purity 98.9% and 229-JaK-142-6, purity 98.7%, received from Cheminova A/S, Lemvig, Denmark) was administered continuously to Sprague-Dawley rats (source: Charles River UK, Ltd., Margate, Kent, England) for up to 104 weeks via their diet. Every dose group comprised 85 male and female animals. 50 rats/sex/dose were allocated to the 104 weeks oncogenicity study and 35 animals/sex/dose to chronic toxicity testing. 15 male and 15 female rats from every chronic toxicity group were killed after 52 weeks, all remaining were dosed up to scheduled termination after 104 weeks. The glyphosate concentrations in the diet were regularly adjusted to achieve nominal dose levels of 0 (control), 10, 100, 300 and 1000 mg/kg bw/day. These dosages were selected on the basis of a previous subchronic feeding study. Rats were kept under standard conditions. The clinical, haematological and clinical chemistry (chronic toxicity groups only) and pathological investigations were performed according to the requirements of the OECD guidelines mentioned above. Organ weights of adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, prostate, salivary glands, spleen, thymus and uterus were determined in all animals subjected to interim sacrifice

and in 10 rats per sex and dose from the oncogenicity study at study termination. It should be noticed that histological examination of sublingual, submaxillary and parotid salivary glands was subsequently extended to include a sufficiently high number of rats from all dose groups after 52 and 104 weeks.
Statistics: Appropriate standard methods like the F-max test, a parametric ANOVA, Student's t-test, Kruskal-Wallis ANOVA and Fisher's Exact Probability test were applied.

Results:
Dietary analysis: The actually achieved compound concentrations in the diet as compared to the nominal values are considered satisfactory. It is stated in the original report, that homogeneity and 21-day stability of glyphosate in the test diet had been confirmed prior to the commencement of the study.
General observations: Survival was not affected by treatment and there were no clinical signs of toxicity which were thought to be related to glyphosate administration. Ophthalmoscopy did not reveal any indications of adverse effects. Body weight gain was reduced in males and females at the top dose level. At the dose levels below, no consistent and clearly dose-related body weight change was to be seen. Food consumption and water intake were not affected.
Haematology, clinical chemistry, urinalysis: Haematological changes were not considered treatment-related although haematocrit and haemoglobin were occasionally increased in high dose males and females. However, a similar increase was also observed at other dose levels, in particular in males receiving 100 mg/kg bw/day and a clear dose response was lacking. In addition, the differences observed were rather small and there was no consistent trend obvious throughout the study. In contrast, clinical chemistry investigations and urinalysis elucidated some changes which could be attributed to compound administration. An increase in alkaline phosphatase activity became most apparent in high dose males and females but was still obvious and occasionally reached statistical significance at the mid dose levels of 300 and 100 mg/kg bw/day, too. The other changes in clinical chemistry were not considered unequivocally treatment-related, however, one should notice the increase in total bilirubin in female rats. Urine pH was consistently decreased in high dose males and tended to be lower from a dose of 100 mg/kg bw/day onwards. However, such an effect was not observed in females.

Table B.5.5.1.1-1: Clinical chemistry findings in male and female (m/f) rats, mean values (n=10/sex/dose)

Dose group (mg/kg bw/d)	0	10	100	300	1000
Alkaline phosphatase (IU/l), males/females					
week 14	287/182	229/158	320/213	334/223	461***/244*
week 25	251/148	272/152	267/201*	306/227**	367***/225**
week 51	308/144	293/143	310/190*	353/195*	403/221**
week 78	258/124	286/139	284/172	351*/207**	414***/186*
week 102	212/190	265/161	287*/193	267/228	365***/286*
Total bilirubin (µmol/l), females					
week 14	1.2	1.7**	1.7**	1.9***	1.9***
week 25	1.3	1.2	1.4	1.9*	1.6
week 51	1.1	1.1	0.9	1.5	2.0
week 78	1.3	1.1	1.8	1.9	2.5*
week 102	1.1	1.4	1.0	1.5	1.5

* statistically significant, p<0.05, ** p<0.01; *** p<0.001

Organ weights; pathology: At interim sacrifice after 52 weeks, absolute liver weight was reduced at dose levels of 1000, 300 and 100 mg/kg bw/day. For males, however, this finding was not confirmed by means of covariance analysis, i.e. with correction for final body weight. At terminal sacrifice, no statistically significant decrease in liver weight was noted any more. In contrast, mean kidney weight was reduced in male groups receiving 100 and 1000 mg/kg bw after 104 weeks but a clear dose response was lacking. A probably treatment-related

impact on salivary gland weight was noted in both sexes at interim kill as shown in table B.5.5.1.1-2. These increases in salivary gland weight were also confirmed by covariance analysis. At study termination, weight of submaxillary (mandibular)/sublingual glands in both sexes and of the parotid salivary gland (females only) tended to be higher at the two upper dose levels. However, as compared to the controls, the difference was rather small, statistical significance was not achieved and there was no clear dose response.

Table B.5.5.1.1-2: Group mean organ weights assumed to be affected by treatment

Dose level (mg/kg bw/d)	0	10	100	300	1000
Absolute liver weight (g) in males/females after 52 weeks	24.63/14.46	22.80/13.17	21.58*/13.01	20.70**/14.18	19.70***/12.04**
Liver weight (g) as corrected for final body weight in males/females after 52 weeks	22.40/14.60	21.62/13.89	21.70/13.15*	21.81/12.87**	21.88/12.34***
Parotid salivary glands, absolute weight (g) in males/females after 52 weeks	0.18/0.25	0.22/0.28	0.28*/0.26	0.32**/0.26	0.38***/0.29
Absolute weight (g) of sublingual and submaxillary (mandibular) salivary glands in males/females after 52 weeks	0.88/0.58	0.84/0.62	0.84/0.61	0.87/0.63	0.99*/0.67**

* statistically significant, p<0.05, ** p<0.01; *** p<0.001

Gross necropsy did not reveal indications of treatment-related non-neoplastic changes. The only remarkable histopathological finding attributed to glyphosate administration was a dose-related increase in the number of animals exhibiting cellular alteration of the parotid and mandibular (submaxillary) salivary glands (see table B.5.5.1.1-3) at the top and both mid dose levels. This alteration was described in the original report as the occurrence of hypertrophic and weakly (mandibular gland) or more deeply (parotid gland) basophilic acinar cells without any evidence of degeneration or other toxic damage. In most cases, the degree of alteration was assessed by the study pathologist as slight or moderate. Changes ot this type were also seen after 52 weeks already. The sublingual salivary gland was apparently not affected.

Neoplasia was present in all groups but there was no relationship with dose in the incidence of any individual tumour or in the incidence of animals with tumours.

Table B.5.5.1.1-3: Incidence of cellular alteration (all degrees) of salivary glands in male and female rats (oncogenicity study, all animals)

Dose level (mg/kg bw/d)	0	10	100	300	1000
No. examined (m/f)	50/50	46/50	49/50	50/50	49/48
Alteration of parotid salivary gland, no. affected (m/f)	7/2	9/8	21**/12**	41***/21***	36***/38***
Alteration of mandibular salivary gland, no. affected (m/f)	7/11	5/8	22***/12	42***/18	31***/26**

** statistically significant, p<0.01; *** p<0.001

Conclusion:
Glyphosate was apparently not oncogenic. Liver and salivary glands were identified possible target organs of glyphosate-related chronic toxicity. On the

basis of organ weight changes, clinical chemical and histological findings, the lowest dose of 10 mg/kg bw/day is considered the NOEL in this chronic feeding study in rats. The NOEL is in compliance with the conclusion of the study authors who had defined a "No Toxicological Effect Level (NTEL)" at this dosage. The notifier established the NOAEL at 300 mg/kg bw/day based on reduced body weight gain in males and increased alkaline phosphatase activity in both sexes at the highest dose level.

B.5.5.1.2 FURTHER FEEDING STUDIES IN RATS

Suresh, T.P. (1996): Final report on combined chronic toxicity and carcinogenicity study in Wistar rats. Rallis Research Centre, Bangalore, India; Study no. TOXI:886.C.C-R. Dates of experimental work: 4 March 1992 - 4 March 1994. This report was submitted by the notifier Feinchemie.

Material and methods:
Test method: OECD guideline 453 (adopted 12 May 1981).
Deviations: None.
GLP: Yes.
Acceptance: The study is considered acceptable.
Test system: Groups of 50 Wistar rats (conventional random breed of the test facility; six weeks old at the start of treatment) per sex and dose were fed diets containing glyphosate (manufactured by EPIC-Schwebda Chemicals, Ltd., Persipolis, Maharashtra, India; purity of the two batches used: 96.8% and 96.0%) for two years. The dose levels chosen on the basis of a previous 90-day rat study were 0 (control), 100, 1000 and 10000 ppm corresponding to an average daily compound intake of 6.3, 59.4 and 595.2 mg/kg bw/day in male animals and of 8.6, 88.5 and 886.0 mg/kg bw/day in females. In addition, a further control group comprising 10 male and 10 female rats and a satellite high dose group comprising 20 animals per sex were subjected to interim sacrifice after 12 months.

Results:
There was no impact of test compound administration on the occurrence of clinical signs, on survival, body weight and body weight gain or food consumption in any of the test groups neither in males nor in females. Haematological and urinalysis parameters were not affected by treatment. The occurring changes occasionally reaching statistical significance were in the expected range of biological variation and were apparently not dose-related. Clinical chemistry investigations revealed some variations but there was no consistent trend throughout the study period and between the sexes. Statistical significance was indicated for the slight dose-dependent increase in ASAT activity in all treated male groups at the 6 month sampling time, for the decrease in gamma-glutamyl tansferase activity in top dose males and the increase in albumin concentration in the mid dose male group at 12 months and for an isolated increase in AP activity in the low dose males at study termination. In high dose females, albumin was decreased at 6 month sampling and ALAT and ASAT activities were significantly increased after 18 months. According to the study report, all these changes were still in the range of historical control data. The only finding which was considered likely to be treatment-related by the Rapporteur was the increase in AP activity in female rats at the mid and high dose levels (see table B.5.5.1.2-1). It should be noticed, however, that a similarly consistent increase was not observed in males.

Table B.5.5.1.2-1: Alkaline phosphatase activity (IU/l; group mean values) in female Wistar rats (n=10)

Dose level (ppm)	0	100	1000	10000
6 month sampling	133	146	152	235*
12 month sampling	141	158	203*	231*
18 month sampling	101	139	197*	194*
24 month sampling	254	274	220	249

* statistically significant (p value not given in the original report)

The incidence of tumours was not increased in this study. In addition, there were no significant changes in the organ weights and, with the possible exception of cataracts in high dose males, no gross or histopathological non-neoplastic findings which could be attributed to glyphosate administration. The incidence of animals with cataracts was 3, 4, 2 and 7 in the male control, low, mid and high dose groups. In females, the respective values were 1, 4, 5 and 4. It should be noticed that no remarkable findings were recorded in salivary glands.

Conclusion:
Based on the increase in AP enzyme activity in females at 1000 ppm, the low dose of 100 ppm (equivalent to a daily intake of 6.3 mg/kg bw in male and of 8.6 mg/kg bw in female rats) is considered the NOEL in this study. This increase might indicate a weak effect on the liver. Because concomitant liver pathology was lacking, this particular as well as the other, more equivocal, changes in clinical chemistry parameters were not considered adverse effects. Hence, the mid dose of 1000 ppm (about 60 mg/kg bw/day) appears to represent the NOAEL in this study. This value was also established by the study author and proposed by the applicant but was based on clinical chemistry findings only. The Rapporteur, in contrast, also considered the slight increase in the incidence of cataracts in high dose males a possible treatment-related effect since a similar observation has been made in the previous rat study reported below.

Stout, L.D. and Ruecker, F.A. (1990): Chronic study of glyphosate administered in feed to albino rats. Monsanto Agricultural Company, St. Louis, Missouri; Laboratory Project no. MSL-10495. Dates of experimental work: 5 August 1987 - 10 August 1989. The study was submitted as part of the joint dossier of Monsanto and Cheminova.

Material and methods:
Test method: As stated in the original report, this study was performed according to U.S. EPA Pesticide Assessment Guidelines, Subdivision F, Guideline no. 83-5. The study design was compliant with OECD guideline 453 (adopted 12 May 1981).
Deviations: The satellite groups comprised 10 animals per sex instead of 20 as recommended in the OECD guideline. The survival had fallen below 50% (varying between 29 and 44%) in all test groups at the time of scheduled termination after 24 months.
GLP: Yes.
Acceptance: The study is considered acceptable.
Test system: Sprague-Dawley (CD) rats (obtained from Charles River Breeding Lab., Portage, Michigan, U.S.A.) were administered glyphosate (lot no. XLH-264, purity 96.5%, manufactured by Monsanto) for two years as admixture in their diet at dose levels of 0, 2000, 8000 and 20000 ppm. The actual daily intake was approximately 89, 362 and 940 mg/kg bw in male animals and 113, 457 and 1183 mg/kg bw in females. Dose groups comprised 60 animals per sex. 10 male and 10 female rats from each of these groups were subjected to interim sacrifice after 12 months.

Results:
Group survival rates were not affected and there were no clinical signs related to treatment except the ophthalmoscopic findings mentioned below. Body weight and body weight gain were not impaired in males. In high dose females, body weight gain was decreased by up to 23% (at 20 months after treatment had started). Due to this reduction, body weight in this group was significantly lower from day 51 until approximately the 20th month although there was no marked difference as compared to the female control group at study termination any more. Food consumption tended to be increased in top dose male rats but this was not considered toxicologically significant. In females, no difference in food consumption was observed between the groups. The variations in haematological and clinical chemistry parameters were not attributed to treatment since they were not consistently noted at more than one timepoint, were within the historical control range and/or did not occur in a dose-related manner. The toxicological relevance of the markedly increased mean value for the activity of AP in the high dose female group at the last sampling point is equivocal since it was mainly due to one animal only. A statistically significant decrease in urine pH in high dose males was assumed to reflect the weak acidity of renally excreted glyphosate. In contrast, ophthalmoscopy and pathology elicited some adverse effects which were or might be treatment-related. An increased incidence of cataractous lens changes was noted in high dose males by two experts prior to study termination and was confirmed by histopathology (see table B.5.5.1.2-2). The more frequent occurrence of these changes although not reaching statistical significance and confined to one sex and the highest dose level was considered an effect of compound administration.

Table B.5.5.1.2-2: Incidence of cataracts and/or lens fiber degeneration in male Sprague-Dawley rats as determined by ophthalmoscopy and histopathology

Investigation	0 ppm (control)	2000 ppm	8000 ppm	20000 ppm
Ophthalmoscopy - 1st expertise	0/15	-	-	5/20
Ophthalmoscopy - 2nd expertise	1/14	-	-	8/19
Histopathology, terminal sacrifice	2/14	3/19	3/17	5/17
Histopathology, all animals	4/60	6/60	5/60	8/60

In high dose males, a slight but statistically significant increase (113% of the respective mean control value) in absolute liver weight after 24 months and in relative liver weight after 52 weeks was noted. This was the only organ weight change which could be related to treatment. There were no remarkable findings at necropsy. Histopathology revealed a higher incidence of animals displaying inflammation of the stomach squamous mucosa reaching statistical significance in mid dose females (see table B.5.5.1.2-3). There were no other non-neoplastic findings that could be attributed to glyphosate administration. It should be noticed that no indication of an histologically visible effect neither on the liver nor on the salivary glands was obtained in this study. However, it is stated in the report that only the submandibular salivary glands have been evaluated microscopically but not the parotid glands.

Table B.5.5.1.2-3: Total incidence of stomach mucosa inflammation in Sprague-Dawley rats

Dose level	0 ppm (control)	2000 ppm	8000 ppm	20000 ppm
Males	2/58	3/58	5/59	7/59
Females	0/59	3/60	9/60**	6/59

** statistically significant, p≤0.01

Generally, the occurrence of tumours was not increased. There was some concern on the frequency of pancreatic islet cell adenoma observed in male rats. The incidence of this tumour was 1/58 (2%), 8/57 (14%), 5/60 (8%) and 7/59 (12%) in

the control, low, mid and high dose male groups reaching statistical significance ($p \leq 0.05$) at 2000 ppm. In females, the respective incidences were 5/60, 1/60, 4/60 and 0/59. According to the study report the historical prevalence in untreated Sprague-Dawley rats had reached 17% just covering the frequency obtained in this study. The increase among treated males was not clearly dose-related and was not significant in the Peto trend test. Furthermore, there was no evidence of pre-neoplastic pancreatic lesions like a higher incidence of hyperplasia. Only one islet cell carcinoma was noted occurring in the male control group. It was concluded, therefore, that the more frequent occurrence of pancreas adenoma in treated male rats was not substance-related.

Conclusion:
The NOEL in this study was 2000 ppm equal to approximately 89 (males) or 113 mg/kg bw/day (females). In contrast, the applicant had established a NOAEL of 8000 ppm since the higher incidence of stomach mucosa inflammation at the mid and high dose levels was not considered treatment-related. Systemic effects of glyphosate administration, i.e. an impact on body weight gain in females, a slight increase in liver weight and the more frequent occurrence of cataractous changes in males were confined to the highest dose level of 20000 ppm. The most likely explanation for the cataracts is the exacerbation of age-related degenerative lens changes by treatment.

A long-term study in Charles River Sprague-Dawley rats **(Lankas, 1981)** was performed at Bio/Dynamics, Inc. (U.S.A.) and submitted by Monsanto to the JMPR of WHO/FAO for evaluation. A brief description and assessment of this study is contained in the 1986 evaluations (Part II - Toxicology) published by FAO in 1987. Groups of 50 male and 50 female rats per dose were fed glyphosate "technical" (purity not stated) for approximately 26 months at dose levels of about 3, 10 and 31 mg/kg bw/day in males and 3.4, 11 or 34 mg/kg bw/day in females. Dietary levels were regularly adjusted to maintain these dosages. According to the evaluation by the JMPR, there were no clinical signs of toxicity and no effects of treatment on mortality. Although there was a trend for reduced body weight in treated males during most of the growth period, this was not considered treatment-related since there were no significant differences at the end of the study. Haematology, clinical chemistry and urinalysis did not provide evidence of toxicity. Organ weights were not affected and there were no remarkable gross or histopathological findings. There was no increase in tumour incidence which could be unequivocally attributed to treatment although a slightly higher number of males bearing interstitial cell tumours of the testes in the top dose group (incidences: 0/15 in the control group, 2/26 in the low and 1/16 in the mid dose group versus 4/26 at the highest dose level at terminal sacrifice) had been noted. In the original study report which was assessed by the German authorities for national registration purposes, it was additionally reported that the total incidence for all male animals on study was 0/50, 3/50, 1/50 and 6/50 in the control, low, mid and high dose groups. However, this tumour is common in aged rats and the incidence in the top dose group only slightly exceeded the historical control range. Furthermore, one should also notice that the occurrence of this tumour was not increased in any of the more recent chronic rat studies described above even at much higher dose levels. The highest dose of about 31 mg/kg bw/day was considered the NOEL in this study. It must be stated that the dose levels used were too low to elicit possible adverse effects related to glyphosate administration. For the lack of any effect dose and because of reporting deficiencies, this study is considered to provide supplementary information only.
Remark:
An interim report after six months of a further chronic study in rats was made available to the Rapporteur by the notifier Alkaloida. However, according to a statement of this company submitted later, the study was interrupted then for economical reasons. The interim report did not reveal findings that were

expected to alter the overall assessment of glyphosate toxicity. Since a 6-month treatment period is not sufficient to evaluate long-term effects in rats, it was not included in this monograph and is not cited in the reference list. Furthermore, the applicant Luxan has indicated that a long-term study in rats was in preparation. A respective protocol was submitted, however, neither the final nor an interim report were available when this monograph was prepared.

B.5.5.2 MOUSE

B.5.5.2.1 LONG-TERM FEEDING STUDY IN CD-1 MICE

Knezevich, A.L. and Hogan, G.K. (1983): A chronic feeding study of glyphosate (Roundup® technical) in mice. Bio/dynamics, Inc., East Millstone, New Jersey; Report no. BDN-77-420, Lab. Project no. 77-2061. Dates of experimental work: 31 March 1980 - 14 March 1982. The study was submitted as part of the joint dossier of Monsanto and Cheminova.
Remark: This study was also made available to the JMPR of WHO/FAO for evaluation. Several notifiers (i.e. Agrichem [Glyphosate Tulip Task Force], Alkaloida, Barclay, Sinon [Shinung], Sanachem) are referring to this evaluation published in 1987 (Pesticide residues in food, 1986 evaluations, Part II - Toxicology).

Material and methods:
Test method: According to the applicants dossier, the study was originally performed according to provisional (proposed) U.S. guidelines for pesticide registration. However, the study design is in compliance with OECD guideline 451 ("Carcinogenicity studies", adopted in 1981).
Deviations: None. However, some examinations (e.g. haematology, histopathology) were carried out in excess of guideline requirements.
GLP: No. When the study was performed, GLP was not compulsory. A quality assurance statement is included in the report.
Acceptance: The study is considered acceptable.
Test system: Groups of 50 male and female CD-1 mice (source: Charles River, Portage, Michigan, U.S.A.) were administered glyphosate (Lot nos. NB 1782608/3 and 1782610/7; purity: 99.7%; supplied by Monsanto, St. Louis, Missouri, U.S.A.) continuously via their diet over a period of nearly two years. Diets were prepared fresh weekly. The dose levels were 0 (control), 1000, 5000 and 30000 ppm. A justification for the choice of these dietary concentrations is not given. The animals were kept under standard conditions and had free access to food and water. The clinical observations as well as body weight and food intake determinations were conducted according to guideline requirements. In addition, the water consumption was measured in 10 or 12 animals for two- or three-day intervals at months 12 and 24. Haematological investigations were performed on 10 mice per sex and dose at months 12 and 18 and on 12 male animals/group and on all surviving females at scheduled termination. Haematology included haemoglobin and haematocrit determination, erythrocyte and platelet count, erythrocyte morphology and differential white blood cell count. At terminal sacrifice, organ weights (adrenals, brain, gonads, heart, kidneys, liver, spleen) were recorded for all animals. Gross *post mortem* examination was performed on all mice including those which died spontaneously, accidentally or were killed moribund prior to study termination. About 40 organs and tissues including all gross lesions and tissue masses from all animals were subjected to histopathology.
Statistics: Appropriate parametric and non-parametric standard methods as Bartlett´s test, ANOVA, Dunnett´s test, Kruskal-Wallis test, Summed rank test (Dunn) and regression analysis were applied.

Results:

Dietary analysis: Analysis of stability, homogeneity and actual concentration of the test compound was the responsibility of the sponsor. The respective additional report prepared by Monsanto was not submitted to the Rapporteur. However, it is stated in the notifier's dossier that glyphosate concentrations in the test diet averaged approximately 95% of the target values throughout the study.

Compound intake: The test substance intake was calculated from individual body weight and food consumption data based on nominal concentrations. A time-weighted average was determined. These values were indicated in the notifier's dossier.

Table B.5.5.2.1-1: Mean actual test compound intake (mg/kg bw/day) throughout the study period

Dose level (ppm)	1000	5000	30000
Male animals	157	814	4841
Female animals	190	955	5874

General observations: Clinical signs of toxicity were not observed in this study. Mortality was low during the first 18 months (i.e. the usual duration of a cancerogenicity study in this species) of the administration period with survival rates greater than or equal to 62% and 68% for males and females, respectively. Thereafter, mortality increased as expected in ageing mice but did not demonstrate any treatment-related effect. The total mortality in males reached 27, 32, 32 and 23 in the control, low dose, mid dose and high dose groups. The respective figures in females were 27, 38, 22 and 23.

No consistent dose-related effects on food consumption and efficiency or water intake became apparent in any of the dose groups although food consumption values showed a considerably high degree of inter-group variation. Mean body weight in high dose males was lower than in the control group being statistically significant at most weighing intervals. The highest difference was noted at study termination (-11%). Occasionally, a statistically significant decrease in body weight was also observed in low and mid dose males, however, a consistent trend and a clear dose response were not obvious. In female mice, body weight was not affected.

Haematology: Laboratory investigations did not provide evidence of a substance-related effect.

Organ weights, pathology: In high dose males, mean absolute and relative weight of the testes was increased although statistical significance was not reached. The toxicological significance of this finding is equivocal since there were no concomitant histological changes. The increase in mean ovarian weight for the top dose female group was mainly due to a single animal with an extremely high individual value.

There was no conclusive evidence of cancerogenicity obtained in this study. The tumour incidence did not significantly increase with dose. The study authors reported a trend towards a more frequent occurrence of lymphoreticular neoplasia in treated mice, in particular in females. However, this was not confirmed at the Rapporteurs' review. In males, the incidence of renal tubule adenoma was marginally increased (0/49, 0/49, 1/50, 3/50 in the control, low, mid and high dose groups, respectively). According to an additional expert statement included in the study report, one tumour of that type was also seen in the male control group. In the absence of any pre-neoplastic kidney lesions suggesting an adverse effect of treatment, this finding was considered spurious.

Two non-neoplastic histological changes affecting the liver and urinary bladder in male mice were assumed to be treatment-related. There was a higher incidence of centrilobular hepatocyte hypertrophy in high dose males and a more frequent occurrence of slight-to-mild bladder epithelial hyperplasia in the mid and high dose, however, strict dose response was lacking.

Table B.5.5.2.1-2: Incidence (total number and percentage) of remarkable histopathological findings in male mice (statistical significance not indicated in the original report)

Dose level (ppm)	0	1000	5000	30000
Liver: centrilobular hepatocyte hypertrophy	9/49 (18%)	5/50 (10%)	3/50 (6%)	17/50 (34%)
Urinary bladder: epithelial hyperplasia	3/48 (6%)	3/46 (7%)	10/47 (21%)	8/47 (17%)

Conclusion:
It can be stated that glyphosate was not oncogenic in this mouse study. Females tolerated a dose as high as 30000 ppm without any adverse effects. In addition, no clinical signs of toxicity became obvious in male mice up to this highest dose level. Based on body weight effects and histological findings in male animals, however, the lowest dose of 1000 ppm (equivalent to 157 mg/kg bw/day) was considered the NOEL in this study by the Rapporteur.
The study authors did not establish a NOEL but it became apparent from the study report that they did not consider the bladder epithelial hyperplasia a treatment-related effect. Accordingly, a NOEL of 5000 ppm is reported in the notifiers dossier.

B.5.5.2.2 FURTHER FEEDING STUDIES IN MICE

Atkinson, C. et al. (1993): Glyphosate 104 week dietary carcinogenicity study in mice. Inveresk Research International Ltd., Tranent, Scotland; IRI Report No. 7793, Project No. 438618. Dates of experimental work: 21 December 1989 - 23 December 1991. The study was submitted as part of the joint dossier of Monsanto and Cheminova.

Material and methods:
Test method: The study was performed according to an U.S.EPA guideline (FIFRA 83-2) and generally complied with OECD guideline 451 ("Carcinogenicity Studies", adopted 12 May 1981).
Deviations: None.
GLP: Yes.
Acceptance: The study is considered acceptable.
Test system: 50 male and 50 female CD-1 mice (source: Charles River, Margate, Kent, UK) per dose group were administered glyphosate (supplied by Cheminova A/S, Lemvig, Denmark; purity 98.6% as indicated by the sponsor or 97.5 - 100.2% as determined by the performing laboratory) via their diet over a period of approximately two years. The dietary levels were adjusted regularly to achieve constant dose levels of 0, 100, 300 and 1000 mg/kg bw/day which had been selected on the basis of subchronic testing. Glyphosate was found to be stable for at least 21 days in the test diet and dietary analysis revealed that homogeneity and actual compound concentration were generally within acceptable limits of ±10%. Clinical, haematological and pathological investigations were performed according to guideline requirements. Organ weights were determined in ten animals per sex and group. Data were analyzed by means of appropriate statistical methods.

Results:
Survival was not impaired in any of the test groups and no treatment-related clinical signs were observed. There were no adverse effects on body weight and food consumption. In contrast, the body weight was increased in high dose males and to a lesser extent also in females throughout a considerable part of the study. In males, this difference was statistically highly significant for the most intervals between study weeks 2 and 56. The total body weight gain when determined for the entire dosing period, however, was similar in all groups in both sexes. Differential blood count revealed no evidence of an effect neither

in males nor in females. Generally, organ weights were not affected. However, there was an increase in absolute thymus weight reaching statistical significance in mid and high dose males (see table B.5.5.2.2-1). The increase was confirmed by covariance analysis. The toxicological significance of this isolated finding is equivocal since concomitant histological findings were not observed. On the other hand, there was a higher number of male mice in the top dose group with a thymus described as "enlarged/firm" at necropsy. It is stated in the report that the organ weight increase in high dose males was due to one animal with an enlarged thymus infiltrated with lymphoma cells.

Table B.5.5.2.2-1: Mean absolute thymus weight (g) in CD-1 mice after 104 weeks

Dose level (mg/kg bw/d)	0	100	300	1000
Male animals	0.02	0.02	0.03**	0.03*
Female animals	0.02	0.05	0.04	0.06

* statistically significant, p<0.05; ** p<0.01

Gross and histopathological examination elicited some variation between dose groups in the incidence of certain lesions. The occurrence of mineral deposits in the brain was significantly increased in high dose males (13/50) as compared to the control group (4/49). It should be noticed that this is a common finding in mice of this age and strain. The incidence of lung masses was slightly higher in the top dose male group (18/50 versus 10/50 in the control). Upon histopathological examination, there was little difference left between these two groups. Hence, this latter finding was not assumed to be treatment-related. There was no significant increase in the occurrence of neoplasia. The observed variations did not show a dose relationship and were still in the range of historical control data.

Conclusion:
It can be concluded that doses of glyphosate as high as 1000 mg/kg bw/day did not produce cancerogenicity in CD-1 mice. Moreover, the animals tolerated even the highest dose without overt signs of toxicity. Accordingly, the applicant established the NOEL at this dose. However, since one can not definitely exclude that the increase in thymus weight and in mineral deposits in the brain were treatment-related, it seems more appropriate to establish a NOAEL at the highest dose of 1000 mg/kg bw/day. This is also in agreement with the study authors who defined a "No Toxicological Effect Level (NTEL)" at this dose.

Bhide, M.B. (1988): Carcinogenicity and chronic toxicity study of glyphosate (technical) of Excel Industries Ltd., Bombay. Indian Inst. of Tox., Bombay, India; Report no. not indicated; submitted to the Rapporteur independently by the companies Barclay and Luxan. Dates of experimental work: not given in the study report.

Material and methods:
Test method: Not applicable.
Deviations: Not applicable. For main deficiencies of the study, see below.
GLP: No. When the study was performed, GLP was not compulsory.
Acceptance: The study is unacceptable for a reliable assessment of cancerogenicity 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.
Test system: 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. Animals were observed for clinical signs and mortality once or twice a day. Mice were palpated for occurrence and size of tumours. Body weight and food consumption were determined once a week during the first three months and every

4 weeks thereafter. Haematological and limited biochemisty investigations were performed on satellite group animals (5/sex/dose) after 9 months and on all surviving mice of the main groups at scheduled termination. Haematology included total red and white blood cell count, haematocrit, haemoglobin, differential white cell count and platelet count. The following biochemical parameters were analyzed: ALAT, blood urea nitrogen, total serum protein and glucose. At necropsy, adrenals, heart, kidneys, liver, spleen and gonads were weighed. Organs and tissues from all mice surviving up to scheduled termination were subjected to histopathology. In contrast to the requirements of OECD guideline 451, some organs were not examined microscopically, e.g. salivary glands, mammary gland and sternum with bone marrow. Summary incidence tables on pathological findings were not included in the report. Statistical analysis of the data was not performed.

Results:
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.

Conclusion:
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 is not 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.

Vereczkey, L. and Csanyi, E. (1992, revised version): 18-month carcinogenicity study of gliphosate in mice. Institute for Drug Research, Budapest, Hungary; study no. 8010; submitted by Alkaloida. Dates of experimental work: not given, however, the original Hungarian report was issued in 1982.

Material and methods:
Test method: Not applicable. To a certain extent, the study design complied with OECD guideline 451 ("Carcinogenicity Studies", adopted 12 May 1981).
Deviations: Only two dose groups were included instead of at least three as required in the guideline.
The number of animals surviving up to scheduled termination and subjected to pathological examination was too small for meaningful evaluation. The mice which had died intercurrently were not examined and the cause of death is unknown.
Following terminal sacrifice, pituitary and thymus were not examined microscopically.
Haematological examination (blood smear evaluation, differential blood cell count) was not performed.
GLP: No. When the study was performed, GLP was not compulsory.

Acceptance: The study is unacceptable for a reliable assessment of cancerogenicity 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 cancerogenicity 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.
Test system: Glyphosate (purity not indicated since the respective supplement was not submitted to the Rapporteur; manufacurer not given) was administered to groups of 50 male and female CFLP/LATI mice (bred in a facility in Gödöllö, 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.

Results:
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.

Conclusion:
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.