B.5.6 REPRODUCTION AND DEVELOPMENTAL TOXICITY

B.5.6.1 REPRODUCTION TOXICITY STUDIES

A number of multi-generation studies was performed in rats to investigate a possible impact of glyphosate (acid) administration on the reproductive performance. The available studies are summarized in table B.5.6.1-1. The scientific value of some of these studies is rather limited since the dose levels tested were much too low and one could not expect the occurrence of any toxic effects.

Table B.5.6.1-1: Multigeneration studies with glyphosate in rats

Type of study/ rat strain	Dose levels	NOEL, parental effects	NOEL, effects on reproduction and offspring	Reference	Submitted by (notifier)
Two generation feeding study, Wistar rat	0-100-1000-10000 ppm	10000 ppm (ca 700-800 mg/kg bw/d)	10000 ppm (ca 700-800 mg/kg bw/d)	Suresh, 1993	Feinchemie
Two generation feeding study, Sprague-Dawley rat	0-1000-3000-10000 ppm	1000 ppm (ca 80/87 mg/kg bw/d in males/ females)	10000 ppm (ca 797/881 mg/kg bw/d in males/ females	Brooker et al., 1992	Monsanto/ Cheminova
One generation dose range feeding study, Sprague-Dawley rat	0-3000-10000-30000 ppm	Not established	Not established	Brooker et al., 1991	Monsanto/ Cheminova
Two generation feeding study, Sprague-Dawley rat	0-2000-10000-30000 ppm	10000 ppm (ca 722/757 mg/kg bw/d in males/ females)	10000 ppm (ca 722/757 mg/kg bw/d in males/ females)	Reyna, 1990	Monsanto/ Cheminova
Three generation feeding study, Wistar rat	0-75-150-300 ppm	300 ppm (ca 15 mg/kg bw/d)	300 ppm (ca 15 mg/kg bw/d)	Bhide, 1988*	Luxan
One generation, oral (gavage) administration prior to mating, during pregnancy and up to day 21 of lactation, Wistar rat	0-5-10 mg/kg bw/d	10 mg/kg bw/d	10 mg/kg bw/d	Bhide, 1988*	Barclay
Three generation feeding study, Wistar rat	0-200-1000-5000 ppm	5000 ppm (approx. 460/ 500 mg/kg bw/d in males/ females)	5000 ppm (approx. 462/ 502 mg/kg bw/d in males/ females)	Antal, 1985	Alkaloida
Three generation feeding study, CD rat	0-3-10-30 mg/kg bw/d	30 mg/kg bw/d	30 mg/kg bw/d	Schroeder and Hogan, 1981*	Monsanto/ Cheminova; referred to also by Agrichem, Barclay, Sinon [Shinung] and Sanachem

* studies providing supplementary information only, mostly due to the low dose levels tested

It has been shown that the active ingredient does not exhibit a specific hazard for reproduction. Weak effects on the offspring were indicated by a reduced pup weight but were confined to dose levels as high as 30000 ppm (Reyna, 1990). Data from a range-finding study by Brooker et al. (1991) suggest that a decrease in mean pup weight could occur at lower dose levels already but this was not confirmed in any of the more comprehensive studies with administration of the test substance for two or three generations. Compound-related effects in the parent animals were similar to those seen in the subchronic and long-term studies and occurred at comparable dose levels.

Further information regarding reproductive effects may be revealed in dominant lethal tests. In one assay of this type in Wistar rats (Suresh, 1992; see section B.5.4.2.2), transient effects on reproduction like a lower implantation and a higher resorption rate were observed as a result of the apparent acute toxicity when single high doses of 1000 and 5000 mg/kg bw were administered by oral gavage to the males. However, from the second pairing onwards, the parameters returned to the range of control values. Thus, also these tests do not provide evidence of primary reproduction toxicity.
It was decided to report the two generation study by Reyna (1990) in detail since effects in both adults and pups were noted in this experiment. The other studies are more briefly described. Two of these studies (Brooker et al., 1991 and 1992) also provide additional information regarding histological changes in the salivary glands. Similar findings have been described in subchronic and long-term studies and are discussed elsewhere in this monograph (see sections B.5.3, B.5.5 and B.5.8). Salivary gland tissue was not examined in the other reproduction studies.

B.5.6.1.1 TWO-GENERATION STUDY IN SPRAGUE-DAWLEY RATS

Reyna, M.S. (1990): Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats. Monsanto Agricultural Company, St. Louis, Missouri; Study no. EHL 88038, Laboratory Project nos. MSL-10387 and/or ML-88-106 (both indicated). Dates of experimental work: 24 October 1988 - 13 October 1989. This study was submitted as part of the joint dossier of Monsanto and Cheminova.

Material and methods:
Test method: No particular method was indicated in the original report. However, the description of the study shows that it was mainly performed in compliance with OECD guideline 416 ("Two-generation reproduction toxicity study", adopted 26 May 1983).
Deviations: None (compared to the abovementioned OECD guideline).
GLP: No formal certificate. When the study was performed, GLP was not compulsory. According to a self-certification of the laboratory, the study was conducted in complicance with GLP regulations.
Acceptance: The study is considered acceptable.
Test system: Groups of 30 male and 30 female Sprague-Dawley rats (Charles River Breeding Laboratory, Portage, Michigan, U.S.A.) were fed diets containing glyphosate (purity: 97.67%, manufacturer: Monsanto) at target concentrations of 0, 2000, 10000 or 30000 ppm. The first parental generation animals (F0) were treated for approximately 11 weeks and then mated to produce the F1 generation. 30 rats/sex/dose group from the F1 generation were similarly exposed (for approximately 14 weeks) and mated twice to produce the F2A and F2B generations. The animals were kept under standard conditions. Diets containing glyphosate were prepared fresh once a week. In life and *post mortem* examinations were carried out in accordance with guideline requirements. All litters were randomly culled to a number of eight pups on lactation day 4. Parental animals and pups not used for further breeding were sacrificed on lactation day 21.
Histopathological examination was performed on all retained tissues (i.e. kidneys, ovaries, pituitary [F1 adults only], prostate, seminal vesicle, skin/mammary gland, testes with epididymes, uterus/vagina and gross lesions) obtained from all control and top dose F0 and F1 adults and one F2B weanling per sex and litter.
Statistics: According to a "decision tree analysis" of the performing laboratory, standard methods like Dunnett's Multiple Comparison test, Bartlett's test, Kruskal-Wallis, Jonckheere's and/or Mann-Whitney tests and uncorrected Chi-Square test were applied.

Results:

Dietary analysis: Regular analyses of the stability, homogeneity and concentration of the test material in the diet revealed satisfactory results.

General observations (parental toxicity): There were some deaths throughout the study which were not attributed to treatment. The only clinical sign probably related to exposure was the frequent occurrence of soft stools in high dose males and females in the F0 and F1 generations. The body weight s of F0 high dose animals gradually decreased (relative to controls) during the pre-mating period and were approximately 8% below controls prior to mating. In the F1 generation, body weight s of high dose males and females remained approximately 8 - 11% below controls throughout the 14-week post-weaning treatment period before mating. During gestation and lactation, maternal body weight s in the top dose groups (F0/F1) tended to remain lower than in the controls. Similarly, high dose animals tended to eat slightly less diet than the control animals. At the low and mid dose levels, body weight (gain) and food consumption were not affected.

Reproductive performance, litter and pup data: Mating, pregnancy and fertility indices were comparable throughout the groups. Likewise, precoital and gestational lengths were not influenced by treatment. However, there was evidence of a decrease in litter size in the high dose groups (see table B.5.6.1.1-1) although the difference was rather small and statistical significance was not reached. This reduction was not accompanied by a significant increase in the number of dead pups per litter. In particular, the outcome of the second mating of the F1 parental animals (F2B litters) shows that the litter size may vary considerably. Thus, it is not clear whether the decrease in litter size at the 30000 ppm dietary level was related to treatment.

Table B.5.6.1.1-1: Mean litter size in two successive generations

Dose level (ppm)	0	2000	10000	30000
F1 litters	13.3	12.6	12.7	11.6
F2A litters	12.0	12.3	11.6	10.8
F2B litters	11.9	10.9	13.2	10.7

Mean pup weight at birth was similar among the groups. Pup survival during the postnatal period was not affected. However, during lactation, body weight gain in high dose pups in all three litters was lower as compared to the control groups (see table B.5.6.1.1-2). At the mid dose level, F2A pups also gained less weight. However, this was not confirmed for the second mating. Thus, only the reduction in the groups receiving 30000 ppm was considered a likely compound-related effect.

Table B.5.6.1.1-2: Mean pup weight (g) after birth and during lactation

Dose level (ppm)	0	2000	10000	30000
F1 litters, day 0	6.12	6.08	6.29	6.30
day 4 (postcull)	9.37	9.30	9.48	9.59
day 14	30.36	29.76	29.97	29.81
day 21	51.99	50.44	49.77	45.93**
F2A litters, day 0	6.13	6.05	6.13	6.29
day 4 (postcull)	9.72	9.41	9.61	9.81
day 14	31.39	30.30	30.38	29.82
day 21	53.35	51.17	50.08*	45.88**
F2B litters, day 0	6.24	6.05	6.17	6.33
day 4 (postcull)	9.78	9.56	9.25	9.67
day 14	30.62	31.27	31.94	28.83*
day 21	50.78	51.83	50.95	43.44**

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

Pathology: Gross and histopathological examination did not reveal evidence of findings which could be attributed to treatment. The only statistically significant organ weight change was a slight increase in testes to body weight ratio in high dose males but this was attributed to their lower terminal body weight .

Conclusion
The mid dose of 10000 ppm was considered the NOEL for both parental and reproductive toxicity in this study. This assessment is based on reduced body weight gain and soft stools in the high dose adults and on a decrease in püp weight gain and equivocally reduced litter size in the group receiving 30000 ppm. The intermediate dietary concentration of 10000 ppm was calculated to correspond to a mean daily compound intake of 722 mg/kg bw for the male rats and of 757 mg/kg bw for the females.

B.5.6.1.2 FURTHER MULTIGENERATION STUDIES IN RATS

Suresh, T.P. (1993): Two generation reproduction study in Wistar rats. Rallis India Ltd., Rallis Agrochemical Research Station, Bangalore, India; Study no. TOXI:885-RP-G2. Dates of experimental work: May 1991 - April 1992. The report was submitted to the Rapporteur by the notifier Feinchemie. This GLP-like study is considered acceptable although an effect dose was lacking.

Material and methods:
Groups of 30 male and 30 female Wistar rats each were fed glyphosate (purity: 96.8%, manufactured by M/S EPIC-Schwebda Chemicals, New Bombay, India) for two successive generations and up to weaning of a third one at dietary levels of 0 (control group), 100, 1000 and 10000 ppm. First parental animals (P0 generation) were treated for 10 weeks before mating. This mating produced the F1 litter from which the second parental generation (P1) was selected to produce the F2 litter which was sacrificed at weaning. Thus, there was only one litter per generation. In life and *post mortem* examinations were performed in compliance with the requirements of OECD guideline 416 (adopted 26 May 1983). The data obtained were analyzed statistically.

Results:
The only common clinical signs observed were respiratory affections like nasal discharge and snuffling. However, there were no remarkable intergroup differences in the occurrence of these symptoms. One dam died of dystocia in the low dose group. One high and one low dose female died during the lactation period. These deaths could not be attributed to treatment. Body weight, body weight gain and food consumption of the parental animals were not adversely affected by compound administration throughout the study. Fertility was not impaired. There was no consistent and dose-related impact of treatment on litter data although mean litter size was reduced in the F1 generation at least at the low and mid dose level. However, there was no clear dose response and the finding was not confirmed in the F2 generation (see table B.5.6.1.2-1).

Table B.5.6.1.2-1: Mean litter size in two successive generations

Dose level (ppm)	0	100	1000	10000
F1 generation	11.3	9.8	9.9	10.4
F2 generation	11.7	10.4	10.9	11.9

Survival rate and growth of the pups were similar among the groups. Gross and histopathology did not reveal findings which could be related to glyphosate administration.

Conclusion:
The highest dose of 10000 ppm can be considered the NOEL for both parental and reproductive toxicity. One can estimate that this dietary level would correspond to a mean daily compound intake of 700 - 800 mg/kg bw/day. [The mean daily intake was not reported for all dietary levels by the study author but for the low dose level of 100 ppm a corresponding average value of 7.7 mg/kg bw/day was given in the original report.] Surprisingly, the notifier as well as the study author had established a NOAEL of "more than 100 ppm". This is, of course, true

but it is not reasonable why the NOEL or at least the NOAEL is not set at the much higher level of 10000 ppm. In the original study report, it is clearly mentioned that the test compound had no adverse effects on reproduction at any of the dose levels tested and that there were no other major toxic effects.

Brooker, A.J.; Homan, B.A.; Hadley, J.C. and Offer, J.M. (1991): Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring. Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; Report no. CHV 42/90619. Dates of experimental work: 11 November 1989 - 12 January 1990. This report was submitted as part of the joint dossier of Monsanto and Cheminova. The GLP-like study is considered acceptable.

Material and methods:
Groups of 10 mated female Sprague-Dawley rats (Crl:CD® (SD) BR VAF/Plus strain) were fed diets containing glyphosate (purity: 98.6%; supplied by Cheminova A/S, Lemvig, Denmark) at concentrations of 0, 3000, 10000 and 30000 ppm from day 3 of pregnancy up to weaning of their offspring. After weaning on day 21 *post partum*, 10 male and 10 female offspring per group were selected and reared on their respective treated diets to six weeks of age. Rats were observed for mortalities and overt signs of toxicity. Body weight, food and water consumption were determined. Pregnancy rate, gestation length and litter data from birth to weaning were recorded. Following sacrifice, all adult rats and pups were subjected to external and internal gross examination. The uterus of each female which gave birth was inspected for implantation sites. The parotid salivary glands of the parent females were preserved and examined histologically. Dietary analysis and statistical analysis of the data obtained were not performed.

Results:
Effects on parental animals included soft feces and increased urination at 10000 and 30000 ppm. One low dose female was sacrificed due to poor condition and apparent dystocia on day 22 of gestation. Since similar cases did not occur at higher dose levels, this death is not considered treatment-related. One high dose female was found dead on day 21. The cause of death could not be identified. Thus, it is not clear whether this death was related to treatment or not. Slight reductions in body weight gain and food consumption and an increase in water intake were observed in high dose animals. Signs of gastrointestinal disturbances (watery and/or dark contents, distended and/or congested stomach, distended caecum) were noted at 10000 and 30000 ppm. Macroscopic (enlarged/firm/congested/swollen) and histopathologic changes in salivary glands were recorded in all treatment groups.
There were neither adverse effects on reproduction parameters nor on survival of pups through weaning. Pup weights were reduced in the high dose group and, to a lesser extent, at 3000 and 10000 ppm. In addition, soft feces and reductions in food consumption and food utilization were recorded in the top dose offspring. Macroscopic salivary gland findings and gastrointestinal disturbances were noted in high dose juvenile rats which had been treated up to six weeks of age.

Conclusion:
A NOEL for reproduction effects could not be established in this range-finding study due to a reduced pup weight in all dose groups. It is noteworthy that effects occurred in this experiment at lower dose levels than in all the reproduction studies.

Brooker, A.J.; Myers, D.P.; Parker, C.A.; Offer, J.M.; Singh, H.; Anderson, A. and Dawe, I.S. (1992): The effect of dietary administration of glyphosate on reproductive function of two generations in the rat. Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; Report no. CHV 47/911129. Dates of experimental work: 29 March 1990 - 22 March 1991. This report was submitted as part of the joint dossier of Monsanto and Cheminova. The GLP-like study is considered acceptable.

Material and methods:
Glyphosate (purity: 99.2%; supplied by Cheminova A/S, Lemvig, Denmark) was administered to groups of Sprague-Dawley rats (Crl:CD® (SD) BR VAF/Plus strain) via their feed at dietary levels of 0, 1000, 3000 and 10000 ppm for two consecutive generations producing two litters each. The dose levels for this experiment were selected on the basis of a preliminary study (Brooker et al., 1991) described above. Dietary analysis revealed that the actually achieved glyphosate concentrations were acceptable throughout the study(within 15% of nominal values at least). F0 parental animals (28/sex/group) were exposed to the test substance for 10 weeks prior to first mating and then continuously through to termination. These rats were mated twice (at an age of 16 and 26 weeks) to produce the F1A and F2B litters. The F1 parental generation (24 animals per sex and dose group) was selected from the F1A litters, reared to maturity and mated at an age of 16 and 27 weeks. Direct treatment of this second generation was considered to commence when these rats were approximately 4 weeks of age. The animals were housed under standard conditions. Observations were carried out in compliance with OECD guideline 416. Some *post mortem* investigations were even done in excess of guideline requirements. Organ weight analyses were conducted on all F0 and F1 adults. All control and high dose F0 and F1 adults as well as any apparently infertile adults from the low and mid dose groups were subjected to histopathological examination of a range of reproductive tract tissues and the pituitary. Microscopic examinations of the salivary glands (identified previously as target organs) were conducted on all control and high dose adults and extended to all treated adults (submaxillary for females only) following the observation of changes at the highest dose level. Data were analyzed statistically.

Results:
There were neither mortalities nor clinical signs occurring which were obviously related to compound administration. At the top dose level of 10000 ppm, a slightly higher food and water consumption was noted in F1 females. Body weight s tended to be somewhat lower in F1 males.
Reproductive performance and duration of pregnancy were not affected by treatment. In the F1 generation, pregnancy rate was less than expected. This was seen in both matings, however, this observation was clearly not related to the dose administered and was also recorded among the control animals. In all four matings, the litter size was somewhat lower at the top dose level as compared to the control groups. However, since this change was apparently not dose-related when the low and mid dose groups were taken into account and since there was no increase in mean pup loss in the groups receiving 10000 ppm, an impact of glyphosate administration was not considered likely. The mean pup weight, the survival rate and growth of the offspring were similar among the groups.
Organ weights were not affected. Pathology did not reveal remarkable findings in the reproductive tract. Treatment-related changes in the parotid salivary gland of males and females and the submaxillary (submandibular) salivary gland of females were apparent at 3000 and 10000 ppm occurring with a dose-related incidence in the F0 as well as in the F1 adults. The typical finding was hypertrophy of acinar cells with prominent granular cytoplasm. The F0 and F1 groups receiving the low dose were not affected.

Conclusion:
Based on salivary gland changes, the NOEL for parental effects was 1000 ppm (equal to approximately 78.9 mg/kg bw/day in the male rats and to 86.9 mg/kg bw/day in the females). [The notifier had additionally established a NOAEL of 3000 ppm (about 237/260 mg/kg bw/day in males/females) on the basis of lower body weight and higher food consumption at the top dose level since the salivary gland findings were not considered adverse.] For effects on reproduction and pups, a NOEL of 10000 ppm (corresponding to 797 mg/kg bw/day in the male animals and to 881 mg/kg bw/day in the females) has been established because no

consistent changes have been observed up to the highest concentration level tested.

Antal, A. (1985): Three-generation reproduction study in rats with the oral administration of glyphosate. Toxicological Laboratory of Plant Protection and Agrochemical Centre, Keszthely, Hungary; Report no. not indicated. Dates of experimental work: 8 March 1982 - 18 March 1984. This report was submitted to the Rapporteur by Alkaloida. When the study was performed, GLP was not compulsory. The study is considered acceptable although its design does not comply with current guideline requirements.

Material and methods:
Glyphosate (purity not indicated, supplied by Alkaloida, Tiszavasvari, Hungary) was fed to Wistar rats for three consecutive generations at dietary levels of 0, 200, 1000 and 5000 ppm. Rats were administered the test substance in their diet for a 12-week period before first mating. Males and females were mated in a sex ratio of 1:1. In the F1 and F2 generations, there were two matings. In contrast, three litters were produced in the F3 generation. The number of paired animals varied between the generations. In the F0 generation, 6 males and 6 females were used. The respective numbers were 12 and 24 for the F1B and F2B generations. These animals were sacrificed on day 28 after parturition. Glyphosate administration was continued until this day. In the F3C generation, 10 animals per sex and dose group were selected by randomisation and examined following an 8-week dosing period and a subsequent 4-week recovery phase. All the rats were daily observed for signs of toxicity. Body weight and food consumption were determined regularly. Litter parameters were recorded. Prior to sacrifice of adult rats, blood was taken for haematological and clinical chemistry investigations. Following sacrifice, the animals were necropsied and organ weights were determined. A limited number of rats (3 or 5 per dose group and generation) was subjected to histopathology.

Results:
No adverse effects of treatment were observed neither in adult rats nor in the pups in any of the groups up to the highest dose of 5000 ppm. There were occasional differences in the parameters indicative of reproductive performance among the groups, however, no consistent and dose-related trend throughout the generations was found.

Conclusion:
The highest dose level of 5000 ppm was considered the NOEL for parental as well as for reproductive toxicity in this study. This dietary concentration was calculated to correspond to a mean daily intake of about 462 mg/kg bw for the male rats and 502 mg/kg bw/day for the females.

There were some more reproduction studies submitted by different notifiers which did not reveal any indications of adverse effects. However, the dose levels used in these experiments appear very low when the toxicological profile of glyphosate is taken into account.

Bhide, M.B. (1988): Report on effect of glyphosate technical of Excel Industies Ltd., Bombay, on fertility and general reproductive performance (Segment I). Indian Institute of Toxicology, Bombay, India; Report no. not indicated. Dates of experimental report: not given.
This report was submitted to the Rapporteur by the notifier Barclay. The study design does not comply with current OECD guidelines. When the study was performed, GLP was not compulsory. The study is considered supplementary only since the dose levels used are much too low for the identification of possible harmful effects of glyphosate administration on reproduction. In addition, the range of investigated parameters was rather limited and there were some reporting deficiencies.

Groups of 10 male and 30 female Wistar rats were administered glyphosate (source: see study title above; purity not reported) once a day by oral (gastric) gavage. The dose levels were 0 (control group, receiving only 0.2 ml of the vehicle corn oil), 5 and 10 mg/kg bw/day. Medication of male rats started approximately 60 days before mating and females were treated for 14 days prior to mating. The animals were mated in a sex ratio of 1:3. After establishing successful mating, males were sacrificed and testes were removed and examined histopathologically. Treatment of female rats was continued during gestation and lactation. Half of the dams were killed after day 13 of pregnancy (not further specified in the report) and uteri and ovaries were examined for living fetuses, early and late deaths or for the number of corpora lutea, respectively. The remaining females were allowed to litter normally. Litter parameters were recorded and survival rate and growth of the pups were observed up to weaning after 21 days. Adult and young animals were daily observed for any toxic symptoms. Body weight and food intake were determined periodically.
There were no adverse effects of glyphosate administration occurring neither in the parent animals nor in the pups. Reproductive performance was not affected. The highest dose of 10 mg/kg bw/day was the overall NOEL in this study.

Bhide, M.B. (1988): Report on effect of pesticides on reproductive process - Segment IV - Three generation reproduction study with albino rats using glyphosate technical of Excel Industries Ltd., Bombay. Indian Institute of Toxicology, Bombay, India; Report no. not indicated. Dates of experimental report: not given.
This report was submitted to the Rapporteur by the notifier Luxan. When the study was performed, GLP was not compulsory. The study is considered supplementary only since the dose levels used are much too low for the identification of possible harmful effects of glyphosate administration on reproduction. In addition, there were some reporting deficiencies. Statistical analyis of the results was not performed or not reported.
Glyphosate (source: see study title above; purity not reported) was given via the diet at dose levels of 0, 75, 150 or 300 ppm to Wistar rats over three generations. In the F0 generation, the groups consisted of 8 male and 16 female animals from which similar groups were produced by selective mating for the F1, F2 and F3 generations. Two litters per generation were delivered. The rats were observed for changes in body weight and food consumption, pregnancy rate, litter size, number of live and stillborn pups and any abnormalities. Testes of F0 males and selected organs and tissues of parental animals in the next two generations were examined histopathologically.
There were no adverse effects of treatment observed in any of the parameters investigated. Thus, a dose level of 300 ppm was considered the NOEL for both parental and reproduction toxicity in this study. Using the usual conversion factor of 20, this concentration would correspond to an approximate daily intake of 15 mg/kg bw/day.

Schroeder, R.E. and Hogan, G.K. (1981): A three generation reproduction study in rats with glyphosate. Bio/dynamics Inc., East Millstone, New Jersey, U.S.A. on behalf of Monsanto; Report no. BDN-77-417, Project no. 77-2063. Dates of experimental work: 14 June 1978 - 9 April 1980.
The report was submitted to the Rapporteur as part of the joint dossier of Monsanto and Cheminova. This study was evaluated by the JMPR of WHO/FAO in 1986 (published in 1987) when more recent studies using much higher glyphosate concentrations were not available yet. It was also referred to by the notifiers Barclay, Sinon [Shinung] and Sanachem. When the study was performed, GLP was not compulsory. The study is considered supplementary since the selected dose levels were too low and, accordingly, an effect dose was not reached.
Glyphosate (supplied by Monsanto, purity not reported) was fed to groups of CD rats (24 females and 12 males per dose level and generation) for three successive generations at dietary concentrations which were regularly adjusted to achieve a mean daily compound intake of 0, 3, 10 and 30 mg/kg bw. Results of

the analysis for stability, homogeneity and proper concentrations of the test compound in the diet were presented in a separate report (Allan et al., 1981) which was also submitted to the Rapporteur and is included in the Reference list. Overall, these investigations revealed satisfactory data.
There were two litters per generation. The extent of in-life and *post mortem* examinations to a large extent complied with current guideline requirements. There were no treatment-related effects on survival, body weight or food consumption. Gross necropsy and histopathological examinations and organ weight determinations did not reveal any evidence of effects related to compound administration. Reproductive parameters were not affected. In the F1B generation, pup survival between lactation days 4 and 21 was significantly lower than the control level for all treated groups. However, this effect was not observed in the F1a or in any other generation and was not considered biologically meaningful. An equivocal increase in pup kidney tubular dilatation occurring at the top dose level was confined to the F3B generation offspring and was not confirmed in the more recent studies described above using much higher dose levels and examining much more pups. Thus, this finding was considered spurious and not related to treatment. The NOEL in this study was 30 mg/kg bw/day for both parental and reproduction toxicity.
Remark:
It should be noticed that the notifier Agrichem [Glyphosate Tulip Task Force] for data on reproduction toxicity refers to a publication by Atkinson (1985, see Reference list) who reported the existence of a three-generation study in rats which had been evaluated by the EPA in 1981. A NOEL of 10 mg/kg bw/day was established. It is not clear whether this is a further study not available to the Rapporteur or if this information relates to the study by Schroeder and Hogan (see above) with a different NOEL set.

B.5.6.1.3 FURTHER INFORMATION ON MALE AND FEMALE REPRODUCTIVE EFFECTS

Published literature
Chan and Mahler (1992) investigated sperm motility, sperm count per gram caudal tissue and testicular spermatid head count as part of terminal examinations in a subchronic study in rats and mice (see section B.5.3.2). In addition, testicular, epididymal and caudal weights were determined. In females, estrous length was studied by means of vaginal cytology over the last two weeks of the dosing period. Male rats experienced a significant decrease (by approximately 20%) in sperm count in the two upper dose groups receiving 25000 and 50000 ppm. The Rapporteur does not consider this reduction a toxic effect since a change of 20% is within the normal variation in the rat. Other parameters indicative of an impact on male fertility were not altered. Top dose female rats had a longer estrous cycle compared to the controls (5.4 days at 50000 ppm versus 4.9 days). The biological significance of this observation is not known. As an isolated finding, it is not considered conclusive to indicate an adverse effect on reproduction. In mice, no changes were seen neither in males nor in females.

B.5.6.2 DEVELOPMENTAL TOXICITY STUDIES

The embryo-/fetotoxic and teratogenic potential of glyphosate was tested in rats, rabbits and mice. The studies in rats and rabbits are summarized in tables B.5.6.2-1 and -2. Overall, it can be concluded that the test compound has a low teratogenic potential. However, adverse effects on the number of viable fetuses and the fetal weight were noted in rats and rabbits at higher dose levels. A reduced ossification and a higher incidence of skeletal and/or visceral anomalies are also indicative of fetotoxicity occurring at doses causing maternal toxicity.

In rats, the lowest NOEL for both maternal and developmental effects was 300 mg/kg bw/day and the lowest LOEL was 1000 mg/kg bw/day. No evidence of teratogenicity was obtained. The most comprehensive rat study (selected to be reported in detail) is that by Brooker et al. (1991) revealing signs of fetotoxicity (evidence of delayed ossification) at 1000 mg/kg bw/day. At the extremly high dosage of 3500 mg/kg bw/day causing overt maternal toxicity including mortality, a higher incidence of skeletal anomalies and reduced ossification were observed. The mean fetal weight was diminished. In another study (Tasker and Rodwell, 1980), the number of viable fetuses per litter was decreased at this dose.

B.5.6.2-1: Teratogenicity studies in rats

Strain	Dose levels (mg/kg bw/d)	NOEL, maternal (mg/kg bw/d)	NOEL, developmental (mg/kg bw/d)	Reference	Submitted by (notifier)
CD	0-300-1000-3500	300	300	Brooker et al., 1991	Monsanto/Cheminova
Wistar	0-1000 (limit test)	1000	1000	Suresh, 1991	Feinchemie
Wistar	0-100-500	500	500	Bhide, 1986*	Luxan; Barclay
CD	0-300-1000-3500	1000	1000	Tasker and Rodwell, 1980	Monsanto/Cheminova; also referred to by Agrichem [Task Force], Barclay, Sinon [Shinung] and Sanachem
CFY	0-22-103-544	544	544	Anonym, 1981**	Alkaloida

* study considered supplementary only
** feeding study with serious reporting deficiencies providing supplementary information only

The outcome of two additional studies in rats (not tabulated above) suggests that the dietary administration of glyphosate did not affect the viability and postnatal development of the pups (Anon., 1981) even when the treatment of the dams was continued during lactation (Bhide, 1988).
In the rabbit studies which were considered acceptable for evaluation purposes (administration by oral gavage), fetal effects were confined to the upper dose levels causing also overt maternal toxicity. In two studies (Suresh, 1993; Brooker et al., 1991), there was limited evidence of an adverse effect on fetal viability but only at doses as high as 450 or 500 mg/kg bw/day. In addition, a slight increase in visceral and/or skeletal anomalies was noted at 500 mg/kg bw/day (Suresh, 1993; Bhide and Patil, 1989) and, to a lesser extent and of more equivocal toxicological significance, at 450 mg/kg bw/day (Brooker et al., 1991). It should be noticed that some visceral anomalies (in particular affecting the heart) were reported repeatedly by different investigators. The lowest maternal NOEL was established at 20 mg/kg bw/day and the lowest NOEL for fetotoxicity at 100 mg/kg bw/day (Suresh, 1993). This most recent rabbit study study is reported in detail. However, the other gavage studies which were described more briefly indicate that rabbits might tolerate higher doses without signs of fetotoxicity. The feeding study in rabbits suggesting a higher percentage of fetal loss at lower dose levels is of limited scientific value due to serious deficiencies. Nontheless, additional information is provided since this is the only available rabbit study using the dietary route. However, it is hardly to understand that an increase in intrauterine mortality would be elicited in a feeding study at doses far below those at which fetal effects were observed in the gavage studies. Thus, it is very doubtful whether this finding was actually related to glyphosate administration. Against the background of the data obtained in more valid, GLP-like studies, it can be concluded that the NOEL for developmental toxicity in rabbits is much higher.

Table B.5.6.2-2: Teratogenicity studies in rabbits

Strain	Dose levels (mg/kg bw/d)	NOEL, maternal (mg/kg bw/d)	NOEL, developmental (mg/kg bw/d)	Reference	Submitted by (notifier)
New Zealand White (NZW)	0-20-100-500	20	100	Suresh, 1993	Feinchemie
NZW	0-50-150-450	50	150	Brooker et al., 1991	Monsanto/Cheminova
NZW	0-125-250-500	250	250	Bhide and Patil, 1989	Barclay; Luxan
Dutch Belted	0-75-175-350	75	350	Tasker, 1980	Monsanto/Cheminova; also referred to by Agrichem [Task Force], Barclay, Sinon [Shinung] and Sanachem
NZW	0-10.5-50.7-255.3	255.3	10.5	Anon., 1981*	Alkaloida

* feeding study with serious reporting deficiencies providing supplementary information only

In mice, a single study (published by Zhu et al., 1984) has shown that there were no signs of developmental toxicity up to the highest dose of 1050 mg/kg bw/day.

B.5.6.2.1 RAT

B.5.6.2.1.1 DEVELOPMENTAL TOXICITY STUDY IN CD RATS

Brooker, A.J.; John, D.M.; Anderson, A. and Dawe, I.S. (1991): The effect of glyphosate on pregnancy of the rat (incorporates preliminary investigation). Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; Report nos. CHV 43 & 41/90716. Dates of experimental work (main study): 16 November 1989 - 12 December 1989. This study was submitted as part of the joint dossier of Monsanto and Cheminova.

Material and methods:
Test method: The study was run in compliance with OECD guideline 414 ("Teratogenicity", adopted 1981). It is also stated in the report that the U.S. EPA (FIFRA), Subdivision F, guideline 83-3 had been followed.
Deviations: None (compared to the abovementioned OECD guideline).
GLP: Yes.
Acceptance: The study is considered acceptable.
Test system: Glyphosate (Batch no. 206-Jak-25-1, purity: 98.6%; supplied by Cheminova A/S, Lemvig, Denmark) was given by gavage to groups of 25 mated female CD rats (Crl:CD® (SD) BR VAF/Plus strain, source: Charles River, St. Aubin les Elbeuf, France) at dose levels of 0 (vehicle control), 300, 1000 and 3500 mg/kg bw/day on days 6 through 15 of pregnancy. [These dosages were chosen by the sponsor on the basis of a preliminary study performed at the same laboratory which was made available to the Rapporteur as part II of the study report.] The test compound was suspended in 1% methylcellulose to achieve a constant dosing volume of 1 ml/100 g bw. Formulations were prepared fresh daily and compound concentrations were regularly adjusted for individual body weights. The dams were killed on gestation day 20. In life and *post mortem* investigations were performed according to guideline requirements. In addition, water intake was measured daily.
Statistics: Statistical analysis was routinely performed on litter data. Significance tests were normally two-tailed. Methods applied were the Kruskal-Wallis test, Fischer's exact test and the non-parametric equivalent of Williams' test.

Results:

Maternal effects: Mortality was confined to the highest dose and clinical signs of toxicity were observed at the two upper dose levels. Two high dose females were sacrificed on day 7 and 13, respectively, following noisy respiration and gasping. Since respiratory signs were apparent in most animals of this group and in few cases at the mid dose level, these deaths were considered treatment-related. One more top dose dam was killed just after a probable intubation error. Post-dosing salivation and loose faeces were further symptoms observed among animals receiving 3500 mg/kg bw/day. During the first two days of treatment, body weight gain was reduced at the top and, although only to a marginal extent, at the mid dose level. Food consumption was slightly decreased in the high dose group during the dosing period but was comparable with controls thereafter. Water intake was increased at 3500 mg/kg bw/day. Necropsy at termination did not reveal findings which could be attributed to treatment.

Litter data and fetal effects: A total of 23, 23, 25 and 22 dams had live young at day 20 in the control, low, mid and high dose groups, respectively. There were no abortions and no total resorptions. Implantation rate, post-implantation loss, litter size and sex ratio were similar in all groups. The only effect was a reduction in mean fetal weight at 3500 mg/kg bw/day. The occurrence of malformations was not significantly increased by treatment. However, the incidence of rib distortion (wavy ribs) was markedly higher in the top dose group. In addition, reduced ossification was seen slightly more frequently at this dose level and also at 1000 mg/kg bw/day. As result, the percentage of fetuses showing skeletal anomalies (variations) was significantly increased at the two upper dose levels. However, the percentage for the mid dose level only slightly exceeded the historical background range (21.9 - 27.2%) whereas the respective control value was atypically low in this study. The fetal effects which were assumed to be treatment-related are summarized in table B.5.6.2.1.1-1.

Table B.5.6.2.1.1-1: Fetal effects attributable to treatment

Dose level (mg/kg bw/d)	0	300	1000	3500
Mean fetal weight (g)	3.96	3.90	3.89	3.71**
Fetuses with distortions affecting thoracic ribs/no. of fetuses examined	1/155	-/143	3/166	28/144
Reduced ossification of one or more cranial centres	3/155	2/143	12/166	10/144
Reduced ossification of sacro-caudal vertebral arches	3/155	8/143	17/166	15/144
Percentage of fetuses with unossified sternebrae	13.7	28.5	17.6	33.8**
Percentage of fetuses showing skeletal variations	11.7	22.6	28.4*	35.7**

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

Conclusion:

The NOEL for maternal toxicity was 300 mg/kg bw/day based on mortality, clinical signs, decreased body weight gain and reduced food and water intake indicating a clear adverse effect of glyphosate administration at 3500 mg/kg bw/day and a possible impact at 1000 mg/kg bw/day. There was no evidence of teratogenicity in this study since the incidence of malformations was not increased. A higher number of skeletal variations in high dose group fetuses suggest some fetotoxicity at a dose where maternal toxicity was also apparent. Delayed ossification could be in line with the lower fetal weight at the top dose level of 3500 mg/kg bw/day. When the historical control data are taken into account, the toxicological significance of delayed ossification at the intermediate dose level is equivocal. Accordingly, the study authors and the notifier had established the developmental NOEL at 1000 mg/kg bw/day since they did not attribute the reduced ossification to treatment. However, since a marginal effect also at 1000 mg/kg bw/day can not be completely excluded, the lowest dose

of 300 mg/kg bw/day is also considered the NOEL for developmental toxicity by the Rapporteur.

B.5.6.2.1.2 FURTHER TERATOGENICITY STUDIES IN RATS

Tasker, E.J. and Rodwell, D.E. (1980): Teratology study in rats. International Research and Developmental Corp., Mattawan, Michigan; Report no. IR-79-016. Dates of experimental work: 16 April 1979 - 12 May 1979.
This study was submitted as a part of the joint dossier of Monsanto and Cheminova. It was evaluated by the JMPR of WHO/FAO in 1986 and is also reported by Atkinson (1985, submitted by Agrichem) who relates to an evaluation of U.S.EPA in 1981. References to this study were also made by the notifiers Barclay, Sinon [Shinung] and Sanachem. The study is considered acceptable although it was conducted before implementation of GLP regulations.
Glyphosate (purity: 98.7%; manufacturer: Monsanto, St.Louis, Missouri) was administered by gavage to groups of 25 mated Charles River CD rat females at dosage levels of 300, 1000 and 3500 mg/kg bw/day. The test material was given once daily on days 6 through 19 of gestation. A control group of the same size received the vehicle, aqueous 0.5% Methocel®, on a comparable regimen. On day 20 of pregnancy, surviving dams were sacrificed and delivered by caesarean section.
No treatment-related abnormal signs were observed in the 300 and 1000 mg/kg bw dose group rats. At 3500 mg/kg bw/day, soft stools, diarrhea, red nasal discharge, reduced activity and rales were noted. Six high dose rats died between gestation days 10 and 17. In addition, a reduced maternal body weight gain was noted at the top dose level throughout the dosing period. This was mainly due to a loss in maternal body weight over the first three days of treatment.
Reproductive and fetal effects were also confined to the top dose level. The mean number of viable fetuses per litter and the mean fetal weight were decreased. There was a significant increase in early resorptions causing a slight increase in total postimplantation loss. In addition, the total number of fetuses with malformations was increased at the highest dose level but the number of affected litters was identical to that in the control groups. Since the incidence and type of malformations were similar to those from historical control data, it was concluded that these findings were not related to treatment. In contrast, the higher number of fetuses with unossified sternebrae in this dose group was considered an adverse effect of compound administration. However, this is rather a developmental variation than a malformation.
Thus, the NOEL for the maternal as well as for fetotoxicity was 1000 mg/kg bw/day in this study.

Suresh, T.P. (1991): Teratogenicity study in Wistar rats. Test compound: Glyphosate technical. Rallis Agrochemical Research Station, Bangalore, India on behalf of Feinchemie; Study no. TOXI: ES.883-TER-R. Dates of experimental work: November/December 1990.
This experiment was performed as a limit test in Wistar rats with only two study groups receiving 0 or 1000 mg/kg bw/day. The report was submitted to the Rapporteur by the notifier Feinchemie. When the study was performed, GLP was not compulsory. A formal GLP certification for the performing laboratory is dated from 1992. The study is considered acceptable.
Glyphosate (purity: 96.8%; manufacturer: EPIC-Schwebda Chemicals, New Bombay, India) was given to 20 mated Wistar rats from day 6 through day 15 of gestation by oral gavage. The limit dose of 1000 mg/kg bw had been selected on the basis of preliminary tests which were also briefly described in the main study report. A control group of 30 mated rats received only the vehicle, i.e. refined peanut oil. The total daily dosing volume was 5 ml/kg bw for both groups. Sacrifice and caesarean section were performed on day 20. Investigations were carried out according to OECD guideline 414 ("Teratogenicity"). Appropriate statistical methods were applied.

There was no evidence of maternal toxicity Mortality and clinical signs of toxicity were not noted. Body weight , body weight gain and food intake were not impaired. There was no impact on reproduction indices or litter data. The incidence of fetal malformations was not increased in the treated group compared to the control. There was limited evidence of a higher incidence of delayed ossification (caudal vertebral arch, forelimb proximal and hindlimb distal phalanges) in the group receiving glyphosate. On the other hand, delayed ossification of other parts of the skeleton, in particular the skull, was more frequently seen in the control group. Thus, there was no clear and consistent impact of test compound administration on the process of ossification. Thus, the NOEL for both maternal and developmental toxicity is assumed to be 1000 mg/kg bw/day.

Bhide, M.B. (1986): Report on effect of glyphosate technical of Excel Industries Ltd., Bombay on reproductive process. Segment II - Teratological study. Indian Institute of Toxicology, Bombay, India; Report no. not indicated. Dates of experimental work: not given.
This report was independently submitted to the Rapporteur by the notifiers Barclay and Luxan. When the study was performed, GLP was not compulsory. This study is considered supplementary only due to serious reporting deficiencies. In addition, only two instead of three dose groups were included. Statistical analysis was apparently not performed.
20 mated female Wistar rats per group were administered glyphosate (source: see study title above; purity not reported) at dose levels of 0 (control, vehicle not stated), 100 and 500 mg/kg bw/day on gestational days 6 through 15 by gavage. Dams were sacrificed and fetuses delivered on day 20.
Neither maternal nor reproduction and fetal effects were observed up to the highest dose tested. Thus, the NOEL for both maternal and developmental toxicity was 500 mg/kg bw/day in this study.

Anonym (1981): Teratological investigation of glyphosate in rats and rabbits. This report on a further teratogenicity study in rats was prepared by the Department of Toxicology, Plant Protection and Agrochemical Centre, Keszthely, Hungary and was submitted by Alkaloida. There is no identification of the author. A study number and the dates of experimental work are lacking. In addition, the description of the study conditions is rather poor. Thus, this study can be at best considered supplementary. However, this is the only teratogenicity study in rats with glyphosate administration via the dietary route and, therefore, is considered to provide additional information. Groups of 12 to 15 pregnant CFY rats were fed glyphosate (source not reported; purity: 96.8%) at dietary concentrations adjusted to achieve nominal dose levels of 20, 100 and 500 mg/kg bw from day 6 to day 18 of gestation. As calculated, the actual dietary levels were 22, 103 and 544 mg/kg bw/day. A control group receiving untreated diet comprised 13 pregnant females. Dams were sacrificed and fetuses delivered and examined on gestation day 20. There was no evidence of maternal toxicity. An impact of treatment on litter data including resorption rate, the number of live fetuses per litter and mean fetal weight was not observed. There were no malformations recorded. It can be concluded that glyphosate at dietary doses of up to 544 mg/kg bw/day did not adversely affect fetal development. This highest dose is assumed to represent a NOEL for both maternal and developmental toxicity in this study.

B.5.6.2.1.3 DEVELOPMENTAL TOXICITY STUDIES IN RATS INCLUDING A POSTNATAL OBSERVATION PERIOD

Additional information concerning developmental toxicity of glyphosate comes from two feeding studies in rats. In these studies, treated females were allowed to deliver their offspring to investigate possible perinatal or postnatal

effects of treatment. In the study mentioned first, administration of glyphosate was even continued up to lactation day 21.

Bhide, M.B. (1988): Effect of glyphosate (technical) of Excel Industries Limited Bombay on reproductive processes. Segment III - Effect on suckling and lactating dams. Indian Institute of Toxicology, Bombay, India; Report no. not indicated. Dates of experimental work: not given.
This report was independently submitted to the Rapporteur by the notifiers Barclay and Luxan. When the study was performed, GLP was not compulsory. This study is considered supplementary since only a limited number of parameters has been investigated and there was no effect dose in this study. The actual compound intake was not calculated. Pathological examination was not performed. Statistical analysis was apparently not conducted.
Groups of 20 mated Wistar rats were fed glyphosate (purity not given, for manufacturer see study title) from day 15 of gestation through day 21 *post partum*. The nominal dose levels were 50 and 100 mg/kg bw/day. A control group of the same size received only the vehicle (0.2 ml of corn oil). Body weight and food consumption of the dams were not affected. Similarly, there was no impact of treatment on litter parameters including pup weight, on survival rate or growth of the pups. Thus, the highest dose of 100 mg/kg bw/day was the NOEL in this study.

Anonym (1981): Teratological investigation of glyphosate in rats and rabbits. Department of Toxicology, Plant Protection and Agrochemical Centre, Keszthely, Hungary, aubmitted by Alkaloida. The authors name, study number and dates of experimental work are lacking. In addition, the description of the study conditions is rather poor. Thus, this study can be at best considered supplementary.
As part of a more comprehensive study (referred to also in sections B.5.6.2.1.2 and B.5.6.2.2.2), ten female CFY rats from each the control and the top dose group were were fed the test substance glyphosate (purity: 96.8%) on days 6 through 18 of pregnancy. The intended dose level was 500 mg/kg bw/day and the calculated actual mean value was 558 mg/kg bw/day. The dams were allowed to deliver their offspring and pups were raised till day 28 of lactation when all animals were sacrificed and subjected to gross and histopathological examination. No effects of glyphosate administration were observed neither in the dams nor in the pups.

B.5.6.2.2 RABBIT

B.5.6.2.2.1 DEVELOPMENTAL TOXICITY STUDY IN NZW RABBITS

Suresh, T.P. (1993): Teratogenicity study in rabbits. Test compound: Glyphosate technical. Rallis Agrochemical Research Station, Bangalore, India; Study no. TOXI: 884-TER-RB. Dates of experimental work: 24 December 1991 - 6 March 1992.

Material and methods:
Test method: OECD guideline 414 ("Teratogenicity", adopted 12 May 1981).
Deviations: None.
GLP: Yes (according to self-certification of the laboratory). It should be noticed that the test facility was inspected by employees of the German GLP Federal Office a few weeks (30 March - 02 April 1992) after this study had been finished. As a result it was confirmed that toxicological studies on rats, rabbits, mice and birds were conducted in compliance with GLP priniciples.
Acceptance: The study is considered acceptable.
Test system: Glyphosate technical (Code no. FSG 03090 H/05 march 1990, Batch no. 60; purity: 96.8%; manufactured by M/S EPIC-Schwebda Chemicals, New Bombay, India) was administered once daily by gavage to mated New Zealand White rabbits (source: Toxicology Department, Rallis Agrochemical Research Station, Bangalore,

India; age: approximately 6 months and above) from day 6 through day 18 of gestation. Dose groups consisted of 26 (control), 17 (low dose) or 16 (mid and high dose) female animals. The dose levels had been selected on the basis of two range-finding studies in non-pregnant and pregnant rabbits, respectively. In the main study, the groups received 0 (vehicle control), 20, 100 or 500 mg glyphosate/kg bw/day. The test compound was suspended in 0.5% aqueous carboxymethyl cellulose to give an equal dosage volume of 2 ml/kg bw. The rabbits were housed individually under standard conditions and had free access to pelleted rabbit diet and drinking water. Duration of the acclimatization period before mating was 10 days. Mating had been performed on a one-to-one basis. Sacrifice of the does and cesarean section were performed on day 28 of gestation. The in life and *post mortem* examinations were carried out in compliance with guideline requirements. Thus, all fetuses were subjected to both visceral and skeletal evaluation.
Statistics: The litter was used as the basic sampling unit. Test methods applied were Bartlett's test, ANOVA, Dunnett's test, Mann-Whitney test, Student's t-test and Chi-square test. The level of significance was $p \leq 0.05$.
Remark: For comparison, results of a further teratogenicity study conducted at this test facility under similar conditions were also presented in the original study report. The test substance ("historical positive control") in this study was acetylsalicylic acid (ASA) administered at a dose level of 200 mg/kg bw/day. Since this monograph is only dealing with glyphosate, the results obtained with ASA are not reported here.

Results:
Maternal effects: The high dose caused respiratory and gastrointestinal signs of toxicity like rales and diarrhea in the does. The death of 8 top dose and 4 mid dose females occurring between study days 7 and 19 was considered compound-related by the study author. At necropsy, lungs, trachea and/or intestine were found to be affected in some of these rabbits. However, in three mid dose and three high dose decedents, no abnormalities were detected. Since maternal body weight at the high dose level was significantly lower on day 0 (day of mating) already, a treatment-related impact on body weight as postulated in the original report was not apparent. The initial difference remained throughout the study and this was also confirmed when the corrected body weight (i.e., minus uterine weight) at term was taken into account. One can only notice that there was no body weight gain (group mean) in high dose rabbits during the treatment period whereas the does in the other groups did gain some weight (0.0 versus 0.1 kg). Food consumption was statistically significantly decreased in the top dose group during the dosing period. Abortion did not occur in any of the dose groups. There was one high dose female showing complete resorption. In all dose groups, some of the does were non-pregnant. At scheduled termination, the number of pregnant rabbits available for examination was 20 (control), 13 (low dose group), 12 (mid dose) and 6 (high dose). The considerable differences were due to the varying number of pregnant animals at start of dosing and to the high mortality at the upper dose levels.

Litter data and fetal effects: There was no consistent, i.e. statistically significant and dose-related impact on litter parameters. The incidence of external malformations was not increased. In contrast, the total number of fetuses with major visceral anomalies (classified as major visceral malformations in the original report) tended to be high in all treatment groups and was significantly increased at the 500 mg/kg level. This was mainly due to the percentage of fetuses with dilated heart which was significantly elevated at all dose levels (see Table B.5.6.2.2.1-1). The incidence of skeletal variations, anomalies and malformations showed a considerable degree of variance between the groups but did not demonstrate a clear dose-response pattern. Only the statistically significant increase in the occurrence of extra 13th rib exhibiting also a dose-related trend might be attributable to treatment.

Table B.5.6.2.2.1-1: Pathological findings in rabbit fetuses

Dose group (mg/kg bw/d)	0	20	100	500
Percentage of fetuses with "dilated heart"	0.0	5.1*	5.2*	17.9*
No. affected/total number of fetuses examined	-	4/78	4/77	5/28
Litters affected/no. of litters	-	3/13	2/12	2/6
Fetuses with major visceral malformations	4/133	6/78	6/77	8/28
Percentage of fetuses with extra 13th rib	0.0	1.3	2.6	3.6*

* statistically significant, p≤0.05

Conclusion:
The NOEL for maternal toxicity was 20 mg/kg bw/day in this study since it can not be excluded that the intercurrent deaths were substance-related. However, one should notice that overt clinical signs of toxicity, impaired food consumption and a possible impact on body weight gain were confined to the top dose level of 500 mg/kg bw/day. With regard to the visceral malformations, the study author concluded that the fetal NOEL was less than the lowest dose of 20 mg/kg bw/day. However, the absolute number of fetuses with the diagnosis "dilated heart" and the number of affected litters is rather small and does not show a marked difference between the treated groups. The relative increase at the top dose level is not easy to assess since the number of litters and fetuses available is much lower than in the other groups. An information concerning the degree of heart dilatation was not given and the consequencies of such a finding in a fetus are equivocal. There was no evidence of other and much more common visceral anomalies in this study. For these considerations, it is rather unlikely that the isolated finding of a dilated heart was really related to glyphosate administration. Nontheless, the figures indicate that a treatment-related effect can not be completely excluded at least at the highest dose level. In addition, the percentage of fetuses with extra 13th rib was statistically significantly higher in this dose group. Thus, the mid dose of 100 mg/kg bw/day is considered the NOEL for fetotoxicity in this study based on a possible substance-related increase in the occurrence of extra ribs and a more frequent observation of heart dilatation.

B.5.6.2.2.2 FURTHER TERATOGENICITY STUDIES IN RABBITS

Tasker, E.J. et al. (1980): Teratology study in rabbits. International Research and Developmental Corp., Mattawan, Michigan; Report no. IR-79-018. Dates of experimental work: 10 April 1979 - 11 May 1979.
This study was submitted as a part of the joint dossier of Monsanto and Cheminova and was also evaluated by the JMPR of WHO/FAO in 1986. It has been also referred to by some further notifiers (Barclay, Sinon [Shinung], Sanachem). The notifier Agrichem [Glyphosate Tulip Task Force] for data on developmental toxicity refers to a publication by Atkinson (1985, see Reference list) who reports the outcome of this study relating to an evaluation of U.S.EPA in 1981. The study is considered acceptable although it was conducted before implementation of GLP regulations.
Artificially inseminated Dutch Belted rabbits (16 or 17 does per group) were administered glyphosate technical (purity 98.7%; manufacturer: Monsanto, St.Louis, U.S.A.) as a 0.5% aqueous suspension in Methocel® at a constant dosing volume of 1 ml/kg bw once daily from gestation days 6 through 27 by oral gavage. The dose levels were 0 (vehicle control), 75, 175 and 350 mg/kg bw/day.
The maternal body weight was not affected. According to the study report, definite signs of maternal toxicity as an increase in the frequency of soft stool, diarrhea and nasal discharge were observed at the highest dose level and to a lesser extent at 175 mg/kg bw/day. However, the actual incidences were not

given. Intercurrent deaths were confined to the treated groups with a total number of 1, 2 and 10 rabbits in the low, mid and high dose group, respectively. For one mid dose and 7 high dose females, the cause of death could not be elicited and one can not exclude that these deaths were treatment-related. Thus, the low dose of 75 mg/kg bw/day was assumed to represent the NOEL for maternal effects rather than 175 mg/kg bw/day as proposed by the notifier. There was no evidence of fetal effects or of an impact on litter parameters up to the highest dose level tested. Hence, a NOEL of 350 mg/kg bw/day was established for developmental toxicity although the number of does available for examination at caesarean section was limited at the top dose level because of the high mortality rate.

Brooker, A.J. et al. (1991): The effect of glyphosate on pregnancy of the rabbit (incorporates preliminary investigations). Huntingdon Research Centre, Huntingdon, Cambridgeshire, England; Project nos. CHV 45 & 39 & 40/901303. Dates of experimental work: 14 December 1989 - 2 March 1990.
This developmental toxicity study in New Zealand White rabbits was submitted as a part of the joint dossier of Monsanto and Cheminova. The GLP-like study is considered acceptable.
Glyphosate (Batch no. 206-Jak-25-1, purity: 98.6%; manufacturer: Cheminova A/S, Lemvig, Denmark) was given once daily by oral gavage to 16 - 20 mated females per group at dose levels of 0 (vehicle control), 50, 150 and 450 mg/kg bw/day from days 7 to 19 of pregnancy. The vehicle was 1% methylcellulose and a constant dosing volume of 5 ml/kg bw was applied. One mortality was recorded in the high dose group following abortion and body weight loss. Gastro-intestinal signs of toxicity and inappetence were observed at doses of 450 and 150 mg/kg bw. Mean food consumption and body weight gain were reduced during the treatment period at these dose levels. Thus, the lowest dose of 50 mg/kg bw/day was considered the NOEL for maternal toxicity in this study. On day 29, at terminal sacrifice, 18, 12, 15 and 13 litters were available for examination in the control, low, mid and high dose groups, respectively. There was a significant increase in embryonic deaths in treated groups compared to controls. Accordingly, the percentage of post implantation loss was elevated. However, a comparison with historical control data from the performing laboratory (Huntingdon) revealed that the incidence in the study control group was atypically low. In addition, a clear dose-related pattern was not demonstrated. On the other hand, it should be noticed that an increase in the occurrence of late embryonic deaths at the top dose level was also observed in a further study in New Zealand White rabbits reported below.

Table B.5.6.2.2.2-1: Summary of post-implantation losses

Dose level (mg/kg bw/d)	0	50	150	450
Total post-implantation loss (given in %)	5.7	19.5*	15.3*	21.0**
Embryonic deaths per litter	0.6	1.8*	1.5*	1.8**
Late embryonic deaths per litter	0.2	0.9	0.5	1.3**

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

It was concluded that no convincing indications of teratogenicity were obtained in this study up to the highest dose of 450 mg/kg bw/day. There was some concern about the more frequent occurrence of fetuses with heart malformations like interventricular septal defect in the high dose group, however, the incidence was still in the range of historical background data. On the other hand, anomalies of the heart have been described in other rabbit teratogenicity studies with glyphosate, too. Thus, a possible effect on the occurrence of visceral anomalies remains equivocal. The study authors and the notifier established the overall NOEL for fetal effects at the top dose level of 450 mg/kg bw. However, since a treatment-related impact on fetal viability at this dosage is considered possible, the mid dose level of 150 mg/kg bw/day is assumed to represent a more reliable NOEL for fetotoxicity.

Bhide, M.B. and Patil, U.M. (1989): Rabbit teratology study with glyphosate technical. Indian Institute of Toxicology, Bombay, India; IIT Project no. 1086. Dates of experimental work: 3 July 1989 - 2 November 1989.
This report was independently submitted to the Rapporteur by the notifiers Barclay and Luxan. GLP: no. The study is considered acceptable although it is not stated in the report whether statistical analysis of the data had been performed.
Following successful mating, 15 New Zealand White rabbits per group were administered glyphosate (Lot no.: 38, purity: 95%; manufacturer: Excel Industries, Bombay, India) once daily from day 6 through day 18 of gestation at dose levels of 0 (vehicle control), 125, 250 and 500 mg/kg bw/day. The test material was suspended in 0.1% aqueous gum acacia to give a dosing volume of 5 ml/kg bw. The does were delivered by caesarean section and sacrificed on day 29. Group mean body weight gain was markedly reduced at the highest dose level during the second part of the treatment period (day 12 through 18) and thereafter until termination. During and after the dosing period, food consumption was slightly reduced in this group. In addition, two high dose females aborted. The maternal NOEL was 250 mg/kg bw/day in this study.
No evidence of fetotoxic or teratogenic effects was obtained up to the mid dose of 250 mg/kg bw/day. However, the number of viable fetuses per litter was decreased at the top dose level and the number of dead implants was increased (see table B.5.6.2.2.2-2).

Table B.5.6.2.2.2-2: Mean number of live and dead fetuses per litter

Dose level (mg/kg bw/d)	0	125	250	500
Viable implants	7.3	8.0	8.0	5.2
Non-viable implants	0.07	0.13	0.27	1.4

In addition, the occurrence of visceral and skeletal malformations and variations was increased in this group as compared to the control and the lower dose groups. The incidences of visceral and skeletal malformations are given in table B.5.6.2.2.2-3.

Table B.5.6.2.2.2-3: Visceral and skeletal malformations; total number and percentage

Dose level (mg/kg bw/d)	0	125	250	500
No. of fetuses examined	109	113	120	78
Heart: ventricular septal defect	0 (0%)	1 (0.89%)	1 (0.83%)	2 (2.56%)
Lungs: postcaval lobe absent	0 (0%)	1 (0.89%)	2 (1.61%)	4 (5.13%)
Kidney(s): absent	1 (0.92%)	2 (1.77%)	2 (1.61%)	6 (7.69%)
Rudimentary 14th rib, unilateral	1 (0.92%)	0 (0%)	2 (1.61%)	5 (6.41%)

Based on the possible impact on the viability of fetuses and on an increased frequency of malformations and variations in the highest dose group, the NOEL for developmental toxicity was established at 250 mg/kg bw/day, too.

Anonym (1981): Teratological investigation of glyphosate in rats and rabbits. This report on a further teratogenicity study in rabbits was prepared by the Department of Toxicology, Plant Protection and Agrochemical Centre, Keszthely, Hungary and was submitted by Alkaloida. There is no identification of the author. A study number and the dates of experimental work are lacking. In addition, the description of the study conditions is rather poor. Thus, this study can be at best considered supplementary. However, since this is the only teratogenicity study in rabbits with glyphosate administration via the dietary route, it is considered to provide additional information (supplementary study).
Groups of 14 to 16 pregnant New Zealand rabbits were fed a diet containing glyphosate (source not reported; purity: 96.8%) on the 6th to the 19th day of gestation. Dietary concentrations were calculated to provide actual dose levels of 10.5, 50.7 and 255.3 mg/kg bw/day. A control group receiving untreated diet comprized 14 pregnant does. The female rabbits were sacrificed and fetuses delivered and examined on gestation day 28. There was no evidence of maternal

toxicity. The only developmental effect was a higher percentage of fetal loss at the two upper dose levels (0.93%, 0.79%, 6.06% and 7.03% in the control, low, mid and high dose groups, respectively) due to a higher number of dead fetuses per dam. There were no malformations noted and mean fetal weight was not affected. It was concluded that glyphosate was not teratogenic in rabbits at dietary doses of up to 255.3 mg/kg bw/day whereas the NOEL for fetotoxicity was 10.5 mg/kg bw/day in this feeding study.

B.5.6.2.3 MOUSE

Published literature

Zhu, Y., Jiang, X. and Tan, G. (1984): Test on the toxicity of a new herbicide: glyphosate. Gui Yan Medical University, China. Published in: Environmental Science, 5 (2), 52-54. An English translation of the original Chinese publication was submitted by the notifier Barclay. The description of experimental work is rather poor and the results were not tabulated. However, additional information on teratological investigations in a third animal species is provided.

20 mated Kuming mouse females were administered glyphosate (manufactured in China, described as "pure") via stomach tube on gestation days 6 through 14 at dose levels of 80, 420 and 1050 mg/kg bw/day. The negative control group of the same size received the vehicle (1% starch solution) only. Benzene hexachloride (positive control substance, 80 mg/kg bw/day) was administered to a fifth group. The mice were sacrificed on pregnancy days 17 or 18 and the fetuses were delivered. There was no evidence of dose-related toxic effects in the groups receiving glyphosate. In particular, there were no signs of structural malformations caused by the test material. In contrast, fetal growth was impaired in the positive control group.

B.5.7 NEUROTOXICITY

No evidence of neurotoxicity of glyphosate was observed in acute, subchronic or chronic studies in rodents and dogs. In goats and cattle, however, central nervous functions were affected after single or repeated administration of high doses of the glyphosate isopropylammonium salt (see B.5.8.2.1). Glyphosate does not belong to substance classes like organophosphates or pyrethroids which are known or suspected to cause delayed neurotoxicity. Hence, studies for delayed neurotoxicity are not considered essential. Nontheless, two neurotoxity studies in hens are available. These studies were not performed according to current guidelines and, therefore, are considered to provide supplementary information only. Both experiments did not reveal convincing evidence of a primary neurotoxic effect in hens, even at a dose causing overt clinical signs.

Bhide, M.B. (1987): Report on a 21 day oral neurotoxicity in domestic hen of glyphosate (technical) of Excel Industries Ltd. Bombay. Indian Institute of Toxicology, Bombay, India; Report no and dates of experimental work not given. The report was submitted by the notifier Barclay.
A neurotoxicity study was conducted with glyphosate technical (purity not reported) in twelve White Leghorn hens at an age of 8 to 10 months. The test method was not specified. Groups of three animals per sex and dose were orally administered daily doses of 0 (control), 250, 500, 1000 mg/kg bw/day glyphosate in corn oil for 21 consecutive days. The hens were examined at least once daily for signs of overt toxicity. Body weights and egg weights were recorded daily. Food consumption was recorded on days 7 and 3 before the study and on days 1, 4, 8, 11, 15, 18 and 21 during the treatment period. Blood samples were collected from alar vein and haematological and biochemical parameters were monitored prior to treatment, on the 11th day of the study and at termination. Spinal cord and sciatic nerve were histologically analysed using Holmes silver stain.
All hens survived the study and did not exhibit any signs of neurotoxicity except one high dose hen showing slight ataxia on day 18. All hens of the highest dose group appeared hunched and lethargic from day 5 to 11. Red liquid and matting of feathers in anogenital region were noticed from day 16 to the end of the study. In the other groups, hens did not exhibit any clinical symptoms. An overall reduction in body weight of about 20% as well as a decrease in food consumption occurred in the highest dose group. A slight reduction in haematological parameters (haemoglobin, packed cell volume and red blood cell count) was also found in this group. Blood chemistry (ALAT, SAP, total serum protein, blood urea nitrogen and plasma cholinesterase), gross pathology and histology did not provide indications of adverse effects. Egg weights were unaffected in all hens.

Bhide, M.B. (1988): Report on a 21 day oral neurotoxicity study in domestic hen of Glycel 41 SL of Excel Industries Ltd. Bombay. Indian Institute of Toxicology, Bombay, India; Report no and dates of experimental work not given. The report was submitted by the notifier Luxan.
A neurotoxicity study as described above (Bhide, 1987) was conducted using Glycel 41 SL technical. Groups of three hens per sex and dose were orally administered daily doses of 0 (control), 400, 800 and 1600 mg glyphosate/kg bw/day in corn oil for 21 days.
All animals survived the study and did not exhibit any signs of neurotoxicity. Clinical signs were confined to the hens of the highest dose group appearing hunched and lethargic from days 6 or 7 onwards. Red liquid and matting of feathers in anogenital region was noticed from day 16 to the end of the study. An overall reduction in body weight of about 23% as well as a decrease in food consumption occurred in the top dose group. A slight reduction in haematological parameters (haemoglobin, packed cell volume and red blood cell count) and a reduction in the number of eggs were also found in this group. Egg weights and blood chemistry were unaffected in all hens. Gross pathology and histology did not provide remarkable findings.

B.5.8 OTHER DATA

B.5.8.1 TOXICOLOGICAL STUDIES ON METABOLITES

B.5.8.1.1 TOXICOLOGICAL PROFILE OF THE METABOLITE AMPA

Aminomethylphosphonic acid (AMPA) is by far the most important metabolite of glyphosate in plants and up to now the only one which had been identified with certainty in mammals (see section B.5.1.2). The toxicological properties of this substance have been investigated on behalf of the companies Monsanto and Cheminova. Acute, subacute, subchronic and teratogenicity studies as well as mutagenicity tests and investigations on the metabolic fate of this metabolite were performed. The conclusion can be drawn that AMPA has a lower toxicity than the parent substance and that the metabolite is neither genotoxic nor teratogenic. Thus, residues of AMPA occurring in plants or plant products are well covered by the ADI value proposed for glyphosate.
It is interesting to mention that AMPA did not cause salivary gland effects as observed with glyphosate even at lower doses in studies performed at the same laboratory.
The studies described below were all submitted to the Rapporteur as part of the joint dossier of Monsanto and Cheminova. They were thoroughly evaluated and the essential information tabulated. Below the tables, the available studies are listed. The study on kinetics and metabolism is reported first but not tabulated.
Remark: Two additional studies on AMPA were submitted by the notifier Zeneca to support the registration of the closely related substance glyphosate-trimesium. These studies (i.e. an acute toxicity study in rats and an Ames test in *S.typhimurium* and *E.coli*) are reported in the toxicological part of the monograph on that compound. The results confirm the low acute toxicity of the metabolite AMPA with an LD50 greater than 5000 mg/kg bw and the lack of a mutagenic potential.

Metabolic fate

Colvin, L.B.; Moran, S.J. and Miller, J.A. (1973): CP 67573 residue and metabolism. Part 11: The metabolism of aminomethylphosphonic acid-^{14}C (CP 50435-^{14}C) in the laboratory rat. Monsanto Agricultural Division, Research Department; Report no. 303. Dates of experimental work not given. The study was evaluated by the JMPR of the WHO/FAO in 1986 (Part II - Toxicology, published in 1987). It is also referred to by the notifier Barclay. When the study was performed, GLP was not compulsory. The study is considered acceptable although there were some reporting deficiencies.
Male Wistar rats received a single dose of approximately 6.7 mg ^{14}C-AMPA by oral gavage and were sacrificed 120 hours after dosing. During this period, urine, faeces and expired gases were collected at 12- or 24-hour intervals and assayed for radioactivity. At sacrifice, blood and selected tissues were examined for radioactive residues.
It was found that a total of approximately 74% of the dose appeared in the faeces and ca 20% in the urine with less that 0.1% expired as $^{14}CO_2$. These figures suggest only limited absorption from the gastrointestinal tract. Elimination is rapid and nearly complete. More than 50% was excreted in the faeces during the first 24 hours following dosing. About 13% of the administered dose were found in the urine within the first 12 hours already. Approximately 0.06% of the total dose only was recovered from the carcass at 120 hours post dose. Liver, kidney and muscle exhibited residues of 6, 6 and 3 ppb, respectively. Chromatographic and spectral data demonstrated that the orally administered AMPA was excreted unchanged in the urine. There was no indication of further metabolism.

Acute toxicity

Table B.5.8.1-1: Summary of acute toxicity studies performed with AMPA

Study type	Species/strain/ number and sex	Dose range	Result	Main effects	Reference
Acute oral toxicity	Albino rat, 10 per sex	5010 - 10000 mg/kg bw	LD50: 8300 mg/kg bw; first dose causing death: 6310 mg/kg bw	Appetite and activity↓, weakness, diarrhea, collapse and death; gastro-intestinal inflammation and liver discoloration	Birch, 1973*
Acute oral toxicity	Sprague-Dawley rat, 5 per sex	5000 mg/kg bw (limit test)	LD50 > 5000 mg/kg bw (no deaths occurring)	Piloerection, diarrhea, subdued behaviour, hunched posture, soiled anal and perigenital areas	Cuthbert and Jackson, 1993a
Acute dermal toxicity	Sprague-Dawley rat, 5 per sex	2000 mg/kg bw (limit test), 24 h exposure	LD50 > 2000 mg/kg bw (no deaths occurring)	No clinical signs noted, no abnormalities detected at necropsy	Cuthbert and Jackson, 1993b
Skin irritation	Albino rabbit, m/f, number not given	500 mg applied	Non-irritant (to intact skin)	-	Birch, 1973*
Eye irritation	Albino rabbit, 1 male, 2 females	100 mg applied	Slightly irritant	Slight to moderate erythema, very slight edema, discharge	Birch, 1973*
Sensitization (Magnusson-Kligman test with preliminary dose ranging test)	Dunkin-Hartley guinea pig, 20 females/group (one control and one treatment group included), 8 females used for range finding procedures	test material concentrations for intradermal induction: 10%; for topical induction: 25%; for challenge: 25%	Not sensitizing	Slight dermal irritation following induction procedures	Cuthbert and Jackson, 1993c

* study providing supplementary information only
Remark: The purity of the test material was not indicated in these studies.

Birch, M.D. (1973): Toxicological investigation of CP 50435. Younger Laboratories Inc., St. Louis, Missouri; Monsanto Project no. Y-73-19. Dates of experimental work: not given. GLP: no. When the study was performed, GLP was not compulsory. The study is considered supplementary since experimental procedures are described very briefly only.

Cuthbert, J.A. and Jackson, D. (1993a): AMPA: Acute oral toxicity (limit) test in rats. Inveresk Research International, Tranent, Scotland; Report no. 8763, IRI Project no. 552409. Dates of experimental work: 01 April 1992 - 15 April 1992. GLP: yes. The study is considered acceptable.

Cuthbert, J.A. and Jackson, D. (1993b): AMPA: Acute dermal toxicity (limit) test in rats. Inveresk Research International, Tranent, Scotland; Report no. 8764, IRI Project no. 552409. Dates of experimental work: 01 April 1992 - 15 April 1992. GLP: yes. The study is considered acceptable.

Cuthbert, J.A. and Jackson, D. (1993c): AMPA: Magnusson-Kligman maximisation test in guinea pigs. Inveresk Research International, Tranent, Scotland; Report no. 8765, IRI Project no. 552409. Dates of experimental work: 01 April 1992 - 01 May 1992. GLP: yes. The study is considered acceptable.

Subacute and subchronic studies

Table B.5.8.1-2: Summary of subacute and subchronic studies with AMPA in rats and dogs

Study type, species, strain, number and sex	Purity of test material	Dose levels	NOEL/NOAEL	Main effects/ Target organs	Reference
14-day feeding study, CD rats, 5 animals/sex and dose	99.9%	0-1000-2000-4000 mg/kg bw/d	NOEL: 2000 mg/kg bw/d	Bw gain (males) and food consumption↓; red colored material in urine of one top dose male	Goldenthal, 1978*
4-week dose range (oral gavage) study, Sprague-Dawley rats, 5 animals/sex and dose	not given	0-10-100-350-1000 mg/kg bw/d	NOEL: 100 mg/kg bw/d	Bw gain (females)↓; kidney weight (males)↑	Heath et al., 1993
90-day feeding study, CD rats, 20 animals/sex and dose	99.96%	0-400-1200-4800 mg/kg bw/d	NOEL: 400 mg/kg bw/d	Mortality, clinical signs (distended abdomen, soft stools); males: bw gain and food consumption↓; clinical chemistry (lactate dehydrogenase↑); epithelial hyperplasia (urinary bladder and kidney pelvis)	Estes et al., 1979
13-week oral (gavage) study, Sprague-Dawley rats, 10 rats/ sex and dose	not given	0-10-100-1000 mg/kg bw/d	NOEL: 1000 mg/kg bw/d	No remarkable findings	Strutt et al., 1993
One-month study in Beagle dogs, capsule administration, 2 animals/sex and dose	94.38%	0-10-30-100-300-1000 mg/kg bw/d	NOEL: 100 mg/kg bw/d	Haematological changes indicative of a mild to moderate anaemia (red blood cell count, haematocrit and haemoglobin↓, reticulocyte count↑); at the top dose level more frequent occurrence of diarrhea	Stout, 1991
90-day study in Beagle dogs, capsule administration, 5 animals/sex and dose	87.8%	0-10-30-100-300 mg/kg bw/d (nominal), actual doses due to low purity: 8.8; 26.3, 87.8, 263 mg/kg bw/d	NOEL: 300 (263) mg/kg bw/d	No remarkable findings	Tompkins, 1991

* study providing supplementary information only

Goldenthal, E.I. (1978): Fourteen day rat feeding study. International Research and Development Corp., Mattawan, Michigan; Report no. IRD-77-309, Study no. 401-026. Dates of experimental work: 25 November 1977 - 9 December 1977. GLP: no. When the study was performed, GLP was not compulsory. The study is considered supplementary since the range of parameters investigated was rather limited, e.g. haematology, blood und urine clinical chemistry and histopathology were not performed.

Heath, J.; Strutt, A.; Iswariah, V. (1993): AMPA 4 week dose range finding study in rats with administration by gavage. Inveresk Research International, Tranent, Scotland; Report no. 7803, IRI Project no. 450860. Dates of experimental work: 20 February 1992 - 19 March 1992. GLP: yes. The study is considered acceptable although histological evaluation was confined to the suspected target organs (urinary bladder and mandibular, sublingual and parotid salivary glands).

Estes, F.L.; Jefferson, N.D.; Blair, M. and Goldenthal, E.I. (1979): 90-day subacute rat toxicity study. International Research and Development Corp., Mattawan, Michigan; Report no. IRD-78-174, Study no. 401-050. Dates of experimental work: 27 June 1978 - 26 September 1978. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable.
Supplemental report to this study:
Lauer, R. (1979): Analysis of animal feed diets in the aminomethylphosphonic acid (AMPA) 90-day subacute rat toxicity study, performed at International Research and Development Corporation. Monsanto, St. Louis; Report no. MSL-0682, project no. 7163. In this report, results of the dietary analysis are given. It has been shown that AMPA was stable in the diet for the required one-week period and that an average of 98.2% of planned the level has been fed over the 90-day study period.

Strutt, A.; Atkinson, C.; Hudson, P. and Snodgrass, E. (1993): AMPA 13 week toxicity study in rats with administration by gavage. Inveresk Research International, Tranent, Scotland; Report no. 7866, IRI Project no. 450876. Dates of experimental work: 16 April 1992 - 17 July 1992. GLP: yes. The study is considered acceptable.

Stout, L.D. (1991): One month study of AMPA administered by capsule to Beagle dogs. Monsanto Environmental Health Laboratory, St. Louis, Missouri; Study no. 90074, Project no. ML-90-186. Dates of experimental work: 04 April 1990 - 03 May 1990. GLP: yes (self-certification of the laboratory). The study is considered acceptable although histopathology was not performed.

Tompkins, E.C. (1991): 90-day oral (capsule) toxicity study in dogs with AMPA. WIL Research Laboratories, Inc., Ashland, Ohio; Report no. WIL-50173, Sponsor no. WI-90-354. Dates of experimental work: 05 September 1990 - 06 December 1990. GLP: yes (self-certification of the laboratory). The study is considered acceptable.
Supplemental report to this study:
Holland, M.E. (1991): Results of the stability analyses of AMPA (aminomethylphosphonic acid) test material used in a 90-day dog study at WIL Laboratories (WI-90-354). Monsanto Environmental Health Laboratory, St. Louis, Missouri; Report no. MSL-11291. The stability of the lot of AMPA used as test material over the study period was shown. In addition, purity values for the test material of 88.3% and 90.6% were reported.

Mutagenicity studies

Table B.5.8.1-3: Summary of mutagenicity tests on AMPA *in vitro* and *in vivo*

Study type	Test system	Purity of test material	Dose range/ Metabolic activation	Result	Reference
Ames test	S.typhimurium strains TA 98, 100, 1535, 1537 and 1538; E.coli strain WP2hcr	99%	10 - 5000 µg/plate, with and without S9 mix	Negative	Shirasu et al., 1980*
Ames test	S.typhimurium strains TA 98, 100, 1535 and 1537; plate incorporation and preincubation assay	99.2%	310 - 5000 µg/plate, with and without S9 mix	Negative	Jensen, 1993a
Mammalian cell gene mutation assay	Mouse lymphoma cells (L5178Y)	99.2%	310 - 5000 µg/ml, with and without S9 mix	Negative	Jensen, 1993b
Rec assay	Bac. subtilis strains M45, H17	99%	20 - 2000 µg/disk	Negative	Shirasu et al., 1980*
UDS test in vitro	Primary rat hepatocytes	94.38%	5 - 5000 µg/ml	Negative for UDS up to 2500 µg/ml; cytotoxicity at higher doses disabling analysis	Bakke, 1991
Bone marrow micronucleus test	NMRI:Bom mice, a total of 15/sex allocated to three groups (5/sex) sacrificed 24, 48 and 72 h post dose; positive and negative control groups also included	99.2%	5000 mg/kg bw, single oral exposure	Negative	Jensen, 1993c
Bone marrow micronucleus test	CD-1 mice, 15/sex and dose, groups of 5/sex/dose killed at 24, 48 and 72 h post dose; positive and negative control groups also included	94.38%	100-500-1000 mg/kg bw, single i.p. injection	Negative	Klier and Stegeman, 1993

* study providing supplementary information only

Shirasu, Y.; Moriya, M. and Ohta, T. (1980): CP50435: Microbial mutagenicity study. Institute of Environmental Toxicology, Kodaira, Tokyo, Japan; Report no. ET-80-402. Dates of experimental work: not given. GLP: no. When the study was performed, GLP was not compulsory. This report includes the description and the results of two experiments, an Ames test and a rec assay. These studies are considered supplementary only due to reporting deficiencies and since a confirmatory experiment has not been performed. Moreover, the rec assay was run without metabolic activation only.

Jensen, J.C. (1993a): Mutagenicity test: Ames Salmonella test with AMPA, batch 286-JRJ-73-4. Scantox, Lille Skensved, Denmark; Laboratory no. 13269. Dates of experimental work: 20 October 1992 - 06 November 1992. GLP: yes (self-certification of the laboratory). Formal certificate of a national authority is lacking. The study is considered acceptable.

Jensen, J.C. (1993b): Mutagenicity test: In vitro mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y). Test compound: AMPA, batch 286-JRJ-73-4. Scantox, Lille Skensved, Denmark; Laboratory no. 13270. Dates of

experimental work: 14 December 1992 - 27 January 1993. GLP: yes (self-certification of the laboratory). Formal certificate of a national authority is lacking. The study is considered acceptable.

Bakke, J.P. (1991): Evaluation of the potential of AMPA to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay using the male F-344 rat. SRI International, Menlo Park, California; SRI Study no. 2495-V01-91, Monsanto Study no. SR-91-234. Dates of experimental work: 20 June 1991 - 18 September 1991. GLP: yes (self-certification of the laboratory). Formal certificate of a national authority is lacking. The study is considered acceptable.

Jensen, J.C. (1993c): Mutagenicity test: Micronucleus test with AMPA, batch 286-JRJ-73-4. Scantox, Lille Skensved, Denmark; Laboratory no. 13268. Dates of experimental work: 26 October 1992 - 12 November 1992. GLP: yes (self-certification of the laboratory). Formal certificate of a national authority is lacking. The study is considered acceptable.

Kier, L.D. and Stegeman, S.D. (1993): Mouse micronucleus study of AMPA. Monsanto Environmental Health Laboratory, St. Louis, Missouri; Project no. EHL-90170/ML-90-404. Dates of experimental work: 20 August 1990 - 24 October 1990. GLP: yes (self-certification of the laboratory). The study is considered acceptable.

Developmental toxicity studies

Table B.5.8.1-4: Summary of developmental toxicity studies on AMPA in rats

Species, strain, number of mated fe-males on test	Dose levels and study design	Purity of test mate-rial	NOEL, maternal toxicity/Main effects if occurring	NOEL, develop-mental toxicity/ Main effects if occurring	Reference
Sprague-Dawley (Crl:CD) rat, 8/group (range-finding study)	0-125-250-500-750-1000 mg/kg bw; oral (gavage) administration from gestation day 6 through 15	94.38%	1000 mg/kg bw/d	1000 mg/kg bw/d	Holson, 1991a
Sprague-Dawley (Crl:CD) rat, 25/group	0-150-400-1000 mg/kg bw; oral (gavage) administration from gestation day 6 through 15	94.38%	150 mg/kg bw/d; clinical signs (incidence of hair loss and mucoid faeces↑) at 400 and 1000 mg/kg; bw gain and food con-sumption↓ (transitional effects) at 1000 mg/kg bw	400 mg/kg bw/d; mean fetal weight slightly but significantly reduced at the highest dose level, no evi-dence of terato-genicity	Holson, 1991b
Sprague-Dawley rat, 25/group	0-100-350-1000 mg/kg bw/d; oral (gavage) ad-ministration from gesta-tion day 6 through 16	not given	1000 mg/kg bw/d	1000 mg/kg bw/d	Hazelden, 1992

Holson, J.F. (1991a): A dose range-finding developmental toxicity study of AMPA in rats. WIL Research Laboratories, Inc., Ashland, Ohio; Report no. WIL-50146, Sponsor no. WI-90-247. Dates of experimental work: 19 June 1990 - 14 July 1990. GLP: yes (self-certification of the laboratory). The study is considered acceptable.

Holson, J.F. (1991b): A developmental toxicity study of AMPA in rats. WIL Research Laboratories, Inc., Ashland, Ohio; Report no. WIL-50159, Sponsor no. WI-90-266. Dates of experimental work: 07 August 1990 - 31 August 1990. GLP: yes (self-certification of the laboratory). The study is considered acceptable.

Supplemental report to this study:
Holland, M.E. (1991): Results of the analyses of corn oil samples from WIL Research Laboratories for AMPA (aminomethylphosphonic acid). Monsanto Environmental Health Laboratory, St. Louis, Missouri; Report no. MSL-10674. Projct no. ML-90-290/EHL 90164. The AMPA concentrations in corn oil dosing solutions used for the main teratology study in rats were determined and found to be 105 - 120% of the target levels.

Hazelden, K.P. (1992): AMPA teratogenicity study in rats. Inveresk Research International, Tranent, Scotland; Report no. 7891, IRI Project no. 490421. Dates of experimental work: 27 February 1992 (arrival of first batch of test animals) - 03 September 1992 (completion of fetal examination). The study was conducted in two successive replicates separated in time by 8 weeks. GLP: yes. The study is considered acceptable.

B.5.8.1.2 ACUTE ORAL TOXICITY OF A FURTHER MINOR METABOLITE

(N-methyl-N-phosphonomethyl)glycine is considered a further minor metabolite of glyphosate occasionally detected in plants and environmental media. The acute oral toxicity of this substance in rats has been investigated on behalf of the notifier Cheminova.

Jacobsen, S.D. (1991): Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats. Scantox, Lille Skensved, Denmark; Laboratory no. 12837. Dates of experimental work: 04 July 1991 - 18 July 1991. GLP: yes (self-certification of the laboratory). Formal certificate of a national authority is lacking. The study is considered acceptable.
5 male and 5 female SPF Wistar rats received the test compound (purity: 97.3%) by oral gavage at a dose level of 5000 mg/kg bw (limit dose). The test substance was administered in 1% carboxymethylcellulose to give a dosing volume of 20 ml/kg bw. A vehicle control group of the same size was also included in this study. Since no animals died throughout the 14-day post-observation period, the LD50 must be in excess of 5000 mg/kg bw. Clinical signs occurring mainly on days 1 and 2 after dosing were piloerection, pinched abdomen and diarrhea. Necropsy after two weeks did not reveal remarkable gross pathological changes.

B.5.8.2 FURTHER TOXICOLOGICAL STUDIES

B.5.8.2.1 TOXICITY OF GLYPHOSATE IN FARM ANIMALS

The acute and subacute toxicity of glyphosate and its isopropylamine salt has been studied in goats and cattle. It can be concluded that toxicity in ruminants is not higher than observed with laboratory animals. When the isopropylamine salt had been administered, the clinical course of poisoning was predominated by neurological signs, in particular in animals which died. In addition, there was evidence of slight renal damage.

Rowe, L.D. (1981/1987): The acute toxicity of glyphosate in female goats. United States Dept. of Agriculture Veterinary Toxicology and Entomology Research Laboratory, Texas; Report no.: VT-80-450, Laboratory report/project no.: 80006. Study terminated: 5 May 1981; study reported: 23 March 1987. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable. This report was submitted as part of the joint dossier of Monsanto and Cheminova.
Four groups consisting of five female Spanish goats were administered single doses of glyphosate acid via stomach tube at dosages of 1980, 3090, 4620, and 10000 mg/kg bw. Water was used as a vehicle to give a constant dosing volume of 500 ml per animal. Four control groups of five goats each were administered

water only. The LD50 (14 days) was calculated to be 3530 mg/kg bw. Clinical signs of toxicity included a decrease in food consumption, loss of body weight, abdominal distress, depression, ataxia, mild diarrhea and recumbency. The minimum dose used already produced mild symptoms (reduced food consumption, diarrhea). Changes of blood urea nitrogen concentration, serum creatinine concentration, and in the number of circulating segmental neutrophils were the most consistent laboratory findings at all dose levels. However, clinical chemistry parameters for surviving animals returned to normal towards the end of the study. No gross lesions which could be attributed to treatment were to be seen. Toxic tubular nephrosis was the only consistent histopathological lesion observed in fatally treated goats.

Rowe, L.D. (1981/1987): The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats. United States Dept. of Agriculture Veterinary Toxicology and Entomology Research Laboratory; Report no.: VT-80-451, Laboratory report/project no.: 80007. Study terminated: 8 May 1981; study reported: 23 March 1987. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable. This report was submitted as part of the joint dossier of Monsanto and Cheminova.
Five groups consisting of five female Spanish goats each were administered single doses of the formulated IPA salt (MON-0139) via stomach tube at dosages of 1400, 4290, 5360, 6700 and 10000 mg/kg bw. Water was used as a vehicle to give a constant dosing volume of 500 ml per animal. Five control groups of five goats each were administered water only. The LD50 (14 days) was calculated to be 5700 mg/kg bw. Signs of toxicity in fatally poisoned goats included decreased food consumption, abdominal distress, ataxia, and recumbency. Toxic signs in surviving goats were a decrease in food consumption, diarrhea, and loss of body weight. The lowest dosage of MON-0139 produced minimal (abdominal distension, evidence of reduced urination) or no toxic signs. Abortion occurred in one case in this group but was also seen in one control goat. The minimum dosage producing definite signs of illness (reduced food consumption, diarrhea) was 4290 mg/kg bw. One fatally poisoned goat (receiving 6700 mg/kg bw) and one surviving goat (4290 mg/kg bw group) each displayed an unusual "collapsing syndrome" of apparent neurological origin approximately 2 days after dosing. Signs of CNS dysfunction including behavioral abnormalities and convulsions were seen at dosages of 4290 mg/kg bw and above. Ischemic hippocampal neurons and focal edema in the hippocampus were observed in the brain of one affected goat. There were no concomitant biochemical or hematological changes. It was stated in the report that clinical entities like this "collapsing syndrome" are often associated with cerebral anoxia. No gross pathologic lesions were seen. Mild to severe tubular nephrosis was the only consistent histopathological lesion observed in fatally poisoned goats.

Rowe, L.D.; Martin, B.W.; Lovering, S.L.; Harvey, R.B. and Peterson, H.D. (1982/1987): The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle. United States Dept. of Agriculture Veterinary Toxicology and Entomology Research Laboratory; Report no.: VT-82-003, Laboratory study no.: 82002. Dates of experimental work: 02 February 1982 - 05 May 1982; study reported: 1987. GLP: no. When the study was performed, GLP was not compulsory. The study is considered acceptable. This report was submitted as part of the joint dossier of Monsanto and Cheminova.
Groups of 3 Brahman-cross heifers were administered MON 0139 at daily doses of 540, 830, 1290 and 2000 mg/kg bw by stomach tube for seven consecutive days. Water was used as a vehicle to give a constant dosing volume of 500 ml. Four control groups of two heifers each were sham-treated with tap water only. The dose levels had been selected on the basis of a preliminary trial.
All animals were at least once daily observed for clinical abnormalities. Body weight and hay consumption were determined regularly. Surviving heifers were observed for 14 - 15 days after the final dose. Blood samples for haematological and biochemical investigations were collected on 3 separate days during the pre-

treatment period and then on days 2, 6, 8, 14 and 21 after first dosing. All heifers were necropsied following unscheduled death or at study termination. Histopathology was confined to slides prepared for liver, kidney and tissues with gross lesions obtained from animals receiving doses of 1290 and 830 mg/kg bw/day and from 6 of the 8 control heifers.
All three top dose heifers died within 1.5 days after receiving the sixth or seventh dose. In these animals, nervous system effects like head tremors, convulsions, ataxia and possible visual impairment and sternal recumbency have been observed. In the group receiving 1290 mg/kg bw/day, one heifer also died after the final dose but without exhibiting signs of nervous dysfunction. Clinical signs of general toxicity included a decrease in food intake, weight loss, diarrhea and behavioural depression and were noted at the two upper dose levels. Diarrhea and decreased food consumption were also seen in the mid dose group receiving 830 mg/kg bw/day. At the two upper dose levels, haematological changes (i.e. elevations in haematocrit, haemoglobin and red blood cell count) were indicative of haemoconcentration probably resulting from diarrhea. An increase in ASAT, creatine phosphokinase and lactate dehydrogenase activity suggests rather muscle than liver damage since there were no histological liver findings. Muscle damage could result from convulsions or prolonged sternal recumbancy. Some further biochemical findings (blood urea nitrogen↑, creatinine↑) could be well due to dehydration but, in particular in the presence of histological kidney lesions, might also provide evidence of renal function impairment. Gross pathology revealed signs of dehydration and of gastro-intestinal irritation at the upper dosages. Kidney and liver to brain weight ratios were increased in these groups. Histopathological findings were confined to the kidneys and consisted of mild to marked tubular vacuolization and nuclear pyknosis in the heifers receiving 1290 mg/kg bw/day. The lowest dose of 540 mg/kg bw/day can be considered a NOEL for toxic effects in this study.

B.5.8.2.2 MECHANISTIC STUDY FOR CLARIFICATION OF SALIVARY GLAND FINDINGS

Effects on salivary glands were observed in one long-term study in rats (see B.5.5.1.1) and in some subchronic studies in rats and mice (see section B.5.3.2). This rare finding was characterized by a histopathological feature described as "cellular alteration" (for explanation of details see the aforementioned sections). In rats, cellular alteration was observed in two strains. It is not clear whether changes of this type were generally a result of glyphosate administration because such effects have not been reported before. However, similar findings, if occurring, would simply not have been detected in many other studies due to methodical reasons, i.e. the lack or the only limited extent of histopathological investigation of salivary glands.

Published literature
Because of morphologic similarities to salivary gland changes observed with the adrenergic agonist isoproterenol, a subacute study was designed to test the hypothesis that the salivary gland effects of glyphosate were also mediated through an adrenergic mechanism (Chan and Mahler, 1992). Groups of four male F344/N rats received glyphosate at a dietary level of 50000 ppm or were fed an untreated control diet. In three of the groups, the adrenergic agonist isoproterenol and/or the antagonist propranolol were administered by continuous subcutaneous infusion by osmotic minipumps. The study design was as follows:
Group 1: control diet, only vehicle (water + 0.1% ascorbate) administered by minipump;
Group 2: glyphosate diet, only vehicle administered by minipump;
Group 3: glyphosate diet, propranolol (1.2 mg/kg bw/d) administered by minipump;
Group 4: control diet, isoproterenol (1.0 mg/kg bw/d) administered by minipump;
Group 5: control diet, isoproterenol and propranolol administered by minipump.

After 14 days of treatment, the left parotid and submandibular/sublingual salivary glands were removed, weighed separately and processed for electron microscopy. The right parotid and submandibular/sublingual salivary glands were removed, sectioned and stained for histological evaluation.
Both isoproterenol and glyphosate induced significant enlargement of the salivary glands, glyphosate having much greater effect than isoproterenol. The parotid was most affected. Propranolol inhibited the effect of both substances on salivary gland weight but not completely in the case of glyphosate. Microscopically, similar changes were induced by glyphosate and isoproterenol consisting of cytoplasmic basophilic change, fine vacuolation and swelling of acinar cells resulting in a relative reduction in the number of ducts present. Glyphosate-treated animals were most severely affected. Propranolol, however, clearly protected the rats from the more severe lesions. Likewise, modest protection of histological effects caused by isoproterenol was seen. Cytoplasmic alteration of the submandibular gland was more subtle and histologically detectable only in glyphosate-treated animals. However, electron microscopy elucidated an effect of isoproterenol on this gland, too. It could not be determined if the serous or mucous glandular acini were selectively affected by glyphosate. No changes were seen in the sublingual glands examined from any group demonstrating target specifity of glyphosate- and isoproterenol-associated lesions to those salivary glands which are mainly innervated by adrenergic fibers.
The authors assume that effects of glyphosate on salivary glands were due to an adrenergic mechanism. The biological significance of this finding is unkown (Chan and Mahler, 1992).

B.5.8.2.3 PHARMACOLOGICAL AND BIOCHEMICAL STUDIES

Pharmacological effects of glyphosate, its salts or the herbicide Roundup® have been studied in different species. Studies of this type are usually not required in Europe for registration of a compound like glyphosate for the use in plant protection. Nontheless, these studies are considered to provide additional information and reported briefly in this monograph, therefore. In addition, efforts have been made to elucidate the mechanism of toxicity of glyphosate products including the examination of toxic effects caused by surfactants.

Original studies

Takahashi, H. and Kakinuma, Y. (1992): Ammonium salt of glyphosate (MON-8750): General pharmacological study. Institute of Environmental Toxicology, Tokyo, Japan; Study no. IET 90-0149/ET-92-15). Dates of experimental work: 04 February 1992 - 02 March 1992. GLP: yes (self-certification of the laboratory). This report was submitted as part of the joint dossier of Monsanto and Cheminova.
The potential effects of MON 8750 (purity: 94.78%) on the nervous, respiratory and circulatory systems were assessed following single i.p. injection to male and female ICR mice at doses up to 5000 mg/kg bw and single i.v. injection to urethane-anesthetized and non-anesthetized male rabbits at doses up to 500 mg/kg bw. At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived i.v. application of 500 mg/kg bw although animals showed some neurological signs. In rabbits which died, heart rate was decreased and ECG changes have been noted. At the next lower dose levels (1250 mg/kg bw in mice or 125 mg/kg bw and 31.3 mg/kg bw in rabbits, respectively), transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. Respiratory rate was increased in surviving rabbits but decreased in anesthetized rabbits which died. It was concluded that an impact on cardiorespiratory functions is involved in acute toxicity. The lethal dose appears to be decreased under anesthesia.

Anonym (not before 1988): Toxicodinamic study of glyphosate in rat. Ministry of Agriculture, Centre of Plant Protection and Agrochemistry, Toxicological Laboratory, Keszthely, Hungary. Study identification and dates of experimental work not given. GLP: no. The report was submitted by the notifier Alkaloida. Half an hour after narcotization with a chloralose-urethan mixture, male Wistar rats were administered a single dose of 5000 mg glyphosate (96% pure)/kg bw by oral gavage. All animals died within 2 - 7 hours. Treatment was followed by a marked decrease in arterial pressure by approximately 50% two hours after treatment as compared to the initial values. There was no clear impact on heart and respiratory rate. ECG and venous pressure changes, if occurring in some animals, were considered incidental and body temperature was not affected.

Bhide, M.B. and Naik, P.Y. (1987): Synergism and potentiation in rats of glyphosate (tech.) of Excel Industries Ltd., Bombay. Study identification and dates of experimental work not given. GLP: no. This report was submitted independently by the notifiers Barclay and Luxan.
Glyphosate was administered to groups of 10 male Wistar rats as a single oral dose of 5000 mg/kg bw at a constant dose volume of 10 ml/kg in corn oil. Simultaneously, the animals received the compound 2,4-D sodium salt at dose levels of 376, 473, 596, 750, 944 or 1189 mg/kg bw. The second compound used for a potentiation experiment was dalapon at doses of 2500 and 5000 mg/kg bw. After simultaneous dosing, the rats were observed for 14 days for toxic symptoms and mortality. According to the study authors, no potentiation has been observed with glyphosate and dalapon. However, two out of ten rats died after application of 5000 mg/kg bw glyphosate and 5000 mg/kg bw dalapon. When both compound were administered alone, no mortalities occurred.
The simultaneous administration of glyphosate and 2,4-D sodium salt caused a markedly higher mortality in all dose groups. In the groups receiving glyphosate and doses of 596 mg 2,4-D sodium salt/kg bw and above, all animals died. When the latter compound was administered alone, 100% mortality has been reached only at 1189 mg/kg bw. Hence, at least an additive acutely toxic effect of glyphosate and 2,4-D can be assumed.

Mizuyama, K. (1987): Irritating effect of glyphosate, surfactant and Roundup on stomach and small intestine in dogs. Dep. of clinical medicine, University of Tsukuba, Japan. The study was submitted as part of the joint dossier of Monsanto and Cheminova.
The IPA salt of glyphosate, Roundup herbicide (41% IPA) and the surfactant MON 0818 (15 % of which is contained in Roundup) and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup® herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.

Published literature
Tai, Yamashita and Walimori (1990) studied the haemodynamic effects of continuous i.v. application of either glyphosate IPA salt, the formulation Roundup or a surfactant (according to the notifier Monsanto/Cheminova who submitted this publication: tallow amine surfactant) in dogs. The impact on cardiovascular functions was studied in groups of five anesthetized and artificially ventilated female Beagle dogs. Duration of i.v. exposure was 60 minutes. A total of 8.2 g glyphosate (IPA salt administration) or 2.8 g glyphosate (Roundup) was injected into the dogs. These amounts would correspond to doses of about 550 - 820 mg/kg bw or 180 - 280 mg/kg bw, respectively, since the body weight of the dogs was 10 to 15 kg.

The surfactant and Roundup significantly reduced the blood pressure, cardiac output and left ventricular stroke work index suggesting a marked effect on circulation. It could be shown that the cardiac depression observed with Roundup was likely due to the surfactant since, in contrast, mean arterial blood pressure was even increased when glyphosate isopropylamine salt had been injected. Similarly, the IPA salt did not cause changes in heart rate or cardiac output. A decrease in blood pH observed in this group could be either due to a direct effect of administration or to metabolic acidosis. In any case, it was not strong enough to affect the circulatory system.

Olorunsogo, Bababunmi and Bassir (1979) investigated the effect of glyphosate on rat liver mitochondria *in vivo*. The IPA salt of glyphosate enhanced the rate of oxigen consumption by the mitochondria isolated from livers of rats sacrificed 5 hours after a single intraperitoneal dose. The reaction medium used was deficient in phosphate acceptor. It was suggested that uncoupling of mitochondrial oxidative phosphorylation was a major lesion in glyphosate intoxication. However, this assumption was questioned by Talbot et al. (1991) since technical problems make the aforementioned results of Oloronsogo et al. difficult to interpret. Therefore, up to now, the mechanism behind glyphosate toxicity is not considered to be sufficiently characterized on a subcellular level.

B.5.9 MEDICAL DATA

Information coming from medical surveillance of plant personnel is rather limited especially when the large scale production and the diversity of manufacturing facilities all over the world is taken into consideration. In addition, some data on fieldworker exposure and few studies in human volunteers have been submitted. Although glyphosate is a widely used herbicidal compound in agriculture and other fields, epidemiological studies on general population exposure are not available.
Despite the low acute toxicity of glyphosate active ingredient there is much experience concerning intoxications of humans who have been orally exposed to formulations in particular Roundup herbicides. Accordingly, there is sufficient information on the effects of poisoning occurring in humans and on medical treatment. There is strong evidence also supported by pharmacological studies that these effects might be rather due to certain by-products like surfactants but not to the active ingredient itself. Following inhalative exposure to formulations containing glyphosate, transient symptoms like headache were occasionally observed but there is no confirmed evidence of serious or long-lasting adverse health effects.

B.5.9.1 MEDICAL SURVEILLANCE ON MANUFACTURING PLANT PERSONNEL

The only more concrete information was submitted as part of the joint dossier of Monsanto and Cheminova and refers to the results of medical surveillance on production and maintenance workers at the Monsanto plant in Antwerp, Belgium. Neither abnormalities nor cases of injury due to exposure with glyphosate acid or formulated IPA salt (including manufacturing intermediates and formulations) have been detected there. The only symptom that has been recorded on one occasion was epistaxis in two workers who had been accidentally exposed to an intermediate of glyphosate. There were no cases neither of skin irritation nor of dermal or respiratory sensitization due to the active ingredient or the formulations. So far this point was addressed by the other notifieres at all, no statement on their actual experience was given.

B.5.9.2 FIELDWORKER EXPOSURE

Published literature
Based on published data, it can be concluded that exposure of workers to the herbicide Roundup under normal conditions is low (Lavy, 1992; Jauhiainen et al. 1991). However, the occurrence of weak clinical symptoms upon inhalative exposure cannot be completely excluded since in one study there was evidence of headache during the spraying period (Jauhiainen et al., 1991).

Lavy et al. (1992) measured the Roundup exposure of 14 conifer seedling workers (applicators, weeders, and scouts) employed at two tree nurseries for years. The authors stated that glyphosate exposure via dislodgeable residue, direct contact during applications or season-long exposure would not pose a threat to human health when used under normal tree nursery conditions.

Also in forest workers, Jauhiainen et al. (1991) determined the occupational exposure to glyphosate during brush saw spraying work. The purpose of this study was to measure the exposure to Roundup as determined from the breathing zone and from urine samples. The possible health effects of glyphosate at the measured exposure levels were also evaluated. A test group of 5 workers (44 to 49 years old) sprayed an average of 9.8 litre of an 8% Roundup® solution each day (6-hour effective) for five consecutive days. A control group of five workers (36 to 54 years old) was also included. Exposure to glyphosate during application was low. For air samples collected midweek during the spraying, glyphosate concentrations

were less than the limit of detection of 1.25 µg/m^3. At the end of the spray period, two air samples were found to contain measurable concentrations of glyphosate (2.8 and 17.5 µg/m^3). At an estimated breathing rate of 1.8 m^3/h, the maximum exposure to glyphosate via inhalation in this study would be 31.5 µg/h. In the urine samples, no traces of glyphosate were detectable. Findings in the medical examinations before and after the exposure did not differ. However, two workers in the exposed group reported headache during the workweek.

The following publication is considered unacceptable for evaluation purposes because the information concerning glyphosate is equivocal. One cannot exclude that exposure to glyphosate formulations may have contributed to the poisoning incidents with unknown chemicals. Therefore, a lack of any risk cannot be postulated with respect to this data.
Griffith, Duncan and Konefal, J. (1985) reported pesticide poisonings among Florida citrus fieldworkers. In a survey of 1811 Florida citrus fieldworkers performed in 1981, 25 pesticide related poisoning incidents involving 29 fieldworkers were analysed. Accidents with glyphosate have not been reported. However, 17 of these cases were caused by unknown agrochemicals.

B.5.9.3 OBSERVATION ON EXPOSURE OF THE GENERAL POPULATION

Exposure may occur through ingestion of residues on treated crops. According to the Rapporteurs database, no epidemiological studies relating to the exposure of the general population to glyphosate or its salts are available.

B.5.9.4 VOLUNTEER STUDIES

Original study
Bhide, M.B. (not dated): Field monitoring studies on human volunteers and live stock with Glycel 41 % SL of Excel Industries Ltd., Bombay. Indian Institute of Toxicology, Bombay, India; Report no.: not given. The study was independently submitted by Luxan and Barclay. Due to reporting deficiencies, the study is considered to provide supplementary information only.
To determine the effects of a glyphosate formulation on human volunteers, Glycel 41 % SL was diluted in water to contain 0.75 % active ingredient. A quantity of 1800 litres (13.5 kg a.i.) was sprayed on fallow land (about 2 hectares) with weeds by 10 volunteers wearing hand gloves, eye goggle, nose-guard, full shirt and pyjama. The spraying commenced early in the morning lasting 8 hours. Furthermore, five male and five female calves were exposed to the accidental drift of the spray. The volunteers and the calves were clinically examined and blood samples were taken before and at the end of the trial and again 1 and 7 days after. No significant changes in respect of clinical, haematological and biochemical parameters were to be seen neither in the humans nor in the animals.

Published literature
Roundup herbicide containing the IPA salt (41 % glyphosate), water and surfactant was evaluated for acute and accumulative irritation and allergic potential in 346 volunteers (Maibach, 1986). The test compound was used undiluted and as a 10% v/v dilution in distilled water. Roundup produced at most only minor skin irritation. The herbicide had no greater irritation potential on intact skin than either an all purpose cleaner, a dishwashing liquid, or a baby shampoo which were also tested in concurrence. When tested on abraded skin, Roundup had a slightly greater incidence of erythema at the 24-hour reading. Statistical analysis of the 21-day cumulative irritation data showed that the herbicide and the baby shampoo were less irritant than the all purpose cleaner and the dishwashing liquid. No evidence of the induction of photoirritation, allergic or photoallergic contact dermatitis was obtained.

B.5.9.5 DIRECT OBSERVATION IN HUMANS (E.G. CLINICAL CASES OF POISONING)

There were no poisoning incidents reported which could be related to glyphosate acid or its sodium or monoammonium salts. The described cases of intoxication were all attributed to IPA salt formulations in particular the widely used herbicide Roundup suggesting that poisoning could be well due to other ingredients of the formulated products. The route of exposure was mostly oral and in rare cases also inhalative or dermal. In addition, there is limited evidence of some phototoxicity of the domestic product "Tumbleweed" containing glyphosate. However, this effect is more likely due the preservative benzisothiazolone. As reported above (see section B.5.9.4), no indications of phototoxicity due to Roundup have been observed in a large number of human volunteers.

Published literature

1. Acute poisoning

Talbot et al. (1991) reviewed and described a large number of cases of acute poisoning with a glyphosate-surfactant herbicide (`Roundup`) occurring in Taiwan. Between 1980 and 1989, 93 cases of intoxication were treated at the Changhua Christian Hospital, Taiwan, of which 90 incidents were attributed to a Monsanto product (containing 41% glyphosate as the IPA salt, 15% surfactant, and water). Besides clinical examination, hematological and biochemical laboratory investigations have been performed. Poisoning due to some other agricultural agents (i.e. organophosphates and, with one exception, paraquat) was excluded by appropriate test methods.

This database includes 13 cases of accidental oral exposure as well as 80 cases of intentional ingestion. In addition, 6 cases of accidental dermal exposure were mentioned, however, it is not clear whether the affected people belong to those treated at the hospital since in sum the number of 93 would be exceeded and dermal exposure was asymptomatic.

74 of the exposed persons showed clinical symptoms of poisoning and there were seven deaths, five of them occurring within 12 hours after ingestion with two persons already dead on arrival at the hospital. The other two victims died within 24 hours or at 38.5 hours following ingestion despite medical care. Two of these mortalities were due to combined intake of Roundup and paraquat or parathion, respectively.

The clinical signs exhibited were classified for severity into four groups (table B.5.9.5-1).

Table B.5.9.5-1: Classification of severity of symptomes observed in acute poisoning with glyphosate-based herbicides

Classification	Description
Asymptomatic	No complaints and no abnormalities on physical or laboratory examination.
Mild	Mainly GIT symptoms (nausea, vomiting, diarrhoea, abdominal pain, mouth and throat pain) that resolved within 24 h. Vital functions were stable, and there was no renal, pulmonary or cardiovascular involvement.
Moderate	GIT symptoms lasting longer than 24 h, GIT haemorrhage, endoscopically verified oesophagitis or gastritis, oral ulceration, hypotension responsive to intravenous fluids, pulmonary dysfunction not requiring intubation, acid-base disturbance, evidence of transient hepatic or renal damage, or temporary oliguria.
Severe	Pulmonary dysfunction requiring intubation, renal failure requiring dialysis, hypotension requiring treatment with pressor amines, cardiac arrest, coma, repeated seizures, or death.

GIT: Gastrointestinal tract

Accidental oral intake was asymptomatic or caused mild oral discomfort as sole sign since only small amounts were ingested and immediately rejected.

Intentional ingestion (80 cases) resulted in gastrointestinal signs observed in 66 % of the patients. Symptoms included sore throat (43 %), dysphagia (31 %) and gastrointestinal haemorrhage (8 %). The average amount of Roundup ingested by non-survivors was in the range of 85 - 200 ml. In contrast, much larger amounts

(500 ml) were reported to have been ingested by some patients showing only mild to moderate symptoms. Deaths following ingestion of the glyphosate herbicide alone were due to a syndrome that involved hypotension, unresponsive to intravenous fluids or vasopressor drugs, and sometimes pulmonary oedema in the presence of normal central venous pressure. The incidence of clinical signs, changes in hematological and clinical chemistry parameters and findings upon further medical examinations is given in table B.5.9.5-2.

Table B.5.9.5-2: Incidence of physical and laboratory findings

Organ/tissue	Number examined	Finding	Number affected
GIT	49*	sore throat	32
		mouth ulcer	32
		dysphagia	23
		epigastric pain	16
		melaena	6
		vomiting	1
Blood	48	leucocytosis	48
		differential count: shift-to-left	1
Lung	17	abnormal chest radiographs	9
		aspiration	1
Heart	13	shock	7
		arrythmia	4
		abnormal ECG	7
Kidney	10	oliguria	3
		haematuria	3
		creatinine increased	9
		blood urea nitrogen increased	0
Liver	14	ASAT increased	14
		ALAT increased	8
CNS	9	confused	3
		coma	6

GIT: Gastrointestinal tract
* endoscopy performed in 23 cases

The authors stated that the symptomatology in this series was generally in agreement with 56 cases including 9 deaths reported from Japan although the incidence of individual effects was different.
It was concluded that the toxic syndrome resulting from the massive ingestion of Roundup consists of gastrointestinal mucosal irritation, hypotension, and pulmonary insufficiency. It was suggested by the authors that those people over 40 years of age, who ingest more than 100 ml, are at the highest risk of a fatal outcome.

Tominack, Conner and Yamashita (1989) discussed the clinical management of Roundup herbicide exposure. To facilitate poisoning control, the authors have reviewed six papers dealing with such intoxications, mainly suicide attempts. It was stated that accidental exposure to small volumes of Roundup herbicide does not produce serious toxic effects. Minor local irritation is responsive to basic first aid care. Large amounts ingested, in contrast, may produce severe gastrointestinal irritation, shock, pulmonary edema, renal shutdown, and possibly death. The following symptoms and findings have been reported after oral ingestion of large amounts: neurologic (drowsiness or stupor), effects on oral mucosa (sore throat, salivation, erythema, ulceration), gastrointestinal (nausea and vomiting, epigastric and abdominal pain, diarrhea), cardiovascular (hypotension), respiratory (asymptomatic hypoxemia, dyspnoea, cough, bronchospasm, cyanosis, aspiration pneumonia, pulmonary edema, and respiratory failure), renal (microscopic abnormalities and proteinuria in urine, increased blood urea nitrogen and serum creatinine concentration), and metabolic (mild fever, metabolic acidosis). Dermal and eye irritation may be caused by direct contact as well as oral or nasal irritation upon inhalation.
Any patient who has ingested more than 0.5 ml of Roundup®/kg bw should be considered for admission to a hospital for observation and monitoring.
Aggressive supportive treatment is suggested for ingestions of large amounts.

Kawamura et al. (1987) reported two cases of glyphosate (Roundup®) poisoning in Japan. Two males (72 and 27 years old) experienced glyphosate poisoning in an attempt of suicide. The patients ingested this herbicide in amounts of 100 ml and 20 ml, respectively. Gastric lavage, fluid therapy and forced diuresis were performed immediately. In one man, glyphosate was not detected in the blood at the third day while it was detected in the urine even at the ninth day. Both men survived and there were no severe toxic effects occurring. It was stated that intensive care including hemodialysis or hemoperfusion might be required if the amount of glyphosate ingested is exceeding 100 ml.

2. Phototoxicity

In the communication mentioned first (Hindson and Diffey, 1984a), the authors reported a phototoxic reaction of a 64-year old untanned Caucasian male to the weedkiller "Tumbleweed" which has become widely used as a domestic product. Subsequent information received from the manufacturer indicated that, in addition to its active ingredient glyphosate, the product contained the preservative benzisothiazolone. The patient was retested to the separate constituents glyphosate and benzisothiazolone. No phototoxic reactions were observed with glyphosate, but the combination of UV-A radiation and benzisothiazolone produced delayed erythema when radiation was in excess of 7 Joule/cm^2. The authors concluded that benzisothiazolone was the phototoxic agent in "Tumbleweed" but not glyphosate as they originally implied (Hindson and Diffey, 1984b).

B.5.9.6 PROPOSED TREATMENT OF POISONING

The medical treatment of poisoned people has already been addressed above (section B.5.9.5). In addition, a published clinical report focussing on different methods to force elimination of Roundup from the body is available. Furthermore, there is one *in vitro* study dealing with the use of adsorbents for control of poisoning in humans.

Takagagi, S. (1987): In vitro adsorption of glyphosate to various adsorbents (cholestylamine, activated charcoal and Kayexalate®). Clinical Medicine Dept., University of Tsukuba, Japan. Report identification and dates of experimental work not given. This study was submitted as part of the joint dossier of Monsanto and Cheminova.

Glyphosate IPA (purity not specified) was mixed with adsorbents in artificial gastric or intestinal juices to determine the adsorbent capacity cf the three compounds mentioned in the title. These mixtures were incubated at 37°C for 24 hours with constant stirring. Glyphosate levels in the supernatant were determined via an HPLC method. At pH 6.8 (artificial intestinal juice), cholestylamine adsorbed approximately 20 % of IPA. At pH 1.2 (artificial stomach juice), no adsorption occurred with cholestylamine. Little or no adsorption occurred with activated charcoal or Kayexalate® at either pH. It was recommended to use cholestylamine as an adsorbent after gastric lavage in poisoning cases with glyphosate IPA in an effort to capture glyphosate that had been transferred to the intestine.

Published literature

Hiraiwa et al. (1990)* compared the effect of hemodialysis, direct hemoperfusion and forced diuresis on glyphosate excretion following oral ingestion of Roundup herbicide (containing approximately 360g/l IPA salt and surfactant). A 62-year old man who ingested about 80 ml of Roundup® herbicide was treated with the methods mentioned above. Hemodialysis and hemoperfusion continued for four hours. It was suggested that hemodialysis is the most effective method to remove glyphosate from blood, if adequate circulation volume and systolic blood pressure are maintained. Diuresis is also considered effective.

* Original publication in Japanese, journal not identified due to lack of translation, English summary available

B.5.10 SUMMARY OF MAMMALIAN TOXICOLOGY AND EVALUATION

B.5.10.1 MAMMALIAN TOXICITY

Toxicokinetics and metabolism

Following oral administration, glyphosate is absorbed from the gastrointestinal tract rapidly but only to a limited extent of not more than 30 - 40%. Percutaneous absorption of glyphosate at least through the intact skin is confined to a small portion of less than 3%. Elimination via faeces and urine is rapid and nearly complete. Distribution into the organs and tissues is limited with generally low residues occurring. There is no evidence of accumulation in the animal body. After a period of 3 to 7 days following oral administration, total body burden accounted for less than 1% of the applied radioactivity. Elimination from bone is slower than from other tissues. By far, the highest residues were measured in this tissue followed by kidney and liver. This pattern of absorption, distribution and elimination was not significantly changed neither by single high doses administered nor by repeated administration of low doses. Similarly, there was no consistent sex influence. Metabolism of glyphosate, if occurring, is very limited. Most of the parent glyphosate is eliminated unchanged. To a little extent of less than 0.5%, aminomethylphosphonic acid (AMPA) which is known to be the major plant metabolite of glyphosate may be formed also in mammals. Studies in rabbits, goats and laying hen revealed a similar pattern of toxicokinetics and metabolism.

Acute toxicity

Glyphosate acid and its salts exhibit a low acute toxicity in laboratory animals by the oral and dermal route with LD50 values greater than 2000 mg/kg bw. General signs of oral intoxication were breathing difficulties, reduced activity, ataxia and convulsions.
The acute inhalation toxicity was also low with LC50 values above the limit test dose of 5 mg/l air per 4 hours obtained for the acid and the isopropylammonium salt (IPA). Toxic symptoms after inhalative exposure included irritation of the upper respiratory tract, hyperactivity, loss of hair, ruffled fur and a slight decrease in body weight but were not consistently observed throughout the studies. First death occurred at a concentration of 1.3 mg/l (4-hcur exposure) when IPA was tested. The ammonium salt was tested up to the maximum attainable concentration of 1.9 mg/l with comparable clinical signs but no deaths occurring. The sodium salt was not tested for inhalation toxicity.
Glyphosate acid and the IPA were more toxic by the intraperitoneal route with LD50 values down to 134 mg/kg bw. The occurrence of late deaths was assumed to be caused by subsequent peritonitis instead of a direct toxic drug effect.
Regarding primary irritation, glyphosate acid and the salts were found to be non-irritant to intact skin and only slightly irritant to abraded skin. Undiluted glyphosate acid was found to be strongly irritant to rabbit eyes requiring classification and labelling. There was markedly less eye irritation observed with the salts.
Neither glyphosate acid nor the salts have shown sensitizing effects in guinea pigs. However, only the acid and the IPA have been tested under the more stringent conditions of the Magnusson-Kligman test.

Short-term toxicity

Subacute and subchronic toxicity studies also revealed a low oral toxicity of the compound. The lowest NOELs observed were 50 mg/kg bw/day in a subacute and about 100 mg/kg bw/day in a 90-day feeding study in rats with first effects occurring in the range of 250 - 300 mg/kg bw/day. In most studies in rats, mice, and dogs, however, higher NO(A)ELs were established. Some lower values found in dogs were obtained in studies of limited scientific value but these rather equivocal findings were not confirmed in more recent experiments using much higher dosages. Changes in clinical chemistry parameters and liver weight

changes might indicate an impact on the liver at least in rats. Soft stools and diarrhea, together with occasionally reduced body weight gain and food consumption, suggest irritation of the gastrointestinal tract. In some oral rat studies and in one experiment with mice, effects on salivary glands were elucidated upon histopathological examination.
Repeated dermal exposure of rabbits and rats to glyphosate did not result in any systemic effects. Dermal irritation was observed only at doses as high as 5000 mg/kg bw/day in rabbits or 1000 mg/kg bw/day in rats.
Subacute inhalative toxicity of glyphosate active ingredient in rats is low. Up to the highest tested concentration of 3.8 mg/l air, neither local nor systemic toxicity was noted upon repeated exposure.

Mutagenicity
Glyphosate was examined for mutagenicity in a wide range of test systems covering all relevant endpoints *in vitro* as well as *in vivo*. Against the background of this large database, it can be concluded that the active ingredient does not exhibit a mutagenic risk to humans. In the vast majority of the studies, glyphosate proved clearly negative. One of the two micronucleus tests using the extremely high and already cytotoxic dose of 5000 mg/kg bw/day revealed a weak increase in the incidence of micronuclei but only in one sex. It should be also taken into consideration that there is no evidence of cancerogenic or teratogenic effects in humans although glyphosate products have been in world-wide use for many years.

Long-term toxicity and cancerogenicity
In long-term studies in rats and mice, no evidence of cancerogenicity was obtained. The lowest NOELs of 10 mg/kg bw/day and only for female animals 100 ppm (approximately 6 mg/kg bw/day) were established in chronic rat studies with first effects occurring in the range of 60 - 100 mg/kg bw/day. Mice appeared less vulnerable. Concerning the non-neoplastic effects upon long-term exposure, considerable differences among the different studies became apparent. In general, there were no adverse effects on survival and no clinical signs of toxicity in any of the chronic studies. Body weight gain was compromized in female Sprague-Dawley rats at the upper dietary level of 20000 ppm, equivalent to a mean daily intake of 1183 mg/kg bw. A lower body weight was also noted in male CD-1 mice receiving the extremely high dietary dose of 30000 ppm (ca 4800 mg/kg bw/day) for two years. A higher activity of alkaline phosphatase and liver weight changes in rats as well as centrilobular hepatocyte hypertrophy in male mice were assumed to reflect a weak effect on the liver. In addition, effects on the eyes and on salivary glands were observed in rats but not consistently seen in all studies. A higher incidence of cataracts was noted in male Wistar rats at 10000 ppm and in male Sprague-Dawley rats at 20000 ppm. In Sprague-Dawley rats, non-neoplastic histological changes accompanied by a higher organ weight occurred in the parotid and mandibular salivary glands in both sexes at 100 mg/kg bw/day and above. Similar microscopic lesions were observed in subchronic studies in rats and mice as well as in multigeneration studies in rats. On the other hand, neither in dogs nor in the long-term mouse studies, effects on salivary glands have been reported. A mechanistic 14-day study in rats suggested the changes to be mediated through an adrenergic mechanism. Local inflammation of gastric mucosa was noted in one study on Sprague-Dawley rats at the high and mid dose levels in both sexes. This finding was most likely due to mucosal irritation and might well correspond to epithelial hyperplasia in the urinary bladder as observed in a long-term mouse study at 5000 ppm and above.

Reproduction toxicity
A number of multigeneration studies in rats did not indicate a specific hazard of glyphosate for reproduction. Weak effects on the offspring as evidenced by a reduced pup weight were confined to dose levels as high as 30000 ppm. Compound-related effects in the parent animals were similar to those seen in the subchronic and long-term studies and occurred at comparable dose levels.

Glyphosate does not cause teratogenicity. Adverse effects on the number of viable fetuses and the fetal weight were noted in rats and rabbits at higher dose levels causing also maternal toxicity. A reduced ossification and a higher incidence of skeletal and/or visceral anomalies at these dosages were also indicative of fetotoxicity. The NOEL for developmental effects was 300 mg/kg bw/day in rats and 350 mg/kg bw/day in rabbits. In rabbits, maternal effects occurred at doses lower than those found effective in developmental toxicity, subacute and subchronic studies in rats and might indicate a higher vulnerability of this species.

Other experimental data
Toxicological studies in laboratory animals did not indicate a specific potential for neurotoxicity. Similarly, neurotoxicity studies in hen did not provide clear evidence of such effects.

Acute and subacute toxicity studies in goats and cattle confirmed the low toxicity of the compound. Clinical symptoms, however, were somehow different from those observed in laboratory animals. When the IPA had been administered at doses causing overt toxicity, the clinical signs were predominated by CNS dysfunction. In addition, slight renal damage was found upon histopathology. In rats, equivocal evidence of an impact on the kidneys was obtained in one subacute study only. A specific hazard for farm animals is not to be expected.

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

Acute toxicity of glyphosate formulations is mediated through an effect on cardiovascular (circulatory) and respiratory functions. A common sign of poisoning is a decrease in arterial blood pressure. There is evidence coming from pharmacological studies that this effect might be rather due to certain surfactants than to the active ingredient itself. This could explain the higher toxicity of some formulations as observed also in cases of human poisoning.

Medical data
Clinical examinations of employees involved in the manufacturing process gave no evidence of adverse health effects as a result of potential exposure to the active ingredient. No data on exposition of general population and no appropriate epidemiological studies are available.
However, there is a number of cases of acute intoxication in humans following accidental and intentional ingestion of glyphosate products. Clinical reports suggest that toxicity of formulations was mediated through an impact on cardiovascular (circulatory) and respiratory functions. The toxic syndrome resulting from massive oral intake consists of gastrointestinal mucosal irritation, hypotension and pulmonary insufficiency.
Following inhalative exposure to formulations containing glyphosate, transient symptoms like headache were occasionally observed but there is no confirmed evidence of serious or long-lasting adverse health effects.
Investigations with a Roundup formulation in human volunteers did not provide evidence of skin irritation, allergic or photoallergic contact dermatitis.

B.5.10.2 ACCEPTABLE DAILY INTAKE (ADI)

The acceptable daily intake should be based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. In the case of glyphosate, the different notifiers have proposed ADI values covering a wide range between 0.05 and 1.75 mg/kg bw (see table B.5.10.2-1). This variance is due to the different studies used as the respective basis

for ADI calculation but may also result from a controversial evaluation of critical studies.

Table B.5.10.2-1: Summary of ADI values proposed by the different notifiers and by the Rapporteur

Notifier	ADI (mg/kg bw)	Toxicological data on which this ADI proposal is based	Remarks of the Rapporteur
Monsanto/ Cheminova	1.75	Teratogenicity study in rabbits, NOEL: 175 mg/kg bw/d.	See discussion below.
Agrichem	0.1	3-generation study in rats, NOEL 10 mg/kg bw/d.	Based on published literature. Study not identified. Much higher NOELs have been established in more recent reproduction studies.
Alkaloida	0.06	12-month study in dogs, NOAEL: 300 ppm (5.79 - 14.62 mg/kg bw/d).	Supplementary study, NOAEL = highest dose tested.
Barclay	0.3	Chronic study in rats (NOEL 31 mg/kg bw/d) and 3-generation study in rats (NOEL 30 mg/kg bw/d) with reference to 1986 JMPR evaluation.	No original studies. In both cases, the NOELs were the highest doses tested. Both studies were considered supplementary only.
Feinchemie	0.05	Chronic study in rats, NOEL: 100 ppm (ca 5.5 mg/kg bw/d).	Interim report conclusion.
Herbex	-	Proposal for an ADI not submitted; appropriate studies not performed.	-
Luxan	0.15	Cancerogenicity study in mice (NOAEL 150 ppm, ca 15 mg/kg bw/d) and 3-generation study in rats (NOEL 300 ppm, ca 15 mg/kg bw/d).	Suppplementary studies. In the reproduction study, NOEL = highest dose tested. Much higher NOELs have been established in more recent long-term and reproduction studies.
Nufarm	-	No toxicological data submitted.	-
Sanachem	0.3	Published literature.	It is assumed that this value refers to the JMPR evaluation in 1986 (i.e. ADI derived from a long-term rat study).
SCC/I.Pi.Ci.	-	Proposal for an ADI not submitted; appropriate studies not performed.	The company refers to the database of other notifiers.
Sinon [Shinung]	0.3	Published literature.	It is assumed that this value refers to the JMPR evaluation in 1986 (i.e. ADI derived from a long-term rat study).
Rapporteur	0.3	Summary of long-term studies in rats.	See discussion below.

A very high ADI of 1.75 mg/kg bw was proposed in the joint dossier of Monsanto and Cheminova and is based on the NOEL for maternal toxicity in a teratogenicity study in rabbits (Tasker, 1980). It is discussed here since it is far outside the range of all the other suggested values. This proposal was not accepted by the Rapporteur for the following reasons:
1. The NOEL for maternal toxicity in the respective study was established by the Rapporteur at 75 mg/kg bw/day instead of 175 mg/kg bw/day (see section B.5.6.2.2.2).
2. If a NOEL of 175 mg/kg bw/day for the abovementioned rabbit study would have been accepted, one could identify some valid studies revealing adverse effects at lower doses. In a recent long-term study in rats (Suresh, 1996), effects occurred in female animals at a dietary dose level of 1000 ppm (ca 60 mg/kg bw/day). The NOELs and LOELs established in a further chronic rat study (Atkinson et al., 1993) and in two other rabbit teratogenicity studies (Suresh, 1993; Brooker et al., 1991) were well below 175 mg/kg bw/day.

Usually, a chronic study is considered most appropriate to derive the ADI. Since the rat proved the most sensitive species upon long-term exposure, it is suggested to establish the ADI for glyphosate on the basis of the chronic toxicity data obtained in rats. Table B.5.10.2-2 gives a summary of the available long-term rat studies.

Table B.5.10.2-2: Summary of long-term studies with glyphosate in rats

Strain/duration	Dose levels	NOEL/LOEL	Target organs/ Main effects	Reference	Submitted by (notifier)
Wistar, 2 years	0-100-1000-10000 ppm	LOEL: 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, bodyweight↓, 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	NOEL: 31 mg/kg bw/d (highest dose)	No treatment-related effects.	Lankas, 1981	Published by WHO/ FAO (1986 JMPR evaluation)

Taking these studies together, one can assume the NOEL for chronic effects of glyphosate in the rat between 10 and 100 mg/kg bw/day. In the study by Suresh (1996), an increase in alkaline phosphatase activity was apparent at the mid dose level of 1000 ppm in female rats. Since this effect was more pronounced at the top dose level and also observed in a further rat study (Atkinson et al., 1993), it is considered treatment-related. Hence, it appears reasonably to establish the LOEL for glyphosate-related long-term effects in rats at approximately 60 mg/kg bw/day. Accordingly, the dose of 31 mg/kg bw/day is assumed to represent a reliable NOEL from which the ADI value can be derived. It should be emphasized that this proposal is not based on one single study only but on the overall assessment of all available long-term studies in rats.

For calculation of the ADI, the usual assessment factor of 100 is considered appropriate since there was no evidence of cancerogenicity, mutagenicity, teratogenicity or any other specific hazard to be expected with glyphosate. Thus, an

ADI of 0.3 mg/kg bw

is proposed.

This value is also considered an appropriate sum ADI for AMPA alone or for AMPA in combination with glyphosate since the parent compound and its main plant and environmental metabolite have very similar chemical structures and since toxicological studies with AMPA have shown a low general toxicity and the lack of any specific hazards.

B.5.10.3 ACCEPTABLE OPERATOR EXPOSURE LEVEL (AOEL)

The acceptable operator exposure level should be established on the basis of the highest dose at which no adverse effect is observed in relevant studies in the most sensitive species. However, in contrast to the setting of an ADI, the AOEL is usually based on mid-term studies (i.e. subacute/ subchronic and reproduction or developmental toxicity studies) since these studies in most cases can be considered a more appropriate model for the actual operator exposure to be expected.

On the basis of their respective studies, the different notifiers proposed oral AOELs for glyphosate varying between 0.4 mg/kg bw/day and 10.0 mg/kg bw/day. In table B.5.10.3-1, the various proposed AOELs, the NOELs serving as basis for the respective proposal and the assessment factors (AF) used to derive the AOEL are listed. It should be noticed that the low oral absorption of glyphosate has not been taken into consideration by any of the notifiers but by the Rapporteur. The assumed values of dermal absorption are also included since they are needed for calculation of the acceptable dermal exposure.

Table B.5.10.3-1: AOEL values for glyphosate as proposed by the different notifiers and by the Rapporteur

Notifier	Relevant study	Reference	NOEL/ NOAEL (mg/kg bw/d)	AF	AOEL proposed (mg/kg bw/d)	Dermal absorption assumed
Monsanto/ Cheminova	Teratogenicity, rabbit (maternal toxicity)	Tasker, 1980 (see B.5.6.2)	175	25	7.0	2.2%
Agrichem [Task Force)	3-generation, rat	Schroeder and Hogan, 1981 (see B.5.6.1)	10	25	0.4	2%
Alkaloida	90-d, rat	Parker, 1993 (see B.5.3.2.1)	125	25	5.0	10%
Barclay	90-d, dog	Bhide, 1985 (see B.5.3.2.3)	250	25	10.0	5%
Feinchemie	90-d, rat	Suresh, 1992 (see B.5.3.2.1)	100	100	1.0	10%
Herbex	-	-	-	-	not proposed	not indicated
Luxan	3-generation, rat; 80-week, mouse	Bhide, 1988 (see B.5.6.1); Bhide, 1988 (see B.5.5)	15 15	25	0.6	10%
Nufarm	No data available					
Sanachem	-	-	-	-	0.5	not indicated
SCC/I.Pi.Ci.	-	-	-	-	not proposed	not indicated
Sinon [Shinung)	-	-	-	-	0.5	not indicated
Rapporteur	Teratogenicity, rabbit (maternal toxicity)	Tasker, 1980 (see B.5.6.2)	75	100	0.2*	3%

* systemic AOEL taking oral absorption into consideration

If the valid mid-term studies are taken into consideration, the lowest effect level was 100 mg/kg bw/day at which evidence of maternal toxicity (i.e. mortality) was obtained in a teratogenicity study in rabbits (Suresh, 1993). In another rabbit developmental toxicity study (Brooker et al., 1991), clinical signs of toxicity occurred at 150 mg/kg bw/day. These findings suggest that the rabbit is the most sensitive species in studies of short or mid-term duration. The assumption of a particular sensitivity of the rabbit is supported by the results of the teratogenicity studies in rats revealing much higher NOELs with maternal effects occurring at doses as high as 1000 mg/kg bw/day and above. Furthermore, the effect doses in the subacute and subchronic studies in rats and mice and in most dog studies were consistently well above 100 mg/kg bw/day, the lowest being in the range of 250-300 mg/kg bw/day. The low NOELs established in a few multigeneration studies (see section B.5.6.1) could not be taken into account since only very low doses had been tested and effect doses were lacking. In two subchronic dog studies (Verecskey and Csanyi, 1981, 1982), rather low NO(A)ELs were determined, too, but these studies were considered supplementary only and the effects observed were not confirmed in more recent dog studies using much higher dose levels. Hence, it is scientifically sound to derive the AOEL primarily from a rabbit teratogenicity study. A summary of the relevant studies is given in table B.5.10.3-2.

Table B.5.10.3-2: Relevant developmental toxicity studies in rabbits

Strain	Dose levels (mg/kg bw/d)	NOEL, maternal (mg/kg bw/d)	NOEL, developmental (mg/kg bw/d)	Reference	Submitted by (notifier)
New Zealand White (NZW)	0-20-100-500	20	100	Suresh, 1993	Feinchemie
NZW	0-50-150-450	50	150	Brooker et al., 1991	Monsanto/Chemi-nova
NZW	0-125-250-500	250	250	Bhide and Patil, 1989	Barclay; Luxan
Dutch Belted	0-75-175-350	75	350	Tasker, 1980	Monsanto/Chemi-nova; also referred to by Agrichem [Task Force], Barclay, Sinon [Shinung] and Sanachem

It is proposed to derive the AOEL from the NOEL of 75 mg/kg bw/day obtained in the study by Tasker et al. (1980) since this is the highest dose at which no signs of maternal toxicity were observed in rabbits. Using an AF of 100, an oral AOEL of 0.75 mg/kg bw/day could be established. However, for calculation of a systemic AOEL, the low oral absorption rate of about 30% (see B.5.1.1) has to be taken into account. This calculation would result in a numeric value of 0.225 mg/kg bw/day which was also used for the formal assessment of operator exposure in this monograph (see B.5.14). For practical reasons, it seems appropriate to establish an approximate value. Thus, an

AOEL of 0.2 mg/kg bw/day (systemic)

is proposed.

Acceptable doses for the dermal and inhalation routes have been calculated and are taken into consideration for assessment of operator exposure (see B.5.14).