Evaluation of the potential genotoxicity of Glyphosate,

Glyphosate mixtures and component surfactants

James M. Parry

Centre for Molecular Genetics and Toxicology

School of Biological Sciences

University of Wales Swansea

Swansea SA2 8PP, UK

Introduction

The available data concerning the potential genotoxic activity of glyphosate, glyphosate mixtures and surfactants have been evaluated and the results of the evaluation are presented in Tables 1 to 14. Each of the tables reviews the data for the three groups of chemicals grouped according to the type of test system used to assess potential genotoxicity, the effect produce and reference to the appropriate data set.

Conclusions

Evaluation of the genotoxicity of Glyphosate

I. Bacterial mutagenicity (Table 1)

Two comprehensive studies (Scantox 10.9.91-A, Li and Long 1988) provide no evidence of mutagenic activity for glyphosate in *Salmonella typhimurium.*

One study of differential DNA repair in the *Bacillus subtilis* rec assay gave negative results.

I conclude that there was no evidence that glyphosate is genotoxic in bacteria.

II. *In vitro* cytogenetic assays (Table 3)

(a) Chromosomal aberrations

Two studies in human and bovine lymphocytes report positive results over dose ranges up to 170μM following exposure for 72 hrs in the absence of S9 mix (Lioi *et al* 1998a, 1998b).

One negative study in human lymphocytes over a dose range of up to 562μg/ml in both the presence and absence of S9 mix and at sampling times of up to 48 hrs (Notox 141918).

Note: the Lioi *et al* studies present a combined data set of experiments from 3 separate donors.

One negative study in *Allium cepa* root tips has been reported.

(b) Sister chromatid exchange

Two studies report positive results in human and bovine lymphocytes over dose ranges of up to 170μM following exposure for 72 hrs in the absence of S9 mix (Lioi *et al* 1998a, 1998b).

Evaluation. There is published evidence that glyphosate shows clastogenic activity following 72 hrs exposure of both bovine and human lymphocytes (Lioi *et al* 1998a, 1998b).

In my view there is a need to repeat the studies of Lioi *et al* to a comprehensive protocol to clarify the potential clastogenic activity of glyphosate.

III. Point mutation in cultured mammalian cells (Table 5)

Negative results are reported in both the Tk assay using mouse lymphoma cells (up to 5000μg/ml) and the HGPRT assay using Chinese hamster cells (up to 22500 μg/ml) in both the presence and absence of S9 mix (Scantox 10.9.91-B, Li and Long 1988).

There is no evidence that glyphosate is a point mutagen in cultured mammalian cells.

IV. In vivo chromosome studies in rodents. (Table 6)

a) Rat bone marrow cytogenetics assay

There is one negative study reported in the bone marrow of rats exposed to 1000mg/kg bw (Li and Long 1988),

b) Mouse bone marrow micronucleus assay.

There are two negative studies at concentrations of up to 5000mg/kg bw available for evaluation (Rank *et al* 1993, Scantox 12.9.91) However, in neither study is there substantive evidence of bone marrow toxicity.

There is one positive study at 300mg/kg with multiple dosing, sampled at 24hrs (Bolognesi *et al* 1997). However, this study only involved the use of 4 animals per dose point however bone marrow toxicity was observed.

Evaluation. There are conflicitng results concerning the bone marrow activity of glyphosate which can only be resolved by repeating the Bolognesi *et al* (1997) study.

V Dominant Lethal Study (Table 10)

There is one negative dominant lethal assay involving exposure of male mice of concentration up to 200mg/kg bw (RD 300, SRRS L1147)

Evaluation. There is no evidence that glyphosate is capable of inducing dominant lethal mutations in mouse male germ cells.

VI Miscellaneous Endpoints (Table 8)

a) **G6PD activity**

Two studies demonstrate increases in G6PD activity (as a marker of a pro-oxidant state) in human and bovine lymphocytes at concentrations of up to 170μM (Lioi *et al* 1998a, 1998b). G6 PD activity was reduced in presence of an antioxidant.

Note : no genetic endpoint was measured in these studies.

b) **Induction of 8-OHdG**

One study demonstrates the production of 8-OHdG (as a marker of oxidative damage) in the liver of mice exposed to glyphosate (Bolognesi *et al* 1997)

c) **Induction of DNA damage measured by alkaline elution**

One study demonstrates the production of single strand breaks in liver and kidney of mice following exposure to 300mg/kg bw of glyphosate (Bolognesi *et al* 1997).

d) **Induction of DNA adducts measured by ^{32}P post - labelling**

One study reports no increase in adducts in the liver and kidneys of mice following exposure to 130 and 270mg/kg of glyphosate (Peluso *et al* 1998)

e) **Hepatocyte DNA repair assay**

One limited study (low concentrations used) reported negative results for its ability of glyphosate to induce repairable DNA assay using rat hepatocytes (Li and Long 1988).

Evaluation. These studies provide some evidence that glyphosate may be capable of inducing oxidative damage under both *in vitro* and *in vivo* conditions

Evaluation of the genotoxicity of Glyphosate mixtures

Bacterial mutagenicity (Table 2)

1) The limited published study (Rank *et al* 1993) showed single dose point increases in mutagenicity of a Glyphosate mixtures in *Salmonella* strains TA98 and TA100.

Four comprehensive studies with glyphosate mixtures of concentration of 31% to 72% (MSL – 11731, MSL – 11729, MSL – 11730, BioAgri G.1.1.050/96) provide no evidence of mutagenic activity in *Salmonella typhimurium.*

Evaluation. In view of the extensive negative data in studies performed to comprehensive protocols I conclude that Glyphosate mixtures are not mutagenic to *Salmonella typhimurium..*

11) **In vitro cytogenetics (Table 4)**

a) Chromosomal aberrations

There are no available studies involving the analysis of the induction of chromosome aberrations in cultured mammalian cells.

There is one published study in *Allium cepa* root tips reporting positive results (described as being indicative of spindle disturbances) at concentrations greater then 720 µg/ml (Rank *et al* 1993).

b) Sister chromatid exchange

There are two studies reporting positive results in human lymphocytes at concentrations from 100µg/ml to 2500µg/ml (Bolognesi *et al* 1997, Vigfusson and Vysa 1980).

Evaluation. The *in vitro* cytogenetic data for glyphosate mixtures are inadequate for evaluation.

IV ***In vivo* mouse bone marrow micronucleus assay (Table 7)**

There are 5 studies in mouse bone marrow which report negative results for micronucleus induction for various mixtures of glyphosate at concentrations of up to 3400mg/kg bw (Rank *et al* 1993, BioAgri C.1.2-60/96, MSL – 11771, MSL7173, MSL – 1172). However, most of the studies provide only limited evidence of bone marrow toxicity.

There is one positive study of a Roundup mixtures at 450mg/kg bw with multiple dosing and sampled at 24 hrs (Bolognesi *et al* 1997). Bone marrow toxicity was reported in this study but only 3 animals were used per dose point.

Evaluation. Conflicting results concerning the bone marrow activity of glyphosate mixtures can only be resolved by repeating the Bolognisi *et al* (1997) study.

V **Drosophila sex linked recessive lethal mutation assays (Table 11)**

One study provides limited evidence that following larval feeding both Roundup and Pondmaster mixtures produced some positive results in spermatocyte broods (Kale *et al* 1995)

Evaluation. Some limited evidence that Glyphosate mixtures are capable of inducing sex linked recessive mutations in the male germ cells of *Drosophila melanogaster*.

VI **Miscellaneous Endpoints (Table9)**

(a) Induction of 8-OHdG

One study demonstrates the production of 8-OHdG (as a marker of oxidative damage) in the liver and kidneys of mice exposed to Roundup mixture (Bolognesi *et al* 1997).

(b) **Induction of DNA damage measured by alkaline elution**

One study demonstrates the production of single strand breaks in the liver and kidney of mice exposed to 300mg/kg bw of Roundup mixture (Bolognesi *et al* 1997)

c) **Induction of DNA adducts measured by ^{32}P post labelling**

One study reports an increase in adducts in the liver and kidneys of mice following exposure to 400, 500 and 600mg/kg bw of Roundup Mixtures (Bolognesi *et al* 1997)

d) **COMET assay**

One study demonstrates the induction of chromosome damage as measures in the COMET assay following exposure of tadpoles to Roundup at concentrations above 27mg/litre (Clements *et al* 1997)

Evaluation. These studies provide some evidence that Roundup mixture produces DNA lesions *in vivo,* probably due to the production of oxidative damage.

Evaluation of the genotoxicity of Surfactants

1) **Bacterial Mutagenicity (Table 12)**

Three comprehensive studies failed to demonstrate any mutagenic activity for the surfactants in bacterial assays (MSL – 10625, MSL – 1538, Hoecht 92.0487).

11) ***In vitro* chromosome aberration assay (Table 13)**

One study failed to demonstrate any significant increase in chromosome aberrations after exposure to Dodigen 4022 at concentrations of up to 6000μg/ml (Hoecht 92.1025).

However, a number of non-significant changes in various parameters were reported. This study should be repeated.

III) **Mouse bone marrow micronucleus assay (Table 14)**

One limited experiment (ML-89-463) produced negative results in mouse bone marrow with MON 0818 at 100mg/kg bw.

Evaluation. The only adequate studies with the surfactants are those involving bacterial mutagenicity assays. There was no evidence that the various surfactants are bacterial mutagens.

Overall Conclusions

1) It is clear from the data provided that with the exception of one limited study (Rank *et al* 1993) there is an extensive range of studies which demonstrate that glyphosate and glyphosate are **not** genotoxic in bacteria.

2) There is published *in vitro* evidence that glyphosate is clastogenic and capable of inducing sister chromatid exchange in both human and bovine lymphocytes (Lioi *et al* 1998a, 1998b).

3) *In vitro* cytogenetic data on glyphosate mixtures are inadequate for evaluation.

4) There are two studies (Scantox 10.9.91, Li and Long 1988) which demonstrate that glyphosate is not a point mutagen in cultured mammalian cells.

5) This is a published study indicating that glyphosate was not clastogenic in rat bone marrow (Li and Long 1988). There are two studies which indicate that glyphosate was not capable of inducing micronuclei in mouse bone marrow (Rank *et al* 1993, Scantox 12.9.99). However, in neither study was there substantive evidence of bone marrow toxicity.

   There is one published study which suggests that glyphosate may be capable of inducing micronuclei in mouse bone marrow when delivered by multiple dosing (Bolognesi *et al* 1997).

6) Five studies report negative results for micronucleus induction in the bone marrow of mice following exposure to glyphosate mixtures. However, these studies provide only limited evidence of bone marrow toxicity. None of the studies were performed to a protocol equivalent to that of Bolognesi *et al* (1997) which gave positive results with glyphosate.

7) There is one dominant lethal study which failed to demonstrate any capacity to induce genotoxicity in mouse male germ cells (RD300, SRRS L1147). However, it should be noted that this is a relatively insensitive methodology.

8) No dominant lethal assay results are available for glyphosate mixtures.

9) No sex-linked recessive lethal assay in *Drosophila* results are available for glyphosate.

10) Following larval feeding, Roundup and Pondmaster mixtures containing glyphosate produced some positive results in spermatocyte broods (Kale *et al* 1995).

11) Glyphosate induced G6PD activity in both bovine and human lymphocytes (Lioi *et al* 1998a, 1998b) and the production of 8-OHdG in mouse liver (Bolognesi *et al* 1997). Both observations indicate that glyphosate may be capable of inducing a pro-oxidant state leading to the formation of the oxidative damage lesion 8-OHdG.

12) A Roundup mixture containing glyphosate was shown to produce 8-OHdG in both the liver and kidneys of mice (Bolognesi *et al* 1997). These observations indicate the Roundup mixture is capable of inducing oxidative damage *in vivo*.

13) Glyphosate failed to induce repairable DNA damage in a limited *in vitro* study in rat hepatocytes (Li and Long 1988).

14) Glyphosate induced single strand breaks *in vivo* in the liver and kidneys of mice (Bolognesi *et al* 1997).

15) Roundup mixture produced single strand breaks *in vivo* in the liver and kidneys of mice (Bolognesi *et al* 1997).

16) Glyphosate mixture but not Glyphosate produced an increase in uncharacterised DNA adducts *in vivo* in the liver and kidneys of mice (Peluso *et al* 1998).

The overall genotoxicity profiles of glyphosate and glyphosate mixtures are illustrated in Figures 1 and 2 respectively.

17) None of the surfactants demonstrated any mutagenic activity in bacteria.

18) There are no adequate data to evaluate the *in vitro* clastogenic activity of surfactants.

19) One limited bone marrow micronucleus assay failed to detect any micronucleus inducing activity with the surfactant MON0818.

Specific evaluation of the genotoxicity of glyphosate

On the basis of the study of Lioi *et al* (1998a and 1998b) I conclude that glyphosate is a potential clastogenic *in vitro*. The study of Bolognesi *et al* (1997) indicates that this clastogenic activity **may** be reproduced *in vivo* in somatic cells. However, the dominant lethal assay (of limited sensitivity) indicates that this genotoxic activity is not reproduced in germ cells. The work of Bolognesi *et al* (1997) and Lioi *et al* (1998a and 1998b) suggests that the genotoxicity observed may be derived from the generation of oxidative damage in the presence of glyphosate.

Specific evaluation of genotoxicity of glyphosate mixtures

In view of the absence of adequate data no evaluation of the clastogenic potential *in vitro* of glyphosate mixtures is possible. In the absence of a micronucleus study to the protocol of that used by Bolognesi *et al* (1997) no adequate assessment of the potential activity of glyphosate mixtures in bone marrow is possible. The available studies do not provide any evidence of genotoxicity in rodent bone marrow. There is some evidence from *Drosophila* to suggest that glyphosate mixtures may have some germ cell activity.

The studies of Bolognesi *et al* (1997) suggests that glyphosate mixtures may be capable of inducing oxidative damage *in vivo*.

Specific evaluation of surfactants

None of the surfactants were capable of inducing mutations in bacteria. No adequate data available to evaluate the *in vitro* or *in vivo* clastogenicity of the surfactants.

Publications utilized in the assessment of the genotoxic activity of glyphosate and glyphosate formulations.

Lioi *et al* (1998a). Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures *in vitro*. *Mutation Research* **403**, 13-20.

Lioi *et al* (1998b). Cytogenetic damage and the induction of pro-oxidant state in human lymphocytes exposed *in vitro* to glyphosate, vinclazolin, atrazine and DPX-E9636. *Environ. Molec. Mutagenesis* **32**, 39-46.

Rank *et al* (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, *Salmonella* mutagenicity test and *Allium* anaphase-telophase test. *Mutation Research* **300**, 29-30.

Bolognesi *et al* (1997). Genotoxic activity of glyphosate and its technical formulation Roundup. *J. Agric. Food Chem.* **45**, 1957-1962.

Kale *et al* (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ. Molec. Mutagenesis* **25**, 148-153.

Vigfusson and Vyse (1980). The effect of the pesticides, Dexon, Captan and Roundup on sister chromatid exchange in human lymphocytes *in vitro*. *Mutation Research* **79**, 53-57.

Clements *et al* (1997). Genotoxicity of select herbicides in *Ranacates beiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (COMET) assay. *Environ. Molec. Mutagenesis* **29**, 277-288.

Peluso *et al* (1998). ^{32}P-postlabelling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ. Mol. Mutagenesis* **31**, 55-59.

Li and Long (1988). An evaluation of the genotoxic potential of glyphosate. *Fundamental and Applied Toxicology* **10**, 537-546.

Reports utilized in the assessment of the genotoxic activity of glyphosate and glyphosate formulations

1. BioAgri G.1.2-60, Micronucleus study with Glifos.
2. BioAgri G.1.1-050/96, Ames/Salmonella assay of Glifos.
3. Hoecht 92.0487, Bacterial mutagenicity assay of Dodigen 4022.
4. Hoechst 92.1024, Chromosome aberration assay of Dodigen 4022 in V79 cells.
5. ML-89-463, Mouse micronucleus assay of MON 0818
6. MSL-1538, Ames/Salmonella assay of MON 8080
7. MSL-10625, Ames/Salmonella assay with surfactant MON 0818.
8. MSL-11729, Ames/Salmonella assay with Roundup MON 2139.
9. MSL-11730, Ames/Salmonella assay of Rodeo.
10. MSL-11731, Ames/Salmonella assay of Direct of MON 14445.
11. MSL-11771, Mouse micronucleus test with Roundup.
12. MSL-11772, Mouse micronucleus study of Rodeo.
13. Notox 141918, Chromosome aberration study of Glyfosaat *in vitro* in human lymphocytes.
14. MSL-11773, Mouse micronucleus study of Direct.
15. RD 300 SRRSL1147, Dominant Lethal Study of glyphosate in mice.
16. Scantox, 12.9.91 Micronucleus test with glyphosate.
17. Scantox, 10.9.91-B, *In vitro* mammalian cell gene mutation test.

Figure 1

Profile of genotoxicity of Glyphosate

Bacteria	- ve
↓	
In vitro cytogenetics	+ ve
↓	
In vitro point mutation in mammalian cells	- ve
↓	
In vivo clastogenicity	2 – ve 1 + ve
↓	
Male germ cell dominant lethal	- ve
↓	
Drosophila sex-linked recessive lethal	?
↓	
Induction of oxidative damage *in vivo*	+ ve
↓	
Induction of single strand breaks *in vivo*	+ ve
↓	
Induction of DNA adducts *in vivo*	- ve

Figure 2

Profile of Genotoxicity of Glyphosate Mixtures

Bacterial Mutagenicity	- ve
↓	
In vitro cytogenetics	?
↓	
In vitro point mutation in mammalian cells	?
↓	
In vivo clastogenicity	- ve
↓	
Male germ cell dominant lethal	?
↓	
Drosophila sex-linked lethal	limited positive
↓	
Induction of oxidative damage *in vivo*	+ ve
↓	
Induction of single strand breaks *in vivo*	+ ve
↓	
Induction of DNA adducts *in vivo*	+ ve

Table 1

Glyphosate

Endpoint	Effect	Cell type	Reference
Glyphosate (206-Jak-25-1) Point Mutation Induction in Ames test	Negative 310 to 2500µg/plate + S9 mix 160 to 2500µg/plate – S9 mix	Salmonella TA 98 TA 100 TA 1535 TA 1537	Scantox 10.9.91-A
Glyphosate Differential sensitivity rec assay	Negative 20 to 2000µg/test disc	*Bacillus subtilis*	Li and Long (1988)
Point mutation induction in Ames test	Negative 10 to 5000µg/plate + and – S9	**Salmonella** TA 98 TA 100 TA 1535 TA 1537 TA 1538 *E. coli* WP2 hcr	Li and Long (1988)

Table 2

Glyphosate Mixtures

Endpoint	Effect	Cell type	Reference
Roundup Point Mutation Induction in Ames Test	Positive minus S9 mix at 360µg/plate	TA 98	Rank *et al* 1993
	Positive in presence of S9 mix at 720µg/plate	TA 100	Rank *et al* 1993
	Note: Single point increases No evidence of dose response		
Direct Mixture (72%)			
Point mutation induction in Ames test	Negative 15 to 1500µg/plate + S9 5 to 500µg/plate –S9	TA 98 TA 100 TA 1535 TA 1537	MSL-11731
Roundup (31%) Point mutation induction in Ames test	Negative 15 to 1500µg/plate + S9 5 to 500µg/plate – S9	TA 98 TA 100 TA 1535 TA 1537	MSL-11729
Roundup Mixtures			
Rodeo (40%) Point Mutation in Ames test	Negative 50 to 5000µg/plate + and – S9 mix	TA 98 TA 100 TA 1535 TA 1537	MSL-11730
Glifos (41%) Point Mutation in Ames test	Negative 1 to 5000µg/plate + and – S9 mix	TA 97a TA 98 TA 100 TA 1535	BioAgri G.1.1-050/96

Table 3

Glyphosate

Endpoint	Effect	Cell type	Reference
Glyphosate-N-(phosphonomethyl) glycine Chromosome aberrations	Positive 5 to 51μM 72 hrs exposure in absence of S9 mix	Human lymphocytes	Lioi *et al* 1998(a)
Sister chromosome exchange	Positive 5 to 51μM 72 hrs exposure in absence of S9 mix	Human lymphocytes	Lioi *et al* 1998(a)
Chromosome aberrations	Positive 17 to 170μM 72 hrs exposure in absence of S9 mix	Bovine lymphocytes	Lioi *et al* 1998(b)
Sister chromosome exchange	Positive 17 to 170μM 72 hrs exposure in absence of S9 mix	Bovine lymphocytes	Lioi *et al* 1998(b)
Note: Lioi *et al* studies indicate data derived from 3 donors combined.			
Glyfosaat Chromosome aberrations	Negative 33 to 237μg/ml –S9 14hrs 56 to 333μg/ml –S9 48hrs 33 to 562μg/ml +S9 24hrs 100 to 562μg/ml +S9 48 hrs	Human lymphocytes	Notox 141918
Note: Reduction in mitotic index in absence of +S9 mix and at 24 hrs in presence of S9 mix.			
Glyphosate isopropylamine salt Cytogenetic changes	Negative	*Allium cepa* root tips	Rank *et al* (1993)

Table 4

Glyphosate Mixture

Endpoint	Effect	Cell type	Reference
Roundup Sister chromatid exchange	Positive at 100μg/ml 72 hrs exposures	Human lymphocytes	Bolognesi *et al* (1997)
Cytogenetic changes	Positive response at concentrations greater than 720μg/litre Characterised as spindle disturbance	*Allium cepa* root tip	Rank *et al* (1993)
Sister chromatid exchange	Small positive increase at 250 and 2500μg/ml	Human lymphocytes	Vigfusson and Vyse (1980)

Table 5

Glyphosate

Endpoint	Effect	Cell type	Reference
Glyphosate (206-Jak-25-1) Tk mutation induction in mammalian cells	Negative 0.65, 1.3, 2.5, 5.0mg/ml –S9 mix 0.52, 1.0, 2.1, 4.2mg/ml +S9 mix	Mouse lymphoma L5178Y	Scantox 10.9.91-B
Glyphosate HGPRT Mutation induction in mammalian cells	Negative 5 to 22.5mg/ml + and – S9 mix	Chinese hamster	Li and Long (1988)

Table 6

Glyphosate

Endpoint	Effect	Cell type	Reference
Glyphosate isopropylamine salt Micronucleus induction	Negative up to 200mg/kg by i.p. injection Note: only 1 dose point gave reduction in PCE/NCE ratio	Mouse bone marrow	Rank *et al* (1993)
Glyphosate (analar grade) Micronucleus induction	Positive response at 300mg/kg at 24hrs Multiple dosing i.p. injection 4 animals analysed Reduction in PCE/NCE ratio	Mouse bone marrow	Bolognesi *et al* (1997)
Glyphosate (206-Jak-25-1) Micronucleus induction	Negative 5000mg/kg at 24, 48, 72hrs No evidence of bone marrow toxicity	Mouse bone marrow	Scantox 12.9.91
Glyphosate Chromosomal aberrations	Negative 1gm/kg sampled at 6, 12, 24hrs	Rat bone marrow	Li and Long (1988)

Table 7

Glyphosate Mixtures

Endpoint	Effect	Cell type	Reference
Roundup (41%) Micronucleus induction	Negative up to 200mg/kg only sampled at 48hrs	Mouse bone marrow	Rank *et al* (1993)
Roundup Micronucleus induction	Positive response at 450mg/kg Multiple dose 3 animals sampled reduction in PCE/NCE ratio	Mouse bone marrow	Bolognesi *et al* (1997)
Glifos (41%) Micronucleus induction	Negative 68, 137, 206mg/kg i.p. delivered 2 x at 24hr intervals Note: Inadequate study	Mouse bone marrow	BioAgri G.1.2-60/96
Roundup 31% Micronucleus induction	Negative 140, 280, 555mg/kg i.p. injection sampled at 24, 48, 72hrs Note: Limited evidence of bone marrow toxicity One male 268 showed increase in micronuclei	Mouse bone marrow	MSL-11771
Direct (72%) Micronucleus induction	Negative 91, 183, 365mg/kg by i.p. sampled at 24, 48, 72hrs Note: Limited evidence of bone marrow toxicity one female 186 183mg/kg at 48hrs showed an increase	Mouse bone marrow	MSL-11773
Rodeo (40%) Micronucleus induction	Negative 850, 1700, 3400mg/kg by i.p. sampled at 24, 48, 72hrs	Mouse bone marrow	MSL-11772

Table 8

Miscellaneous Endpoints

Glyphosate, N- (phosphonomethyl)glycine

Endpoint	Effect	Cell type	Reference
G6PD activity	Increase in activities 5 to 51μM	Human lymphocytes	Lioi *et al* 1998(a)

Note, increase in G6PD activity reduced by presence of antioxidant N-acetyl cysteine, but not eliminated.

G6PD activity	Increase in activity 17 to 170μM	Bovine Lymphocytes	Lioi *et al* 1998(b)

Note, increase in G6PD activity reduced by presence of antioxidant N-acetyl cysteine, but not eliminated

Glyphosate (Analar Grade)

Induction of 8-OHdG	Increase in 8–OHdG in liver	Mice *In vivo*	Bolognesi *et al* (1997)
Induction of DNA damage measured by alkaline elution	Increase in single-strand breaks in liver and kidney at 4 hrs following 300mg/kg	Mice *In vivo*	Bolognesi *et al* (1997)

Glyphosate isopropylammonium salt.

Induction of DNA adducts measured by ^{32}P post-labelling	Negative no increase in adducts in liver and kidney at 130 and 270mg/kg	Mice *In vivo*	Peluso *et al* (1998)

Table 8 continued

Glyphosate

Hepatocyte DNA repair assay	Negative 12.5ng to 125µg/ml	Rat Hepatocytes	Li and Long (1988)

Note Very low concentrations used, study adds very little value to the analysis of the potential genotoxicity of Glyphosate.

Table 9

Miscellaneous Endpoints

Glyphosate Mixtures

Endpoint	Effect	Cell type	Reference
Roundup (41%) Mon 35050			
Induction of 8-0HdG	Increase in 8–0HdG in Liver and Kidney	Mice *In vivo*	Bolognesi *et al* (1997)
Induction of DNA damage measured by alkaline elution	increase in single-strand breaks in Liver and Kidney at 4hrs following 300mg/kg	Mice *In vivo*	Bolognesi *et al* (1997)
Induction of DNA adducts measured by ^{32}Ppost-labelling Note. Adducts were not characterised	increase in adducts in liver and kidney at 400, 500 and 600mg/kg	Mice *In vivo*	Peluso *et al* (1998)
Roundup			
COMET assay	Positive results observed at concentrations above 27mg/ litre	Tadpoles of *Rana catosbeiana*	Clements *et al* 1997

Table 10

Glyphosate

Endpoint	Effect	Cell type	Reference
Dominant Lethal Study	Negative Small reduction in viable foetuses in week 1 at 800mg/kg, week 3 at 2000mg/kg Increase in late reabsorptions at week 8 at 200mg/kg	Mouse male gametes exposed Effect measured in embryos	RD300 SRRS L1147

Table 11

Glyphosate Mixtures

Endpoint	Effect	Cell type	Reference
Roundup Sex linked recessive lethal mutations	Positive result in Spermatocyte broods At 1μg/ml.	*Drosophila melanogaster* Larval exposure	Kale *et al* (1995)
Pondmaster Sex linked recessive lethal mutations	Positive result in spermatocyte broods at 0.1μg/ml	*Drosophila melanogaster* larval exposure	Kale *et al* (1995)

Table 12

Surfactant

Endpoint	Effect	Cell type	Reference
Surfactant MON 0818			
Point Mutation induction in Ames test	Negatives 1 to 100µg/plate +S9 0.3 to 30µg/plate –S9	**Salmonella** TA 98 TA 100 TA 1535 TA 1537	MSL – 10625
Surfactant MON 8080			
Point Mutation induction in Ames test	Negatives 0.003 t0 3µl /plates + ad – S9 mix	**Salmonella** TA 98 TA 100 TA1535 TA 1537	MSL – 1538
Surfactant Dodigan 4022			
Point Mutation Induction in Ames test	Negatives 4 to 10,000 µg/plats in both presence and absence at S9 Mix	**Salmonella** TA 98 TA 100 TA 1535 TA 1537 TA 1538 *E. coli* WP2uvrA	Hoecht 92.0487

Table 13

Surfactant Dodigen 4022

Endpoint	Effect	Cell type	Reference
In vitro chromosome aberrations	Complex set of results – None significant Concentration range 600 to 6000µg/ml sampled at 7, 18 and 28hrs **Mitotic index minus S9** decreased at 7hrs increased at 18hrs decreased at 28hrs **Mitotic index plus S9** decreased at 7hrs increased at 18hrs no change at 28hrs **Polyploidy minus S9** decreased at 7hrs decreased at 18hrs increased at 28hrs **Polyploidy plus S9** decreased at 7hrs decreased at 18hrs increased at 28hrs **Aberrations minus S9** increased at 7hrs no change at 18hrs increased at 28hrs **Aberrations plus S9** increased at 7hrs no change at 18hrs increased at 28hrs	Chinese hamster V79	Hoecht 92.1024

Note: Experiments are difficult to interpret and should have been repeated.

Table 14

Surfactant MON 0818

Endpoint	Effect	Cell type	Reference
Micronucleus induction Note – limited experiment	Negatives 100mg/kg by I.p. sampled at 24 and 48 hrs No evidence of animal or bone marrow toxiety	Mouse Bone marrow	ML-89-463

in vitro ✓cocoamin → tallow
alkyl sulfat > ED
quat.
etheramin

Key Issues concerning the potential genotoxicity of glyphosate, glyphosate formulations and surfactants; recommendations for future work.

James M. Parry

Centre for Molecular Genetics and Toxicology
School of Biological Sciences
University of Wales Swansea
Swansea SA2 8PP, UK

Key Questions

1. Is glyphosate an *in vitro* clastogen? Can the positive studies of Lioi *et al* (1998a, 1998b) be reproduced?
2. Is glyphosate an *in vivo* clastogen? Can the positive studies of Bolognesi *et al* (1997) be reproduced?
3. If glyphosate is an *in vitro* and *in vivo* clastogen, what is its mechanism of action and does the mechanism lead to other types of genotoxic activity *in vivo* such as point mutation induction?
4. Does glyphosate produce oxidative damage?
5. Can we explain the reported genotoxic effects of glyphosate on the basis of the induction of oxidative damage?
6. If glyphosate is an *in vivo* genotoxin is its mechanism of action thresholded? Under what conditions of exposure are the antioxidant defences of the cell overwhelmed?
7. Are there differences in the genotoxic activities of glyphosate and glyphosate formulations?
8. Do any of the surfactants contribute to the reported genotoxicity of glyphosate formulations?

Deficiencies in the Data Set

1. No adequate *in vitro* clastogenicity data available for glyphosate formulations.

2. No bone marrow micronucleus study of glyphosate available using multiple dosing and adequate animal numbers.

3. No studies available demonstrating the effects of anti-oxidants upon the induction of genotoxic endpoints by glyphosate.

4. No adequate *in vitro* or *in vivo* clastogenicity data for surfactants used in glyphosate formulations.

Actions Recommended

a) Provide comprehensive *in vitro* cytogenetic data on glyphosate formulations.

b) On the assumption that the reported *in vitro* positive clastogenic data for glyphosate is due to oxidative damage determine the influence of antioxidants. Evaluate the clastogenic activity of glyphosate in the presence and absence of a variety of antioxidant activities. Such a study should also incorporate glyphosate formulations to clarify the validity of reports of differences in activity. I recommend that both a) and b) should be undertaken using the *in vitro* micronucleus assay in human lymphocytes. The *in vitro* micronucleus assay would provide a more cost-effective method for evaluating a large number of experimental variables. Same as screen chrom ab

c) Evaluate the induction of oxidative damage *in vivo* and determine the influence of the antioxidant status of the animals. Determine the exposure concentrations of glyphosate which overwhelm the antioxidant status of tissues.

d) Perform an *in vivo* bone marrow micronucleus assay with multiple dosing with adequate numbers of animals to determine whether the work of Bolognesi *et al* (1997) can be reproduced.

e) I am making no recommendation to repeat any of the sister chromatid exchange studies. Chromosomal aberration data will always take priority over SCE data so I

see no point in repeating SCE studies as they involve an endpoint which is poorly defined and doesn't lead to genetic changes.

f) In view of the increasing appreciation of the value of the COMET assay as marker of tissue-specific damage I recommend the consideration of its use in any *in vivo* studies performed. The COMET assay would provide the ability to determine whether damage is produced in a wide range of tissues following glyphosate exposure. Such studies would also indicate whether the COMET positive results for glyphosate formulations in tadpoles (Clements *et al* 1997) are reproduced in mammals. In view of the data on oxidative damage (Bolognesi *et al* 1997) I would recommend COMET assays in the liver and kidney of mice if the oxidative data are confirmed as indicated under c).

g) I do not recommend any transgenic point mutation assay at this time. There is no available evidence that glyphosate is a point mutagen and the relatively low sensitivity of the transgenic assay means that negative results would have little value in the assessment of the hazard and risk of glyphosate exposures.

h) I do not recommend any studies of DNA adduct induction at this time. Such a study would only be of value if the adducts formed were characterised which would require major efforts. If the adducts reported by Peluso *et al* (1998) are the result of oxidative damage they are likely to be of the same type as those produced in the absence of glyphosate exposure by background oxidative damage.

i) Provide comprehensive *in vitro* data on the surfactants.

My overall view is that if the reported genotoxicity of glyphosate and glyphosate formulations can be shown to be due to the production of oxidative damage then a case could be made that any genetic damage would be thresholded. Such genetic damage would only be biologically relevant under conditions of compromised antioxidant status. If such an

oxidative damage mechanism is proved then it may be necessary to consider the possibility of susceptible groups within the human population.

If the genotoxic activity of glyphosate and its formulations is confirmed it would be advisable to determine whether there are exposed individuals and groups within the human population. If such individuals can be identified then the extent of exposure should be determined and their lymphocytes analysed for the presence of chromosome aberrations. In such populations micronucleus studies would probably only be of value in aspleenic individuals.

Comments on Parry Evaluation of
Glyphosate and Glyphosate Formulation Potential Genotoxicity.
Larry Kier
September 18, 1999

There is no summary evaluation in the initial section and no overall conclusions are presented on the genotoxicity of glyphosate or glyphosate formulations.

Although the summary says most studies (i.e. unpublished reports) were conducted according to OECD guidelines, this is clearly not the case for several published studies cited but this is not mentioned in the evaluation.

The depth of analysis of the studies is rather superficial. The analysis of the unpublished reports appears to be much more thorough than analysis of the published reports.

Ames tests--There are numerous published and unpublished negative Ames studies with glyphosate that contradict the reported positive findings of Rank et al. The evaluation doesn't go into any depth on the quality of the Rank et al. data in comparison with the other reports. (e.g., reproducibility or testing at equivalent doses).

Micronucleus--There is no analysis of the possible significance of differences in protocol between Bolognesi et al. and the other negative studies. In particular, what are the implications of multiple dosing (actually 2 doses) compared with a single dose. How many instances of clear positive/negative differences exist for these two protocols?

There is no conclusion about what the data say about glyphosate. The published studies are presented as some evidence of genotoxicity and the reports are presented as giving no evidence.

There is mixing of glyphosate and formulations in the analysis.

What's the significance of one animal showing an increase in micronuclei noted for micronucleus studies of Roundup and Direct? Apparently the conclusion is that these studies are negative, but if that is the case why mention single animal results. Are these considered significant?

There appears to be no evaluation of the significance of different endpoints--e.g. comet in tadpoles, oxidative damage, in vivo vs. in vitro. etc. These are all apparently considered as equivalent in this evaluation.

It's not clear how these data and reports lead to a concern about stability of glyphosate formulations.

WRATTEN, STEPHEN J [FND/1000]

To: [redacted] FARMER, DONNA R [FND/1000]
Cc: KIER, LARRY D [NCP/1000]; HEYDENS, WILLIAM F [FND/1000]; [redacted]
Subject: Comments on Parry write-up

[redacted] and Donna

I was somewhat disappointed in the Parry report, not particularly from his conclusions but just the way they're presented. The style and rather casual lack of completeness and preciseness would make it hard to circulate this around to anyone as supporting information. Has he ever worked with industry before on this sort of project?

I will mail the marked-up paper back to you, but some other general comments need to be made:

1. It is odd that the one study by BioAgri is discussed right on the first page in rather extensive detail but none of the others are. I understand that he didn't like this one, but it is still strange to read this way.

2. The whole report could benefit from a couple of introductory paragraphs about what he was asked to do and what he received as far as reports. Did he have all the Monsanto reports as well as the literature articles? Was he asked to compare these, evaluate the methods, explain the differences, identify any faults, or what?

3. Some where the report needs to identify the full citations of each report evaluated and give the full Literature references for the public documents. Also the test material should be clearly identified, ideally by both MON number and brand name if needed, but at least to say which are glyphosate and which are formulations - this is done, sort of, but not as clearly as I'd like. Separate tables would be good.

4. He has an odd way of starting all conclusions with a negative - ie., points 2, 3, and 4 on page 3. Couldn't the sentence structure be modified to be less awkward? When he says "no data were provided..." time and again, it makes it sound as though he was suspicious that there were data but he didn't get them. I know this is not the intent, but it could be cleaned up.

5. Table 1 seems to state repeatedly that "there was no evidence of xxx mutagenicity". It would be more powerful if it said "there was convincing evidence that glyphosate does not act as a xxx mutagen". "no evidence of" is a very weak way of stating a conclusion.

6. He says very little about the literature reports. So little that one almost forgets about them. Can he not provide some critique about their quality and methodology as compared to the Monsanto reports? Are they included in or excluded from the statement in the first paragraph sentence "these studies were performed to a high standard and to OECD recommended guidelines"? In the section entitled "Assessment of the published..." on p. 2, I am hard-pressed to find any assessment. It is almost merely a listing of what everyone already knew from casually reading the abstract.

7. In his conclusions (p. 2), do the "studies evaluated" (line 2) include the literature reports or not? IN other words, is he saying that none of the studies (Monsanto plus literature) had evidence of glyphsoate genotoxic potential, or is he limiting this conclusion to the Monsanto studies?

8. Of course we know there were no data of the type listed in points 2, 3, and 4 on p. 3. We didn't need him to tell us that. The key point is whether the conclusions of Bolognesi, and Rank can be discounted on the basis of the strength and number of studies at hand, or whether their experiments need to be repeated independently to credibly refute the findings. Of course we knew that the latter would be the most convincing approach, but we need him to make any arguments that can be made on the data we have.

Overall, I guess we have his recommendation of studies that could be used to strengthen the database on p. 4. , but that is about it. I do not see that he has stuck his neck out on anything at all controversial, and therefore, there is little value in the write-up as written that could be useful. Hope it didn't cost much. Perhaps this is too harsh, and I don't know what your proposal to him was, but I guess I would expect more than this of a Professor.

Steve

PRIFYSGOL CYMRU ABERTAWE
Ysgol y Gwyddorau Biolegol
Parc Singleton, Abertawe, SA2 8PP

UNIVERSITY OF WALES SWANSEA
School of Biological Sciences
Singleton Park, Swansea, SA2 8PP

[redacted]
Monsanto Europe
Parc Scientific Fleming
Rue Laid Burnait 5
B-1348 Louvain-La-Neuve
Belgium

18 August 1999

Dear [redacted]

You find enclosed my evaluation of the package of studies provided by yourself, which studied the genotoxicity of glyphosate, its various formulations and surfactants. I apologise for the time taken for the evaluation, but as I explained previously, I had a sudden urgent request from UK government to evaluate the genotoxicity of growth promoting hormones used in beef production.

Please let me know if there are any parts of my evaluation and recommendations, which you would like, clarified.

Yours Sincerely

JM

Professor James M. Parry

P.S. as a personal point - I don't really understand why you use contract labs, your "in house" studies are usually better and easier to follow

Tel [redacted] Fax [redacted]

An analysis of potential genotoxicity of glyphosate and its various formulations

Professor James M. Parry, Centre for Molecular Genetics and Toxicology, School of Biological Sciences, University of Wales Swansea, Swansea SA2 8PP.

Evaluation

The various studies provided have been individually evaluated and these evaluations are summarised in Table 1. In general, these studies were performed to a high standard and to OECD recommended guidelines. Table 1 has been separated into glyphosate itself, glyphosate formulations and some component surfactants and assessments for each study provided.

I have major reservations concerning one of the studies, which are discussed below:-

General Comments of BioAgri G.1.2-60/96

Rather confusing report-relating doses used to fraction of LD50, which is reported to be 250mg/kg b.w. My assumption from the report is that the top doses of 206mg/kg are 75% of the LD50. This is in marked contrast to the top dose of 555mg/kg used in study MSL-1171.

In general this is a poor study, for example; the measurement of the PCE/total erythrocyte ratio is normally determined by counting 500 or 1000 erythrocytes which are classified during counting into PCE or NCE, in this study they count erythrocytes until they reach 1000 of **either** NCE **or** PCE. This results in major differences between individual animals, for example in the control males one animal has a ratio of 0.31 whereas another has a ratio of 0.66, with such variation it would be difficult to demonstrate a significant change in bone marrow toxicity. The positive control chemical cyclophosphamide only gave a positive result in one animal of each sex; thus we cannot be assured that ~~the method was being correctly applied~~. In addition,

there was a very high level of bone marrow toxicity in one female exposed to cyclophosphamide.

Assessment of the published studies of glyphosate and its formulations

The published information on glyphosate and its formulations provide some evidence for genotoxic activity i.e. some evidence of:

a) Ames test positive results (Rank *et al* 1993)

b) Induction of sister chromatid exchange in cultured human lymphocytes (Bolognesi *et al* 1997).

b) Induction of sister chromatid exchanges and chromosome aberrations in cultured bovine lymphocytes (Lioi *et al* 1998).

c) Positive response in mouse bone marrow micronucleus assay (Bolognesi *et al* 1997).

d) Evidence for the induction of oxidative damage in rodent liver and kidney (Bolognesi *et al* 1997).

could we distinguish which are gly and which are formulations?

Conclusions of the studies provided

The overall results of the studies are combined together in Table 2. This table illustrates that in none of the studies evaluated ~~was there~~ contained convincing evidence that glyphosate had genotoxic potential. However, there are a number of deficiencies in the studies provided:-

1) A complete data package as generally recognised by international regulatory agencies i.e.

   a) Bacterial mutagenicity assay.

   b) *In vitro* cytogenetics assay.

   c) *In vitro* point mutation assay.

d) Rodent bone marrow micronucleus assay

was only provided for glyphosate itself.

2) For none of the formulations of glyphosate was any *in vitro* cytogenetic or mouse lymphoma point mutation date provided.

3) No *in vivo* rodent bone marrow study was provided which is completely comparable to the published study of Bolognesi *et al* (1997).

4) No data was supplied which addresses the question of the potential oxygen radical damaging activity of glyphosate.

word order is odd.

The range of bacterial mutagenicity studies provided were all performed to a high standard and are consistently negative. Thus, they do not provide any support for the observations of Rank *et al* (1993) that glyphosate is a bacterial mutagen.

None of the rodent bone marrow micronucleus studies provide evidence that glyphosate is a potential rodent bone marrow genotoxin. However, I should emphasise that there is no study directly comparable to the positive study of Bolognesi *et al* (1997).

There was no evidence from the studies provided that glyphosate was clastogenic and/or mutagenic in cultured mammalian cells. However, equivalent data is not available for glyphosate formulations.

There was no evidence in any of the studies evaluated that the various surfactants used in glyphosate formulations were potential genotoxins.

Remaining unresolved problems concerning the genotoxicity of glyphosate

The main question to be addressed is: When studied using methodologies, which measure a recognised genetic endpoint, are glyphosate containing products genotoxic? The areas lacking in clarity are:-

1) *In vitro* chromosome damaging activity. There are some published positives and the studies evaluated here are not comprehensive for various formulations.

2) There is a limited published bone marrow positive study (Bolognesi *et al* 1997). However, no equivalent study was available for evaluation.

3) Some published evidence is available that glyphosate is capable of inducing oxidative damage. No studies were provided which address this potential activity. No information is provided to indicate whether formulation influences this activity.

4) The confusing picture of the activity of the various formulations of glyphosate reported in the literature suggests that there may be differences in the way samples are stored. The studies on the various surfactants do not clarify the matter of the reported activity of formulations.

Recommendations for further work

To clarify the remaining problems I recommend the following additional studies:-

1) A definitive *in vivo* bone marrow micronucleus study with multiple dosing to repeat the basic protocol used in the Bolognesi *et al* (1997) study.

2) A comprehensive *in vitro* cytogenetics study. I would recommend an *in vitro* micronucleus study in which all the formulations of glyphosate could be evaluated and the potential effects of antioxidants determined.

3) In view of the differences in the responses of the various formulations reported in the published papers it would be of value to evaluate the stability of the formulations and its influence on genotoxic activity.

19-8-99.

Include full study info please
citation

Table 1a

Review of Genotoxicity Data on Glyphosate Formulations and Surfactants

MSL-11731
Salmonella mutagenicity assay of **Direct** (72% Glyphosate)

TA98, TA100, TA1535, TA1537
15 to 1500μg/plate in presence of S9 mix
5 to 500μg/plate in absence of S9 mix

Some toxicity observed above ...

There was no evidence that **Direct** was a bacterial mutagen.

MSL-11729
Salmonella mutagenicity assay of **Roundup** (31% Glyphosate)

TA98, TA100, TA1535, TA1537
15 to 1500μg/plate in presence of S9 mix
5 to 500μg/plate in absence of S9 mix

Some toxicity observed above ...

There was no evidence that **Roundup** was a bacterial mutagen.

Scantox 10.9.91
Salmonella Mutagenicity assay of **Glyphosate** (206-Jak-25-1)

TA98, TA100, TA1535, TA1537
310, 630, 1300, 2500μg/plate in presence of S9 mix
160, 310, 630, 1300, 2500μg/plate in absence of S9 mix

Toxicity observed above..

There was no evidence that **Glyphosate** was a **Salmonella** mutagen.

MSL-11730
Salmonella Mutagenicity assay of **Rodeo** (40% **Glyphosate**)

TA98, TA100, TA1535, TA1537
50 to 5000μg/plate in presence and absence of S9 mix

No toxicity observed

There was no evidence that **Rodeo** was a **Salmonella** mutagen.

Table 1b

BioAgri Report G.1.1-050/96
Salmonella Mutagenicity assay of **GLIFOS**

TA97a, TA98, TA100, TA1535
1 to 5000μg/plate in presence and absence of S9 mix

In all cases **Glifos** reduced the number of bacterial colonies counted

There was no evidence that **Glifos** was a **Salmonella** mutagen.

Scantox Report 10.9.91
In vitro Mammalian cell mutation assay in mouse lymphoma L5178Y cells **Glyphosate** (206 Jak-25-1)

0.63, 1.3, 2.5, 5mg/ml without metabolic activation
0.52, 1.0, 2.1, 4.2mg/ml with S9 mix

No evidence thaat **Glyphosate** produced any increase in mutation in either the presence or absence of S9 mix.

Notox Project 141918
In vitro chromosome aberrations in cultured human lymphocytes, **Glyfosaat**

33, 56, 100, 133, 178, 237μg/ml without S9 mix, 14 hrs fixation
56, 100, 133, 178, 237, 333μg/ml without S9 mix, 48 hrs fixation
33, 56, 100, 133, 178, 237, 333, 562μg/ml with S9 mix, 24 hrs fixation
100, 133, 178, 237, 333, 562μg/ml with S9 mix, 48 hrs fixation
not all doses were scored

Reduction in mitotic index in absence of S9 mix and at 24 hrs fixation in presence of S9 mix

No evidence of any increase in chromosome aberrations in the study.

Scantox Report 12.9.91
Mouse micronucleus **Glyphosate** (206-Jak-25-1)

Oral exposure 5000mg/kg bw sampled at 24, 48 and 72 hrs

Evidence for overall toxicity not clear from report
No evidence of bone marrow toxicity
No evidence that Glyphosate produced an increase in micronuclei in bone marrow.

Table 1c

BioAgri Report G.1.2-60/96
Mouse micronucleus **GLIFOS** (41% **Glyphosate**)

68, 137 and 206mg/kg by i.p. injection delivered twice .... at 24 hr intervals - said to relate to 75, 50 and 25% of LD50 for mice

No evidence of bone marrow toxicity
No evidence that **GLIFOS** produced an increase in micronuclei in bone marrow.
Inadequate study see text.

MSL-1171
Mouse Micronucleus **Roundup** Formulation (31% **Glyphosate**)

140, 280, 555mg/kg - sampled at 24, 48, 72 hrs single i.p. injection

Evidence of toxicity in high dose group
No evidence of increase in micronucleated erythrocytes
However limited evidence of bone marrow toxicity at doses...
No evidence of increases in micronuclei in overall data
One animal Male 268 showed increase in micronuclei

MSL-11773
Mouse Micronucleus **Direct** Formulation (72% **Glyphosate**)

91, 183 and 365mg/kg sampled at 24, 48, 72 hrs single i.p. injection

Evidence of toxicity in high dose group
This experiment used the same control and positive controls as MSL-11771
No evidence of bone marrow toxicity
No evidence of increases in micronuclei in overall data
One animal female 186 183mg/kg at 48 hrs showed an increase

MSL-11772
Mouse Micronucleus **Rodeo** Formulation (40% **Glyphosate**)

850, 1700, 3400mg/kg sampled at 24, 48, 72 hrs single i.p. injection

Toxicity observed. Some evidence of bone marrow toxicity at what levels
No evidence of increases in micronuclei

This experiment used the same control and positive controls as 11771 and 11773.

Table 1d

RD300 SRRSL 1147
Mouse dominant lethal study, **Glyphosate**

200, 800, 2000mg/kg bw by gavage

Large comprehensive study involving mating for up to 8 weeks
Clear response from positive control

Small reduction in viable foetuses in week 1 at 800mg/kg, week 3 at 2000mg/kg and an increase in late reabsorptions at 200mg/kg at week 8

I conclude that glyphosate was not a dominant lethal inducing chemical under the experimental conditions used.

MSL-10625
Salmonella Assay of **Mon 0818** Surfactant

TA98, TA100, TA1535, TA1537 in presence and absence of S9 mix. 1 to 100μg/plate in presence of S9
0.3 to 30μg/plate in absence of S9 mix

No evidence that **MON 0818** is mutagenic in Salmonella.

MSL-1538
Salmonella Assay of **MON 8080** Surfactant

TA98, TA100, TA1535, TA37 in presence and absence of S9 mix
0.003 to 3μl/plate in absence of S9 mix and presence of S9 mix

No evidence that MON 8080 is mutagenic in **Salmonella**.

Hoecht 92.0487
Salmonella and **Escherichia coli** assay of **Dodigan 4022** Surfactant

TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* WP2 uvrA
4 to 10,000μg/plate in both presence and absence of S9 mix

No evidence that **Dodigen 4022** is mutagenic in the bacterial test strains used.

Table 1e

Hoechst 92.1024
In vitro chromosome aberrations in Chinese hamster V79 cells **Dodigen 4022** surfactant

Without metabolic activation	6000μg/ml for 7 hrs 6000, 3000, 600μg/ml for 18 hrs 6000μg/ml for 28 hrs
With S9 mix	6000μg/ml for 7 hrs 6000, 3000, 600μg/ml for 18 hrs 6000μg/ml for 28 hrs

This is a rather confusing dataset

Mitotic index	decreased at 7 hrs increased at 18 hrs decreased at 28 hrs	in absence of S9 mix
	decreased at 7 hrs increased at 18 hrs no change at 28 hrs	in presence of S9 mix
Polyploidy	decreased at 7 hrs decreased at 18 hrs decreased at 28 hrs	in absence of S9 mix
	decreased at 7 hrs decreased at 18 hrs increased at 28 hrs	in presence of S9 mix
Aberrations	increased at 7 hrs no change at 18 hrs increased at 28 hrs	in absence of S9 mix
	increased at 7 hrs no change at 18 hrs increased at 28 hrs	in presence of S9 mix

None of increases significant

Overall experiments are difficult to interpret and should have been repeated.

Table 1f

KHL 89182/ML-89-46
Mouse Micronucleus Test, **MON 0818** surfactant

100mg/kg sampled at 24 and 48 hrs by i.p. injection

No evidence of animal toxicity, no bone marrow toxicity
No evidence of increases in micronuclei

Limited experiment doesn't really tell us very much.

Evaluation of Publications

1) Li & Long (1988). Glyphosate tested in bacterial mutagenicity rec-assay, Chinese hamster gene mutation assay, hepatocyte DNA repair assay, and *in vivo* cytogenetic assay.
- negative results reported for all assays.

2) Vigfusson and Vyse (1980). Roundup evaluated for its ability to induce sister chromatid exchange in cultured human lymphocytes.
Small increase reported at 250μg/ml.

3) Kale *et al* (1995). Roundup and Pondmaster evaluated in *Drosophila* sex linked recessive lethal.
Positive result observed in spermatocyte broods at concentration of 1μgm/ml of **Roundup** and 0.1μg/ml of **Pondmaster**.

4) Clements *et al* (1997). Roundup evaluated in the COMET assay in tadpoles of *Rana catesbeiana*.
Positive results observed at concentrations above 27mg/litre.

Table 2a

Overview Genotoxicity of Glyphosate

Preparation	Test System	Result
Glyphosate (206-Jak-25-1)	**Salmonella** Assay up to 2500μg/plate	-
	In vitro mouse lymphoma mutation assay up to 5mg/ml	- ve
	Mouse micronucleus oral dosing 5000mg/kg bw	- ve
Glyphosate (Techical)	Dominant lethal 200, 800, 2000mg/kg bw by gavage	- ve
Glyphosaat (assumed to be glyphosate)	*In vitro* cytogenetics up to 562μg/ml	- ve

Table 2b

Formulations

Preparation	Test System	Result
Rodeo (40% Glyphosate)	**Salmonella** Assay up to 5000μg/plate	- ve
	Mouse micronucleus 850, 1700, 3400mg/kg bw i.p. injection	- ve
GLIFOS (41% Glyphosate)	**Salmonella** assay up to 5000μg/plate very toxic	ve
	Mouse micronucleus 68, 127, 206mg/kg bw 2 x i.p. injections	- ve Inadequate study
Roundup (31% Glyphosate)	**Salmonella** assay up to 1500mg/plate	- ve
	Mouse micronucleus 140, 280, 555mg/kg bw i.p. route	- ve
Direct (72% Glyphosate)	**Salmonella** assay up to 1500μg/plate	- ve
	Mouse micronucleus 91, 183, 365mg/kg bw i.p. route	ve

Table 2c

Surfactants

Preparation	Test System	Result
Dodigen 4022 Surfactant	Bacterial Mutagenicity Assay up to 10,000μg/plate	- ve
	In vitro cytogenetics up to 6000μg/ml	Poor study difficult to evaluate
Mon 0818 Surfactant	**Salmonella** Mutagenicity Assay up to 100μg/plate	ve
	Mouse Micronucleus 100mg/kg bw i.p.	- ve
Mon 8080 Surfactant	**Salmonella** Mutagenicity Assay up to 3μl per plate	- ve