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An Evaluation of the Genotoxic Potential of Glyphosate[1]

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An Evaluation of the Genotoxic Potential of Glyphosate. LI, A. P., AND LONG, T. J. (1988). *Fundam. Appl. Toxicol.* **10**, 537–546. The potential genotoxicity of glyphosate, the active ingredient in Roundup herbicide, was tested in a variety of well-established *in vitro* and *in vivo* assays including the *Salmonella typhimurium* and *Escherichia coli* WP-2 reversion assays, recombination (rec-assay) with *Bacillus subtilis*, Chinese hamster ovary cell gene mutation assay at the hypoxanthine/guanine phosphoribosyl transferase gene locus, hepatocyte primary culture/DNA repair assay, and *in vivo* cytogenetics assay in rat bone marrow. No genotoxic activity was observed in the assays performed. The data suggest that glyphosate should not pose a genetic risk to man.

Glyphosate (*N*-phosphonomethyl glycine) is an effective herbicide, acting primarily as a competitive inhibitor of 5-enolpyruvoylshikimic acid-3-phosphate synthase (Amrhein *et al.*, 1980), an enzyme essential to the synthesis of aromatic amino acids in plants. Due to the absence of this enzyme in animals, glyphosate would be expected to be selectively toxic to plants. The animal toxicology data for glyphosate support this expectation. The acute oral LD50 of glyphosate is reported to be approximately 4–6 g/kg in rat and rabbit (Atkinson, 1985). On the basis of these and other acute toxicity study results (Monsanto, 1985), glyphosate is therefore generally considered to be essentially nontoxic to mammalian species. It is considered to be essentially nontoxic to blue gill, carp, daphnia, duck, fiddler crab, harlequin, quail, and shrimp, and considered only slightly toxic to Atlantic oyster and trout (Atkinson, 1985).

Because of the broad-spectrum, nonselective herbicidal activity of glyphosate, it is widely used in the agricultural, industrial, and residential sectors where potential human exposure to the chemical may occur. To investigate whether exposure to glyphosate would pose genetic risk to the human population, we studied the genotoxic potential of glyphosate in a variety of well-established *in vitro* and *in vivo* assays: the *Salmonella typhimurium*/histidine and *Echerichia coli* WP-2 reversion assays, rec-assay in *Bacillus subtilis*, the Chinese hamster ovary cell/hypoxanthine guanine phosphoribosyl transferase (CHO/HGPRT) gene mutation assay, rat primary hepatocyte/DNA repair assay, and *in vivo* bone marrow cytogenetics assay in rats.

MATERIALS AND METHODS

Test chemicals. A single lot of glyphosate (lot XHJ-64) was used in the battery of genotoxicity assays. The lot was synthesized by the Monsanto Agricultural Company and had a purity of approximately 98%.

1. Microbial Genotoxicity

a. Salmonella/histidine reversion assay. The plate-incorporation procedures of Ames *et al.*, 1975 were used.

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Glyphosate was tested for mutagenicity with five *his*- strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, and TA98 for the detection of frame-shift mutations; and TA100 for the detection of base-pair substitution mutations) using the plate-incorporation assay in both the absence and presence of Aroclor 1254-induced rat liver homogenate supernatant (S9) as described by Ames *et al.*, 1975. Glyphosate was dissolved in distilled water and tested at doses of 10, 50, 100, 500, 1000, and 5000 μg/plate. Distilled water was used as the solvent control. 2-Aminoanthracene (10 μg/plate) was used as the promutagen positive control for all five strains. β-propiolactone (50 μg/plate), AF-2 (2-(2-furyl)3-(5-nitro-2-furyl) acrylamide), (0.05–0.25 μg/plate), 9-aminoacridine (200 μg/plate), and 2-nitrofluorene (50 μg/plate) were used as direct-acting positive controls. The statistical significance of the dose–response relationship was determined using linear-regression analysis without further data transformation at the significance level of $p \leq 0.05$.

b. E. coli WP2 reverse mutation. The tryptophan-*hcr* strain of *E coli* WP2 was used to test for glyphosate mutagenicity in both the absence and presence of S9 using the plate-incorporation procedure as described for the *Salmonella*/histidine reversion assay (Ames *et al.*, 1975; Dunkel *et al.*, 1984). The assay was performed simultaneously with the *Salmonella*/histidine reversion assay using the same doses of glyphosate. Distilled water was used as the solvent control. The positive controls for testing in the presence and absence of S9 were 0.25 μg/plate AF-2 and 10 μg/plate 2-aminoanthracene, respectively. The statistical significance of the dose–response relationship was determined using linear-regression analysis without further data transformation at the significance level of $p \leq 0.05$.

c Rec-assay. The normal (H17, rec+) and recombination deficient (M45, rec−) strains of *B. subtilis* were used. Stock cultures were stored at −80°C. After the stock cultures were melted, both strains were streaked on a B-II agar plate with micropipets. The start points of the two streaks were placed close to but not contacting each other. Solutions (<10 mg/ml) and suspensions (>10 mg/ml) of glyphosate were prepared using sterilized distilled water. A disk of filter paper (diameter = 1 cm) soaked with 0.02 ml of the glyphosate solutions or suspensions was placed on the start points of the two streaks.

After an overnight incubation at 37°C, the length of inhibitory zones was measured and compared between the recombination proficient and deficient strains. The test doses were 20, 100, 200, 500, 1000, and 2000 μg glyphosate per disk. Distilled water was used as the negative solvent control. Kanamycin (10 μg/disk) and mitomycin (0.1 μg/disk) were used as positive controls.

2. *Mammalian Genotoxicity in Vitro*

a. CHO/HGPRT gene mutation. The CHO cell line (K_1BH_4) originally obtained from Dr. A. W. Hsie of Oak Ridge National Laboratory was used. The performance of the assay was consistent with the published guidelines (Li, 1985; Li, *et al.*, 1987). For cytotoxicity determination CHO cells were seeded in 25-cm² plastic culture flasks at 0.5×10^6 cells per flask in growth medium 18–24 hr before treatment. On the day of treatment, medium was changed to 2.5 ml Ham's F12 medium without serum, with or without S9. An equal volume of 2× solutions of glyphosate (dissolved directly in medium without serum) was then added. After incubation of 3 hr at 37.5 ± 2°C, the treatment medium was discarded. The cells were washed with 5 ml of Hanks' balanced salt solution and the cells were removed from the flasks by trypsinization and counted. Three samples of 200 cells were plated per sample for determination of cloning efficiency. The plates were returned to incubation for 7–9 days. The colonies which developed were fixed with 70% methanol, stained with 10% Giemsa, and counted by hand. Cytotoxicity was expressed as relative survival:

$$\text{Cloning efficiency (C.E.)} = \frac{\text{No. of colonies}}{\text{No. of cells plated}}$$

$$\text{Relative survival (R.S.)} = \frac{\text{C.E. (treated)}}{\text{C.E. (control)}}.$$

For mutagenicity determination, the cells were processed as described above for cytotoxicity determination, except that in addition to plating 200 cells for cloning efficienty, 10^6 cells were plated in 10 ml of subculture medium (hypoxanthine-free Ham's F12 medium (K. C. Biological), supplemented with 10% dialyzed newborn calf serum). The cells were subcultured every 2–3 days for the 7–9 days for the expression of mutant phenotype. Mutant selection was performed as previously described (Li, 1981) using selective medium consisting of hypoxanthine-free Ham's F12 medium, supplemented with 10 μM 6TG and 5% dialyzed newborn calf serum. A total of 10^6 cells per sample were plated in 100-mm plates (5 plates, 2×10^5 cells per plate) in 8 ml of selective medium per plate for mutant selection. A total of 200 cells per sample in triplicate were placed in 2 ml of selective medium without 6TG for the determination of cloning efficiency (C.E.). The plates were then incubated for 8–12 days. The colonies which developed were fixed, stained, and counted. Results were expressed as mutant frequency (M.F.):

$$\text{M.F.} = \frac{\text{No. of mutant colonies}}{\text{No. of cells plated}} \times \frac{1}{\text{C.E.}}.$$

Aroclor 1254-induced rat liver homogenate (S9) commercially purchased from Litton Bionetics was used as an exogenous activation system. The S9–cofactor, consisting of 50 mM sodium phosphate (pH 7.5), 4 mM NADP, 5 mM glucose 6-phosphate, 30 mM KCl, 10 mg $MgCl_2$, 10 mM $CaCl_2$, and different amounts of liver S9, was used. The different concentrations of S9 in this report represent the percentage of S9 (v/v) in the S9–cofac-

TABLE 1

MUTAGENICITY TESTING OF GLYPHOSATE IN *Escherichia coli* WP2 *hcr* AND *Salmonella typhimurium* STRAINS TA1535, TA100, TA1537, TA1538, AND TA98

Treatment (μg/plate)		S9 mix	Revertants/plate**					
			WP2 *hcr*	TA1535	TA100	TA1537	TA1538	TA98
Solvent control (water)		−	20	6	167	9	10	24
			24	14	129	10	13	23
Glyphosate	10	−	22	2	130	3	17	27
			21	5	160	7	24	28
	50	−	12	5	151	5	15	33
			25	5	159	6	15	40
	100	−	18	4	143	8	17	20
			20	5	160	8	24	20
	500	−	21	3	118	11	7	31
			26	1	143	9	15	24
	1000	−	15	9	87	10	18	21
			18	12	120	10	12	23
	5000	−	*	6	58	3	6	10
			*	6	87	3	7	3
Solvent control (water)		+	17	6	139	7	8	22
			22	5	140	5	11	16
Glyphosate	10	+	25	4	110	3	16	19
			18	1	135	3	11	23
	50	+	27	9	123	7	13	21
			22	5	131	9	17	26
	100	+	33	5	129	11	18	9
			17	7	115	6	14	20
	500	+	28	3	138	12	15	19
			30	3	111	5	7	26
	1000	+	29	11	97	11	20	15
			24	4	88	7	11	23
	5000	+	25	5	51	6	11	19
			34	7	36	3	15	22
2-Aminoanthracene	10	−	23	8	179	18	23	40
	10	+	98	376	>3000	370	>3000	>3000
			79	335	>3000	388	>3000	>3000
Positive control		−	1672[a]	315[b]	1024[c]	>10000[d]	>3000[e]	326[f]
			2272	358	1150	>10000	>3000	296[f]

[a] 0.25 μg/plate AF-2.
[b] 50 μg/plate *p*-propiolactone.
[c] 0.05 μg/plate AF-2.
[d] 200 μg/plate 9-aminoacridine.
[e] 50 μg/plate 2-nitrofluorene.
[f] 0.1 μg/plate AF-2.
* Inhibition of bacterial growth was observed.
** No statistically significant ($p \leq 0.05$) dose–response relationship was observed for glyphosate treatment based on linear-regression analysis.

TABLE 2

GENOTOXICITY TESTING OF GLYPHOSATE IN THE *Bacillus subtilis* REC-ASSAY

Treatment (μg/disk)	Inhibitory zone (mm) M45 (*rec*−)	Inhibitory zone (mm) H17 (*rec*+)	Difference* (mm)
Control (H_2O)	0	0	0
Glyphosate			
20	0	0	0
100	0	0	0
200	0	0	0
500	0	0	0
1000	0	0	0
2000	0	0	0
Kanamycin (10)	7	5	2
Mitomycin C (0.1)	11	0	11

* No statistically significant ($p \leq 0.05$) dose–response relationship observed for glyphosate treatment based on linear-regression analysis.

tor mixtures. One milliliter of the S9–cofactor mixture was added to 4 ml of medium for cytotoxicity or mutagenicity testing.

Mutagenicity data were analyzed according to the statistical method of Snee and Irr (1981), designed specifically for the CHO/HGPRT mutation assay. Mutant frequency values were transformed according to the equation $Y = (X + 1)^{0.15}$, where Y = transformed mutant frequency and X = observed mutant frequency. Student's t test was then used to compare treatment data to solvent control data. The Snee and Irr analysis also allowed the determination of the dose–response relationship as linear, quadratic, or higher-order. A computer program obtained from Joe Irr (DuPont) and incorporated into the Monsanto computer by Alan Dickinson (Monsanto) was used.

Two independent mutagenicity experiments were performed. In the first experiment, glyphosate at concentrations of 5, 17.5, and 22.5 mg/ml was tested in the absence of S9 and in the presence of 1, 2, 5, and 10% S9 (v/v S9 in cofactor mixture), 1 ml of S9/cofactor mixture was added to a final volume of 5 ml of treatment medium. In the second experiment, 2 to 20 mg/ml and 5 to 25 mg/ml of glyphosate were tested in the absence and presence of 5% S9, respectively. Glyphosate solutions were prepared in medium (Ham's F12) and neutralized to a pH of 7.0 to 7.4 using 1 N sodium hydroxide. Medium was used as the solvent control. Ethyl methanesulfonate (EMS, 200 μg/ml) and benzo[a]pyrene (BaP, 2 μg/ml) were used as positive controls for testing in the absence and presence of S9.

b Hepatocyte/DNA repair The assay of Williams (1976) using noninduced primary rat hepatocytes was used to test the ability of glyphosate to induce unscheduled DNA synthesis in mammalian cells. Hepatocyte primary cultures were initiated from an adult male Fischer 344 rat as previously described (Williams, 1976) and seeded onto 25-mm-round coverslips (Thermanox in William's Modified Eagle's medium, WME) and incubated at 37°C for 2 hr. The coverslips were then washed with 1 ml of medium.

Immediately after washing, various levels of glyphosate negative control (pyrene), control solvent (DMSO), positive control benzo[a]pyrene (BaP), and 10 μCi/ml tritiated thymidine ([^{3}H]TdR), 60–80 Ci/mM, were added to the culture in 2 ml WME. The hepatocytes were incubated at 37°C for a total of 18–20 hr in the presence of the test chemical and in [^{3}H]TdR–WME. Each coverslip was removed from its well and processed as previously described (Williams, 1976). Results of the HPC/DNA repair test were quantified by determining the net increase in nuclear grains induced by a test chemical. An Artek Model 880 electronic counter with microscopic attachment was used for grain counting. Only those cells which were viable at the time of fixation, indicated by swollen nuclei (as a result of incubation in the hypotonic sodium citrate), and those evenly coated with emulsion were scored. Between 5 and 20 cells randomly selected from each quadrant of the coverslip were counted. Counts were obtained for each nucleus; background was determined by counting three nuclear-sized areas adjacent to the nucleus. Net nuclear grain counts were calculated by subtracting the highest cytoplasmic count from the nuclear count. The scoring method was designed to avoid false positives by choosing the highest cytoplasmic count of each cell as a background. Results of individual experiments were reported as the mean ± standard deviation of net grain counts for triplicate coverslips.

Seven concentrations of glyphosate ranging from 12.5 ng/ml to 125 μg/ml were tested. Glyphosate was dissolved in 0.1 N sodium hydroxide for testing. The solvent control for glyphosate-treated cultures was 0.1 N sodium hydroxide at 1% of final volume. The negative controls were DMSO (1%) and pyrene (50 μM). BaP at 50 μM was used as the positive control. Student's t test was used to determine the statistical significance of differences in the net grain count per nucleus between the treatment groups (test chemical and positive control groups) and solvent control, using a significance level of $p \leq 0.05$.

3. *Mammalian Genotoxicity in Vivo: In Vivo Bone Marrow Cytogenetics*

The assay was performed as recommended by Preston *et al* (1981). Glyphosate was dissolved in Hanks' bal-

TABLE 3

CYTOTOXICITY AND MUTAGENICITY OF GLYPHOSATE IN CHO CELLS AT VARIOUS CONCENTRATIONS OF AROCLOR 1254-INDUCED RAT LIVER S9

Treatment	Cytotoxicity[a] (R.S.)	Mutagenicity Mutant frequency[b] $\times 10^{-6}$
A. No S9		
Medium control	1.00	7.4
Glyphosate		
5 mg/ml	1.10	7.1
17.5 mg/ml	0.64	14.4
22.5 mg/ml	0.11	5.3
Positive control (EMS, 0.2 mg/ml)	0.92	163.7*
B. 1% S9		
Medium control	1.00	5.9
Glyphosate		
5 mg/ml	1.12	4.3
17.5 mg/ml	0.69	11.6
22.5 mg/ml	0.25	15.3
Positive control (BaP, 2 μg/ml)	0.74	353.1*
C. 2% S9		
Medium control	1.00	7.1
Glyphosate		
5 mg/ml	1.00	8.6
17.5 mg/ml	0.77	8.1
22.5 mg/ml	0.44	10.2
(BaP, 2 μg/ml)	0.49	185.9*
D. 5% S9		
Medium control	1.00	4.4
Glyphosate		
5 mg/ml	1.08	6.4
17.5 mg/ml	0.84	6.9
22.5 mg/ml	0.67	8.5
(BaP, 2 μg/ml)	0.46	121.2*
E. 10% S9		
Medium control	1.00	9.1
Glyphosate		
5 mg/ml	1.02	10.5
17.5 mg/ml	0.37	16.3
22.5 mg/ml	0.18	9.7
(BaP, 2 μg/ml)	0.35	95.3*

[a] Survival relative to solvent control (average of triplicate treatment).
[b] Mutants per 10^6 clonable cells (average of duplicate treatments).
* Statistically significant response ($p \leq 0.05$).

anced salt solution (HBSS) and neutralized to a pH of 7.0 with 1 N sodium hydroxide. The glyphosate solution was then administered by intraperitoneal (ip) injection to 18 male and 18 female Sprague–Dawley rats at a dosage level of 1000 mg/kg body wt. Two additional groups of 18 male and 18 female rats were administered HBSS as the solvent control or 25 mg/kg of cyclophosphamide as the positive control. Six animals of each sex for each of the three groups were terminated at 6, 12, and 24 hr after treatment. Two hours prior to termination, colchicine (2 mg/kg) was administered to all animals to arrest cells in metaphase.

Immediately after termination, bone marrow cells were collected from both femurs of each animal and pro-

TABLE 4

CYTOTOXICITY AND MUTAGENICITY OF GLYPHOSATE IN CHO CELLS IN THE ABSENCE OR PRESENCE OF 5% AROCLOR 1254-INDUCED RAT LIVER S9

Treatment	Cytotoxicity[a] (R.S.)	Mutagenicity Mutant frequency[b] $\times 10^{-6}$
A. No S9		
Medium control	1.00	11.3
Positive control (EMS, 0.2 mg/ml)	0.92	135.4*
Glyphosate		
2 mg/ml	0.99	3.5
5 mg/ml	0.93	11.3
10 mg/ml	0.90	10.8
15 mg/ml	1.04	20.8
20 mg/ml	0.38	10.1
B. 5% S9		
Medium control	1.00	7.7
Positive control (BaP)	0.47	76.8*
Glyphosate:		
10 mg/ml	1.15	5.7
15 mg/ml	0.99	13.1
20 mg/ml	1.13	9.9
25 mg/ml	0.46	13.1

[a] Survival relative to solvent control (average of triplicate treatment).
[b] Mutants per 10^6 clonable cells (average of duplicate treatments).
* Statistically significant response ($p \leq 0.05$).

cessed for slide preparation. When possible, 50 cells per animal (300 cells per treatment per time period) were examined for chromosome aberrations. Student's t test was used to determine the statistical significance of the difference between treatment groups (test chemical and positive control groups) and the vehicle control group, using a significance level of $p \leq 0.05$.

RESULTS

1. *Microbial Mutagenicity*

a. Salmonella/histidine reversion assay. *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98 were treated with 10 to 5000 μg/plate of glyphosate in both the presence and absence of S9. No statistically significant induction of revertants above solvent control levels and no significant dose–response relationship were observed. The positive controls yielded the expected positive responses (Table 1).

b. WP2 reversion assay. As in the *Salmonella*/histidine reversion assay, no significant induction of revertants attributed to glyphosate treatment and no significant dose–response relationship were observed. The positive controls yielded the expected positive responses (Table 1).

c. Rec-assay. While the positive controls demonstrated differential inhibition of the growth of the recombination deficient strain (M45) versus the recombination proficient strain (H17), no growth inhibition was observed for either strain at glyphosate concentrations of 20 to 2000 μg/disk (Table 2).

2. *Mammalian Genotoxicity in Vitro*

a. CHO/HGPRT gene mutation. Two individual experiments were performed for the

TABLE 5

GENOTOXICITY TESTING OF GLYPHOSATE IN THE RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY

Treatment	Net grains per nucleus (±SD)
Glyphosate (mg/ml):	
1.25×10^{-5}	1.4 ± 0.5
6.25×10^{-5}	0.1 ± 0.1
1.25×10^{-4}	0.1 ± 0.2
6.25×10^{-4}	0.1 ± 0.2
1.25×10^{-3}	0.0 ± 0.0
1.25×10^{-2}	0.2 ± 0.2
1.25×10^{-1}	0.1 ± 0.1
0.1 N NaOH (1%) (solvent control)	0.3 ± 0.1
DMSO (1%) (negative control)	0.3 ± 0.5
Pyrene (50 μM) (negative control)	0.4 ± 0.4
BaP (50 μM)	22.9 ± 9.7*
Cell culture control	0.2 ± 0.3

* Statistically significant response ($p \leq 0.05$).

evaluation of glyphosate in CHO cells. In the first experiment, glyphosate at 5, 17.5, and 22.5 mg/ml was tested for mutagenicity in the absence or presence of 1, 2, 5, or 10% S9. Significant cytotoxicity (over 50% cell killing) was observed for 22.5 mg/ml glyphosate in the absence of S9 and in the presence of 1, 2, and 10% S9, and for 17.5 mg/ml glyphosate in the presence of 10% S9. No statistically significant mutagenic response due to glyphosate treatment was observed at any of the S9 levels (Table 3). In the second experiment, CHO cells were treated with 2 to 20 mg/ml of glyphosate in the absence of S9, and with 5 to 25 mg/ml of glyphosate in the presence of 5% S9. Significant cytotoxicity was observed for 20 mg/ml and 25 mg/ml glyphosate for treatment in the absence and presence of S9, respectively. As observed in the first experiment, none of the glyphosate treatment groups had a statistically significant higher mutant frequency than the solvent control and no statistically significant dose–response relationship (linear, quadratic, or higher-order) between dose and mutant frequency was observed (Table 4). The positive controls EMS and BaP yielded the expected positive responses in both experiments. A decrease in mutagenicity of BaP with increasing S9 concentration as reported earlier (Li, 1984) was observed. The decrease was probably a result of detoxification (e.g., GSH conjugation) of the active BaP metabolites by the liver S9.

b. Hepatocyte/DNA repair. Based on the protocol used, the limit of glyphosate solubility was reached at a stock-solution concentration of 12.5 mg/ml. This was translated to a maximum test concentration of 125 μg/ml. For the seven doses of glyphosate ranging from 12.5 ng/ml to 125 μg/ml, no cytotoxicity nor statistically significant increase in net grains/nucleus above the solvent control was observed. The positive control BaP yielded the expected high net grains/nucleus value (Table 5).

3. Mammalian Genotoxicity in Vivo: Rat Bone Marrow Cytogenetics

Chromatid-type aberrations were observed in both the solvent control and glyphosate treatment groups at low frequencies. Chromatid deletions, the most frequent category, was observed at a frequency of approximately 1%. No statistically significant increases in either chromosomal aberrations or achromatic lesions (gaps) were observed in the glyphosate-treated groups at any time point studied. The expected high frequencies of chromosomal aberrations were observed for the positive control groups (Table 6).

As no general toxicity was observed for the male and female rats after glyphosate treatment at the various time points, it was necessary to demonstrate the availability of glyphosate to bone marrow cells. In a separate study, using ^{14}C radiolabeled glyphosate, glyphosate was shown to reach the rat bone marrow with a peak level at 0.5 hr after ip dosing and with a half-life for elimination in excess

TABLE 6

CHROMOSOMAL ABERRATION FREQUENCIES OBSERVED IN BONE MARROW CELLS FROM RATS TREATED WITH GLYPHOSATE (1 g/kg)

	Number of cells	Normal	Chromatid deletions	Chromatid interchanges	Chromatid intrachanges	Achromatic lesions	Percentage aberrant cells
A. 6-hr sampling time							
Vehicle control							
Male	300	296	3	1	0	0	1.3
Female	300	292	4	0	0	5	2 7
Total	600	588	7	1	0	5	2.0
Glyphosate							
Male	300	293	3	0	0	6	2 3
Female	300	291	3	0	0	6	3 0
Total	600	584	6	0	0	12	2.7
B. 12-hr sampling time							
Vehicle control							
Male	300	297	1	0	0	2	1.0
Female	275	271	1	0	0	1	1 5
Total	575	568	2	0	0	3	1.2
Glyphosate							
Male	300	294	3	0	0	3	2.0
Female	277	270	2	0	0	6	2.5
Total	577	564	5	0	0	9	2 3
C. 24-hr sampling time							
Vehicle control							
Male	300	296	1	0	0	3	1.3
Female	265	259	3	0	0	5	2.3
Total	565	555	4	0	0	8	1.8
Glyphosate							
Male	192	190	2	0	0	0	1 0
Female	300	289	5	0	0	6	3 7
Total	492	479	7	0	0	6	2.6
Cyclophosphamide (positive control)							
Male	256	148	217	76	6	34	42 2*
Female	21	16	14	1	0	3	23.8*
Total	277	164	231	77	6	37	40 8*

* Statistically significant response ($p < 0.05$)

of 7.6 hr. The peak value in bone marrow was calculated to be approximately 400 ppm, with a corresponding plasma value of 2000 ppm (Ridley, 1983).

DISCUSSION

The herbicide glyphosate was evaluated in a battery of genotoxicity assays. The genotoxicity endpoints used include microbial gene mutation, *in vitro* mammalian gene mutation, *in vitro* UDS, and *in vivo* bone marrow cytogenetics. The rec-assay was found not to be suitable for evaluation of glyphosate. The assay is based on differential cytotoxicity between the repair proficient and repair deficient strain of *B. subtilis*, and under our experimental conditions, glyphosate was not cytotoxic to either strain. Negative results were obtained for all the other assays. On the basis of our findings, we conclude that glyphosate is nongenotoxic.

Vigfusson and Vyse (1980) previously reported that glyphosate at high concentrations caused a statistically significant increase in sister-chromatid-exchange (SCE) in human lymphocytes *in vitro*, and concluded that the

chemical is "at most weakly mutagenic based on the SCE test." The validity of those results is questionable as no dose–response relationship was observed. Further, the significance of SCE as an indicator for genotoxicity is controversial. In a recent article, Tennant *et al.* (1987) showed that a variety of chemicals positive in the *in vitro* SCE assay were noncarcinogenic in rodent bioassays, nonmutagenic in the *Salmonella* mutation assay, and nonclastogenic in cultured mammalian cells. The negative results with an extensive battery of *in vitro* and *in vivo* assays reported here are consistent with the negative findings for glyphosate by others employing the *Salmonella*/histadine reversion assay (Chiao *et al.*, 1980; Njagi and Gopalan, 1980; Wildeman and Nazar, 1982; Shirasu *et al.*, 1982), the *E. coli* WP2 hcr reverse mutation assay (Shirasu *et al.*, 1982), and the *Drosophila* sex-linked recessive lethal assay (Gopalan and Njagi, 1981). In addition, glyphosate has been shown not to interfere with the repair of uv-induced DNA damage (Ohta *et al.*, 1986).

Results reported here and those reported by other researchers therefore provided evidence that glyphosate should not pose a genetic risk to man.

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