**Supplemental Information – Genotoxicity Expert Panel Review**

**Appendix B**

**Supplementary information for unpublished regulatory studies cited in Williams et al. (2000)**

**Table 1. Bacterial Reversion and Rec Assays**

**Report Reference:** Shirasu et al. (1980)

**Author/Study Director:** Y. Shirasu

M. Moriya

T. Ohta

**Year:** 1980

**Title:** [AMPA]: MICROBIAL MUTAGENICITY STUDY

**Assay:** Bacterial Reverse Mutation

**Report Identification Number:** None

**Report Guideline Statement:** None

**Test Material:** Aminomethylphosphonic acid (99%)

**Report Conclusion:** The microbial mutagenicity testing was performed on AMPA. This compound was negative in ‘the repair test (rec-assay) with *Bacillus subtilis* H17 (rec+) and M45 (rec-) and in the reverse mutation tests with or without a liver metabolic activation system employing *Escherichia coli* WP2 hcr and *Salmonella typhimurium* TA series (TA1535, TA1537,TA1538, TA1OO and TA98) as tester strains.

**Control Materials:**

**Negative (vehicle):** Distilled water

**Positive:** See summary tables

**Metabolic Activation:** Aroclor-1254 induced rat liver homogenate 30% in S9 Mix

**Summary data tables**

**Experiment 1**

**Plate Incorporation**

**Two replicate plates**

| Substance | Amt/Plate  (µg) | Revertants/Plate | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | WP2 hcr | TA98 | TA1538 | TA100 | TA1535 | TA1537 |
| *Without S9* |  |  |  |  |  |  |  |
| Veh. Cont.a |  | 13, 10 | 29, 31 | 11, 6 | 96, 98 | 6, 8 | 5, 6 |
| Test Mat. | 10 | 18, 18 | 16, 30 | 7, 3 | 120, 124 | 4,6 | 8, 7 |
|  | 50 | 11, 14 | 27, 30 | 7, 9 | 129, 141 | 4, 2 | 1, 1 |
|  | 100 | 20, 9 | 21, 17 | 4, 4 | 108, 137 | 8, 3 | 6, 7 |
|  | 500 | 22, 14 | 16, 30 | 7, 9 | 106, 124 | 11, 4 | 5, 4 |
|  | 1000 | 20, 14 | 24, 26 | 7, 6 | 84, 138 | 6, 2 | 4, 8 |
|  | 5000 | 12, 13 | 23, 31 | 8, 5 | 107, 81 | 15, 6 | 4, 8 |
| Pos. Cont.b |  | 1304, 1476 | 212, 223 | >3000, >3000 | 588, 648 | 694, 684 | >10000, >10000 |
| *With S9* |  |  |  |  |  |  |  |
| Veh. Cont.a |  | 12, 6 | 16, 22 | 10, 13 | 102, 107 | 4, 5 | 10, 4 |
| Test Mat. | 10 | 11, 11 | 13, 19 | 6, 14 | 102, 105 | 7, 9 | 2, 9 |
|  | 50 | 12, 8 | 20, 12 | 10, 4 | 91, 91 | 5, 2 | 2, 5 |
|  | 100 | 16, 10 | 19, 19 | 7, 7 | 81, 83 | 7, 5 | 14, 1 |
|  | 500 | 10, 21 | 21, 16 | 5, 13 | 79, 103 | 5, 4 | 3, 7 |
|  | 1000 | 11, 21 | 14, 16 | 9, 9 | 97, 96 | 3, 5 | 9, 5 |
|  | 5000 | 17, 11 | 12, 17 | 6, 7 | 83, 99 | 2, 4 | 4, 4 |
| Pos. Cont.b |  | 52, 50 | 212, 223 | >3000, >3000 | >3000, >3000 | 164, 232 | 128, 204 |
| Pos. Cont.  –S9b |  | AF-2  (0.25 µg) | AF-2  (0.1 µg) | 2-NF  (50 µg) | AF-2  (0.05 µg) | β-P  (50 µg) | 9-AA  (200 µg) |
| Pos. Cont. +S9b |  | 2-AA  (10 µg) | 2-AA  (10 µg) | 2-AA  (10 µg) | 2-AA  (10 µg) | 2-AA  (10 µg) | 2-AA  (1.25 µg) |

a Vehicle control: water

b Pos. Cont.--Positive Control with positive controls and amounts per plate indicated at the bottom of the table: AF-2, food additive; 2-NF, 2-nitrofluorene; β-P, β-propiolactone, 9-AA, 9-aminoacridine; 2-AA, 2-aminoanthracene

**Rec Assay**

***B. subtilis* H17 (repair proficient) and M45 (repair deficient)**

**Disk Assay**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Substance | Amt/Disk (µg) | Inhibitory Zone (mm) | | Difference (mm) |
|  |  | M45 | H17 |  |
| ***Without S9*** |  |  |  |  |
| Vehicle Cont.a |  | 0 | 0 | 0 |
| Test Material | 20 | 0 | 0 | 0 |
|  | 100 | 0 | 0 | 0 |
|  | 200 | 0 | 0 | 0 |
|  | 500 | 0 | 0 | 0 |
|  | 1000 | 0 | 0 | 0 |
|  | 2000 | 0 | 0 | 0 |
| Pos. Cont.b | Kan | 6 | 5 | 1 |
|  | MMC | 9.5 | 2 | 7.5 |

a Vehicle control: water

b Pos. Cont.—Kan, 10 µg/disk kanamycin; MMC, 0.1 µg/disk mitomycin C

**Report Reference:** Kier et al. (1992a)

**Author/Study Director:** L.D. Kier (study director)

S.D. Stegeman

J.G. Costello

S. Schermes

**Year:** 1992

**Title:** Ames/Salmonella Mutagenicity Assay of ROUNDUP® Herbicide Formulation

**Assay:** Bacterial Reverse Mutation

**Report Identification Number:** MSL-11729

**Report Guideline Statement:** None

**Test Material:** Roundup® Herbicide Formulation (31% glyphosate acid equivalent)

**Report Conclusion:** The test sample, Roundup® Herbicide Formulation, was concluded not to be mutagenic towards any of the *Salmonella typhimurium* test strains used (TA98, TAl 00, TA1535, and TA1537) in the presence or absence of an Aroclor 1254-induced rat liver homogenate metabolic activation system (S-9 Mix).

**Control Materials:**

**Negative (vehicle):** Distilled water

**Positive:** See summary tables

**Metabolic Activation:** Aroclor-1254 induced rat liver homogenate 10% in S9 Mix

**Summary data tables**

**Experiment 1**

**Plate Incorporation**

**Three replicate plates (treatment and vehicle control)**

| Substance | Amt/Plate (µg) | Revertants/Plated  Mean ± Std. Dev. | | | |
| --- | --- | --- | --- | --- | --- |
|  |  | TA98 | TA100 | TA1535 | TA1537 |
| ***Without S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 25.9 ± 9.6 | 136.7 ± 12.2 | 16.0 ± 5.6 | 8.6 ± 3.0 |
| Test Mat. | 5 | 29.3 ± 4.0 | 126.0 ± 2.0 | 14.7 ± 3.1 | 7.0 ± 1.0 |
|  | 15 | 24.3 ± 11.0 | 120.7 ± 24.2 | 13.7 ± 6.1 | 5.7 ± 1.5 |
|  | 50 | 26.3 ± 7.2 | 131.7 ± 17.6 | 13.7 ± 2.1 | 5.7 ± 2.1 |
|  | 150 | 29.7 ± 5.7 | 106.7 ± 13.3 | 10.7 ± 2.1 | 7.0 ± 1.0 |
|  | 500 | 19.7 ± 5.7Tb | T | T | 6.0 ± 2.8T |
| Pos. Cont.c | level 1 | 44 | 276 | 114 | 19 |
|  | level 2 | 99 | 1190 | 428 | 181 |
|  | level 3 | 191 | 1500 | 1940 | 1970 |
| ***With S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 36.9 ± 4.3 | 155.5 ± 9.9 | 13.7 ± 4.4 | 9.4 ± 3.6 |
| Test Mat. | 15 | 33.3 ± 3.1 | 151.0 ±19.3 | 10.3 ± 1.2 | 8.3 ± 1.5 |
|  | 50 | 28.7 ± 3.2 | 144.7 ± 24.8 | 10.7 ± 5.1 | 9.7 ± 3.2 |
|  | 150 | 32.0 ± 9.2 | 142.7 ± 9.3 | 12.3 ± 1.5 | 9.7 ± 5.7 |
|  | 500 | 28.7 ± 2.3 | 111.3 ± 11.0T | 9.3 ± 1.5 | 7.7 ± 4.0T |
|  | 1500 | 24.0 ± 5.6T | T | T | T |
| Pos. Cont.c | level 1 | 102 | 202 | 55 | 24 |
|  | level 2 | 316 | 1930 | 302 | 56 |
|  | level 3 | 726 | 2200 | 1060 | 201 |
| **Pos. Cont. –S9c** |  | 4-NQNO (0.02, 0.1, 0.2) | 4-NQNO (0.02, 0.1, 0.2) | NaNO2 (500, 2500, 5000) | 9-AA (10, 50, 100) |
| Pos. Cont. +S9c |  | 2-AAF (3, 15, 30) | B[a]P (0.2, 1, 2) | 2-AA (1, 5, 10) | **2-AA** (1, 5, 10) |

a Vehicle control: water

b T, toxicity

c Pos. Cont.--Positive Control with positive controls and amounts per plate in µg indicated at the

bottom of the table: 4-NQNO, 4-nitroquinoline-N-oxide; NaNO2, sodium nitrite; 9-AA, 9-aminoacridine; 2-AAF, 2-acetylaminofluorene; B[a]P, benzo[a]pyrene; 2-AA, 2-aminoanthracene

**d** \*, p<0.05; \*\*, p<0.01 Statistically significant) differences between treatment and vehicle control

group using within levels pooled variance and a one-sided t-test with log10 transformed revertants/plate. No statistically significant (p<0.05) dose responses observed using regression analysis and log10 transformed dose levels and revertants/plate.

**Experiment 2**

**Plate Incorporation**

**Three replicate plates (treatment and vehicle control)**

| Substance | Amt/Plate (µg) | Revertants/Plated  Mean ± Std. Dev. | | | |
| --- | --- | --- | --- | --- | --- |
|  |  | TA98 | TA100 | TA1535 | TA1537 |
| ***Without S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 18.6 ± 6.0 | 110.9 ± 21.0 | 12.3 ± 5 .0 | 7.0 ± 1.2 |
| Test Mat. | 5 | 22.0 ± 2.0 | 115.7 ± 6.8 | 12.0 ± 4.4 | 7.3 ± 1.2 |
|  | 15 | 22.0 ± 5.2 | 124.3 ± 11.0 | 12.0 ± 4.0 | 7.7 ± 1.2 |
|  | 50 | 22.0 ± 4.6 | 115.0 ± 10.5 | 11.3 ± 4.9 | 7.0 ± 1.7 |
|  | 150 | 22.0 ± 5.0 | 125.3 ± 15.8 | 11.7 ± 4.9 | 7.3 ± 0.6 |
|  | 500 | 20.3 ± 1.5Tb | T | T | T |
| Pos. Cont.c | level 1 | 30 | 136 | 149 | 26 |
|  | level 2 | 71 | 1030 | 536 | 68 |
|  | level 3 | 272 | 1890 | 1890 | 321 |
| ***With S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 27.4 ± 4.9 | 134.6 ± 16.6 | 12.4 ± 2.8 | 9.2 ± 2.9 |
| Test Mat. | 15 | 32.0 ± 4.4 | 123.0 ± 7.0 | 13.3 ± 4.7 | 6.3 ± 0.6 |
|  | 50 | 34.0 ± 5.2\* | 134.0 ± 24.2 | 12.7 ± 3.2 | 5.7 ± 0.6 |
|  | 150 | 32.3 ± 5.9 | 109.3 ± 22.2 | 9.3 ± 2.5 | 6.0 ± 2.8 |
|  | 500 | 35.0 ± 2.0\*\* | 101.0 ± 26.0T | 6.7 ± 1.2T | 7.0 ± 2.6T |
|  | 1500 | 24.7 2.1 | T | T | T |
| Pos. Cont.c | level 1 | 85 | 176 | 93 | 22 |
|  | level 2 | 234 | 447 | 431 | 80 |
|  | level 3 | 761 | 1470 | 708 | T |
| Pos. Cont.  –S9c |  | 4-NQNO  (0.02, 0.1, 0.2) | 4-NQNO (0.02, 0.1, 0.2) | NaNO2 (500, 2500, 5000) | 9-AA (10, 50, 100) |
| Pos. Cont. +S9c |  | 2-AAF (3, 15, 30) | B[a]P (0.2, 1, 2) | 2-AA (1, 5, 10) | 2-AA (1, 5, 10) |

a Vehicle control: water

b T, toxicity

c Pos. Cont.--Positive Control with positive controls and amounts per plate indicated at the

bottom of the table: 4-NQNO, 4-nitroquinoline-N-oxide; NaNO2, sodium nitrite; 9-AA, 9-aminoacridine; 2-AAF, 2-acetylaminofluorene; B[a]P, benzo[a]pyrene; 2-AA, 2-aminoanthracene

**d** \*, p<0.05; \*\*, p<0.01 Statistically significant) differences between treatment and vehicle control

group using within levels pooled variance and a one-sided t-test with log10 transformed revertants/plate. No statistically significant (p<0.05) dose responses observed using regression analysis and log10 transformed dose levels and revertants/plate.

**Experiment 3**

**Plate Incorporation**

**Three replicate plates (treatment and vehicle control)**

|  |  |  |
| --- | --- | --- |
| Substance | Amt/Plate (µg) | Revertants/Plated Mean ± Std. Dev. |
|  |  | TA98 |
| ***With S9*** |  |  |
| Veh. Cont.a |  | 31.3 ± 12.7 |
| Test Mat. | 250 | 19.0 ± 4.4 |
|  | 500 | 20.7 ± 5.8 |
|  | 1000 | 16.3 ± 9.0T |
| Pos. Cont.c | level 1 | 63 |
|  | level 2 | 342 |
|  | level 3 | 991 |
|  |  |  |
| Pos. Cont. –S9c |  |  |
| Pos. Cont. +S9c |  | 2-AAF (3, 15, 30) |

a Vehicle control: water

b T, toxicity

c Pos. Cont.--Positive Control with positive controls and amounts per plate indicated at the

bottom of the table: 4-NQNO, 4-nitroquinoline-N-oxide; NaNO2, sodium nitrite; 9-AA, 9-aminoacridine; 2-AAF, 2-acetylaminofluorene; B[a]P, benzo[a]pyrene; 2-AA, 2-aminoanthracene

**d \***Statistically significant (\*, p<0.05; \*\*, p<0.01) differences between treatment and vehicle control

group using within levels pooled variance and a one-sided t-test with log10 transformed revertants/plate. No statistically significant (p<0.05) dose responses observed using regression analysis and log10 transformed dose levels and revertants/plate.

**Report Reference:** Kier et al. (1992b)

**Author/Study Director:** L.D. Kier (study director)

S.D. Stegeman

J.G. Costello

S. Schermes

**Year:** 1992

**Title:** Ames/Salmonella Mutagenicity Assay of RODEO®

**Assay:** Bacterial Reverse Mutation

**Report Identification Number:** MSL -11730

**Report Guideline Statement:** None

**Test Material:** Rodeo® Herbicide Formulation) (40% glyphosate acid equivalent)

**Report Conclusion:** The test sample, RODEO, was concluded not to be mutagenic towards any of the *Salmonella typhimurium* test strains used (TA98, TA1 00, TAI 535, and TA1 537) in the presence or absence of an Aroclor ‘1254-induced rat liver homogenate metabolic activation system (S-9 Mix).

**Control Materials:**

**Negative (vehicle):** Distilled water

**Positive:** See summary tables

**Metabolic Activation:** Aroclor-1254 induced rat liver homogenate 10% in S9 Mix

**Summary data tables**

**Experiment 1**

**Plate Incorporation**

**Three replicate plates (treatment and vehicle control)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Substance | Amt/Plate (µg) | Revertants/Platec  Mean ± Std. Dev. | | | |
|  |  | TA98 | TA100 | TA1535 | TA1537 |
| ***Without S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 26.9 ± 9.6 | 138.7 ± 12.2 | 16.8 ± 5.6 | 8.8 ± 3.8 |
| Test Mat. | 50 | 21.0 ± 6.2 | 111.0 ± 17.1 | 11.7 ± 3.2 | 5.7 ± 2.5 |
|  | 150 | 25.7 ± 1.5 | 116.3 ± 12.3 | 11.0 ± 3.0 | 4.3 ± 0.6 |
|  | 500 | 29.0 ± 8.5 | 124.0 ± 24.0 | 11.3 ± 0.6 | 3.7 ± 0.6 |
|  | 1500 | 24.3 ± 6.0 | 99.7 ± 18.9 | 12.3 ± 2.1 | 5.7 ± 2.5 |
|  | 5000 | 21.7 ± 3.2 | 73.3 ± 12.3 | 8.0 ± 2.6 | 3.3 ± 1.2 |
| Pos. Cont.b | level 1 | 44 | 276 | 114 | 19 |
|  | level 2 | 99 | 1190 | 428 | 181 |
|  | level 3 | 191 | 1500 | 1940 | 1970 |
| ***With S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 36.9 ± 4.3 | 155.8 ± 9.9 | 13.7 ± 4.4 | 9.4 ± 3.6 |
| Test Mat. | 50 | 34.3 ± 5.8 | 150.3 ± 20.8 | 10.3 ± 4.2 | 8.7 ± 0.6 |
|  | 150 | 40.0 ± 5.6 | 149.0 ± 3.6 | 11.7 ± 2.5 | 7.3 ± 4.0 |
|  | 500 | 37.3 ± 9.9 | 144.7 ± 17.8 | 12.7 ± 2.9 | 7.0 ± 0.0 |
|  | 1500 | 27.0 ± 7.8 | 151.3 ± 16.2 | 9.3 ± 1.2 | 9.0 ± 1.0 |
|  | 5000 | 21.0 ± 7.9 | 136.7 ± 14.3 | 7.7 ± 0.6 | 7.0 ± 1.0 |
| Pos. Cont.b | level 1 | 102 | 202 | 55 | 24 |
|  | level 2 | 316 | 1930 | 302 | 56 |
|  | level 3 | 726 | 2200 | 1060 | 201 |
| **Pos. Cont. –S9b** |  | 4-NQNO  (0.02, 0.1, 0.2) | 4-NQNO (0.02, 0.1, 0.2) | NaNO2 (500, 2500, 5000) | 9-AA (10, 50, 100) |
| Pos. Cont. +S9b |  | 2-AAF (3, 15, 30) | B[a]P (0.2, 1, 2) | 2-AA (1, 5, 10) | 2-AA(1, 5, 10) |

a Vehicle control: water

b Pos. Cont.--Positive Control with positive controls and amounts per plate in µg indicated at the

bottom of the table: 4-NQNO, 4-nitroquinoline-N-oxide; NaNO2, sodium nitrite; 9-AA, 9-aminoacridine; 2-AAF, 2-acetylaminofluorene; B[a]P, benzo[a]pyrene; 2-AA, 2-aminoanthracene

**c** \*, p<0.05; \*\*, p<0.01 Statistically significant) differences between treatment and vehicle control

group using within levels pooled variance and a one-sided t-test with log10 transformed revertants/plate. No statistically significant (p<0.05) dose responses observed using regression analysis and log10 transformed dose levels and revertants/plate.

**Experiment 2**

**Plate Incorporation**

**Three replicate plates (treatment and vehicle control)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Substance | Amt/Plate (µg) | Revertants/Platec  Mean ± Std. Dev. | | | |
|  |  | TA98 | TA100 | TA1535 | TA1537 |
| ***Without S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 18.8 ± 6.8 | 110.9 ± 21.8 | 12.3 ± 5.0 | 7.0 ± 1.2 |
| Test Mat. | 50 | 26.7 ± 2.5\* | 123.0 ± 9.5 | 14.3 ± 4.0 | 7.3 ± 0.6 |
|  | 150 | 20.7 ± 8.6 | 110.7 ± 12.1 | 11.3 ± 2.5 | 7.0 ± 2.0 |
|  | 500 | 22.0 ± 5.3 | 121.3 ± 26.8 | 13.3 ± 1.2 | 7.0 ± 1.0 |
|  | 1500 | 21.0 ± 2.6 | 112.7 ± 15.6 | 13.7 ± 7.4 | 6.0 ± 3.0 |
|  | 5000 | 14.3 ± 3.8 | 93.0 ± 10.5 | 12.7 ± 1.5 | 5.3 ± 2.5 |
| Pos. Cont.b | level 1 | 30 | 136 | 149 | 26 |
|  | level 2 | 71 | 1030 | 436 | 68 |
|  | level 3 | 272 | 1890 | 1010 | 321 |
| ***With S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 27.7 ± 4.9 | 134.6 ± 16.6 | 12.4 ± 2.8 | 9.2 ± 2.9 |
| Test Mat. | 50 | 28.3 ± 3.1 | 147.0 ± 18.2 | 12.7 ± 1.3 | 7.3 ± 2.1 |
|  | 150 | 32.7 ± 5.8 | 147.7 ± 21.5 | 11.7 ± 5.5 | 5.3 ± 1.5 |
|  | 500 | 30.3 ± 7.5 | 137.0 ± 20.5 | 11.0 ± 2.6 | 7.3 ± 2.3 |
|  | 1500 | 29.7 ± 3.5 | 135.3 ± 11.0 | 11.7 ± 4.7 | 7.7 ± 3.2 |
|  | 5000 | 31.0 ± 2.0 | 129.0 ± 11.4 | 11.7 ± 2.5 | 5.3 ± 1.5 |
| Pos. Cont.b | level 1 | 85 | 176 | 93 | 22 |
|  | level 2 | 234 | 447 | 431 | 80 |
|  | level 3 | 761 | 1470 | 708 | T |
| **Pos. Cont. –S9b** |  | 4-NQNO  (0.02, 0.1, 0.2) | 4-NQNO (0.02, 0.1, 0.2) | NaNO2 (500, 2500, 5000) | 9-AA (10, 50, 100) |
| Pos. Cont. +S9b |  | 2-AAF (3, 15, 30) | B[a]P (0.2, 1, 2) | 2-AA (1, 5, 10) | 2-AA (1, 5, 10) |

a Vehicle control: water

b Pos. Cont.--Positive Control with positive controls and amounts per plate in µg indicated at the

bottom of the table: 4-NQNO, 4-nitroquinoline-N-oxide; NaNO2, sodium nitrite; 9-AA, 9-aminoacridine; 2-AAF, 2-acetylaminofluorene; B[a]P, benzo[a]pyrene; 2-AA, 2-aminoanthracene

**c** \*, p<0.05; \*\*, p<0.01 Statistically significant) differences between treatment and vehicle control

group using within levels pooled variance and a one-sided t-test with log10 transformed revertants/plate. No statistically significant (p<0.05) dose responses observed using regression analysis and log10 transformed dose levels and revertants/plate.

**Report Reference:** Kier et al. (1992c)

**Author/Study Director:** L.D. Kier (study director)

S.D. Stegeman

J.G. Costello

S. Schermes

**Year:** 1992

**Title:** Ames/Salmonella Mutagenicity Assay of []

Direct® Herbicide Formation)

**Assay:** Bacterial Reverse Mutation

**Report Identification Number:** MSL -11731

**Report Guideline Statement:** None

**Test Material:** Direct® Herbicide Formulation (72% glyphosate acid equivalent)

**Report Conclusion:** The test sample, Direct® Herbicide Formulation, was concluded not to be mutagenic towards any of the *Salmonella typhimurium* test strains used (TA98, TAI 00, TA1 535, and TA1 537) in the presence or absence of an Aroclor 1254-induced rat liver homogenate metabolic activation system (S-9 Mix).

**Control Materials:**

**Negative (vehicle):** Distilled water

**Positive:** See summary tables

**Metabolic Activation:** Aroclor-1254 induced rat liver homogenate 10% in S9 Mix

**Summary data tables**

**Experiment 1**

**Plate Incorporation**

**Three replicate plates (treatment and vehicle control)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Substance | Amt/Plate (µg) | Revertants/Plated  Mean ± Std. Dev. | | | |
|  |  | TA98 | TA100 | TA1535 | TA1537 |
| ***Without S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 26.9 ± 9.6 | 138.7 ± 12.2 | 16.8 ± 5.6 | 8.8 ± 3.8 |
| Test Mat. | 5 | 21.3 ± 6.0 | 127.3 ± 21.8 | 11.7 ± 2.5 | 8.0 ± 1.0 |
|  | 15 | 28.3 ± 3.1 | 120.3 ± 4.9 | 10.7 ± 3.6 | 4.7 ± 1.2 |
|  | 50 | 26.0 ± 10.5 | 120.3 ± 15.2 | 14.7 ± 3.8 | 5.0 ± 1.0 |
|  | 150 | 32.3 ± 10.3 | 90.7 ± 16.0 | 11.7 ± 3.5 | 7.0 ± 2.6 |
|  | 500 | T | T | T | 6.7 ± 1.5T |
| Pos. Cont.c | level 1 | 44 | 276 | 114 | 19 |
|  | level 2 | 99 | 1190 | 428 | 181 |
|  | level 3 | 191 | 1500 | 1940 | 1970 |
| ***With S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 36.9 ± 4.3 | 155.8 ± 9.9 | 13.7 ± 4.4 | 9.4 ± 3.6 |
| Test Mat. | 15 | 28.0 ± 9.5 | 142.7 ± 15.4 | 10.7 ± 2.1 | 6.3 ± 1.2 |
|  | 50 | 27.7 ± 6.7 | 153.3 ± 37.1 | 8.3 ± 2.5 | 8.7 ± 3.5 |
|  | 150 | 33.7 ± 11.0 | 111.0 ± 7.0 | 9.0 ± 2.0 | 8.3 ± 2.5 |
|  | 500 | 24.0 ± 8.7 | 89.3 ± 13.7T | 7.0 ± 1.0T | 7.3 ± 0.6T |
|  | 1500 | 28.0 ± 0.0T | T | T | 6.3 ± 2.5T |
| Pos. Cont.c | level 1 | 102 | 202 | 55 | 24 |
|  | level 2 | 316 | 1930 | 302 | 56 |
|  | level 3 | 726 | 2200 | 1060 | 201 |
| **Pos. Cont. –S9c** |  | 4-NQNO (0.02, 0.1, 0.2) | 4-NQNO (0.02, 0.1, 0.2) | NaNO2 (500, 2500, 5000) | 9-AA (10, 50, 100) |
| Pos. Cont. +S9c |  | 2-AAF (3, 15, 30) | B[a]P (0.2, 1, 2) | 2-AA (1, 5, 10) | 2-AA(1, 5, 10) |

a Vehicle control: water

b T, toxic

c Pos. Cont.--Positive Control with positive controls and amounts per plate in µg indicated at the

bottom of the table: 4-NQNO, 4-nitroquinoline-N-oxide; NaNO2, sodium nitrite; 9-AA, 9-aminoacridine; 2-AAF, 2-acetylaminofluorene; B[a]P, benzo[a]pyrene; 2-AA, 2-aminoanthracene

**d** \*, p<0.05; \*\*, p<0.01 Statistically significant) differences between treatment and vehicle control

group using within levels pooled variance and a one-sided t-test with log10 transformed revertants/plate. No statistically significant (p<0.05) dose responses observed using regression analysis and log10 transformed dose levels and revertants/plate.

**Experiment 2**

**Plate Incorporation**

**Three replicate plates (treatment and vehicle control)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Substance | Amt/Plate (µg) | Revertants/Plated  Mean ± Std. Dev. | | | |
|  |  | TA98 | TA100 | TA1535 | TA1537 |
| ***Without S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 18.8 ± 6.8 | 110.9 ± 21.8 | 12.3 ± 5.0 | 7.0 ± 1.2 |
| Test Mat. | 5 | 26.3 ± 8.5 | 122.7 ± 10.0 | 7.7 ± 0.6 | 8.3 ± 2.1 |
|  | 15 | 20.7 ± 9.5 | 125.0 ± 26.9 | 7.0 ± 1.4 | 6.3 ± 1.5 |
|  | 50 | 21.0 ± 2.6 | 122.3 ± 9.3 | 10.7 ± 2.5 | 8.0 ± 2.6 |
|  | 150 | 21.7 ± 4.6 | 91.0 ± 4.4 | 10.3 ± 1.3 | 6.3 ± 1.5 |
|  | 500 | T | T | T | 6.7 ± 1.5T |
| Pos. Cont.c | level 1 | 30 | 136 | 149 | 26 |
|  | level 2 | 71 | 1030 | 436 | 68 |
|  | level 3 | 272 | 1890 | 1010 | 321 |
| ***With S9*** |  |  |  |  |  |
| Veh. Cont.a |  | 27.4 ± 4.9 | 134.6 ± 16.6 | 12.4 ± 2.8 | 9.2 ± 2.9 |
| Test Mat. | 15 | 28.7 ± 2.3 | 144.3 ± 21.5 | 9.3 ± 2.5 | 8.7 ± 2.1 |
|  | 50 | 30.0 ± 3.6 | 143.3 ± 24.0 | 9.3 ± 2.3 | 13.0 ± 5.7 |
|  | 150 | 32.0 ± 4.4 | 124.0 ± 5.0 | 10.3 ± 2.1 | 8.3 ± 2.1 |
|  | 500 | 26.3 ± 4.0 | 85.0 ± 41.9T | 10.0 ± 2.8T | 7.7 ± 0.6T |
|  | 1500 | 24.0 ± 8.5T | T | T | T |
| Pos. Cont.c | level 1 | 85 | 176 | 93 | 22 |
|  | level 2 | 234 | 447 | 431 | 80 |
|  | level 3 | 761 | 1470 | 708 | T |
| **Pos. Cont. –S9c** |  | 4-NQNO (0.02, 0.1, 0.2) | 4-NQNO (0.02, 0.1, 0.2) | NaNO2 (500, 2500, 5000) | 9-AA (10, 50, 100) |
| Pos. Cont. +S9c |  | 2-AAF (3, 15, 30) | B[a]P (0.2, 1, 2) | 2-AA (1, 5, 10) | 2-AA(1, 5, 10) |

a Vehicle control: water

b T, toxic

c Pos. Cont.--Positive Control with positive controls and amounts per plate in µg indicated at the

bottom of the table: 4-NQNO, 4-nitroquinoline-N-oxide; NaNO2, sodium nitrite; 9-AA, 9-aminoacridine; 2-AAF, 2-acetylaminofluorene; B[a]P, benzo[a]pyrene; 2-AA, 2-aminoanthracene

**d** \*, p<0.05; \*\*, p<0.01 Statistically significant) differences between treatment and vehicle control

group using within levels pooled variance and a one-sided t-test with log10 transformed revertants/plate. No statistically significant (p<0.05) dose responses observed using regression analysis and log10 transformed dose levels and revertants/plate.

**Table 2. *In Vitro* Mammalian Cell Assays**

**Report Reference:** van de Waart (1995)

**Author/Study Director:** E.J. van de Waart

**Year:** 1995

**Title:** Evaluation of the Ability of Glyfosaat to Induce Chromosomal Aberrations in Cultured Peripheral Human Lymphocytes

**Assay:** *In Vitro* Mammalian Cell Chromosome Aberration Assay

**Report Identification Number:** Project 141918

**Report Guideline Statement:** OECD 473 adopted May 26, 1983

**Test Material:** GLYFOSAAT **(**Glyphosate) (96%)

**Report Conclusion:** It is concluded that GLYFOSAAT is not clastogenic in human lymphocytes under the experimental conditions described in this report.

**Control Materials**

**Negative (vehicle):** dimethyl sulfoxide

**Positive :** mitomycin C and cyclophosphamide

**Test system:** Blood samples were taken from healthy adult male volunteers by venapuncture. Donor ages and average generation times were 38 (15. 0 hours), 28 (15.0 hours) and 28 (14.9 hours) for the pilot study and experiments 1 and 2, respectively. Blood samples were stored between 4°and 25°C. Within 4 hours after withdrawal lymphocyte

**Treatment/Harvest**: Lymphocyte cultures were established by addition of 0.4 mL of blood to 5 mL of F10 culture medium and 0.1 mL of 9 mg/mL of phytohemagglutinin. After culture for 48 hours test substances were administered to duplicate cultures. Cultures treated in the presence of S9 Mix were treated for 3 hours and then treatment medium was replaced with fresh medium and incubation continued for 20-22 hours or 44-46 hours. Cultures treated in the absence of S9 Mix were treated for 24 or 48 hours. During the last 3 hours of culture cell division was arrested with 0.5 µg/mL colchicine.

**Metabolic Activation:** S9 was prepared from male Wistar rats treated by i.p. injection with 500 mg/kg Aroclor 1254. Animals were sacrificed 5 days after treatment and a 9000xg (S9) supernatant was prepared from livers. S9 Mix contained 50% S9 and 0.2 mL of S9 Mix was added to 5.3 mL of culture medium for metabolic activation treatment.

**Main Study Toxicity Results:** Significant decreases in mitotic index were observed at maximum dose levels tested except for 48 hours with S9.

**Summary data tables:**

Expt 1. Summary table for 24 hour sampling time without S9 mix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Treatment Level | Number of Cells with Aberrations (-gaps)a | | | % Control Metaphases/100 Cells |
|  |  | Culture A | Culture B | Total |
| Solvent Control DMS0 | 0.9% | 2 | 1 | 3 | 100 |
| Glyphosate | 33 µg/mL | 1 | 1 | 2 | 96 |
| Glyphosate | 100 µg/mL | 1 | 0 | 1 | 78 |
| Glyphosate | 237 µg/mL | 2 | 1 | 3 | 47 |
| Mitomycin C | 0.2 µg/mL | 27 | 23 | 50\*\*\* | 44 |

a Results are for number of aberrant cells per 100 cells scored for each duplicate culture excluding gaps.

\*\*\* Statistically different from control, p <0.001 by chi-square test

Expt 1. Summary table for 48 hour sampling time without S9 mix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Treatment Level | Number of Cells with Aberrations (-gaps)a | | | % Control Metaphases/100 Cells |
|  |  | Culture A | Culture B | Total |
| Solvent Control DMS0 | 0.9% | 1 | 0 | 1 | 100 |
| Glyphosate | 237 µg/mL | 0 | 0 | 0 | 65 |
| Mitomycin C | 0.2 µg/mL | 32 | 35 | 67\*\*\* | 83 |

a Results are for number of aberrant cells per 100 cells scored for each duplicate culture excluding gaps.

\*\*\* Statistically different from control, p <0.001 by chi-square test

Expt 1. Summary table for 24 hour sampling time with S9 mix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Treatment Level | Number of Cells with Aberrations (-gaps)a | | | % Control Metaphases/100 Cells |
|  |  | Culture A | Culture B | Total |
| Solvent Control DMS0 | 0.9% | 1 | 3 | 4 | 100 |
| Glyphosate | 237 µg/mL | 0 | 0 | 0 | 101 |
| Glyphosate | 333 µg/mL | 1 | 1 | 2 | 89 |
| Glyphosate | 562 µg/mLb | 1 | 3 | 4 | 55 |
| Cyclophosphamide | 15 µg/mL | 37 | 16 | 53\*\*\* | 33 |

a Results are for number of aberrant cells per 100 cells scored for each duplicate culture excluding gaps.

b Precipitate observed

\*\*\* Statistically different from control, p <0.001 by chi-square test

Expt 1. Summary table for 48 hour sampling time with S9 mix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Treatment Level | Number of Cells with Aberrations (-gaps)a | | | % Control Metaphases/100 Cells |
|  |  | Culture A | Culture B | Total |
| Solvent Control DMS0 | 0.9% | 1 | 0 | 1 | 100 |
| Glyphosate | 562 µg/mLb | 0 | 0 | 0 | 121 |

a Results are for number of aberrant cells per 100 cells scored for each duplicate culture excluding gaps.

\*\*\* Statistically different from control, p <0.001 by chi-square test

Expt 2. Summary table for 24 hour sampling time without S9 mix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Treatment Level | Number of Cells with Aberrations (-gaps)a | | | % Control Metaphases/100 Cells |
|  |  | Culture A | Culture B | Total |
| Solvent Control DMS0 | 0.9% | 0 | 0 | 0 | 100 |
| Glyphosate | 33 µg/mL | 0 | 0 | 0 | 84 |
| Glyphosate | 237 µg/mL | 3 | 0 | 3 | 61 |
| Glyphosate | 333 µg/mL | 1 | 1 | 2 | 34 |
| Mitomycin C | 0.2 µg/mL | 25 | 26 | 51\*\*\* | 43 |

a Results are for number of aberrant cells per 100 cells scored for each duplicate culture excluding gaps.

\*\*\* Statistically different from control, p <0.001 by chi-square test

Expt 2. Summary table for 24 hour sampling time with S9 mix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Treatment Level | Number of Cells with Aberrations (-gaps)a | | | % Control Metaphases/100 Cells |
|  |  | Culture A | Culture B | Total |
| Solvent Control DMS0 | 0.9% | 2 | 1 | 3 | 100 |
| Glyphosate | 333 µg/mL | 2 | 3 | 5 | 93 |
| Glyphosate | 422 µg/mL | 2 | 0 | 2 | 78 |
| Glyphosate | 562 µg/mLb | 0 | 1 | 1 | 85 |
| Mitomycin C | 0.2 µg/mL | 26 | 27 | 53\*\*\* | 36 |

a Results are for number of aberrant cells per 100 cells scored for each duplicate culture excluding gaps.

b Precipitate observed

\*\*\* Statistically different from control, p <0.001 by chi-square test

**Report Reference:** Bakke (1991)

**Author/Study Director:** J. P. Bakke

**Year:** 1991

**Title:** 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

**Assay:** *In Vitro* Primary Hepatocyte UDS

**Report Identification Number:** 2495-V01-91

**Report Guideline Statement:** U.S. EPA FIFRA Guidelines, Subdivision F

OECD Guideline 473, adopted 26 may 1983

**Test Material:** AMPA (94.38%)

**Report Conclusion:** UDS levels did not increase above those of the negative and solvent controls in either experiment after treatment of the hepatocytes with AMPA. Therefore, on the basis of our criteria for a positive response, AMPA is negative in the *in vitro* rat hepatocyte DNA repair assay.

**Control Materials**

**Negative (vehicle):** culture medium

**Positive :** 2-acetylaminofluorene

**Test System:** Primary hepatocytes isolated from male Fischer-344 rats

**Treatment:** Approximately 19 hours

**Metabolic Activation:** No exogenous metabolic activation system

**Main Study Toxicity Results:** Cytotoxicity was determined by microscopic observation and was used to determine scoring at treatments just below those exhibiting toxicity.

**Summary data table:**

**Experiments 1 and 2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment | Experiment 1 |  | Experiment 2 |  |
|  | Net Grainsa  Mean ± SE | % Cells  in Repair | Net Grainsa  Mean ± SE | % Cells  in Repair |
| control medium | -15.3 ± 2.7 | 3 | -12.6 ± 2.5 | 2 |
| AMPA (µg/mL |  |  |  |  |
| 5 | -17.3 ± 1.4 | 0 | -11.1 ± 0.9 | 1 |
| 10 | -14.1 ± 2.5 | 6 | -12.7 ± 2.2 | 2 |
| 50 | -13.9 ± 3.1 | 1 | -13.0 ± 1.9 | 1 |
| 100 | -17.8 ± 3.1 | 2 | -12.3 ± 1.5 | 1 |
| 250 | not tested |  | -11.5 ± 1.3 | 2 |
| 500 | -18.6 ± 2.7 | 0 | -11.8 ± 1.1 | 2 |
| 1000 | -13.1 ± 2.2 | 0 | -11.6 ± 1.2 | 2 |
| 2500 | -12.9 ± 1.8 | 1 | -8.8 ± 1.5 | 1 |
| 3800 | not tested |  | toxic |  |
| 5000 | toxic |  | toxic |  |
| 2-AAF (3 (µg/mL) | 9.4 ± 3.7 | 63 | 19.7 3.0 | 85 |

a Net grains in nucleus.

**Table 3. *In Vivo M*ammalian Assays**

**Report Reference:** Kier et al. (1992d)

**Author/Study Director:** L. D. Kier (study director)

L. J. Flowers

M.B. Huffman

**Year:** 1992

**Title:** Mouse Micronucleus Study of

Roundup® Herbicide Formation

**Assay:** Mouse Bone Marrow Erythrocyte Micronucleus

**Report Identification Number:** MSL-11771

**Report Guideline Statement:** None

**Test Material:** Roundup® Herbicide Formulation (31% glyphosate, acid equivalent)

**Report Conclusion Statement:** Based on the observations and findings of this study, it is concluded that Roundup® herbicide formulation is not genotoxic in *vivo* in mouse bone marrow cells under the experimental conditions of the study.

**Control Materials:**

**Negative (vehicle):** 0.9% saline

**Positive:** cyclophosphamide

**Test System:** 8 to 12 week old male and female CD-I mice

**Exposure route:** i.p. injection (10 mL/kg body weight)

**Animals per Treatment Group:** 5 males and 5 females treated and scored for all groups except 18 males and 22 females treated for high dose group and 5 males and 5 females were scored for each time point for high dose group.

**Treatment/Harvest:** Single dose

Cells were harvested at 24, 48, and 72 hours post dosing for test material and vehicle control treated animals and at 24 hours post dosing for positive control treated animals

**Main Study Toxicity Results:** Dose levels for the main study were selected based on toxicity range finding study data. The maximum dose selected for testing in the micronucleus experiment was 555 mg/kg body weight (a dose greater than 80% of the combined calculated LD50 of 643 mg/kg). Other doses selected were approximately 1/2 (280 mg/kg body

weight) and 1/4 (140 mg/kg body weight) of the maximum dose.

In the main micronucleus experiment, ROUNDUP herbicide formulation was toxic to the male and female mice dosed at the 555 mg/kg treatment level as evidenced by clinical signs and death. Three deaths were observed in the high dose level group (2/18 males and 1/22 females). No deaths were observed in other treatment or control groups. Clinical signs of toxicity (listlessness and/or unresponsiveness) were observed in high dose males and females up to 48 hours after dosing. At the 72 hour time point all remaining high dose level male and female mice appeared normal. All animals in the mid and low dose groups appeared normal throughout the experiment. All positive and vehicle control animals also appeared normal throughout the experiment.

Statistically significant decreases in mean body weight were observed for the high dose male group animals sacrificed at the 48, and 72 hour time points. A statistically significant decrease in mean body weight was observed for the male mid (dose group sacrificed at the 72 hour time point.

A statistically significant decrease in the PCE/total erythrocyte ratio was observed for the high dose male group sacrificed at the 48 hour time point.

**Cells Scored:** 1000 polychromatic erythrocytes/animal for micronucleated PCE’s (500 each for two scorers)

1000 erythrocytes/animal for PCE/erythrocytes (500 each for two scorers)

Slides of bone marrow cells were coded prior to distribution and slides were scored without knowledge of the treatment or control group to which the slides belonged.

**Summary Data Table**

**Mouse Micronucleus Study - Mean Data**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment Group | Dose  Amount /kg bwb | Harvest Timec | Sex | Micronucleated PCE’s  per 1000 PCE  Mean ± Std. Dev. | PCE’s/ Total Erythrocyte Ratio  Mean ± Std. Dev.d |
| Negative Control (Vehicle)a | 10 mL | 24 Hours | Female | 0.8 ± 1.1 | 0.48 ± 0.05 |
|  | Male | 1.4 ± 0.5 | 0.43 ± 0.04 |
| Roundup® Formulation | 140 mg | 24 Hours | Female | 1.0 ± 1.4 | 0.52 ± 0.08 |
|  | Male | 0.8 ± 0.8 | 0.49 ± 0.03 |
| Roundup® Formulation | 280 mg | 24 Hours | Female | 0.2 ± 0.4 | 0.52 ± 0.06 |
|  | Male | 2.2 ± 0.8 | 0.50 ± 0.04 |
| Roundup® Formulation | 555 mg | 24 Hours | Female | 1.4 ± 0.9 | 0.51 ± 0.05 |
|  | Male | 1.8 ± 3.0 | 0.40 ± 0.05 |
| Cyclophosphamide | 40 mg | 24 Hours | Female | 25.6 ± 7.8\*\* | 0.51 ± 0.04 |
|  | Male | 29.2 ± 8.4\*\* | 0.49 ± 0.06 |
| Negative Control (Vehicle)a | 10 mL | 48 Hours | Female | 0.8 ± 0.8 | 0.53 ± 0.07 |
|  | Male | 1.2 ± 2.2 | 0.49 ± 0.04 |
| Roundup® Formulation | 140 mg | 48 Hours | Female | 1.0 ± 1.2 | 0.49 ± 0.03 |
|  | Male | 1.6 ± 2.5 | 0.50 ± 0.05 |
| Roundup® Formulation | 280 mg | 48 Hours | Female | 0.6 ± 0.9 | 0.56 ± 0.03 |
|  | Male | 1.0 ± 1.2 | 0.48 ± 0.06 |
| Roundup® Formulation | 555 mg | 48 Hours | Female | 0.8 ± 1.3 | 0.49 ± 0.08 |
|  | Male | 1.6 ±1.5 | 0.37 ± 0.02\*\* |
| Negative Control (Vehicle)a | 10 mL | 72 Hours | Female | 1.8 ± 1.3 | 0.52 ± 0.10 |
|  | Male | 2.4 ± 1.1 | 0.54 ± 0.09 |
| Roundup® Formulation | 140 mg | 72 Hours | Female | 1.6 ±0.5 | 0.59 ± 0.07 |
|  | Male | 0.8 ± 0.4 | 0.61 ± 0.11 |
| Roundup® Formulation | 280 mg | 72 Hours | Female | 1.0 ± 1.0 | 0.61 ± 0.10 |
|  | Male | 1.4 ± 1.7 | 0.59 ± 0.11 |
| Roundup® Formulation | 555 mg | 72 Hours | Female | 0.2 ± 0.4 | 0,56 ± 0.17 |
|  | Male | 2.0 ± 0.7 | 0.56 ± 0.07 |

a 0.9% saline

b Single dose administered by i.p. injection

c Hours after dose administration

d Note that common negative and positive controls were used for Kier et al. 1992d, 1992e and 1992f.

\*p < 0.05; \*\*p < 0.01 by one-sided Dunnett’s test. Square root transformed data used for statistical analysis of micronucleated PCE

**Report Reference:** Kier et al. (1992e)

**Author/Study Director:** L. D. Kier (study director)

L. J. Flowers

M.B. Huffman

**Year:** 1992

**Title:** Mouse Micronucleus Study of

Rodeo® Herbicide Formation

**Assay:** Mouse Bone Marrow Erythrocyte Micronucleus

**Report Identification Number:** MSL-11772

**Report Guideline Statement:** None

**Test Material:** Rodeo® Herbicide Formulation (40% glyphosate, acid equivalent)

**Report Conclusion Statement:** Based on the observations and findings of this study, it is concluded that Rodeo® herbicide formulation is not genotoxic in *vivo* in mouse bone marrow cells under the experimental conditions of the study.

**Control Materials:**

**Negative (vehicle):** 0.9% saline

**Positive:** cyclophosphamide

**Test System:** eight to twelve week old male and female CD-I mice

**Exposure route:** i.p. injection (10 mL/kg body weight)

**Animals per Treatment Group:** 5 males and 5 females treated and scored for all groups except 18 males and 18 females treated for high dose group and 5 males and 5 females were scored for each time point for high dose group.

**Treatment/Harvest:** Single dose

Cells were harvested at 24, 48, and 72 hours post dosing for test material and vehicle control treated animals and at 24 hours post dosing for positive control treated animals

**Main Study Toxicity Results:** Dose levels for the main study were selected based on toxicity rangefinding study data. The maximum dose selected for testing in the micronucleus experiment was 3400 mg/kg body weight (approximately 80% of the combined calculated LD50 of 4239 mg/kg as determined by the Probit method) and other doses selected were approximately 1/2 (1700 mg/kg body weight) and 1/4 (850 mg/kg body weight) of the maximum dose.

The high dose level was an acceptable maximum dose level as judged by several measures. This dose level was approximately 80% of the LD50 determined in toxicity rangefinder experiments and induced a ‘low incidence of death in high dose level group females (1/18 treated). Clinical signs of toxicity were observed in male and female mice of both the high and mid dose levels and body weight effects were observed in high dose level males at 72 hours after dosing. Additionally, a reduction in the PCE/erythrocyte ratio compared to control values was observed in the high dose level female group sacrificed at 48 hours after dosing suggesting effects on the bone marrow.

**Cells Scored:** 1000 polychromatic erythrocytes/animal for micronucleated PCE’s (500 each for two scorers)

1000 erythrocytes/animal for PCE/erythrocytes (500 each for two scorers)

Slides of bone marrow cells were coded prior to distribution and slides were scored without knowledge of the treatment or control group to which the slides belonged.

**Summary Data Table**

**Mouse Micronucleus Study - Mean Data**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment Group | Dose  Amount /kg bwb | Harvest Timec | Sex | Micronucleated PCE’s  per 1000 PCE  Mean ± Std. Dev. | PCE’s/ Total Erythrocyte Ratio  Mean ± Std. Dev. |
| Negative Control (Vehicle)a | 10 mL | 24 Hours | Female | 0.8 ± 1.1 | 0.48 ± 0.05 |
|  | Male | 1.6 ± 0.9 | 0.43 ± 0.04 |
| Roundup® Formulation | 850 mg | 24 Hours | Female | 1.4 ± 1.7 | 0.52 ± 0.03 |
|  | Male | 0.6 ± 0.5 | 0.49 ± 0.04 |
| Roundup® Formulation | 1700 mg | 24 Hours | Female | 1.6 ± 1.7 | 0.50 ± 0.03 |
|  | Male | 1.2 ± 1.3 | 0.51 ± 0.03 |
| Roundup® Formulation | 3400 mg | 24 Hours | Female | 2.0 ± 1.6 | 0.49 ± 0.03 |
|  | Male | 1.6 ± 1.1 | 0.48 ± 0.08 |
| Cyclophosphamide | 40 mg | 24 Hours | Female | 25.6 ± 7.8\*\* | 0.51 ± 0.04 |
|  | Male | 29.2 ± 8.4\*\* | 0.49 ± 0.06 |
| Negative Control (Vehicle)a | 10 mL | 48 Hours | Female | 0.8 ± 0.8 | 0.53 ± 0.07 |
|  | Male | 1.2 ± 2.2 | 0.49 ± 0.04 |
| Roundup® Formulation | 850 mg | 48 Hours | Female | 2.0 ± 1.0 | 0.52 ± 0.11 |
|  | Male | 0.8 ± 1.3 | 0.49 ± 0.01 |
| Roundup® Formulation | 1700 mg | 48 Hours | Female | 0.8 ± 0.4 | 0.54 ± 0.04 |
|  | Male | 1.4 ± 0.9 | 0.49 ± 0.02 |
| Roundup® Formulation | 3400 mg | 48 Hours | Female | 0.6 ± 0.9 | 0.40 ± 0.05\* |
|  | Male | 1.4 ± 1.1 | 0.44 ± 0.05 |
| Negative Control (Vehicle)a | 10 mL | 72 Hours | Female | 1.8 ± 1.3 | 0.52 ± 0.10 |
|  | Male | 2.4 ± 1.1 | 0.54 ± 0.09 |
| Roundup® Formulation | 850 mg | 72 Hours | Female | 1.4 ± 0.5 | 0.65 ± 0.06 |
|  | Male | 2.6 ± 1.5 | 0.65 ± 0.13 |
| Roundup® Formulation | 1700 mg | 72 Hours | Female | 0.6 ± 0.5 | 0.59 ± 0.09 |
|  | Male | 1.4 ± 1.7 | 0.64 ± 0.15 |
| Roundup® Formulation | 3400 mg | 72 Hours | Female | 1.6 ± 2.1 | 0.66 ± 0.06 |
|  | Male | 1.4 ± 1.1 | 0.57 ± 0.03 |

a 0.9% saline

b Single dose administered by i.p. injection

c Hours after dose administration

\*p < 0.05; \*\*p < 0.01 by one-sided Dunnett’s test. Square root transformed data used for statistical analysis of micronucleated PCE

**Report Reference:** Kier et al. (1992f)

**Author/Study Director:** L. D. Kier (study director)

L. J. Flowers

M.B. Huffman

**Year:** 1992

**Title:** Mouse Micronucleus Study of Direct® Herbicide Formulation

**Assay:** Mouse Bone Marrow Erythrocyte Micronucleus

**Report Identification Number:** MSL-11773

**Report Guideline Statement:** None

**Test Material:** DIRECT® Herbicide Formulation (72% glyphosate acid equivalent)

**Report Conclusion Statement:** Based on the observations and findings of this study, it is concluded that DIRECT herbicide formulation is not genotoxic *in vivo* in mouse bone marrow cells under the experimental conditions of the study.

**Control Materials:**

**Negative (vehicle):** 0.9% saline

**Positive:** cyclophosphamide

**Test System:** eight to twelve week old male and female CD-I mice

**Exposure route:** i.p. injection (10 mL/kg body weight)

**Animals per Treatment Group:** 5 males and 5 female treated and scored for all groups except 18 males and 18 females treated and 5 males and 5 females were scored for each time point for high dose group.

**Treatment/Harvest:** Single dose

Cells were harvested at 24, 48, and 72 hours post dosing for test material and vehicle control treated animals and at 24 hours post dosing for positive control treated animals

**Main Study Toxicity Results:** Dose levels for the main study were selected based on

toxicity range finding study data. The maximum dose selected for testing in the micronucleus experiment was 365 mg/kg body weight (a dose greater than 80% of the combined calculated LD50 1 of 436 mg/kg). Other doses selected were approximately 1/2 (183 mg/kg body

weight) and 1/4 (91 mg/kg body weight) of the maximum dose.

In the micronucleus experiment DIRECT herbicide formulation was toxic to male and female mice in the mid and high dose levels. One death was observed in the high dose level female group (1/18 treated). No deaths were observed in any other treatment or control groups. Clinical signs of listlessness were observed in high dose level male and female mice immediately and 3-5 hours after dosing. Listlessness was also observed in two mid dose level female mice immediately after dosing and in four male mice at 24 hours after dosing.

**Cells Scored:** 1000 polychromatic erythrocytes/animal for micronucleated PCE’s (500 each for two scorers)

1000 erythrocytes/animal for PCE/erythrocytes (500 each for two scorers)

Slides of bone marrow cells were coded prior to distribution and slides were scored without knowledge of the treatment or control group to which the slides belonged.

**Summary Data Table**

**Mouse Micronucleus Study - Mean Data**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment Group | Dose  Amount /kg bwb | Harvest Timec | Sex | Micronucleated PCE’s  per 1000 PCE  Mean ± Std. Dev. | PCE’s/ Total Erythrocyte Ratio  Mean ± Std. Dev. |
| Negative Control (Vehicle)a | 10 mL | 24 Hours | Female | 0.8 ± 1.1 | 0.48 ± 0.05 |
|  | Male | 1.6 ± 0.9 | 0.43 ± 0.04 |
| Direct® Formulation | 91 mg | 24 Hours | Female | 0.6 ± 0.9 | 0.57 ± 0.05 |
|  | Male | 0.6 ± 0.5 | 0.49 ± 0.04 |
| Direct® Formulation | 183 mg | 24 Hours | Female | 1.4 ± 2.1 | 0.51 ± 0.05 |
|  | Male | 2.0 ± 1.6 | 0.49 ± 0.05 |
| Direct® Formulation | 365 mg | 24 Hours | Female | 1.2 ± 0.8 | 0.45 ± 0.09 |
|  | Male | 1.0 ± 1.0 | 0.49 ± 0.06 |
| Cyclophosphamide | 40 mg | 24 Hours | Female | 25.6 ± 7.8\*\* | 0.51 ± 0.04 |
|  | Male | 29.2 ± 8.4”\* | 0.49 ± 0.06 |
| Negative Control (Vehicle)a | 10 mL | 48 Hours | Female | 0.8 ± 0.8 | 0.53 ± 0.07 |
|  | Male | 1.2 ± 2.2 | 0.49 ± 0.04 |
| Direct® Formulation | 91 mg | 48 Hours | Female | 1.0 ± 1.4 | 0.48 ± 0.08 |
|  | Male | 1.2 ± 1.8 | 0.47 ± 0.06 |
| Direct® Formulation | 183 mg | 48 Hours | Female | 1.8 ± 3.0 | 0.53 ± 0.04 |
|  | Male | 1.0 ± 0.7 | 0.51 ± 0.07 |
| Direct® Formulation | 365 mg | 48 Hours | Female | 0.6 ± 0.5 | 0.49 ± 0.08 |
|  | Male | 1.2 ± 0.8 | 0.50 ± 0.08 |
| Negative Control (Vehicle)a | 10 mL | 72 Hours | Female | 1.8 ± 1.3 | 0.52 ± 0.10 |
|  | Male | 2.4 ± 1.1 | 0.54 ± 0.09 |
| Direct® Formulation | 91 mg | 72 Hours | Female | 1.4 ± 1.1 | 0.56 ± 0.06 |
|  | Male | 2.6 ± 1.7 | 0.60 ± 0.04 |
| Direct® Formulation | 183 mg | 72 Hours | Female | 1.2 ± 1.3 | 0.63 ± 0.06 |
|  | Male | 2.0 ± 1.6 | 0.59 ± 0.07 |
| Direct® Formulation | 365 mg | 72 Hours | Female | 1.8 ± 0.8 | 0.60 ± 0.04 |
|  | Male | 0.8 ± 0.4 | 0.65 ± 0.03 |

a 0.9% saline

b Single dose administered by i.p. injection

c Hours after dose administration

\*p ≤ 0.05; \*\*p ≤ 0.01 by one-sided Dunnett’s test. Square root transformed data used for statistical analysis of micronucleated PCE

**Report Reference:** Kier and Stegeman (1993)

**Author/Study Director:** L. D. Kier (study director)

S.D. Stegeman

**Year:** 1993

**Title:** Mouse Micronucleus Study of AMPA

**Assay:** Mouse Bone Marrow Erythrocyte Micronucleus

**Report Identification Number:** MSL-13243

**Report Guideline Statement:** None

**Test Material:** aminomethylphosphonic acid (AMPA) (94.38%)

**Report Conclusion Statement:** The observations and findings of this study indicate that AMPA does not induce micronuclei *in vivo* in mouse bone marrow cells under the experimental conditions of the study.

**Control Materials:**

**Negative (vehicle):** corn oil

**Positive:** cyclophosphamide

**Test System:** seven to ten week old male and female CD-1 mice

**Exposure route:** i.p. injection (10 mL/kg body weight)

**Animals per Treatment Group:** 5 males and 5 females per group and time point

**Treatment/Harvest:** Single dose

Cells were harvested at 24, 48, and 72 hours post dosing for test material and vehicle control treated animals and at 24 hours post dosing for positive control treated animals

**Main Study Toxicity Results:** The selection of the maximum dose for the micronucleus experiment was based on the calculated combined LD50 value of 1357.7 mg/kg and on the observed signs of toxicity in the treated males and females. The maximum dose level, 1000 mg/kg, was approximately 74% of the combined LD50 value and was selected as a single dose that might insure a reasonable chance to achieve observable signs of toxicity but allow survival of the treated animals through the 72 hour time point. Two additional lower doses (100 and 500 mg/kg body weight) were also selected for testing.

In the main micronucleus experiment, toxicity was observed in the mid and high dose level male and female groups. Statistically significant decreases in mean body weight change were observed for the mid (500 mg/kg) and high (1000 mg/kg) dose level male groups sacrificed 48 hours after dosing, and for the mid dose level female group sacrificed 24 and 72 hours after dosing. The mean body weight changes observed for the treated males sampled at 48 hours exhibited a dose-response pattern. An increase in mean weight loss was observed as the treatment level increased with the highest two doses (3-4 fold over concurrent control values) giving statistically significant decreases when compared to vehicle controls. Clinical signs of listlessness were observed at 500 and 1000 mg/kg treatment levels for both sexes; however, the male treated groups had significantly more observations than the female treated groups. No deaths or clinical signs were observed in any of the other AMPA treated groups or control groups (vehicle and positive control). No statistically significant decreases in mean PCE/total erythrocyte ratio were observed for any of the AMPA treated groups or control groups.

**Cells Scored:** 1000 polychromatic erythrocytes/animal for micronucleated PCE’s (500 each for two scorers)

1000 erythrocytes/animal for PCE/erythrocytes (500 each for two scorers)

Slides of bone marrow cells were coded prior to distribution and slides were scored without knowledge of the treatment or control group to which the slides belonged.

**Summary Data Table**

**Mouse Micronucleus Study - Mean Data**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment Group | Dose  Amount /kg bwb | Harvest Timec | Sex | Micronucleated PCE’s  per 1000 PCE  Mean ± Std. Dev. | PCE’s/ Total Erythrocyte Ratio  Mean ± Std. Dev. |
| Negative Control (Vehicle)a | 10 mL | 24 Hours | Female | 1.0 ± 1.4 | 0.40 ± 0.10 |
|  | Male | 0.2 ± 0.4 | 0.40 ± 0.05 |
| AMPA | 100 mg | 24 Hours | Female | 0.8 ± 0.8 | 0.48 ± 0.06 |
|  | Male | 0.2 ± 0.4 | 0.42 ± 0.07 |
| AMPA | 500 mg | 24 Hours | Female | 2.0 ± 2.9 | 0.38 ± 0.12 |
|  | Male | 0.1 ± 0.3 | 0.43 ± 0.05 |
| AMPA | 1000 mg | 24 Hours | Female | 0.8 ± 0.8 | 0.41 ± 0.06 |
|  | Male | 0.8 ± 1.3 | 0.42 ± 0.09 |
| Cyclophosphamide | 40 mg | 24 Hours | Female | 12.0 ± 12.3\* | 0.48 ± 0.05 |
|  | Male | 18.3 ± 10.9\*\* | 0.43 ± 0.06 |
| Negative Control (Vehicle)a | 10 mL | 48 Hours | Female | 0.4 ± 0.9 | 0.50 ± 0.07 |
|  | Male | 0.6 ± 1.3 | 0.48 ± 0.05 |
| AMPA | 100 mg | 48 Hours | Female | 0.2 ± 0.4 | 0.42 ± 0.08 |
|  | Male | 0.0 ± 0.0 | 0.44 ± 0.07 |
| AMPA | 500 mg | 48 Hours | Female | 0.2 ± 0.4 | 0.49 ± 0.02 |
|  | Male | 0.6 ± 0.9 | 0.54 ± 0.09 |
| AMPA | 1000 mg | 48 Hours | Female | 0.0 ± 0.0 | 0.45 ± 0.08 |
|  | Male | 0.2 ± 0.4 | 0.46 ± 0.07 |
| Negative Control (Vehicle)a | 10 mL | 72 Hours | Female | 0.0 ± 0.0 | 0.52 ± 0.04 |
|  | Male | 0.2 ± 0.4 | 0.56± 0.02 |
| AMPA | 100 mg | 72 Hours | Female | 1.6 ± 1.1\* | 0.63 ± 0.09 |
|  | Male | 0.0 ± 0.0 | 0.51 ± 0.02 |
| AMPA | 500 mg | 72 Hours | Female | 0.8 ± 0.8 | 0.62 ± 0.04 |
|  | Male | 0.0 ± 0.0 | 0.50 ± 0.07 |
| AMPA | 1000 mg | 72 Hours | Female | 0.4 ± 0.9 | 0.58 ± 0.06 |
|  | Male | 0.0 ± 0.0 | 0.51 ± 0.03 |

a corn oil

b Single dose administered by i.p. injection

c Hours after dose administration

\*p < 0.05; \*\*p < 0.01 by one-sided Dunnett’s test. Square root transformed data used for statistical analysis of micronucleated PCE.

Historical control data for 72 h time point females: number 45 (9 studies); mean ± s.d.: 1.356 ± 1.569; range: 0.00 - 2.40

**References**

Bakke JP. 1991. Evaluation of the potential of AMPA to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay using the male F344 rat. [Unpublished Report]. Menlo Park (CA): SRI International. Regulatory Study. (Report Identification Number: SR-91-234).

Kier LD, Stegeman S. 1993. Mouse micronucleus study of AMPA. [Unpublished Regulatory Study]. (Report Identification Number: MSL-13243).

Kier LD, Stegeman SD, Costello JG, Shermes S. 1992a. Ames/Salmonella mutagenicity assay of MON 2139 (Roundup® herbicide formulation). [Unpublished Regulatory Study]. (Report Identification Number: MSL-11729).

Kier LD, Stegeman SD, Costello JG, Shermes S. 1992b. Ames/Salmonella mutagenticity assay of RODEO®. [Unpublished Regulatory Study]. (Report Identification Number: MSL-11730).

Kier LD, Stegeman SD, Costello JG, Shermes S. 1992c. Ames/Salmonella mutagenticity assay of MON 14445 Direct® herbicide formation. [Unpublished Regulatory Study]. (Report Identification Number: MSL-11731).

Kier LD, Flowers LJ, Huffman MB. 1992d. Mouse micronucleus study of Roundup® herbicide formation. [Unpublished Regulatory Study]. (Report Identification Number: MSL-11771).

Kier LD, Flowers LJ, Huffman MB. 1992e. Mouse micronucleus study of Rodeo® herbicide formation. [Unpublished Regulatory Study]. (Report Identification Number: MSL-11772).

Kier LD, Flowers LJ, Huffman MB. 1992f. Mouse micronucleus study of Direct® herbicide formulation. [Unpublished Regulatory Study]. (Report Identification Number: MSL-11773).

Shirasu Y, Moriya M, Ohta T. 1980. [Aminomethylphosphonic acid]: microbial mutagenicity study. [Unpublished Report].

van de Waart EJ. 1995. Evaluation of the ability of glyphosate to induce chromosome aberrations in cultured peripheral human lymphocytes. [Unpublished Report]. NOTOX, The Netherlands. (Report Identification Number: Project 141918.

Williams GM, Kroes R, Munro IC. 2000. Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. Regul Toxicol Pharmacol. 31:117‑165.