Monsanto

TOXICOLOGY

MAC/Env. & Public Affairs/EHL
(CO./DIV./DEPT./LOCATION)

Final REPORT
(TYPE OF REPORT)

MAR 7/1992

REPORT NO.: MSL-11773

JOB/PROJECT NO.: EHL 91202/91206, ML-91-436/ML-91-439

DATE: February 25, 1992

(MON 14445)

TITLE: Mouse Micronucleus Study of DIRECT® Herbicide Formulation

AUTHORS:

ABSTRACT:
The potential for DIRECT herbicide formulation to induce chromosomal effects was tested in a mouse bone marrow micronucleus assay. DIRECT herbicide formulation was administered by intraperitoneal injection to groups of male and female CD-1 mice at target doses of 91, 183, and 365 mg/kg body weight for the low, mid and high dose groups, respectively. Negative control groups were treated with vehicle only (0.9% saline, 10 ml/kg body weight) and positive control groups were treated with cyclophosphamide (40 mg/kg body weight). Mouse bone marrow from DIRECT herbicide formulation and vehicle control groups was sampled at 24, 48 and 72 hours after dosing. A single sampling time of 24 hours after dosing was used for the cyclophosphamide positive control group. Slides of bone marrow cells were made from five animals/sex/time point for each group and scored for the occurrence of micronucleated polychromatic erythrocytes (micronucleated PCE) and PCE/erythrocyte ratios.
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REPT. NO.: MSL-11773 AUTHORS:
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The highest DIRECT herbicide formulation dose level, 365 mg/kg body weight, was observed to be toxic to treated male and female mice and was clearly an appropriate maximum dose level for the micronucleus assay. This dose level was more than 80% of the combined LD50 estimated from toxicity rangefinder experiments (436 mg/kg) and induced a low incidence of death in the high dose level female group (1/18 treated females) in the micronucleus assay. Clinical signs of toxicity (listlessness) were observed in high dose level male and female mice and in a few mid dose level male and female mice. Statistically significant body weight effects were observed in high dose level male mice at 48 hours after dosing. No statistically significant decreases in the PCE/total erythrocyte ratios were observed for any of the DIRECT herbicide formulation treated groups.

DIRECT herbicide formulation did not induce increases in the frequency of micronucleated PCEs. No statistically significant increases in micronucleated PCE frequencies compared to control values were observed in any of the dose level groups at any of the time points. Significant increases in mean micronucleated PCE frequencies were observed for the cyclophosphamide treated animals demonstrating the ability of the study conditions to detect micronucleus induction.

The observations and findings of this study indicate that DIRECT herbicide formulation does not exhibit in vivo mammalian genotoxicity in mouse bone marrow cells under the experimental conditions of this study.

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FINAL REPORT

Mouse Micronucleus Study of DIRECT® Herbicide Formulation

Study Numbers: ML-91-436/ML-91-439
EHL Study Numbers: 91202/91206

Authors:

Study Director:

Ph.D, Study Director, Science Fellow — 2/25/92 Date

B.A., Sr. Research Biologist — 2/25/92 Date

B.A., Research Biologist — 2/25/92 Date

Ph. D., Manager — 2/25/92 Date
Analytical, Biochemical and Genetic Toxicology

SUMMARY

The potential for DIRECT herbicide formulation to induce chromosomal effects was tested in a mouse bone marrow micronucleus assay. DIRECT herbicide formulation was administered by intraperitoneal injection to groups of male and female CD-1 mice at target doses of 91, 183, and 365 mg/kg body weight for the low, mid and high dose groups, respectively. Negative control groups were treated with vehicle only (0.9% saline, 10 ml/kg body weight) and positive control groups were treated with cyclophosphamide (40 mg/kg body weight). Mouse bone marrow from DIRECT herbicide formulation and vehicle control groups was sampled at 24, 48 and 72 hours after dosing. A single sampling time of 24 hours after dosing was used for the cyclophosphamide positive control group. Slides of bone marrow cells were made from five animals/sex/time point for each group and scored for the occurrence of micronucleated polychromatic erythrocytes (micronucleated PCE) and PCE/erythrocyte ratios.

The highest DIRECT herbicide formulation dose level, 365 mg/kg body weight, was observed to be toxic to treated male and female mice and was clearly an appropriate maximum dose level for the micronucleus assay. This dose level was more than 80% of the combined LD50 estimated from toxicity rangefinder experiments (436 mg/kg) and induced a low incidence of death in the high dose level female group (1/18 treated females) in the micronucleus assay. Clinical signs of toxicity (listlessness) were observed in high dose level male and female mice and in a few mid dose level male and female mice. Statistically significant body weight effects were observed in high dose level male mice at 48 hours after dosing. No statistically significant decreases in the PCE/total erythrocyte ratios were observed for any of the DIRECT herbicide formulation treated groups.

DIRECT herbicide formulation did not induce increases in the frequency of micronucleated PCEs. No statistically significant increases in micronucleated PCE frequencies compared to control values were observed in any of the dose level groups at any of the time points. Significant increases in mean micronucleated PCE frequencies were observed for the

cyclophosphamide treated animals demonstrating the ability of the study conditions to detect micronucleus induction.

The observations and findings of this study indicate that DIRECT herbicide formulation does not exhibit *in vivo* mammalian genotoxicity in mouse bone marrow cells under the experimental conditions of this study.

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INTRODUCTION

The study was designed to evaluate the potential of the test material, DIRECT herbicide formulation, to induce chromosome effects in an *in vivo* mammalian assay, the mouse bone marrow micronucleus assay.

The *in vivo* micronucleus assay has been found to be sensitive to a variety of chemical agents. The assay has been reviewed by the EPA Gene-Tox program (Heddle et al., 1983 and Mavournin et al., 1990). It is generally accepted that induction of micronucleus formation in the assay is indicative of either clastogenic effects or malsegregation of chromosomes. An advantage of this assay is that it evaluates effects on somatic cells of mice that are treated *in vivo* and thus is relevant to prediction of potential *in vivo* mammalian effects (MacGregor et al., 1987).

This study was conducted at the Monsanto Company Environmental Health Laboratory (645 S. Newstead, St. Louis, MO 63110). This study was sponsored by the Monsanto Agricultural Company. The protocols for studies 91202 (toxicity rangefinding study) and 91206 (micronucleus study) were signed by the study director on November 12, 1991 and November 19, 1991 respectively. Experimental work for study 91202 was initiated on November 13, 1991 and completed on November 20, 1991. Experimental work for study 91206 was initiated on November 19, 1991 and completed on December 26, 1991.

MATERIALS

Test Materials

Identification and composition of the test material sample is given below:

Name:	DIRECT® Herbicide Formulation
Identification:	Lot PSGF-10001
EHL Code:	T910112
Percent Active Ingredient:	72% glyphosate (acid equivalent)
Sample storage:	Sample was stored at room temperature as advised by the sponsor.
Appearance:	Off white granules

Solutions or suspensions of the test material were made using 0.9% saline as the solvent on the day of treatment. The positive control used was commercial grade cyclophosphamide (Sigma Chemical Company, lot 19F-0254).

Animals

The animals used were eight to twelve week old male and female CD-1 mice (Source: Charles River Laboratories Inc., Portage, MI.). Upon receipt, the animals were quarantined for a minimum of seven days. Only animals considered to be normal were released from quarantine and used for testing. Prior to testing, the mice were uniquely identified using ear tags and corresponding cage cards. The animals were housed two per cage prior to dosing and subsequently one per cage after dosing. The animals were housed in stainless steel cages with stainless steel mesh bottoms.

Animals were selected for the different test (or control) groups by a computer-generated randomization scheme. Water (supplied by the public water system of St. Louis, MO) was provided *ad libitum* via an automatic watering system. Purina Certified Laboratory Rodent Chow No. 5002 (Trademark of Purina Mills Inc., St. Louis, Mo.) was used as the diet and was provided ad libitum. This diet has been

determined to be nutritionally acceptable for the maintenance of laboratory rodents and has been certified by the manufacturer not to contain contaminants likely to interfere with the study. The animals were housed in rooms designed to routinely maintain a 12-hour light cycle, a temperature between 64 and 79 °F, and relative humidity between 40 and 70%. There were no excursions in animal room environmental conditions which had any obvious impact on the results of the study.

METHODS AND EXPERIMENTAL DESIGN

Administration of Test Chemical

Animals were treated by a single intraperitoneal injection of 0.9% saline (vehicle control, 10 ml/kg body weight), or DIRECT herbicide formulation in 0.9% saline (10 ml of solution/kg body weight) or cyclophosphamide in 0.9% saline (positive control, 10 ml of solution/kg body weight).

Animal Observations

During the study, all animals were observed for visible toxic effects and mortality immediately after dose administration, 3-5 hours after dosing, and daily thereafter for up to 72 hours after treatment. Animals were weighed at the time of treatment (all experiments) and at the time of sacrifice for bone marrow extraction (main experiment).

Preliminary Experiments for Dose Selection

In the initial rangefinding experiment, two mice of each sex were treated by intraperitoneal injection with the test material at doses of 1000 and 5000 mg/kg body weight. Vehicle control animals were dosed with an appropriate volume of 0.9% saline. Based on the

results of the first experiment doses of 250, 500, 750 and 1000 mg/kg body weight were tested in a subsequent rangefinding experiment. A third experiment was conducted with doses of 310 370 and 430 mg/kg body weight. An additional animal per sex was added to the second rangefinding experiment to evaluate the effect of the test doses on the ability to score for PCE/total erythrocyte ratio. Animals added for evaluating treatment effects on slide quality were sacrificed approximately 24 hours after treatment.

Mouse Micronucleus Experiment :

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 365 mg/kg body weight (a dose greater than 80% of the combined calculated LD50 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. Doses were administered once. Groups of at least fifteen males and fifteen females were used for each dose level. Animals were sacrificed for micronucleus evaluation (five animals/sex/group) at 24, 48 and 72 hours after dosing. Vehicle control groups of fifteen males and females were treated with 0.9% saline only. Concurrent positive control groups of five males and five females were treated with 40 mg/kg cyclophosphamide and sacrificed 24 hours after treatment. Design of the mouse micronucleus experiment is summarized in Table 1.

Table 1

Design of the Mouse Micronucleus Assay

Treatment Group	Number of Mice Treated		Number of Mice to be Sacrificed at the Specified Time Following Treatment					
			24 hours		48 hours		72 hours	
	Male	Female	Male	Female	Male	Female	Male	Female
High Dose *	18	22	5	5	5	5	5	5
Mid Dose	15	15	5	5	5	5	5	5
Low Dose	15	15	5	5	5	5	5	5
Vehicle Control	15	15	5	5	5	5	5	5
Positive Control	5	5	5	5	-	-	-	-

* Additional animals added to the high dose to assure adequate survivors at time of sacrifice for bone marrow extraction.

Extraction of Bone Marrow Cells and Slide Preparation

All animals were sacrificed by cervical dislocation and their femora were removed. Each bone was opened at the end and the bone marrow was flushed with approximately 2 ml of fetal bovine serum into a centrifuge tube. Bone marrow from both femora of each animal were pooled for slide preparation. The suspension was centrifuged to remove the serum. Portions of the remaining cells were placed on clean glass microscope slides and smears were prepared. Two slides were initially prepared for each sample and the remaining cell suspension was stored refrigerated to prepare additional slides if needed. Following preparation of the smears the slides were allowed to air dry overnight. The slides were stained using a HemaTek II slide staining machine and a Wright-Giemsa Stain Pak which includes stain, buffer and rinse solutions.

Scoring of Slides

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. For each animal, two scorers evaluated: a) 500 total erythrocytes for polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) and b) 500 PCEs for micronucleated polychromatic erythrocytes (MN PCEs). PCEs and NCEs were distinguished by different staining properties. Micronuclei were identified as uniform, darkly stained, round or oval shaped bodies found in the cytoplasm of PCEs. Bodies in PCEs which were refractile, improperly shaped or stained, or which were not in the focal plane of the cell, were not scored as micronuclei. PCEs containing more than one micronucleus were scored as a single micronucleated PCE. In a few cases significant discordance in MN PCE frequency were initially observed between two slide scorers (e.g. a difference of 4 or more MN PCE per 500 PCEs scored where one or both MN PCE frequencies were less than 10/500). In these cases slides were rescored to determine if the discordance was reproducible and the rescored values were used for reporting and analysis. The slides to be rescored were evaluated without knowledge of the treatment group to which the slides belonged. Scoring data were used to calculate, for each animal, the ratio of PCEs to total erythrocytes (PCEs plus NCEs) per 1000 erythrocytes and the number of MN PCEs per 1000 PCEs.

<u>Statistical Analysis</u>

LD50 estimates were calculated using the Probit method on toxicity rangefinder data. The individual test animal was used as the individual unit for analysis of micronucleated PCE frequency and PCE/erythrocyte ratio and body weight change. Micronucleated PCE frequencies observed for each animal were transformed as the square root prior to analysis (Snedecor and Cochran, 1967; MacGregor et al., 1987). PCE/total erythrocyte ratios were not transformed. Dunnett's test (one sided) was used for comparison of treatment group and positive control values with vehicle control values (Dunnett, 1955). A critical value of $p \leq 0.05$ was used for statistical significance.

Data Evaluation

To determine whether a statistically significant response is treatment related the following criteria are considered: (a) whether there are dose and time-dependent effects that are consistent with a treatment-induced response and (b) the degree of the response in relation to both concurrent and historical negative and positive control data.

RESULTS

Results of the rangefinding experiments are summarized in Appendix I, Table 1. In the rangefinding experiments DIRECT herbicide formulation was found to be toxic to male and female CD-1 mice at 250 mg/kg body weight and greater as indicated by clinical signs of toxicity and was lethal to male and female CD-1 mice at 430 and 370 mg/kg body weight, respectively, and greater. The combined LD50 was calculated to be 436 mg/kg body weight using the Probit method.

Based on these results, 365 mg/kg (greater than 80% of the combined LD50 value) was selected as a maximum dose that would insure a reasonable probability of observing signs of toxicity but allow survival of the treated animals through the 72 hour time point. Two additional lower doses (91 and 183 mg/kg body weight) were also selected for testing.

Results of the micronucleus experiment are summarized in Appendix I, Tables 2 - 5 with individual animal data in Appendix II, Tables 1-3. 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. Animals in the low dose level group and positive and vehicle control animals appeared normal throughout the experiments. A statistically significant decrease in mean body weight compared to control values was observed in the high dose level males sacrificed at 48 hours. No statistically significant decreases in mean body weight compared to control values were observed in any of the other treatment groups. No statistically significant decreases in mean PCE/total erythrocyte ratios compared to control values were observed in any of the treatment groups.

No statistically significant increases in mean micronucleated PCE frequencies were observed in any of the DIRECT herbicide formulation treated groups when compared to corresponding vehicle control groups. The positive control (cyclophosphamide) yielded the expected positive responses in micronucleus induction indicating the adequacy of the experimental conditions.

DISCUSSION

DIRECT herbicide formulation was tested in a micronucleus assay in male and female CD-1 mice at dose levels of 91, 183 and 365 mg/kg body weight with sacrifice times of 24, 48 and 72 hours. The high dose level was an acceptable maximum dose level as judged by several measures. The high dose level was more than 80% of the estimated LD50 and induced a low incidence of death (1/18) in high dose level females. Clinical signs of toxicity were observed in high dose level male and female mice .

At the dose levels tested, which included an appropriate maximum dose level, no statistically significant increases in the mean micronucleated PCE frequencies were observed in any of the DIRECT herbicide formulation treated animals when compared to vehicle control animals. The positive control (cyclophosphamide) yielded expected positive responses in micronucleus induction indicating the adequacy of the experimental conditions.

CONCLUSION

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.

GENERAL INFORMATION

The protocol, raw data, and final report for this study are located in the archives of the Environmental Health Laboratory.

For testing efficiency, the positive and saline vehicle control animals used in this study were also used for separate studies that were performed concurrently. This procedure in no way adversely affected the results of this assay.

SUPERVISORY PERSONNEL:

[redacted], Ph.D., Manager
Analytical, Biochemical and Genetic Toxicology

[redacted] D.V.M., Dipl. A.C.V.P.
Pathology and Research Studies Director

[redacted], Ph.D.
Director, Environmental Health Laboratory

TRADEMARKS

The following registered trademarks are used in this report:

ITEM	*REGISTERED TRADEMARK OF:*
DIRECT	Monsanto Agricultural Company, St. Louis, MO
RODENT CHOW	Purina Mills, Inc., St. Louis, MO
CD-1 Mice	Charles River Laboratories Inc., Portage, MI.

REFERENCES

Dunnett, C.W (1955). A multiple comparison procedure for comparing several treatments with a control. Jour. Am. Stat. Assoc. 50, 1096-1121.

Heddle, J. A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W. and Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res. 123, 61-118.

MacGregor, J.T., Heddle, J.A., Hite, M., Margolin, B. H., Ramel C., Salamone, M.F., Tice, R.R. and Wild, D. (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. Mutation Res. 189, 103-112.

Mavournin K.H., Blakey D.H., Cimino M.C., Salamone M.F., and Heddle J.A. (1990). The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res. 239, 29-80.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods, 6th edition, 223-226 and 325-327, Iowa State Press, Ames, IO.

S&EH QUALITY ASSURANCE AUDIT STATEMENT

Study Number: 91202/91206
ML-91-436/ML-91-439

Protocol Amendments: None

Study Title: Mouse Micronucleus Study of Direct® Herbicide Formulation

Dates of Inspections and Communication of Findings:

91202	91206
November 15, 1991	November 19, 1991
January 29, 1992	December 06, 12, 1991
February 03, 18, 21, 1992	January 29, 1992
	February 03, 18, 21, 1992

Quality Assurance Review Conducted by:

91202	91206
[redacted]	[redacted]

Results: The Quality Assurance review indicates the final report accurately presents the raw data as developed during the study. There appears to be no significant deviation from applicable GLP regulations that adversely affected study quality or integrity.

[redacted]
Quality Assurance Director

FEBRUARY 25, 1992
Date

Statement of Compliance

To the best of our knowledge, these studies were conducted in general accordance with the U.S. Environmental Protection Agency Good Laboratory Practice (GLP) Standards; the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) GLP Standards; and the OECD GLP Principles, with the following exceptions:

1. Characterization of test and control substances was not conducted according to the standards as part of these studies.

2. Test and control substance concentrations and homogeneity in carrier were not confirmed.

3. The stability of test and control substances, neat and after mixing with carrier were not determined. Mixtures of test substances with carrier were prepared on each day of use.

[redacted] 2/25/92

Study Director Date

[redacted] 2/25/92

Laboratory Management Date

APPENDIX I
Data Summary

APPENDIX I - TABLE 1
SUMMARY OF TOXICITY RANGEFINDER RESULTS FOR DIRECT HERBICIDE FORMULATION

Dose mg/kg	Number Treated		Number of Deaths												
			0-5 Hours		Day 1		Day 2		Day 3		Day 4		Total		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Combined [a]
250	3	3	–	–	–	–	–	–	–	–	–	–	0	0	0/4 [b]
310	2	2	–	–	–	–	–	–	–	–	–	–	0	0	0/4
370	2	2	–	–	–	–	–	–	–	1	–	–	0	1	1/4
430	2	2	–	–	2	1	–	–	–	–	–	–	2	1	3/4
500	3	3	–	–	2	1	–	–	–	–	1	1	3	2	5/6
750	3	3	1	1	2	1	–	–	–	–	–	1	3	3	6/6
1000	5	5	5	3	–	1	–	–	–	–	–	–	5	4	9/10
5000	2	2	2	2	–	–	–	–	–	–	–	–	2	2	4/4

a Number of deaths / total number of animals treated.
b One animal per sex sacrificed for erythrocyte evaluation at 24 hours after dosing.

APPENDIX 1 - TABLE 1 (continued)
SUMMARY OF TOXICITY RANGEFINDING RESULTS FOR DIRECT HERBICIDE FORMULATION

Dose (mg/kg)	Observations - Males
250	One of the treated males appeared normal immediately after dosing, at 3-5 hours after dosing and at 24 hours after dosing at which time this animal was sacrificed for erythrocyte evaluation. The second male appeared listless immediately after dosing and then appeared normal at the 3-5 hour observation and through day 4. The third animal appeared normal immediately after dosing through day 4.
310	The treated males appeared normal immediately after dosing and at the 3-5 hour observation. These animals appeared listless on day 1 and then appeared normal on days 2 through 4.
370	One male appeared normal immediately after dosing and at the 3-5 hour observation. This animal appeared listless on days 1 and 2 then appeared normal on days 3 and 4. The second treated male appeared normal immediately after dosing, then appeared listless at the 3-5 hour observation and on days 1 and 2. This male then appeared normal on days 3 and 4.
430	The two treated males appeared normal immediately after dosing the appeared listless at the 3-5 hour observation. Both animals were found dead on day 1.
500	Two of the treated males appeared listless immediately after dosing and appeared unresponsive at the 3-5 hour observation. These two animals were found dead on day 1. The third treated male appeared listless immediately after dosing, appeared normal at the 3-5 hour observation, appeared listless on days 1 and 2 then appeared unresponsive on day 3. This animal was found dead on day 4.
750	One of the treated males was found dead 3-5 hours after dosing. One of the treated males appeared listless and had rough (raised fur) immediately after dosing. This animal was found dead on day one. The third animal was found dead on day one.
1000	In the initial rangefinding experiment two males were normal immediately after dosing and found dead 3-5 hours after dosing. In the second rangefinding experiment three males were listless with raised fur, immediately after dosing and found dead 3-5 hours after dosing.

APPENDIX 1 - TABLE 1 (continued)
SUMMARY OF TOXICITY RANGEFINDING RESULTS FOR DIRECT HERBICIDE FORMULATION

Dose (mg/kg)	Observations - Males
5000	In the initial rangefinding experiment, the treated males died immediately after dosing.

APPENDIX 1 - TABLE 1 (continued)
SUMMARY OF TOXICITY RANGEFINDING RESULTS FOR DIRECT HERBICIDE FORMULATION

Dose (mg/kg)	Observations - Females
250	One female appeared normal immediately after dosing then appeared listless at the 3-5 hour observation. At 24 hours after dosing this animal appeared normal and was sacrificed for erythrocyte evaluation. The remaining two animals appeared normal throughout the experiment.
310	One female normal through day 1, appeared listless on day 2 then appeared normal through day 4. One animal appeared normal throughout the experiment.
370	One of the treated females appeared listless through day 1, then normal through day 4. One appeared listless though day 1, unresponsive on day 2 and found dead on day 3.
430	One appeared normal immediately after dosing, appeared listless at the 3-5 hour observation and was found dead on day 1. The second appeared normal immediately after dosing, appeared listless for the remainder of the experiment.
500	One treated female appeared unresponsive at the 3-5 hour observation and was found dead on day one. One female appeared listless through day one and then appeared normal. One female appeared normal until day one then appeared listless. This animal was found dead on day 4.
750	One of the females appeared listless immediately after dosing and was found dead at the 3-5 hour observation. One of the treated females appeared listless immediately after dosing. This animal appeared unresponsive at 3-5 hours after treatment and was found dead on day 1. The remaining female appeared listless immediately after dosing and through day 2. On day 3 this animal appeared unresponsive and was found dead on day 4.
1000	In the initial experiment the two treated females appeared listless immediately after dosing and were found dead at the 3-5 hour observation. In the second experiment, one treated female appeared listless with rough fur immediately after dosing and was found dead at the 3-5 hour observation. A second treated female appeared listless with rough fur immediately after dosing and appeared unresponsive at the 3-5 hour observation. This animal was found dead on day 1. The remaining female appeared listless with rough fur immediately after dosing and appeared listless at the 3-5 hour observation. This animal appeared normal for the remainder of the experiment.

APPENDIX 1 - TABLE 1 (continued)
SUMMARY OF TOXICITY RANGEFINDING RESULTS FOR DIRECT HERBICIDE FORMULATION

Dose (mg/kg)	Observations - Females
5000	In the initial rangefinding experiment, the treated females died immediately after dosing.

APPENDIX I - TABLE 2
SUMMARY OF MICRONUCLEUS ASSAY RESULTS FOR DIRECT HERBICIDE FORMULATION
MEAN BODY WEIGHT CHANGE AND ANIMAL OBSERVATIONS

Dose	Sex	Number Treated	Mean Body Weight Change (g) ± Standard Deviation			Deaths
			24 Hours	48 Hours	72 Hours	
Saline Vehicle Control	Male	15	- 0.5 ± 0.6	- 0.4 ± 0.7	0.8 ± 2.0	0
	Female	15	- 1.2 ± 0.3	- 0.8 ± 0.3	- 0.4 ± 0.4	0
DIRECT 91 mg/kg	Male	15	2.6 ± 1.1	3.0 ± 0.5	2.3 ± 0.9	0
	Female	15	- 0.7 ± 0.8	- 0.1 ± 0.4	- 0.4 ± 1.0	0
DIRECT 183 mg/kg	Male	15	1.0 ± 1.8	1.8 ± 0.7	1.5 ± 1.1	0
	Female	15	- 0.7 ± 0.5	- 1.0 ± 0.6	- 0.4 ± 1.1	0
DIRECT 365 mg/kg	Male	18	- 1.8 ± 0.7	- 1.4 ± 0.6 *	- 0.5 ± 1.7	0
	Female	18	- 1.2 ± 0.7	- 0.5 ± 0.5	- 0.3 ± 1.1	1
Cyclo-phosphamide (40 mg/kg)	Male	5	- 1.3 ± 0.6			0
	Female	5	- 0.6 ± 0.4			0

*p ≤ 0.05; ** p ≤ 0.01 by one-sided t-test.

APPENDIX I - TABLE 2 (continued)
SUMMARY OF MICRONUCLEUS ASSAY RESULTS FOR DIRECT HERBICIDE FORMULATION
ANIMAL OBSERVATIONS

Dosing observations - In the high dose group, one male and two females appeared listless immediately after dosing. In the mid dose group, two females appeared listless immediately affter dosing.

3-5 hour observations - In the high dose group, nine males and three females appeared listless. All other treated animals appeared normal at 3-5 hours after treatment.

24 hour observations - In the high dose group, five males appeared listless, one female appeared listless and one female was found dead. In the mid dose group, four males appeared listless.

All remaining animals in the high and mid dose groups appeared normal through 72 hours and all animals in the low dose groups appeared normal throughout the experiments. All positive and vehicle control animals also appeared normal throughout the experiments.

APPENDIX I - TABLE 3
SUMMARY OF MICRONUCLEUS ASSAY RESULTS FOR DIRECT HERBICIDE FORMULATION
PCE RATIO AND MICRONUCLEUS DATA FOR LOW DOSE ANIMALS

Harvest Time (Hours)	Sex	Number	Mean PCE / Total Erythrocyte Ratio ± Standard Deviation			Mean Micronucleated PCE / 1000 PCE ± Standard Deviation		
			Vehicle Control	Test Material	Positive Control	Vehicle Control	Test Material	Positive Control
24	Male	5	0.43 ± 0.04	0.49 ± 0.04	0.49 ± 0.06	1.6 ± 0.9	0.6 ± 0.5	29.2 ± 8.4**
	Female	5	0.48 ± 0.05	0.57 ± 0.05	0.51 ± 0.04	0.8 ± 1.1	0.6 ± 0.9	25.6 ± 7.8**
48	Male	5	0.49 ± 0.04	0.47 ± 0.06		1.2 ± 2.2	1.2 ± 1.8	
	Female	5	0.53 ± 0.07	0.48 ± 0.08		0.8 ± 0.8	1.0 ± 1.4	
72	Male	5	0.54 ± 0.09	0.60 ± 0.04		2.4 ± 1.1	2.6 ± 1.7	
	Female	5	0.52 ± 0.10	0.56 ± 0.06		1.8 ± 1.3	1.4 ± 1.1	

* $p \leq 0.05$; ** $p \leq 0.01$ by one-sided t-test. Square root transformed data used for statistical analysis of micronucleated PCE.

APPENDIX I - TABLE 4
SUMMARY OF MICRONUCLEUS ASSAY RESULTS FOR DIRECT HERBICIDE FORMULATION
PCE RATIO AND MICRONUCLEUS DATA FOR MIDDLE DOSE ANIMALS

Harvest Time (Hours)	Sex	Number	Mean PCE / Total Erythrocyte Ratio ± Standard Deviation			Mean Micronucleated PCE / 1000 PCE ± Standard Deviation		
			Vehicle Control	Test Material	Positive Control	Vehicle Control	Test Material	Positive Control
24	Male	5	0.43 ± 0.04	0.49 ± 0.05	0.49 ± 0.06	1.6 ± 0.9	2.0 ± 1.6	29.2 ± 8.4**
	Female	5	0.48 ± 0.05	0.51 ± 0.05	0.51 ± 0.04	0.8 ± 1.1	1.4 ± 2.1	25.6 ± 7.8**
48	Male	5	0.49 ± 0.04	0.51 ± 0.07		1.2 ± 2.2	1.0 ± 0.7	
	Female	5	0.53 ± 0.07	0.53 ± 0.04		0.8 ± 0.8	1.8 ± 3.0	
72	Male	5	0.54 ± 0.09	0.59 ± 0.07		2.4 ± 1.1	2.0 ± 1.6	
	Female	5	0.52 ± 0.10	0.63 ± 0.06		1.8 ± 1.3	1.2 ± 1.3	

* $p \leq 0.05$; ** $p \leq 0.01$ by one-sided t-test. Square root transformed data used for statistical analysis of micronucleated PCE.

APPENDIX I - TABLE 5
SUMMARY OF MICRONUCLEUS ASSAY RESULTS FOR DIRECT HERBICIDE FORMULATION
PCE RATIO AND MICRONUCLEUS DATA FOR HIGH DOSE ANIMALS

Harvest Time (Hours)	Sex	Number	Mean PCE / Total Erythrocyte Ratio ± Standard Deviation			Mean Micronucleated PCE / 1000 PCE ± Standard Deviation		
			Vehicle Control	Test Material	Positive Control	Vehicle Control	Test Material	Positive Control
24	Male	5	0.43 ± 0.04	0.49 ± 0.06	0.49 ± 0.06	1.6 ± 0.9	1.0 ± 1.0	29.2 ± 8.4**
	Female	5	0.48 ± 0.05	0.45 ± 0.09	0.51 ± 0.04	0.8 ± 1.1	1.2 ± 0.8	25.6 ± 7.8**
48	Male	5	0.49 ± 0.04	0.50 ± 0.08		1.2 ± 2.2	1.2 ± 0.8	
	Female	5	0.53 ± 0.07	0.49 ± 0.08		0.8 ± 0.8	0.6 ± 0.5	
72	Male	5	0.54 ± 0.09	0.65 ± 0.03		2.4 ± 1.1	0.8 ± 0.4	
	Female	5	0.52 ± 0.10	0.60 ± 0.04		1.8 ± 1.3	1.8 ± 0.8	

* $p \leq 0.05$; ** $p \leq 0.01$ by one-sided t-test. Square root transformed data used for statistical analysis of micronucleated PCE.

APPENDIX II
Individual Test Results

Table 1	Body Weight Table for Micronucleus Experiment with DIRECT Herbicide Formulation
Table 2	Slide Scoring Data for the Micronucleus Experiment with DIRECT Herbicide Formulation (PCE/erythrocyte Ratio and Micronucleated PCE's)
Table 3*	Animal Number Assignments for the Micronucleus Experiment with DIRECT Herbicide Formulation

*Note: Animals were initially assigned individual animal lot numbers and those selected for study were subsequently also assigned study numbers. Appendix II, Table 1 reports individual weight data using the assigned study number (without the study number prefix). Slide scoring data were collected using the animal lot number to preclude knowledge of the treatment or control group by the scorers and the data in Appendix II, Table 2 are presented using the individual animal lot numbers (without the lot number prefix). Appendix II, Table 3 presents the lot number and corresponding assigned study number for the individual animals in Appendix II, Tables 1 and 2 to permit comparison of the data on an individual animal basis.

APPENDIX II - TABLE 1
BODY WEIGHT TABLE FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

24 HOUR MALES

Group	Animal Number	Time of Sacrifice [a] (hr)	Body Weight (g)		
			Pretest	Final	Difference
10 ml/kg	M91 001	24	32.7	32.5	-0.2
Sterile Saline	M91 002	24	31.3	30.2	-1.1
Males	M91 003	24	35.1	33.9	-1.2
	M91 004	24	30.8	30.5	-0.3
	M91 005	24	32.0	32.3	0.3
91 mg/kg	M01 001	24	30.0	31.9	1.9
DIRECT ®	M01 002	24	32.8	35.7	2.9
Males	M01 003	24	32.8	34.0	1.2
	M01 004	24	28.3	32.0	3.7
	M01 005	24	30.7	34.2	3.5
183 mg/kg	M02 001	24	25.6	29.0	3.4
DIRECT ®	M02 002	24	30.3	31.9	1.6
Males	M02 010	24	28.0	29.0	1.0
	M02 011	24	34.2	34.7	0.5
	M02 014	24	32.5	31.0	-1.5
365 mg/kg	M03 001	24	35.4	33.3	-2.1
DIRECT ®	M03 004	24	34.5	31.7	-2.8
Males	M03 005	24	36.9	35.8	-1.1
	M03 015	24	35.6	34.4	-1.2
	M03 017	24	32.0	30.3	-1.7
40 mg/kg	M04 001	24	33.8	32.4	-1.4
Cyclophosphamide	M04 002	24	35.6	33.6	-2.0
Males	M04 003	24	33.9	32.5	-1.4
	M04 004	24	36.3	35.1	-1.2
	M04 005	24	34.6	34.2	-0.4

[a] Hours after treatment.

APPENDIX II - TABLE 1 (continued)
BODY WEIGHT TABLE FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

48 HOUR MALES

Group	Animal Number	Time of Sacrifice [a] (hr)	Body Weight (g)		
			Pretest	Final	Difference
10 ml/kg	M91 006	48	33.3	32.3	-1.0
Sterile Saline	M91 007	48	33.8	32.6	-1.2
Males	M91 008	48	35.8	35.6	-0.2
	M91 009	48	33.0	32.7	-0.3
	M91 010	48	31.7	32.2	0.5
91 mg/kg	M01 006	48	24.8	28.2	3.4
DIRECT ®	M01 007	48	29.3	32.9	3.6
Males	M01 008	48	30.1	32.7	2.6
	M01 009	48	28.2	31.1	2.9
	M01 010	48	24.0	26.6	2.6
183 mg/kg	M02 003	48	33.5	35.0	1.5
DIRECT ®	M02 004	48	26.2	28.5	2.3
Males	M02 005	48	28.5	31.0	2.5
	M02 006	48	28.3	30.2	1.9
	M02 007	48	33.6	34.4	0.8
365 mg/kg	M03 002	48	30.2	28.7	-1.5
DIRECT ®	M03 003	48	34.1	33.6	-0.5
Males	M03 006	48	29.3	27.7	-1.6
	M03 007	48	31.0	29.7	-1.3
	M03 008	48	28.1	26.0	-2.1

[a] Hours after treatment.

APPENDIX II - TABLE 1 (continued)
BODY WEIGHT TABLE FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

72 HOUR MALES

Group	Animal Number	Time of Sacrifice [a] (hr)	Body Weight (g)		
			Pretest	Final	Difference
10 ml/kg	M91 011	72	26.2	30.5	4.3
Sterile Saline	M91 012	72	29.9	29.5	-0.4
Males	M91 013	72	27.9	28.2	0.3
	M91 014	72	31.9	31.9	0.0
	M91 015	72	36.2	36.1	-0.1
91 mg/kg	M01 011	72	30.2	31.4	1.2
DIRECT ®	M01 012	72	29.8	33.4	3.6
Males	M01 013	72	29.0	30.9	1.9
	M01 014	72	28.0	30.3	2.3
	M01 015	72	28.5	31.0	2.5
183 mg/kg	M02 008	72	32.5	34.8	2.3
DIRECT ®	M02 009	72	34.1	35.1	1.0
Males	M02 012	72	35.4	37.1	1.7
	M02 013	72	31.4	31.3	-0.1
	M02 015	72	31.5	34.2	2.7
365 mg/kg	M03 009	72	34.9	36.0	1.1
DIRECT ®	M03 010	72	29.6	27.2	-2.4
Males	M03 011	72	32.9	32.3	-0.6
	M03 012	72	35.7	33.7	-2.0
	M03 013	72	37.1	38.3	1.2

[a] Hours after treatment.

APPENDIX II - TABLE 1 (continued)
BODY WEIGHT TABLE FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

24 HOUR FEMALES

Group	Animal Number	Time of Sacrifice [a] (hr)	Body Weight (g)		
			Pretest	Final	Difference
10 ml/kg	F91 001	24	25.5	24.6	-0.9
Sterile Saline	F91 002	24	27.4	26.0	-1.4
Females	F91 003	24	27.9	26.5	-1.4
	F91 004	24	30.1	28.6	-1.5
	F91 005	24	28.8	27.9	-0.9
91 mg/kg	F01 001	24	26.4	25.8	-0.6
DIRECT ®	F01 002	24	24.3	24.9	0.6
Females	F01 003	24	26.0	25.5	-0.5
	F01 004	24	24.4	23.2	-1.2
	F01 005	24	31.4	29.8	-1.6
183 mg/kg	F02 001	24	26.7	25.7	-1.0
DIRECT ®	F02 002	24	26.5	25.4	-1.1
Females	F02 003	24	27.1	25.9	-1.2
	F02 004	24	28.6	28.6	0.0
	F02 005	24	28.3	27.9	-0.4
365 mg/kg	F03 001	24	27.6	26.2	-1.4
DIRECT ®	F03 003	24	30.8	29.6	-1.2
Females	F03 009	24	23.9	23.8	-0.1
	F03 013	24	32.4	30.4	-2.0
	F03 014	24	29.4	28.2	-1.2
40 mg/kg	F04 001	24	28.7	28.5	-0.2
Cyclophosphamide	F04 002	24	29.0	28.7	-0.3
Females	F04 003	24	28.4	27.5	-0.9
	F04 004	24	29.0	28.4	-0.6
	F04 005	24	27.6	26.5	-1.1

[a] Hours after treatment.

APPENDIX II - TABLE 1 (continued)

BODY WEIGHT TABLE FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

48 HOUR FEMALES

Group	Animal Number	Time of Sacrifice [a] (hr)	Body Weight (g)		
			Pretest	Final	Difference
10 ml/kg	F91 006	48	27.4	26.5	-0.9
Sterile Saline	F91 007	48	26.5	26.0	-0.5
Females	F91 008	48	32.6	31.9	-0.7
	F91 009	48	24.7	23.5	-1.2
	F91 010	48	24.2	23.6	-0.6
91 mg/kg	F01 006	48	24.7	24.9	0.2
DIRECT ®	F01 007	48	28.1	27.8	-0.3
Females	F01 008	48	33.1	32.4	-0.7
	F01 009	48	28.3	28.6	0.3
	F01 010	48	30.2	30.2	0.0
183 mg/kg	F02 006	48	23.5	22.2	-1.3
DIRECT ®	F02 007	48	29.2	28.2	-1.0
Females	F02 008	48	26.5	26.5	0.0
	F02 009	48	34.6	33.2	-1.4
	F02 010	48	30.9	29.4	-1.5
365 mg/kg	F03 002	48	25.5	25.1	-0.4
DIRECT ®	F03 004	48	27.5	27.3	-0.2
Females	F03 005	48	26.0	24.8	-1.2
	F03 006	48	25.5	24.7	-0.8
	F03 007	48	24.0	24.1	0.1

[a] Hours after treatment.

APPENDIX II - TABLE 1 (continued)

BODY WEIGHT TABLE FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

72 HOUR FEMALES

Group	Animal Number	Time of Sacrifice [a] (hr)	Body Weight (g)		
			Pretest	Final	Difference
10 ml/kg	F91 011	72	27.0	27.0	0.0
Sterile Saline	F91 012	72	27.0	26.6	-0.4
Females	F91 013	72	30.4	29.5	-0.9
	F91 014	72	31.8	31.4	-0.4
	F91 015	72	25.6	25.5	-0.1
91 mg/kg	F01 011	72	29.6	28.7	-0.9
DIRECT ®	F01 012	72	28.8	28.0	-0.8
Females	F01 013	72	24.7	24.8	0.1
	F01 014	72	25.1	26.2	1.1
	F01 015	72	30.4	28.8	-1.6
183 mg/kg	F02 011	72	27.7	26.1	-1.6
DIRECT ®	F02 012	72	29.8	29.7	-0.1
Females	F02 013	72	33.2	31.6	-1.6
	F02 014	72	23.5	24.1	0.6
	F02 015	72	26.3	26.9	0.6
365 mg/kg	F03 008	72	30.9	30.7	-0.2
DIRECT ®	F03 010	72	28.9	28.0	-0.9
Females	F03 011	72	29.0	29.2	0.2
	F03 015	72	26.0	28.0	2.0
	F03 016	72	25.2	25.6	0.4

[a] Hours after treatment.

APPENDIX II - TABLE 2

Slide Scoring Data for the Micronucleus Experiment with **DIRECT®** Herbicide Formulation
(PCE/Erythrocyte Ratio and Micronucleated PCE's)
24 Hour Males

Group	Animal Number	Time Hrs.	PCE/Erythrocyte Ratio [a]			Micronucleated PCE [b]			
			Sld. 1	Sld. 2	Mean	Sld.1	Sld. 2	Combined	
10 ml/kg	M 038	24	0.456	0.510	0.483	0	2	2	
Sterile Saline	M 177	24	0.352	0.396	0.374	1	0	1	
Males	M 202	24	0.464	0.372	0.418	1	0	1	
	M 209	24	0.476	0.432	0.454	0	1	1	
	M 232	24	0.442	0.370	0.406	1	1	2	
91 mg/kg	M 159	24	0.498	0.560	0.529	0	1	1	
DIRECT®	M 165	24	0.486	0.546	0.516	0	1	1	
Males	M 214	24	0.378	0.478	0.428	0	0	0	
	M 262	24	0.500	0.468	0.484	0	0	0	
	M 291	24	0.472	0.536	0.504	0	1	1	
183 mg/kg	M 097	24	0.510	0.522	0.516	3	1	4	
DIRECT®	M 176	24	0.534	0.562	0.548	1	0	1	
Males	M 259	24	0.496	0.486	0.491	2	1	3	
	M 280	24	0.478	0.452	0.465	1	1	2	c
	M 296	24	0.390	0.440	0.415	0	0	0	
365 mg/kg	M 160	24	0.502	0.496	0.499	0	1	1	
DIRECT®	M 169	24	0.526	0.622	0.574	0	0	0	
Males	M 253	24	0.426	0.460	0.443	0	0	0	
	M 254	24	0.494	0.536	0.515	2	0	2	
	M 292	24	0.438	0.390	0.414	1	1	2	
40 mg/kg	M 112	24	0.400	0.434	0.417	7	9	16	
Cyclophosphamide	M 179	24	0.544	0.602	0.573	13	18	31	
Males	M 194	24	0.434	0.452	0.443	21	18	39	
	M 233	24	0.510	0.560	0.535	12	20	32	c
	M 240	24	0.472	0.474	0.473	18	10	28	

Note: Refer to the end of APPENDIX II Table 2 for all footnote codes.

APPENDIX II - TABLE 2 (continued)

Slide Scoring Data for the Micronucleus Experiment with **DIRECT®** Herbicide Formulation
(PCE/Erythrocyte Ratio and Micronucleated PCE's)
48 Hour Males

Group	Animal Number	Time Hrs.	PCE/Erythrocyte Ratio [a]			Micronucleated PCE [b]		
			Sld. 1	Sld. 2	Mean	Sld.1	Sld. 2	Combined
10 ml/kg	M 066	48	0.436	0.404	0.420	0	0	0
Sterile Saline	M 117	48	0.484	0.462	0.473	0	0	0
Males	M 161	48	0.510	0.508	0.509	0	0	0
	M 257	48	0.558	0.500	0.529	2	3	5
	M 294	48	0.542	0.502	0.522	0	1	1
91 mg/kg	M 090	48	0.476	0.442	0.459	2	2	4
DIRECT®	M 155	48	0.567 [d]	0.570	0.568	0	0	0
Males	M 164	48	0.488	0.310	0.399	0	0	0
	M 220	48	0.444	0.446	0.445	1	1	2
	M 252	48	0.486	0.474	0.480	0	0	0
183 mg/kg	M 126	48	0.600	0.580	0.590	1	0	1
DIRECT®	M 185	48	0.456	0.464	0.460	1	0	1
Males	M 229	48	0.532	0.494	0.513	1	1	2
	M 235	48	0.606	0.560	0.583	0	0	0
	M 302	48	0.418	0.434	0.426	1	0	1
365 mg/kg	M 058	48	0.572	0.482	0.527	1	0	1
DIRECT®	M 069	48	0.592	0.492	0.542	0	0	0
Males	M 152	48	0.452	0.508	0.480	1	0	1
	M 166	48	0.408	0.320	0.364	1	1	2
	M 216	48	0.606	0.544	0.575	1	1	2

Note: Refer to the end of APPENDIX II Table 2 for all footnote codes.

APPENDIX II - TABLE 2 (continued)

Slide Scoring Data for the Micronucleus Experiment with **DIRECT®** Herbicide Formulation
(PCE/Erythrocyte Ratio and Micronucleated PCE's)
72 Hour Males

Group	Animal Number	Time Hrs.	PCE/Erythrocyte Ratio [a]			Micronucleated PCE [b]		
			Sld.1	Sld. 2	Mean	Sld.1	Sld. 2	Combined
10 ml/kg Sterile Saline Males	M 125	72	0.616	0.598	0.607	3	1	4
	M 203	72	0.514	0.436	0.475	1	1	2
	M 225	72	0.674	0.534	0.604	3	0	3
	M 266	72	0.440	0.420	0.430	0	1	1
	M 306	72	0.612	0.596	0.604	1	1	2
91 mg/kg DIRECT® Males	M 188	72	0.600	0.596	0.598	1	1	2
	M 207	72	0.640	0.578	0.609	0	0	0
	M 239	72	0.607 d	0.698	0.652	2	0	2
	M 267	72	0.504	0.620	0.562	3	0	3
	M 271	72	0.576	0.570	0.573	1	0	1
183 mg/kg DIRECT® Males	M 141	72	0.568	0.478	0.523	1	2	3
	M 163	72	0.662	0.722	0.692	2	0	2
	M 210	72	0.656	0.558	0.607	1	0	1
	M 284	72	0.576	0.438	0.507	2	0	2
	M 303	72	0.630	0.568	0.599	0	0	0
365 mg/kg DIRECT® Males	M 139	72	0.535 d	0.756	0.645	0	0	0
	M 154	72	0.634	0.668	0.651	1	0	1
	M 192	72	0.622	0.734	0.678	0	1	1
	M 228	72	0.606	0.696	0.651	1	0	1
	M 276	72	0.616	0.600	0.608	1	0	1

Note: Refer to the end of APPENDIX II Table 2 for all footnote codes.

APPENDIX II - TABLE 2 (continued)

Slide Scoring Data for the Micronucleus Experiment with **DIRECT®** Herbicide Formulation
(PCE/Erythrocyte Ratio and Micronucleated PCE's)
24 Hour Females

Group	Animal Number	Time Hrs.	PCE/Erythrocyte Ratio [a]			Micronucleated PCE [b]		
			Sld. 1	Sld. 2	Mean	Sld. 1	Sld. 2	Combined
10 ml/kg Sterile Saline Females	F 195	24	0.536	0.482	0.509	0	0	0
	F 213	24	0.543 d	0.408	0.476	2	0	2
	F 217	24	0.602	0.508	0.555	0	0	0
	F 234	24	0.436	0.468	0.452	0	0	0
	F 303	24	0.406	0.446	0.426	0	2	2
91 mg/kg DIRECT® Females	F 145	24	0.667 d	0.598	0.633	1	1	2
	F 147	24	0.616	0.528	0.572	1	0	1
	F 185	24	0.636	0.578	0.607	0	0	0
	F 205	24	0.484	0.518	0.501	0	0	0
	F 285	24	0.602	0.468	0.535	0	0	0
183 mg/kg DIRECT® Females	F 179	24	0.632	0.514	0.573	1	4	5
	F 236	24	0.488	0.414	0.451	0	0	0
	F 246	24	0.466	0.512	0.489	0	1	1
	F 258	24	0.556	0.528	0.542	0	0	0
	F 267	24	0.496	0.472	0.484	0	1	1
365 mg/kg DIRECT® Females	F 192	24	0.302	0.350	0.326	0	1	1
	F 193	24	0.610	0.534	0.572	0	0	0
	F 227	24	0.382	0.446	0.414	1	0	1
	F 262	24	0.440	0.468	0.454	2	0	2
	F 273	24	0.498	0.504	0.501	1	1	2
40 mg/kg Cyclophosphamide Females	F 220	24	0.598	0.490	0.544	18	16	34
	F 245	24	0.478	0.482	0.480	5	10	15 c
	F 256	24	0.572	0.498	0.535	19	13	32 c
	F 264	24	0.418	0.474	0.446	10	11	21 c
	F 269	24	0.536	0.534	0.535	15	11	26 c

Note: Refer to the end of APPENDIX II Table 2 for all footnote codes.

APPENDIX II - TABLE 2 (continued)

Slide Scoring Data for the Micronucleus Experiment with **DIRECT®** Herbicide Formulation
(PCE/Erythrocyte Ratio and Micronucleated PCE's)
48 Hour Females

Group	Animal Number	Time Hrs.	PCE/Erythrocyte Ratio [a]			Micronucleated PCE [b]		
			Sld. 1	Sld. 2	Mean	Sld.1	Sld. 2	Combined
10 ml/kg	F 167	48	0.552	0.534	0.543	0	1	1
Sterile Saline	F 187	48	0.612	0.564	0.588	0	0	0
Females	F 200	48	0.428	0.434	0.431	1	1	2
	F 248	48	0.500	0.524	0.512	0	0	0
	F 255	48	0.614	0.574	0.594	1	0	1
91 mg/kg	F 003	48	0.510	0.504	0.507	0	0	0
DIRECT®	F 152	48	0.536	0.570	0.553	0	0	0
Females	F 199	48	0.488	0.510	0.499	0	0	0
	F 280	48	0.476	0.522	0.499	0	2	2
	F 306	48	0.318	0.360	0.339	2	1	3
183 mg/kg	F 057	48	0.598	0.422	0.510	0	0	0
DIRECT®	F 180	48	0.448	0.562	0.505	0	0	0
Females	F 186	48	0.608	0.602	0.605	3	4	7
	F 214	48	0.520	0.522	0.521	0	0	0
	F 278	48	0.508	0.534	0.521	0	2	2
365 mg/kg	F 001	48	0.358	0.394	0.376	0	0	0
DIRECT®	F 056	48	0.512	0.562	0.537	1	0	1
Females	F 238	48	0.496	0.538	0.517	0	0	0
	F 277	48	0.440	0.492	0.466	0	1	1
	F 279	48	0.560	0.594	0.577	0	1	1

Note: Refer to the end of APPENDIX II Table 2 for all footnote codes.

APPENDIX II - TABLE 2 (continued)

Slide Scoring Data for the Micronucleus Experiment with **DIRECT®** Herbicide Formulation
(PCE/Erythrocyte Ratio and Micronucleated PCE's)
72 Hour Females

Group	Animal Number	Time Hrs.	PCE/Erythrocyte Ratio [a]			Micronucleated PCE [b]		
			Sld. 1	Sld. 2	Mean	Sld.1	Sld. 2	Combined
10 ml/kg	F 235	72	0.396	0.384	0.390	1	1	2
Sterile Saline	F 240	72	0.544	0.542	0.543	1	0	1
Females	F 241	72	0.564	0.478	0.521	1	0	1
	F 281	72	0.614	0.720	0.667	0	1	1
	F 290	72	0.500	0.450	0.475	1	3	4
91 mg/kg	F 024	72	0.428	0.512 d	0.470	3	0	3
DIRECT®	F 183	72	0.504	0.634	0.569	0	1	1
Females	F 194	72	0.572	0.513 d	0.543	0	0	0
	F 223	72	0.616	0.594	0.605	0	2	2
	F 302	72	0.652	0.570	0.611	1	0	1
183 mg/kg	F 004	72	0.554	0.574	0.564	0	1	1
DIRECT®	F 202	72	0.652	0.802	0.727	0	3	3
Females	F 225	72	0.648	0.668	0.658	1	1	2
	F 259	72	0.586	0.612	0.599	0	0	0
	F 312	72	0.608	0.620	0.614	0	0	0
365 mg/kg	F 010	72	0.610	0.718	0.664	0	1	1
DIRECT®	F 117	72	0.608	0.578	0.593	2	1	3
Females	F 127	72	0.638	0.600	0.619	1	1	2
	F 197	72	0.604	0.554	0.579	0	1	1
	F 265	72	0.600	0.496	0.548	2	0	2

Note: Refer to the end of APPENDIX II Table 2 for all footnote codes.

APPENDIX II - Table 2 (Footnotes)

a Ratio scored per 500 erythrocytes (PCEs and NCEs) for each slide except as noted in (d) and mean ratio of both slides (equivalent to ratio for 1000 erythrocytes).
b Micronucleated PCE scored per 500 PCEs for each slide and combined micronucleated PCEs for 1000 PCEs scored.
c Significantly discordant scoring results observed in initial scoring, slides were re-scored and rescored value used in analysis.
d Total PCE/NCE not equal to 500, but between 490 and 510. Ratio was calculated to reflect the actual number of PCE/NCE counted.

APPENDIX II - TABLE 3

ANIMAL NUMBER ASSIGNMENTS FOR THE MICRONUCLEUS EXPERIMENT WITH DIRECT® HERBICIDE FORMULATION

Group	Study Number	Lot Number
10 ml/kg	F91 001	F 234
Sterile Saline	F91 002	F 217
Females	F91 003	F 213
	F91 004	F 303
	F91 005	F 195
	F91 006	F 255
	F91 007	F 167
	F91 008	F 200
	F91 009	F 248
	F91 010	F 187
	F91 011	F 241
	F91 012	F 240
	F91 013	F 235
	F91 014	F 290
	F91 015	F 281
91 mg/kg	F01 001	F 185
DIRECT®	F01 002	F 145
Females	F01 003	F 285
	F01 004	F 147
	F01 005	F 205
	F01 006	F 199
	F01 007	F 306
	F01 008	F 003
	F01 009	F 280
	F01 010	F 152
	F01 011	F 024
	F01 012	F 183
	F01 013	F 223
	F01 014	F 302
	F01 015	F 194
183 mg/kg	F02 001	F 246
DIRECT®	F02 002	F 236
Females	F02 003	F 258
	F02 004	F 267
	F02 005	F 179
	F02 006	F 278
	F02 007	F 180
	F02 008	F 186
	F02 009	F 214

APPENDIX II - TABLE 3 (Continued)
ANIMAL NUMBER ASSIGNMENTS FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

Group	Study Number	Lot Number
	F02 010	F 057
	F02 011	F 312
	F02 012	F 225
	F02 013	F 259
	F02 014	F 004
	F02 015	F 202
365 mg/kg DIRECT® Females	F03 001	F 193
	F03 002	F 056
	F03 003	F 192
	F03 004	F 279
	F03 005	F 238
	F03 006	F 001
	F03 007	F 277
	F03 008	F 010
	F03 009	F 227
	F03 010	F 265
	F03 011	F 197
	F03 013	F 273
	F03 014	F 262
	F03 015	F 117
	F03 016	F 127
40 mg/kg Cyclophosphamide Females	F04 001	F 264
	F04 002	F 269
	F04 003	F 220
	F04 004	F 256
	F04 005	F 245
10 ml/kg Sterile Saline Males	M91 001	M 232
	M91 002	M 202
	M91 003	M 038
	M91 004	M 177
	M91 005	M 209
	M91 006	M 117
	M91 007	M 066
	M91 008	M 257
	M91 009	M 294
	M91 010	M 161
	M91 011	M 225

APPENDIX II - TABLE 3 (Continued)
ANIMAL NUMBER ASSIGNMENTS FOR THE MICRONUCLEUS EXPERIMENT WITH DIRECT® HERBICIDE FORMULATION

Group	Study Number	Lot Number
	M91 012	M 306
	M91 013	M 203
	M91 014	M 125
	M91 015	M 266
91 mg/kg	M01 001	M 159
DIRECT®	M01 002	M 262
Males	M01 003	M 291
	M01 004	M 214
	M01 005	M 165
	M01 006	M 155
	M01 007	M 164
	M01 008	M 252
	M01 009	M 220
	M01 010	M 090
	M01 011	M 267
	M01 012	M 207
	M01 013	M 239
	M01 014	M 271
	M01 015	M 188
183 mg/kg	M02 001	M 097
DIRECT®	M02 002	M 280
Males	M02 003	M 235
	M02 004	M 302
	M02 005	M 229
	M02 006	M 126
	M02 007	M 185
	M02 008	M 303
	M02 009	M 141
	M02 010	M 259
	M02 011	M 296
	M02 012	M 284
	M02 013	M 210
	M02 014	M 176
	M02 015	M 163
365 mg/kg	M03 001	M 292
DIRECT®	M03 002	M 058
Males	M03 003	M 166
	M03 004	M 254
	M03 005	M 253
	M03 006	M 069

APPENDIX II - TABLE 3 (Continued)
ANIMAL NUMBER ASSIGNMENTS FOR THE MICRONUCLEUS EXPERIMENT WITH **DIRECT®** HERBICIDE FORMULATION

Group	Study Number	Lot Number
	M03 007	M 152
	M03 008	M 216
	M03 009	M 228
	M03 010	M 192
	M03 011	M 154
	M03 012	M 276
	M03 013	M 139
	M03 015	M 160
	M03 017	M 169
40 mg/kg Cyclophosphamide Males	M04 001	M 233
	M04 002	M 112
	M04 003	M 194
	M04 004	M 240
	M04 005	M 179