Fiche

C09614

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

JUL 2 2 1992

MEMORANDUM

SUBJECT: Glyphosate - List A Chemical for Reregistration - Rereview of Toxicology Studies for Acceptability

Caswell No.: 661A
Project No.: 1-0904
ID No.: 103601
Task Hours: 488

FROM: William Dykstra, Ph.D. William Dykstra 5/3/91
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Jay Ellenberger, PM 50
Reregistration Branch
Special Review and Reregistration Division (H7508C)

for THRU: Roger L. Gardner, Section Head Pamela M. Hurley 5/14/91
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

R. G. 7/15/92

K.B. 7/14/92

Requested Action

The following studies need to be rereviewed to determine their acceptability: 81-1; 81-2; 82-2; 83-1a; 83-3a; 83-3b; 83-4; 84-2a; 84-2b; and 84-4 (other genotoxic effects).

Printed on Recycled Paper

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/14/85
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Roger Gardner, Section Head for Brenda M. Hunley 5/14/85
Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: 84-2(b); Cytogenetics In Vivo TOX Chem. No.: 661A

Accession No.: 251737 MRID No.: 00132683

Test Material: Glyphosate Technical; 98.7% Purity

Synonyms: EHL Sample No. TA830044

Study Number: ML-83-236

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Environmental Health Lab, St. Louis, MO

Title of Report: In Vivo Bone Marrow Cytogenetics Study of Glyphosate in Sprague-Dawley Rats.

Author: A.P. Li

Report Issued: October 20, 1983

Conclusions:

Glyphosate did not induce significant clastogenic effects in rats under conditions of the study which was limited to the assay of a single dose level of 1000 mg/kg. Cyclophosphamide at 25 mg/kg caused a highly significant number of chromosomal aberrations demonstrating the sensitivity of the assay. Under the conditions of the study, glyphosate did not cause any fatalities or other signs of toxicity. Monsanto addressed previous issues in the letter of November 26, 1984 (memorandum of W. Dykstra of March 12, 1985, attached).

Classification: Acceptable

Special Review Criteria (40 CFR 154.7): N/A

Note: Dr. Irving Mauer, geneticist, screened this mutagenicity study for acceptability. The DER is based on part of a Dynamac review.

00006

Review:

Quality Assurance Statement - Present and dated October 21, 1983.

Test Material - The test material was identified as glyphosate (EHL Sample No. TA830044), a white powder having a purity of 98.7 percent.

Materials and Methods:

Preparation of Test Material - The test material was suspended in Hank's buffered salt solution (HBSS) at a concentration of 100 mg/mL and was neutralized to pH 7.0. Solutions were prepared no more than 24 hours before use. A volume of 10 mL/kg was used for ip dosing.

Controls - Cyclophosphamide, the positive control, was dissolved in HBBS (25 mg/mL). One mL/kg (25 mg/kg) was used for dosing. A volume of 10 mL/kg HBSS, the solvent control, was administered intraperitoneally (ip) to the control animals.

Animals - The animals used in the study were male and female Sprague-Dawley rats [CD(SD)BR] from Charles River Breeding Laboratories, which were approximately 9 weeks old at the time of dosing. Water and Purina Laboratory Chow were provided ad libitum except at the fasting period 14 to 24 hours prior to dosing. Animals were maintained in individual cages in rooms maintained at 70 to 74 'F, a relative humidity of 35 to 60 percent, and on a 12-hour light/dark cycle.

Experimental Design - Rats (18/sex/group) were fasted for 14 to 24 hours and then injected ip with (i) solvent (HBSS), (ii) glyphosate (1 g/kg), or (iii) cyclophosphamide (25 mg/kg). Six animals of each sex and group (control, test group, and positive control group) were sacrificed at 6, 12, and 24 hours. Two hours before sacrifice each rat was injected ip with 2 mg/kg colchicine. Sacrifice was by CO_2 asphyxiation and spinal cord severance.

Preparation of Bone Marrow Cells - Marrow was aspirated from each femur into a 5 mL syringe containing 2 mL HBSS. The contents were added to 5 mL of HBSS in a plastic centrifuge and maintained at 37 'C until the slides were prepared.

Slide Preparation - The cells were pelleted by centrifugation (700 x g. 10 min), suspended in 1 mL of hypotonic KCl (0.075 M), and incubated at 37 'C for 30 minutes. The cells were then fixed with an equal volume of Corney's solvent (3/1, v/v methanol glacial acetic acid). The pellet was resuspended in 4 mL of fresh cold fixative and one to two drops of each suspension placed on a clean wet slide. The slides were air dried, stained for 15 to 20

minutes in a 2 percent Giesma solution, rinsed with water, and again air dried.

Scoring of Slides - The slides were scored by three persons in Dr. Julian Preston's laboratory (Oak Ridge National Laboratory). Approximately 50 mitotic cells (300/treatment) were scored for chromosomal aberrations. The following data were recorded:

Number of cells scored
Number of cells with normal chromosome numbers
Chromosome-type aberrations (dicentric, ring, deletions)
Chromatid-type aberrations (chromatid deletions, isochromatid deletions, interchanges, intrachanges)
Achromatic lesions (gaps)
Number of aneuploid cells
Location of cells with aberrations

Statistical Analysis - The Student's t-test was used for data analysis, in which dosing with the test material or positive control was compared to the solvent control.

Results:

The frequency of chromatid-type aberrations was low in both solvent control and glyphosate treated groups (Table 1).

Table 1. Chromatid-Type Aberrations

Time	Control	Glyphosate
6 hours	7/588[a]	6/544
12 hours	2/588[a]	5/564
24 hours	4/555[a]	7/479

[a]Number of aberrations/number of mitotic cells examined. Data for males and females was combined by this reviewer.

There were no chromosomal-type aberrations in marrow cells in either solvent controls or the glyphosate group.

The positive control group was scored only at 24 hours. Because of extreme cytotoxicity, only 21 cells were available for scoring in females and 256 cells in males. There was a high incidence of chromatid type aberrations (231/277 chromatid deletions, 71/231 chromatid interchanges, and 6/277 chromatid intrachanges).

Conclusions and Recommendations

New DER's are attached for each of the studies which have been rereviewed for acceptability. The results of the rereview are summarized below.

Technical Glyphosate

Study	Results	Classification (Core-Grade)
81-1	Toxicity Category III	Minimum
81-2	Toxicity Category IV	Minimum
82-2	NOEL = 1000 mg/kg/day	Guideline
83-1a	NOEL = 31 mg/kg/day	Minimum
83-3a (Rats)	Negative for Terata: Developmental NOEL = 1000 mg/kg Maternal NOEL = 1000 mg/kg	Guideline
83-3b (Rabbit)	Negative for Terata: Developmental NOEL = 350 mg/kg Maternal NOEL = 175 mg/kg	Minimum
83-4	NOEL = 10 mg/kg/day	Minimum
84-2a	Negative for HGPRT/CHO	Acceptable
84-2b	Negative for *in vivo* Rat Cytogenetics	Acceptable
84-2a	Negative for Ames Assay (Two studies)	Acceptable
84-2b	Negative for Mouse Dominant Lethal	Unacceptable
84-4	Negative for Rec-Assay in *B. subtilis*	Acceptable
84-4	Negative for DNA Repair in Rat Hepatocytes	Unacceptable

009614

Reviewed by: William Dykstra, Ph.D. William Dykstra 4/9/91
Review Section I , Toxicology Branch I (H7509C)
for Secondary Reviewer: Roger Gardner, Section Head Pamela M. Hurley 5/14/91
Review Section I, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

Study Type: 81-1, Acute oral, rats

TOX Chem No. 661A
MRID No.: 00067039

Accession Number: N/A

Test Material: Glyphosate, technical; sample No. 96

Synonyms: Roundup, Rodeo, Polado; CP67573-3

Study Number: Monsanto Project No. Y-70-90

Sponsor: Monsanto Company
St. Louis, MO 63129

Testing Facility: Younger Laboratories
St. Louis, MO

Title of Report: Toxicological Investigation of: CP67573-3

Author: Melvin D. Birch

Report Issued: September 18, 1970

Conclusion: The test material was prepared as a 25.0% aqueous solution-suspension. Four groups of male and female (a total of 5 rats/group) Sprague-Dawley young rats received single, oral doses by gavage of 3160, 3980, 5010 and 6310 mg/kg of test material. Observation was for 7 days. Mortality was 1/5, 2/5, 3/5 and 5/5 for the four groups.

LD_{50} (Both sexes) = 4320 mg/kg (3930 - 4750 mg/kg)

Classification: Core-Minimum Toxicity Category III

Special Review Criteria: (40 CFR 154.7) N/A

00003

009614

Review

1. Acute Oral, Rat: Toxicological Investigation of: CP67573-3 (Younger Laboratories, Melvin D. Birch, 9/18/70)

Test Material: glyphosate, technical; Sample No. 96

The test material was prepared as a 25.0% aqueous solution-suspension. Four groups of male and female (a total of 5 rats per group) young Sprague-Dawley rat received single, oral doses by gavage of 3160, 3980, 5010 and 6310 mg/kg of test material. Observation was for seven days.

Results: LD_{50} (Both sexes) = 4320 mg/kg; 95% C.L. (3930-4750 mg/kg) Method of E.J. deBeer for LD_{50}

As shown below, mortality was 1/5, 2/5, 3/5 and 5/5

THE ORAL LD_{50} OF 'CP 67573-3' FOR RATS

Sample Fed As A 25.0% Aqueous Solution-Suspension

Animal No. - Sex	Weight Grams	Dose Mg./Kg.	Fate
1 - Female	225	3160	Survived
2 - Male	200	3160	Survived
3 - Female	200	3160	Died
4 - Male	210	3160	Survived
5 - Female	205	3160	Survived
6 - Male	200	3980	Survived
7 - Female	215	3980	Died
8 - Male	220	3980	Survived
9 - Female	195	3980	Survived
10 - Male	205	3980	Died
11 - Female	200	5010	Died
12 - Male	200	5010	Survived
13 - Female	190	5010	Died
14 - Male	200	5010	Died
15 - Female	200	5010	Survived
16 - Male	215	6310	Died
17 - Female	205	6310	Died
18 - Male	215	6310	Died
19 - Female	200	6310	Died
20 - Male	235	6310	Died

Survival Time: Several hours to six days

Toxic Signs: Reduced activity and reduced appetite (three to seven days in survivors), lethargy, diarrhea, increasing weakness,

collapse and death.

Necropsy: Hemorrhagic lungs and liver and gastrointestinal inflammation (acute in some cases).

Comment: There were no signed statements of Quality assurance or GLP's. However, the report was signed by Melvin D. Birch of the testing lab. Although this study does not fulfill all details of a Subpart F (1982), 81-1, Guidelines Study, the compound, glyphosate, based on mortality data in this study, falls clearly within Toxicity Category III

Classification: Core-Minimum

C09614

Reviewed by: William Dykstra, Ph.D. William Dykstra 4/9/91
Section I, Tox. Branch I, IRS, H7509C
for Secondary reviewer: Roger Gardner, Section Head Pamela McHurley 5/14/91
Section I, Tox. Branch I, IRS, H7509C

DATA EVALUATION REPORT

STUDY TYPE: 81-2, Acute Dermal, Rabbits TOX. CHEM.NO. 661A

ACCESSION NUMBER: N/A MRID NO. 00067039

TEST MATERIAL: Glyphosate, Technical; sample 96

SYNONYMS: CP 67573-3; Roundup.

STUDY NUMBER (s): Monsanto Project No. Y-70-90

SPONSOR: Monsanto Co., St. Louis, MO

TESTING FACILITY: Younger Laboratories, St. Louis, MO

TITLE OF REPORT: Toxicological Investigation of CP-67573-3

AUTHORS (s): Melvin D. Birch

REPORT ISSUED: 9/18/70

CONCLUSIONS: LD_{50}> 7940 mg/kg (females) and 5010 mg/kg (males)

Two NZW rabbits, one male and one female, were used in the study.

One male young NZW, 1.8 kg BW, received a dermally applied dose of 5010 mg/kg of glyphosate technical, as a 50% aqueous paste, on the fur clipped trunk under occlusive wrap for 24 hours. One NZW young female rabbit, 1.8 kg BW received 7940 mg/kg under similar occlusion for 24 hours.

There were no deaths in the study during the 14 day observation period. There were no clinical signs and no abnormal necropsy findings.

Although there are obviously insufficient numbers of rabbits used, technical glyphosate is clearly in toxicity category IV and would undoubtedly exceed the 2.0 g/kg limit dose for dermal toxicity guidelines.

Classification: Core-minimum Toxicity Category IV

00036

Review

1. Acute Dermal LD_{50} - Rabbits (Toxicological Investigations of CP 67573-3; Younger Laboratories; Monsanto Project No. Y-70-90; 9/18/70)
Test Material: glyphosate, technical; Sample No. 96

Two NZW rabbits, one male and one female, were used in the study. The male rabbit and female rabbit each weighed 1.8 kg BW. The male and female rabbits received, as a 50.0 % aqueous paste, dermally applied doses of technical glyphosate (male rabbit received 5010 mg/kg and female rabbit received 7940 mg/kg) on the fur clipped trunk under an occlusive wrap for 24 hours. Observations were for 14 days. Lack of sample prevented further testing.

Results: There were no deaths.

LD_{50} > 7940 mg/kg (female)
LD_{50} > 5010 mg/kg (male)

These results are shown below as presented in the report:

Sample Applied As A 50.0% Aqueous Suspension

Animal No. - Sex	Weight Kg.	Dose Mg./Kg.	Weight Change 5 Days Later (Kg)	Fate
1 Male	1.8	5010	0.1	Survived
2 Female	1.8	7940	0.0	Survived

Toxic Signs: None observed

Necropsy: No abnormal findings reported

Classification: Core-Minimum Toxicity Category IV

009614

Disk 3 HES TC661C

009614

Reviewed by: William Dykstra, Ph.D. William Dykstra 5/14/91
Section I, Tox. Branch I, IRS, H7509C
for Secondary reviewer: Roger Gardner, Section Head Pamela M. Hurley 5/14/91
Section I, Tox. Branch I, IRS, H7509C

DATA EVALUATION REPORT

STUDY TYPE: 82-2, 21 Day Dermal, Rabbit TOX. CHEM. NO.: 661A
MRID No. 00098460

ACCESSION NUMBER: N/A

TEST MATERIAL: glyphosate technical, white powder

SYNONYMS: Roundup, Rodeo, Polado

STUDY NUMBER(s): IRDC, No. 401-168

SPONSOR: Monsanto Co., St. Louis, MO

TESTING FACILITY: IRDC, Mattawan, MI

TITLE OF REPORT: 21-day dermal toxicity study in rabbits

AUTHOR(s): Dale E. Johnson, Study Director, 3/16/82

REPORT ISSUED: March 10, 1982

Technical glyphosate was tested in a 21-day dermal study in rabbits at the following dose levels: 0, 100, 1000 and 5000 mg/kg/day both with intact and abraded skin.

CONCLUSIONS: The NOEL is 1000 mg/kg/day (mid-dose). The LEL is 5000 mg/kg/day and the effects were (1) very slight erythema and edema observed visually, but not microscopically, in both sexes of intact and abraded skin of treated rabbits in comparison to controls; (2) food consumption was consistently decreased in two female high-dose rabbits during the study to a greater extent than in controls and (3) LDH was statistically significantly decreased in both sexes at the high-dose, but this was not considered a toxicologically significant finding (Clinical Guide to Laboratory Tests, N.W. Tietz, 1983, W.B. Saunders Co.).

Classification: Core-guideline

Testing Guideline Satisfied: 82-2

00009

009614

Review

1. 21-day dermal toxicity study in rabbits (IRDC No. 401-168; 3/10/82)

Test Material: glyphosate, technical; white powder; purity not given; source: Monsanto Company.

Quality Assurance Statement: Signed by Barry W. Benson, B.S., Director of Quality Assurance, March 10, 1982. In addition, a statement was provided by the Study Director that GLP's were followed.

Animals: Sixty-two male and 62 female NFW rabbits, young adults, were purchased from Davidson's Mill Farm, Jamesburg, N.J. and were acclimated to the IRDC laboratory for 14 to 16 days prior to initiation of the study.

Rabbits were individually housed and fed Purina Certified Rabbit Chow #5322 and water ad libitum. Animals were observed daily and placed into study groups based upon sex and body weight and randomized selection.

Methods Forty male (2359-2883g) and forty female (2344-2955g) rabbits were assigned to the following treatment groups

		Dose Level	Number of animals and skin preparation			
Group	Test Material	Mg/kg	Male I	Male A	Female I	Female A
I	control	0	5	5	5	5
II	glyphosate, tech.	100	5	5	5	5
III	glyphosate, tech.	1000	5	5	5	5
IV	glyphosate, tech.	5000	5	5	5	5
I = Intact A = Abraded						

Approximately 30% of the body surface of the trunk of each rabbit was shaved free of hair to begin the study and as often as needed. Twice each week, immediately prior to administration of the test material, the dorsal skin of one-half of the rabbits was abraded.

The test material was moistened (made pasty) with physiological saline and evenly applied onto the shaved skin surface of the rabbits. The test material was held in place by occlusion for six hours per day, five days per week for 3 consecutive weeks. The test material was washed off after each 6 hour exposure period. The rabbits wore collars to avoid ingestion of the test material during the entire study.

00210

009614

Observations:

1. **Toxic Signs and skin Reaction**

The rabbits were observed once daily for toxic signs and skin reaction.

Results - There were no compcund-related signs of systemic toxicity. Dermal irritation, consisting of slight erythema and edema, was observed. Scores of 0.5 for edema and erythema in intact skin and scores of 0.5 to 1.0 for edema and erythema in abraded skin were observed at 5000 mg/kg/day. These outwardly observable skin reactions were not detected microscopically. There was no dermal irritation at 100 or 1000 mg/kg/day.

2. **Mortality** - Rabbits were observed twice daily for mortality.

Results: There were no deaths during the study.

3. **Body Weight** - Body weights were obtained twice weekly during the study.

Results: There were no statistically significant changes in body weight or body weight gain in treated male and female intact or abraded rabbits in comparison to controls. The following tables, taken from the report, show the body weight changes in intact and abraded rabbits

Means, standard Deviations, N and Significance
Body Weights Changes (grams), Abraded

Study Period	0 mg/kg (Control) M	0 mg/kg (Control) F	100 mg/kg M	100 mg/kg F	1000 mg/kg M	1000 mg/kg F	5000 mg/kg M	5000 mg/kg F
Initiation - Term								
Ave.	298	353	207	299	535	520	356	338
S.D.	236.1	306.8	179.0	275.9	202.6	279.2	187.7	305.1
N	5	5	5	5	5	5	5	5

Means, Standard Deviations, N and Significance
Body Weight Changes (grams), Intact

Study Period	0 mg/kg (Control) M	0 mg/kg (Control) F	100 mg/kg M	100 mg/kg F	1000 mg/kg M	1000 mg/kg F	5000 mg/kg M	5000 mg/kg F
Initiation - Term								
Ave.	341	410	281	154	409	354	177	237
S.D.	146.2	110.1	114.1	239.3	187.2	218.0	110.6	482.6
N	5	5	5	5	5	5	5	5

4. Food Consumption - Visual estimates of food consumption were made daily for each rabbit.

Results: Although decreases (and some increases) were randomly noted in all groups on several occasions, there did appear to be a treatment-related effect in decreased food consumption in treated females at 5000 mg/kg in comparison to controls. At 5000 mg/kg/day intact skin, treated females had consistently lower food consumption in two rabbits (#14390 and 14428) in comparison to controls.

5. Clinical Pathology Studies - Blood was collected from the ear vein of each rabbit at day 21 for evaluation of hematology and clinical chemistry studies.

a. Hematology -

X		X	
X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count*	X	Mean corpuscular volume (MCV)
X	Reticulocyte count		

Results - There were no dose-related, statistically significant differences between control and treated male and female rabbits. Although occasional statistically significant findings did occur, based on their lack of dose-response, they were not considered compound-related. Therefore, there were no compound-related hematological findings in treated male and female rabbits in comparison to controls.

009614

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*		
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		

Results - Lactate dehydrogenase (LDH) was significantly reduced in high-dose males and females in comparison to controls. The values (IU/L) were 250,169,291 and 76* for C,L,M and HD males and 189, 149, 258 and 28* for C,L,M. and HD females (*P<0.05). However, decreases in lactate dehydrogenase are not of toxicological significance. Therefore, there were no compound-related, toxicologically significant findings in clinical chemistries in treated males and females in comparison to control. Other statistically significant findings were also not considered toxicologically significant.

6. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and they were CHECKED (X) tissues were collected for histological examination. The (XX) organ in addition were weighed.

00013

X		X		X	
	Digestive system		Cardiovasc./ Hemat.		Neurologic
	Tongue		Aorta*		Brain*
	Salivary glands*	W	Heart*		Periph. nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)*
	Stomach*		Lymph nodes*	W	Pituitary*
	Duodenum*		Spleen*		Eyes (optic n.)*
	Jejunum*		Thymus*		Glandular
	Ileum*		Urogenital	W	Adrenals*
	Cecum*	XX	Kidneys*		Lacrimal gland
	Colon*		Urinary bladder*		Mammary gland*
	Rectum*	XX	Testes*	W	Parathyroids*
XX	Liver*	X	Epididymides	W	Thyroids*
	Gall bladder*		Prostate		Other
	Pancreas*		Seminal vesicle		Bone*
	Respiratory	XX	Ovaries		Skeletal Muscle*
	Trachea*	X	Uterus*	X	Skin (treated & untreated)
	Lung*			X	All gross lesions and masses

W: Weighed but not examined microscopically.

Results

a. Organ weight -

There were no statistically significant differences between absolute and relative organ weights of male and female treated rabbits in comparison to controls.

b. Gross pathology -

There were no compound-related macroscopic observations in treated male and female rabbits in comparison to controls. The findings which were observed occurred in control as well as treated groups and were not dose-related. Most findings occurred as single animal observations.

c. Microscopic pathology -

1) Non-neoplastic

No compound-related microscopic lesions were observed in treated male and female tissues examined in comparison to controls, including treated and untreated skin from both sexes. The findings which did occur were not dose-related. In treated skin and untreated skin, the most common finding with respect to incidence and grade was trace to mild dermal inflammatory cell infiltrate. Although, one mid-dose female had trace necrosis in treated skin, untreated skins of 3 male rabbits from the mid-dose and one from the high-dose also showed mild focal necrosis. Therefore, this finding was not compound-related. Additionally, testes of male rabbits from the control and test groups showed trace to mild seminiferous tubule degeneration. This was not compound-related but probably due to non-specific stress.

7. Statistics: Body weights (terminal), hematolcgical and biochemical parameters (day 21) and absolute and relative organ weights (terminal sacrifice) were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate c-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

All statistical analyses compared the treatment groups with the control groups, by sex.

009614

Disk 4 HES Rabbit66.1A

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/13/91
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Roger Gardner, Section Head Pamela M. Hurley 5/13/91
for Section I, Toxicology Branch I - IRS (H7509C) R.G. 7/2/91

DATA EVALUATION REPORT 009614

Study Type: 83-1, Chronic Toxicity - Rat TOX Chem. No.: 661A

Accession Number: MRID No.: 00093879

Test Material: Glyphosate, technical; 98.7% purity; Lot XHJ-64; white powder

Synonyms: Roundup

Study Number: Bio/Dynamics, Inc., Project No. 77-2062

Sponsor: Monsanto Company

Testing Facility: Bio/Dynamics, Inc.
East Millstone, NJ

Title of Report: A Lifetime Feeding Study of Glyphosate in Rats.

Authors: George P. Lankas, Study Director, December 15, 1981

Report Issued: December 23, 1981

Conclusions:

Male and female rats were fed glyphosate in the diet for 26 months at the following dose levels: 0, 3, 10, and 31 mg/kg/day.

The NOEL for chronic toxicity was 31 mg/kg/day (HDT). There was no MTD in the study and therefore the study does not qualify as a carcinogenicity study. Nevertheless, oncogenic issues relating to C-cell thyroid carcinomas in females and interstitial cell testicular tumors were observed and have been fully addressed. The carcinogenic potential was negative up to 31 mg/kg/day (HDT).

Classification: Core-Minimum (for chronic toxicity only)

Special Review Criteria (40 CFR 154.7): N/A

A. Materials:

1. Test Compound - Glyphosate, technical; Description: white powder; Batch No.: XHJ-64; Purity: 98.7 percent; Contaminants: List in CBI appendix.

2. Test Animals - Species: Albino Rat; Strain: Sprague-Dawley CD; Age: 28 days; Weight - Males: 124 g, Females: 102 g; Source: Charles River, Wilmington, MA 01887.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (mg/kg/day)	Main Study 26 Months Male	Main Study 26 Months Female	Interim Sacrifice Months Male	Interim Sacrifice Months Female
1 Control	0	50	50	--	--
2 Low (LDT)	3	50	50	--	--
3 Mid (MDT)	10	50	50	--	--
4 High (HDT)	31	50	50	--	--

2. Diet Preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at day 1 and day 7 of each feed preparation.

Results - Diet analyses results show analytical levels were within ± 16 percent of nominal concentrations for all three dose levels during the entire study. Additionally, glyphosate was stable in the basal diet for the 1-week period of use with assays ranging from 97.4 to 116 percent with a mean value of 104 percent.

Homogeneity analyses of the top, middle, and bottom of the 30, 100, and 300 ppm diets showed the percents of planned diet averaged 95.8, 97.0, and 99.9 percent, respectively. The coefficients of variation were less the 6.3 percent for each dose level measurement.

3. Animals received food (Purina Lab Chow) and water ad libitum.

4. Statistics - Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios, and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically significant differences from control were set at $p < 0.05$. Statistical methods are attached (Appendix A).

009614

5. Quality assurance was signed by Craig Lamb on September 23, 1981.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality and weekly for detailed physical examination.

There were no compound-related toxic signs. The most frequent observations were alopecia, lacrimation, nasal discharge, and rales and occurred at comparable frequencies between control and treated rats of both sexes.

Results

Mortality (Survival) - Survival was approximately 80 to 90 percent through month 20. The study was terminated at month 26 when survival reached 30 percent in control males and high-dose females. The following Table from the report summarizes survival results.

Mortality[a]

Group mg/kg/day	Initial No. on Test	Month:	Number of Animals 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Total
Males																													
I 0.00	50		0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	2	1	2	1	4	4	1	8	2	4	3	35
II 3.05	50		0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	2	0	2	1	2	7	5	2	0	24
III 10.30	50		0	0	0	1	0	0	0	1	1	0	0	0	0	0	2	0	0	1	0	1	4	3	5	8	4	3	34
IV 31.45	50		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	5	2	4	4	6	1	24
Females																													
I 0.00	50		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	4	2	11	3	5	3	32
II 3.37	50		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	2	6	0	5	7	1	27
III 11.22	50		0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	2	1	4	1	5	2	2	0	22
IV 34.02	50		0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	4	3	2	3	4	6	1	6	3	35

[a] Includes animals dying spontaneously, accidentally, or killed in a moribund condition.

00019

2. Body Weight - They were weighed weekly for 14 weeks, then biweekly for remainder of study.

Results

Body Weights[a]

	Males			Dose (ppm)	Females			
Week	0	30	100	300	0	30	100	300
0	182± 10	182± 13	183± 11	183± 12	141± 10	138± 8	139± 9	137± 9
26	547± 53	547± 54 (100%)[b]	546± 51 (100%)	536± 46 (98%)	294± 32	293± 31 (100%)	288± 28 (98%)	287± 31 (98%)
52	664± 79	655± 75 (99%)	650± 68 (98%)	634± 64 (95%)	366± 57	356± 51 (97%)	347± 51 (95%)	354± 56 (97%)
78	724±104	725± 96 (100%)	699± 85 (97%)	691± 79 (95%)	427± 94	404± 71 (95%)	406± 65 (95%)	420± 87 (98%)
104	693±101	689± 88 (99%)	702± 96 (101%)	691± 89 (100%)	453±103	432±101 (95%)	438± 73 (97%)	444± 83 (98%)
T[c]	694±135	675±113 (97%)	664±113 (96%)	692± 94 (100%)	457±127	456± 91 (100%)	438± 81 (96%)	448±101 (98%)

[a] Data excerpted from submitted study. Values are mean ± std. dev., calculated by the investigators.
[b] Percent of control, calculated by reviewer.
[c] T = termination, week 110 for males, 112 for females.

There were no meaningful statistically significant or dose-related decreases in body weight or decreased body weight gains during the course of the study. The maximum decreased body weight ranged 2 to 6 percent less in treated males in comparison to controls during the intermediate months of the study. For females, these differences were statistically significant during months 20 and 21, but not dose-related. These minimal differences in body weight at such a late time period (> 3 months) and the lack of effect on animal survival are considered to not be toxicologically significant.

3. Food Consumption and Compound Intake - Consumption was determined weekly for first 14 weeks and biweekly thereafter, and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results - Food Consumption - Food consumption was comparable between control and treated rats of both sexes. Based on body weight and food consumption data, diets containing glyphosate were adjusted to achieve dietary levels of 3.05, 10.30, and 31.45 mg/kg/day in males and 3.37, 11.22, and 34.02 mg/kg/day in females.

4. Ophthalmological examinations were not performed.

5. Blood was collected before treatment and at 4, 8, 12, 18, and 24 months for hematology and clinical analysis from 10/sex/group animals. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*		Mean corpuscular (HGB conc. (MCHC)
X	Platelet count*		Mean corpuscular volume (MCV)

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium*	X	Albumin*
	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorus*	X	Cholesterol*
X	Potassium*	X	Globulins
	Sodium*	X	Glucose* (fasting)
	Enzymes	X	Total bilirubin* & direct bilirubin
X	Alkaline phosphatase	X	Total protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*		
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		

c. Urinalysis - Urine was collected from fasted animals at 4, 12, 18, and 24 months. The CHECKED (X) parameters were examined.

---

*Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

X		X	
X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

Results - Hematological, clinical chemistries, and urinalysis evaluations at 4, 8, 12, 18, and 24 months did not indicate any compound-related effects. The occasional statistically significant finding in a parameter was either not dose-related, within the range of historical controls, not consistently occurring over time, or was without toxicological significance.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenals*
X	Cecum*	XX	Kidneys*	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
	Rectum*	XX	Testes*	X	Parathyroids*
XX	Liver*	X	Epididymides	XX	Thyroids*
X	Gallbladder*	X	Prostate		Other
X	Pancreas*	X	Seminal vesicle	X	Bone* and bone marrow
	Respiratory	XX	Ovaries	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin
X	Lung*			X	All gross lesions and masses
				X	Blood smear
				X	Head
				X	Harderian gland

*Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

009614

Results

a. Organ Weight - There were no statistically significant, dose-related intergroup differences in absolute and organ to body weight ratios and organ to brain weight ratios in male and female treated rats in comparison to controls. Therefore, there were no compound-related effects in organ weight.

b. Gross Pathology - There were no compound-related effects in gross pathology. The postmortem findings occurred sporadically or were found in both control and treated rats and were not considered related to treatment.

c. Microscopic Pathology

1. Nonneoplastic

Microscopic examination revealed lymphocytic hyperplasia of the thymus occurring at statistically significant incidences in the mid- and high-dose female rats.

A statistical analysis was previously conducted "Test for Significance of Differences Between Proportions" (February 5, 1982).

Lymphocytic Hyperplasia

ppm	No. RESP	Total	% +/- 2(SD)	One Tail P Statistic Fisher's
0.000	5	25	20.00 +/- (17.63)	
30.000	13	32	40.63 +/- (18.58)	0.084
100.000	18	37	48.65 +/- (17.46)	0.020
300.000	17	34	50.00 +/- (18.28)	0.017

Test for a linear trend is not significant.

This lesion was not considered compound-related for the following reasons:

a) This lesion is known to occur spontaneously in older rats and is quite variable in the thymus.

b) There was no appreciable difference in the incidence of this lesion in the spleen, a much less variable indicator for lymphocytic hyperplasia.

c) The severity was similar for control and treated rats, ranging from minimal to moderate.

A clear dose response was not evident and there were no changes in the hematology parameters in treated animals which would confirm the findings of the relationship of these lesions to treatment.

2) Neoplastic

Males - The interstitial cell tumor in the testis of male rats was observed in the following groups as shown below:

Group I (control) 0/50
Group II (low dose) 3/50
Group III (mid-dose) 1/50
Group IV (high dose) 6/50

The occurrence of testicular interstitial tumors of 12 percent (6/50) in the high-dose group is statistically significant ($p = 0.013$).

To further examine these results, the historical control data for interstitial cell tumor of the testes were compiled. These control data include only those lifetime feeding studies with Charles River Sprague-Dawley rats conducted by Bio/Dynamics, Inc. which were tested concurrently with the present study, i.e., were completed within 9 months of termination of the present study, and lasted at least 24 months. For all male animals on test, the high-dose group incidence in the present study is 12 percent (6/50) and was slightly higher than the highest concurrent control incidence of 7 percent (5/75) and higher than the overall incidence of 4.5 percent (24/535).

Additional historical control data were obtained from Charles River Breeding Laboratories (Patricia Lang, 1985) from 24-month studies conducted between 1977 and 1985 using Sprague-Dawley rats provided by Charles River Breeding Laboratories. The data consisted of 11 groups of control animals from various laboratories.

Location & Tumor	No. Exam	No. Tumor	Percent	Range
Testis	880			
Interstitial cell tumor (B)		31	3.5	0 - 12.0
Interstitial cell tumor (M)		1	0.1	0 - 1.1
Interstitial cell tumor (NOS)		23	2.6	0 - 9.1

Individual studies are shown below.

Expanded Table of Testicular Tumors in CD® Rats: 24 Months

Tumor	Group A	B	C	D	E	F	G	H	I	J	K
N =	80	80	86	75	75	100	90	55	89	75	75
Interstitial cell tumor (B)	--	--	2	2	2	6	4	--	--	9	6
Interstitial cell tumor (M)	--	--	--	--	--	--	1	--	--	--	--
Interstitial cell tumor (NOS)	3	7	--	--	--	--	--	5	8	--	--

It can be seen from the Charles River Data Base that the upper end of the range reaches 12.0 percent which was the incidence level in the high-dose glyphosate group.

In view of the totality of data, Toxicology Branch (TB) agrees with the study pathologist, Dr. Martin G. Robl of EPL who states in the report: "The significance, if any, of the 12% incidence of interstitial cell tumor in the testis in the high dose group of male rats in this study in comparison to control group is not known. It may represent a biological variation in this strain of rats. The incidence of interstitial cell tumor in the testis in Group II and Group III of this study was similar to the incidence observed in the control groups of male rats in the other concurrent studies and did not appear to be related to the administration of the test compound in this study."

TB concluded that glyphosate was not carcinogenic to interstitial cells (Leydig cells) of the testes of male rats.

Females - It was observed that there was an increased incidence of C-cell carcinomas in female rats at the high dose in comparison to controls.

Incidence (Percent) of Sprague-Dawley Females Bearing Thyroid C-Cell Tumors of All Animals Examined

Group	Control	Low Dose	Mid Dose	High Dose
Tumor	0	3 (mg/kg/day)	10	30
Adenoma	5/47 (11)	3/49 (6)	6/50 (12)	3/47 (6)
Carcinoma	1/47 (2)	0/49 (0)	2/50 (4)	6/47 (13)
Adenoma or Carcinoma	6/47 (13)	3/49 (6)	8/50 (16)	9/47 (19)

The above table shows that the percent incidence of carcinomas for all female animals examined is 2 percent in the controls and 13 percent in the high-dose animals. Additionally, the percent incidence of adenoma and carcinoma combined in Table II shows that the controls (13%) are comparable to the high-dose (19%).

The time-to-tumors data also shows that the latency of tumors is not affected by treatment. Thyroid weights showed no treatment-related increases and thyroid tumors were not grossly observed except for female rat #831 which had thyroid carcinoma.

Time-to-Tumor Data of Animals/Moribund Sacrifice and Died on Study/Sprague-Dawley Female Thyroid Tumors

Group I - Controls

Animal Number	Tumors	Days	Weeks
225	Adenoma	702	100.3
229	Adenoma	629	89.9
234	Adenoma	699	100.0

Group II - Low-Dose

Animal Number	Tumors	Days	Weeks
443	Adenoma	703	100.4

Group III - Mid-Dose

Animal Number	Tumors	Days	Weeks
618	Adenoma	748	106.9
638	Adenoma	605	86.4
641	Carcinoma	677	96.7

Group IV - High-Dose

Animal Number	Tumors	Days	Weeks
803	Adenoma	689	98.4
820	Carcinoma	751	107.3
822	Adenoma	751	107.3
831	Carcinoma	778	111.1
834	Carcinoma	734	104.9
835	Carcinoma	652	93.1

The following table presents the Bio/Dynamics thyroid C-cell tumor historical control data on female Charles River albino (CD) rats.

Bio/Dynamics Thyroid C-Cell Tumor Historical Control Data:
Female Charles River Albino (CD) (Sprague-Dawley Rats)

Incidence (Percent) of Females Bearing Thyroid C-Cell Tumors All Animals Sacrificed Post 12 Months

Study	Adenoma or Carcinoma	Adenoma	Carcinoma
B			
Group A*	10/58 (17)	10/58 (17)	0/58 (0)
Group B	7/59 (12)	6/59 (10)	1/59 (2)
C			
Group A	5/59 (8)	5/59 (8)	0/59 (0)
Group B	6/85 (10)	6/58 (10)	0/58 (0)
I			
Group A	9/57 (16)	6/57 (11)	3/57 (5)
Group B	6/55 (11)	5/55 (9)	1/55 (2)
J			
Group A	2/58 (3)	2/58 (3)	0/58 (0)
Group B	0/55 (0)	0/55 (0)	0/55 (0)
L	1/53 (2)	1/53 (2)	0/53 (0)

*Studies #B, C, I, and J had two control groups per study, identified as Group A or B.

The historical control data from Bio/Dynamics presented above shows that the percent incidence of carcinomas varied from 0 to 5 percent, whereas

the percent incidence of adenomas or carcinomas varied from 0 to 17 percent.

With respect to the Charles River Breeding Laboratories Data Base (Patricia Lang, 1985) from 24-month studies conducted between 1977 and 1985 using Sprague-Dawley rats provided by Charles River Breeding Laboratories, the data consisted of 11 groups of control animals from various laboratories.

Location & Tumor	No. Exam.	No. Tumor	Percent	Range
Thyroid gland	869			
C-cell adenoma		36	4.1	0 - 13.5
Medullary carcinoma		10	1.2	0 - 4.0

It can be seen that the range of carcinomas is from 0 to 4.0 percent, similar to Bio/Dynamics.

Expanded Table of Thyroid Tumors in CD® Rats: 24 Months

Tumor	Group A	B	C	D	E	F	G	H	I	J	K
N =	78	80	86	75	74	98	90	55	86	73	74
Follicular cell adenoma	1	--	1	--	--	--	--	1	--	--	1
Follicular cell carcinoma	--	--	--	--	--	2	--	5	4	--	--
C-cell adenoma	1	1	1	4	3	8	--	--	--	8	10
Medullary carcinoma	--	--	2	3	2	3	--	--	--	--	--
Carcinoma, undifferent	--	--	--	--	--	--	5	--	--	--	--
Adenoma, (NOS)	1	--	--	--	--	--	2	--	--	--	--

Literature sources of C-cell thyroid tumors have been researched and provide the following information in Tables I through VI.

A spontaneous incidence of 22 percent C-cell tumors in Sprague-Dawley rats has been reported as shown in Table I (Table 12.2, Page 1056).

Tables I and II present the incidence of C-cell tumors in various strains of rats from published literature.

009614

Table I: Tumors of Rat Strains Thyroid, Parafollicular Cell*

Strain	Average Incidence (%)	(Months)	Comments
Buffalo	25	> 24	Increase with age
Fisher	22	> 24	Increase with age
Long-Evans	12 - 45	> 24	Increase with age
OM	33	> 24	Increase with age
Sprague-Dawley	22	> 24	Increase with age
Wistar	19	> 24	Increase with age

*Benvischke et al., Reference 1

Also, Tables I and II show the spontaneous incidence of C-cell tumors in other strains of rats.

Table II: Pathology of Aging Rats*

Summary of the Incidence of Medullary Thyroid Carcinomas and Metastases of Medullary Thyroid Carcinomas in Aging BN/Bi, WAG/Rij, and (WAG x BN) F_1 Rats.*

Strain	Sex	No. Examined	No. with Medullary Thyroid Carcinoma	Percent	Mean Age (Range) in Months	No. Medullary Thyroid Carcinoma with Metastases	Age (in months) of Rats with Metastatic Medullary Thyroid Carcinomas
Bn/Bi	Female	236	15	6	33 (17-38)	2	35, 38
	Male	74	7	9	27 (15-34)	0	
WAG/Rij	Female	101	47	47	35 (26-46)	5	35 (32-39)
	Male	124	41	33	23 (9-29)	1	29
F_1	Female	68	11	16	31 (17-38)	3	25, 27, 28
	Male	67	20	29	34 (22-42)	3	28, 30, 38

*Burek (1978), Reference 2

These references show a high spontaneous incidence of C-cell carcinomas in various strain of female rats.

00329

Other specific literature sources revealed the following information. Thompson and Hunt (1963) showed the following results:

Table III: Summary of Spontaneous Tumors Observed Upon Reexamination of Serial Sections of Selected Tissues from 177 (63 Males, 114 Females) Sprague-Dawley Rats

Type of Tissue and Tumor	No. of Organs Examined	Number of Tumors				Age in Days
		Single Section		Serial Section		
		Male	Female	Male	Female	
Thyroid light cell adenoma	140	4	5	24	31	300-960

The following quote is taken from their 1963 publication and illustrates the increase in tumors found by serial sectioning. "As depicted in Table 1, (Table III, above) a total of 55 lightcell adenomas (24 males, 31 females) were encountered upon re-examination of serial tissue sections of 140 thyroid glands (54 males, 86 females). Only nine of these tumors (four males, five females) were originally observed in single random tissue sections of the thyroid glands of 177 rats (63 males, 114 females). All the nodules were of similar histologic structure, being composed of epithelial cells with lepto-chromatic nuclei, surrounded by a pale, slightly eosinophilic cytoplasm. Mitotic figures were not common, and the cells tended to be organized into lobules. Follicles were not formed by the tumor cells. However, small colloid filled follicles were frequently seen within the substance of these tumors, but were thought to represent normal thyroid follicles which had become encompassed as the tumors enlarged. These nodules varied in size from a microscopic collection of light-cells to large nodules which almost completely replaced the thyroid gland. The smaller nodules were always observed in the central portion of the gland; never occurring at the periphery or in the isthmus. The nodules were frequently encountered in both lobes of the thyroid. The age range of the rats in which light-cell adenomas were observed was 300 to 960 days with a mean of 637 days."

Mackenzie and Garner (1973) presented the following information which shows the difficulty in assessing endocrine adenomas and carcinomas.

"A neoplasm was defined as a lesion with cellular architectural change; it expanded and compressed surrounding tissue noticeably. Size of tumor was not a criterion, if compressed tissue was demonstrable. Many tumors were microscopic and found on a single random section of each organ. No attempt was made to cut deeper into the blocks available, on the chance that additional small neoplasms might be uncovered. The criterion used to diagnose malignancy was the evidence of growth by invasion and/or metastasis. As the material submitted was often inadequate to demonstrate invasion, those tumors morphologically similar to known malignant tumors of the same type were also considered malignant. Neoplasms of the endocrine system, however, could not be classed accurately as benign or malignant by histology, and these are simply called adenomas."

MacKenzie and Garner (1973) examined six sources of rats and found the following results:

Table IV: Sources of Rats

Source and Identification	Number of Rats	Remarks
Sprague-Dawley, Inc. (Sprague-Dawley).	258	Colony originated in 1929. Closed colonies, random breeding.
Charles River, Inc. (Charles River - SD).	535	Original stock from Sprague-Dawley, Inc. Selectively random bred.
Holtzman, Inc. (Holtzman - SD).	208	Nucleus stock from Sprague-Dawley in 1946. Closed colony selectively random bred.
Diablo Animal Laboratories (Diablo-SD).	217	Nucleus stock from Holtzman, Inc. (Sprague-Dawley strain). Maintained closed colony, selectively random bred.

009614

Table IV: Sources of Rats (cont'd)

Source and Identification	Number of Rats	Remarks
Locally bred (Osborne-Mendel).	131	Nucleus stock from Food and Drug Administration Washington, DC. Bred as closed colony for 2 years for project.
Locally bred (Oregon)	673	Closed colony for over 30 years. Random breeding. Original stock of unknown origin.

Table V: Tumors and Organs of Origin in 2082 Rats of 6 Sources*

Tumors	Sprague-Dawley	Holtzman-SD	Charles River-SD	Diablo-SD	Osborne-Mendel	Oregon	Total
Number of Rats	258	268	535	217	131	673	2082
Thyroid:							
Light-cell adenoma	15	9	12	8	2	3	49
Follicular cell carcinoma			2				2

*MacKenzie and Garner (1973), Reference 7

Suzuki et al. (1979) showed the following results:

Table VI: Incidence and Location of Spontaneous Endocrine Tumors in Sprague-Dawley Rats Surviving for More Than 2 Years

Sex	Effective No. of Animals	No. of Tumor-Bearing Animals	Thyroid Medullary Carcinoma
Male	42	36 (86)	33 (79)*
Female	39	28 (72)	19 (49)

*Numbers in parentheses indicate percentage (%).

Suzuki et al. (1979) show a high incidence of medullary thyroid carcinomas (49%) in female Sprague-Dawley rats.

00332

D. References:

1. K. Benvischke, F.M. Garner, and T.C. Jones; PATHOLOGY OF LABORATORY ANIMALS (1978); Volume II; editors, Springer-Verlag pages 1231-1232.

2. J.D. Burek; PATHOLOGY OF AGING RATS (1978); CRC Press, Inc.; page 33.

3. A LIFETIME FEEDING STUDY OF GLYPHOSATE IN RATS; BDN-77-416; January 7, 1981.

4. AN ADDENDUM TO A LIFETIME FEEDING STUDY OF GLYPHOSATE IN RATS: Special Report MSL-2009; January 26, 1983.

5. H. Suzuki, U. Mohr, and G. Kimmerle, SPONTANEOUS ENDOCRINE TUMORS IN SPRAGUE-DAWLEY RATS; (1979); J. Cancer Res. Clin. Oncol. 95. 187-1961.

6. Thompson, S.W. and R.D. Hunt; SPONTANEOUS TUMORS IN THE SPRAGUE-DAWLEY RAT: Incidence rates some types of neoplasms as determined by serial section versus single section technics (1963) Ann. NY. Acad. Sci. 108:832-845.

7. MacKenzie, W.F. and F.M. Garner; COMPARISON OF NEOPLASMS IN SIX SOURCES OF RATS: (1973); J. Natl. Cancer Institute 50:1243-1257.

Consulting EPL pathologists, Dr. Martin G. Pobl and Dr. William E. Ribelin, addressed the issue of the increased incidence of C-cell carcinoma in high-dose female rats.

In a November 9, 1982 letter from Dr. Ribelin to Dr. Oleson of Monsanto, the following was stated:

"You recently asked me to send you a note regarding my interpretation of the significance of the incidence of thyroid C-cell (light cell) carcinomas in the high-dose level rats on the Bio/dynamics study of Roundup.

"The segregation of thyroid, and many other organs, proliferative lesions into hyperplasia, adenoma, and carcinoma will vary among pathologists. Indeed, when one considers the rat is merely a surrogate for man then the distinction between these three classes becomes even more nebulous. Carcinomas do not appear instantly but commence at stages when they are generally recognized only as hyperplasias, progress sometimes to adenomas, then occasionally proceed to adenocarcinomas. Thus, if one were dealing with a carcinogenic phenomena one would expect also an increase in C-cell hyperplasias and adenomas in the treated

group. This is not the case here. The percentage of both hyperplasias and adenomas is greater in the control females than in the high dose level females.

"If one combines the proliferative C-cell lesions of these groups in this study the following results:

Group	1 (Control)	4
Examined	47	47
Hyperplasia	19	18
Adenoma	5	3
Carcinoma	1	6
Total	25	27
Percent	53.2	57.4

"I find these differences insignificant and cannot ascribe any treatment effect from this data."

In a November 29, 1982 letter from Dr. Robl to Dr. Oleson of Monsanto, Dr. Robl states the following:

"This is in reply to your recent inquiry to EPL about 'A Lifetime Feeding Study of Glyphosate (Roundup® Technical) in Rats,' Bio/dynamics Project Number M-6, 77-2062 dated July 17, 1981, regarding c-cell changes in the thyroid. This letter also confirms our telephone conversation of November 18, 1982.

"I have reviewed the incidence of proliferative changes regarding thyroid c-cell changes in rats on this study. When evaluating proliferative changes in the endocrine system of rats for possible carcinogenic effects, the evaluation should include the comparison of the incidence of all the proliferative changes including hyperplasias, adenomas, and carcinomas. Granted, there is some difference in incidence of adenomas and carcinomas among some of the test groups in comparison to the control groups. However, the overall combined incidence of all the proliferative changes in the treated groups of animals is quite similar to the incidence in the control groups.

"If a carcinogenic effect was present, it would be expected that there would be a dose-related change in all aspects of proliferative changes. This was not evident when the incidence of all the proliferative changes of the c-cell was evaluated. The lung is often one of the most common sites for metastatic foci of c-cell tumors in the rat. Metastatic foci are a true

indication of malignancy in tumors. There were no metastatic foci present in the lungs of rats on this study.

"For reasons I have noted, it is my opinion that there does not appear to be a treatment-related effect upon the proliferative changes in the thyroid c-cell in this study."

Dr. Kasza recommended that the thyroid slides be reevaluated by Dr. Capen, an EPA consultant pathologist.

Relative to the Capen review, Dr. Kasza presented the following evaluation and conclusions; "Dr. Charles C. Capen, D.V.M., Ph.D.; Diplomate, American College of Veterinary Pathologists, has completed his investigation and basically he confirmed the diagnoses of the sponsor's pathologist; the tabulated results of Dr. Capen's investigation is shown below.

"Histopathologic Evaluation of Thyroid Glands
From Female Sprague-Dawley (C/D) Rats
Lifetime Feeding of Glyphosate

Thyroid Lesion*	Control (n = 47)	Low Dose (n = 49)	Medium Dose (n = 50)	High Dose (n = 47)
C-Cell Hyperplasia (Nodular and/or Diffuse)	19 (40%)	26 (53%)	25 (50%)	18 (38%)
C-Cell Adenoma	5 (11%)	3 (6%)	7** (14%)	3 (6%)
C-Cell Carcinoma	1 (2%)	0 (%)	1 (2%)	5*** (11%)

(n =) Number of thyroids available for microscopic evaluation.

*Diagnostic criteria used for thyroid C-cell lesions are given below.

**One previously diagnosed C-cell carcinoma (81-1168/603) was interpreted to be a C-cell adenoma according to criteria below.

***One previously diagnosed C-cell adenoma (81-1447/822) was interpreted to be multinodular chief cell hyperplasia of parathyroid gland; one C-cell carcinoma (81-1454/820) was interpreted to be a C-cell adenoma; one C-cell carcinoma (81-1454/824) was interpreted to be a C-cell adenoma; one C-cell adenoma (81-1231/828) was interpreted to be a C-cell carcinoma according to criteria below.

"The following are diagnostic criteria used for the interpretation of thyroid C-cell lesions in the rat:

"1. C-(parafollicular) cell hyperplasia: A nodular and/or diffuse increase of C-cells between thyroid follicles and/or within the follicular basement membrane. The C-cells appear normal with an abundant, lightly eosinophilic, granular cytoplasm and a round-to-oval nucleus with finely stippled chromatin. Cell boundaries often are indistinct. Solid accumulations of C-cells are less than the size of a colloid-distended follicle. C-cells (1-2 cell layers thick) within the basement membrane may compress individual thyroid follicles.

"2. C-(parafollicular) cell adenoma: Discrete, expansive mass or nodule of C-cells larger than a colloid-distended thyroid follicle. Adenomas are well-circumscribed or partially encapsulated from adjacent follicles that often are compressed to varying degrees. C-cells have an abundant cytoplasmic area that stains lightly eosinophilic and a round-to-oval nucleus with finely stippled chromatin. C-cells may be subdivided by fine connective tissue septae and capillaries into small clusters.

"3. C-(parafollicular) cell carcinoma: Extensive proliferation of C-cells with enlargement of one or both thyroid lobes. There is evidence of intrathyroid and/or capsular invasion by the proliferating C-cells, often with areas of hemorrhage and necrosis within the neoplasm. The malignant C-cells often are more pleomorphic (cuboidal, oval, spindle-shaped) than with the benign proliferative lesions and have indistinct boundaries of the lightly eosinophilic cytoplasmic area. Mitotic figures may be numerous in the more anaplastic carcinomas."

Dr. Kasza continued, "We concluded from his review that some tumor diagnoses were changed mainly from malignant to benign. This indicated that the interpretation of benign and malignant neoplasms in the thyroid of rats sometimes varies according to individual pathologists.

"Furthermore, a group of pathologists recently initiated a simplified method* to establish oncogenicity related to chemicals. Although this system has not yet received general acceptance, many highly competent pathologists agree with it. This system advocates grouping of neoplasms to determine he incidence in final analysis. The grouping of neoplasms took place on the consideration of their histogenetic origin.

---

*Working Paper entitled "Guidelines for Combining Benign and Malignant Neoplasms As An Aid In Determining Evidence of Carcinogenicity" (Attachment 8) discussed at the National Toxicology Program, (NTP) Board of Scientific Counselors' Meeting, September 23 and 24, 1982.

According to this recommendation the C-cell adenomas and C-cell carcinomas in rats should be combined in order to establish oncogenicity. This recommendation was based on findings in Fisher 344 rats; however, we have no reason to believe that diagnostic criteria would be any different for the strain (Sprague-Dawley) used in the glyphosate study. We agree with the recommendation of NTP. In addition, we also consider that the differentiation between benign and malignant C-cell tumors can somewhat differ based on varying criteria of individual pathologists.

"Considering the above-mentioned two facts (Dr. Capen's diagnoses and the National Toxicology Program recommendation) we feel that we should combine thyroid benign and malignant C-cell tumors in order to evaluate the oncogenic potential of glyphosate in this rat lifetime study. When the combined incidence is compared there are no statistically significant differences between control (6/47) and test groups (3/49, 8/50, and 8/47)."

Based on all of the above information, Toxicology Branch concluded that C-cell thyroid carcinomas in high-dose female rats were not compound-related.

Attachment

009614

R:62817:Dykstra:LHED-7:KEVRIC:04/22/91:PERM:EK/CL:WO:CL
R:62824:Dykstra:LHED-7:KEVRIC:05/09/91:06/07/91:CL:WO:CL

00033

GLYPHOSATE Sh # 103601

Page_____ is not included in this copy.

Pages 39 through 40 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

009614

Reviewed by: William Dykstra, Ph.D. William Dykstra 5/14/91
Section 1, Tox. Branch I, IRS, H7509C
for Secondary reviewer: Roger Gardner, Section Head Pamela M. Hurley 5/14/91
Section I, Tox. Branch I, IRS, H7509C

DATA EVALUATION REPORT

STUDY TYPE: 83-3, teratology rat TOX. CHEM. NO.: 661A

ACCESSION NUMBER: N/A MRID No. 00046362

TEST MATERIAL: glyphosate, technical; 98.7 purity; Lot XHJ-64; white powder

SYNONYMS: Roundup

STUDY NUMBER (s): IRDL No. 401-054

SPONSOR: Monsanto Co., St. Louis, MO

TESTING FACILITY: IRDL, Mattawan, MI

TITLE OF REPORT: Teratology Study in Rats

AUTHOR(s): Dean E. Rodwell, Director of Teratology

REPORT ISSUED: March 21, 1980

Technical glyphosate was tested in a developmental toxicity study in rats at the following dose levels: 0, 300, 1000 or 3500 mg/kg bw/day.

CONCLUSIONS: The developmental NOEL is 1000 mg/kg/day, (mid-dose). The development LEL is 3500 mg/kg/day (high-dose). Although the findings at 3500 mg/kg/day include more malformed fetuses (10) than in the controls (3), the number of litters with malformed fetuses was the same (3) for both groups. Therefore, this was not considered an effect. The effects were an increase in the number of litters and fetuses with unossified sternebrae, and a decrease in fetal body weight at the LEL.

The maternal NOEL IS 1000 mg/kg/day. The maternal LEL was 3500 mg/kg/day and the effects were 28% decrease in body weight gain, toxic signs, and six deaths.

Classification: Core-guideline

Special Review Cr eria (40 CFR 154.7) N/A

Testing Guideline Satisfied: 83-3 (rat)

00041

009614

Review

1. A Teratology Study in Rats
(IRDC No. 401-504; March 21, 1980)

Test material: glyphosate, technical; 98.7% purity;
Lot XHJ-64; white powder; source: Monsanto Co.

A Quality Assurance Statement was signed by Barry W. Benson on 3/20/80. This study was conducted prior to the publication of the EPA GLP's.

Animals: Approximately 14 week old Charles River COBS SD CD rats (The Charles River Breeding Labs, Inc., Portage, Michigan) were used in this study. All rats were individually housed in a controlled environment and fed Purina Rodent Laboratory Chow #5001 and tap water ad libitum. One female Sprague-Dawley rat was mated to one male Sprague-Dawley rat. The day that mating was detected (copulatory plug on vaginal sperm) was designated day 0 of gestation.

Randomized groups of 25 mated Sprague-Dawley rats were dosed daily during days 6-19 of gestation at a constant volume of 10 ml/kg with 0 (control, vehicle: 0.5% aqueous methocel), 300, 1000 or 3500 mg/kg BW of test material. Individual doses were determined from individual body weights on gestation day 6. No rationale was given on the selection of dose levels. The dosages were prepared daily as a suspension with a magnetic stirring bar maintaining the suspension during dosing.

Methods:

1. Observations
Dams were observed daily for toxic sign, deaths, moribundity. Deceased animals were necropsied.

Results:

There were no compound-related maternal effects at 300 and 1000 mg/kg/day. At the high-dose of 3500 mg/kg/day, all of the dams (except three) were observed at least once to display diarrhea, soft stool, breathing rattles, inactivity, and red matter in the region of nose, mouth, forelimbs, or dorsal head. There were six deaths. One each on gestation day 10 and 17 and two each on gestation day 11 and 12. The cause of death could not be determined.

2. Body weight, caesarean section, maternal and fetal observations

Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20.

On gestation day 20, all surviving females were sacrificed and the uterus was excised and weighed. The locations of viable, nonviable fetuses, early and late resorptions, and the total number of implantations and corpora lutea were counted. The abdominal and visceral cavities of dams were examined for gross

00042

lesions.

All fetuses were individually weighed and examined for external malformations. Each fetus was sexed. One half of the fetuses from each litter were placed in Bouin's fixative for subsequent visceral examination by the Wilson method. The other half of the fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by the Dawson method for skeletal examination.

Results

1. Body weight. There were no compound-related effects in maternal body weight at 300 and 1000 mg/kg/day. At 3500 mg/kg/day, there was a 28.5% decrease in body weight gain during the 0-20 day test period, primarily due to a body weight loss during gestation days 6-9. These results are shown below.

Summary of Group Mean Maternal Body Weights and Body Weight Change

Day of Gestation	Control (0 mg/kg/day)		Technical Glyphosate 300 mg/kg/day		1000		3500	
	Group Mean Maternal Body Weights							
	Mean	S.D.	Mean	S.D	Mean	S.D.	Mean	S.D.
0	270	±14.4	270	±22.8	274	±15.9	261	±16.8
6	297	±16.9	295	±22.9	302	±18.5	288	±15.8
9	305	±17.7	303	±23.7	307	±16.5	275	±19.4
12	318	±19.3	316	±26.2	322	±17.7	299	±21.4
16	350	±19.1	346	±32.6	352	±21.3	326	±25.3
20	416	±23.1	403	±47.0	416	±22.1	373	±33.1
	Group Mean Maternal Body Weight Change (grams)							
0 to 6	27	-	25	-	28	-	27	-
6 to 9	8	-	8	-	5	-	-13	-
9 to 12	13	-	13	-	15	-	24	-
12 to 16	32	-	30	-	30	-	27	-
16 to 20	66	-	57	-	64	-	47	-
0 to 20	146	-	133	-	142	-	112	-

S.D. - Standard Deviation
- Not applicable

2. Caesarean Section Results

At 300 and 1000 mg/kg/day, there were no toxicologically significant effects in mean number of viable fetuses, late or early resorptions, postimplantation loss, corpora lutea, the fetal sex distribution or fetal body weight. The decreases observed at 300 mg/kg/day in viable fetuses/dam and total implantations per dam were not considered compound-related since they were not dose-related. At 3500 mg/kg/day, the following

effects were noted: Statistically significant decreases in viable fetuses/dam and total implantations/dam and mean fetal body weight. These findings are shown below:

Summary of Group Mean Maternal and Fetal Observations at Cesarean Section

Technical Glyphosate (mg/kg/day)

	0			300			1000			3500		
	No.	%	S.D.	No.	%	S.D.	No.	%	S.D.	No.	%	S.D.
Animal on Study:	25	-	-	25	-	-	25	-	-	25	-	
Animals that were gravid:	22	88.0	-	20	80.0	-	21	84.0	-	23	92.0	
Animals that died:	0	0.0	-	0	0.0	-	0	0.0	-	6	24.0	
Mongravid:	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	
Gravid:	0	0.0	-	0	0.0	-	0	0.0	-	6	100.0	
Animals examined at Cesarean Section:	25	100.0	-	25	100.0	-	25	100.0	-	19	76.0	-
Mongravid:	3	12.0	-	5	20.0	-	4	16.0	-	2	10.5	-
Gravid:	22	88.0	-	20	80.0	-	21	84.0	-	17	89.5	-
Dams with resorptions only:	0	0.0	-	0	0.0	-	0	0.0	-	1	5.9	-
Dams with Viable fetuses:	22	100.0	-	20	100.0	-	21	100.0	-	16	94.1	-
Viable Fetuses/Dam:	14.4	-	±1.26	11.9*	-	±4.36	14.3	-	±2.08	11.5*	-	±4.72
Post Implantation loss/dam:	0.6	-	±0.90	0.2	-	±0.52	0.5	-	±0.81	1.2	-	±1.25
Total implantation /Dam:	15.0	-	±1.11	12.1**	-	±4.45	14.8	-	±2.21	12.8*	-	±3.77
Corpora Lutea/Dam:	15.9	-	±1.67	15.2	-	±3.30	16.1	-	±1.81	14.8	-	±1.64
Fetal sex distribution - Male:	159	50.3	-	119	50.2	-	168	56.0	-	97	49.5	-
-Female:	159	49.0	-	118	49.8	-	132	44.0	-	99	50.5	-
Mean fetal body weight (grams):	3.0	-	±0.21	3.7	-	±0.66	3.6	-	±0.19	3.2**	-	±0.34

*Significantly different from control group, mean p<0.05.

**Significantly different from control group, mean p<0.01.

S.D. - Standard deviation

- Not applicable

009614

3. Fetal Morphological observations

There were no malformations in the 300 and 1000 mg/kg/day groups. Three control litters (dam #19999, 20002 and 20016) and three litters from the high-dose (dams # 20083, 20091 and 20096) had malformed fetuses. Additionally, an increase in the number of litters and fetuses with unossified sternebrae was observed at 3500 mg/kg/day. Although the same number of litters with the malformed fetuses occurred in the control and high-dose group, several fetuses with either the anomaly classified as dwarfism or bent tails were found in single litters. As a result, there were more malformed fetuses in the high-dose group (10 fetuses) than in the controls (3 fetuses). Bent tail and dwarfism have occurred in several fetuses in a single litter from TRDC historical controls. These results are shown below.

Summary of the Incidence of Fetal Malformations and Developmental and Genetic Variations					
Glyphosate (mg/kg/day)		Control		Technical	
1000	3500	(0 mg/kg/day)		300	
No. of litters examined:	32	10	21	16	
No. of fetuses examined externally:	316	237	300	196	
No. of fetuses examined vicerally:	155	119	150	97	
No. of fetuses examined skeletally:	161	118	150	99	

00045

Malformations Observed	Fetuses No.	%	Litters No.	%	Fetuses No.	%	Litters No.	%	Fetuses No.	%	Litters No	%	Fetuses No.	%	Litters No.	%
1 mm vesicle over posterior fontenelle:													1	0.5	1	6.3
Brain Anomaly:	1	0.3	1	4.5												
Dwarfism[3]													3	1.5	1	6.3
Rib forked:	1	0.3	1	4.5												
Tail threadlike on anus:	1	0.3	1	4.5												
Tailbent													6	8.1	1	6.3
TOTAL MALFORMATION		No.	%			No	%			No	%			No	%	
Fetuses with soft tissue malformation		2	0.5			0	0.			0	0.			1	3.5	
Fetuses with skeletal malformations:		1	0.3			0	0.0			0	0.0			9	4.6	
TOTAL fetuses with malformations:		3	0.9			0	0.			0	0.			10	5.1	
Litters with soft tissue malformations:		2	9.1			0	0.			0	0.			2	12.3	
Litters with skeletal malformations:		1	4.5			0	0.			0	0.			2	12.3	
Total Litters with malformations:		3	13.6			0	0.			0	0.			3	18.9	

009614

Developmental and Genetic Variations Observed	Fetuses No	%	Litters No	%	Fetuses No.	%	Litters No	%	Fetuses No	%	Litters No		Fetuses No	%	Litters No	%
27 presacral vertebrae					1	0.8	1	5.3								
14th rudimentary rib(s)	1	11.1	9	40.9	19	16.1	8	40.6	25	16.7	14	50.7	13	13.1	8	50.0
7th cervical rib:	1	0.6	1	4.5	1	0.8	1	5.3								
Hyoid unossified:	2	1.2	2	9.1					6	1.7	3	14.3				
Reduced Ossification of Skull:	1	0.6	1	4.5	2	1.7	2	10.5	1	0.7	1	4.8				
Sternebrae #5 and/or #6 unossified:	13	8.1	8	34.4	7	5.9	5	6.3	17	11.3	8	38.1	18	18.1	21	68.8
Other Sternebrae unossified:	1	0.6	1	4.5									5	6.1	3	18.2
Retroesophageal right subclavian:													1	1.3	2	6.3
Renal papilla not developed and/or distended ureter	3	1.9	3	13.5	1	0.8	1	5.0	4	2.7	3	14.3	4	4.1	4	15.3
* P<0.05																

The following table gives historical control data for this particular strain of rat in the same testing laboratory.

00047

009614

IRDC HISTORICAL CONTROL DATA/ Charles River CD® (SD) Rats	
Summary of the Incidence of Malformations and of Developmental and Genetic Variations	
No. of litters examined	524
Total no. of fetuses examined externally:	6955[b]
Total no. of fetuses examined skeletally:	4351
Total no. of fetuses examined viscerally:	2602
Malformations Observed	No. of Fetuses (Litters)
Thread-like tail, small anus	1 (1)
Rib anomalies:	14 (3)
Anophthalmia or microphthalmia:	4 (4)
Scoliosis:	1 (1)
Malformed mandible:	1 (1)
Bent tail:	5 (1)
Multiple anomalies:	2 (2)
Fatal anasarca:	1 (1)
Diaphragmatic hernia:	1 (1)
Fused sternebrae:	1 (1)
Great vessel anomalies:	1 (1)
Dwarfism:	5 (1)
Tympanic ring malformed or absent:	3 (1)
Total no. of fetuses (litters) with malformations:	38 (27)
Variations - Developmental and Genetic Observed	
27 presacral vertebrae:	31 (27)
25 presacral vetebrae:	2 (2)
12 full pair of ribs with 13th rudimentary rib(s) or 13th unilateral full rib:	7 (7)
14th rudimentary rib(s):	796 (306)
14th full rib(s)	13 (12)
7th cervical rib(s)	8 (7)
Extra ossification distal to 14th rib:	1 (1)
Scapula variation:	1 (1)
Sternebrae misaligned:	3 (3)
Sternebrae #5 and/or #6 unossified	550 (249)
Other sternebrae unossified	31 (26)
Entire sternum unossified	2 (2)
Skull reduced in ossification:	58 (37)

(Variations - Developmental and Genetic Observed) cont'd pg 9)

00248

Variations - Developmental and Genetic Observed (cont'd)	No. of Fetuses (Litters)
Hyoid unossified:	48 (31)
Vertebrae reduced in ossification:	5 (5)
Metacarpals or metatarsals unossified:	1 (1)
Entire skeleton reduced in ossification:	1 (1)
Renal papillae not developed and/or distended ureter:	53 (46)
Pubis unossified:	2 (2)

[b] Includes two fetuses that were sent to histology and were not included in the number of fetus examined skeletally or viscerally.

STATISTICAL ANALYSIS

All statistical analyses compared the treatment groups to the control group, with the level of significance at $p<0.05$.

The male to female fetal sex distribution and the number of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel to judge significance of differences.

The mean number of viable fetuses, total implantatons, corpora lutea and mean fetal body weights were compared by analysis of variance (one-way classification), Bartlett's test for homogenity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

DISCUSSION:

1. Maternal Toxicity: decrease in body weight gain, toxic signs and death.

2. Developmental Toxicity: decreases in fetal body weight, increase in the number of litters and fetuses with unossified sternebrae, decreases in viable fetuses/dam and decreases in total implantations/dam.

Study Deficiencies: No major deficiencies.

Core Classification: Core Guideline Data
Maternal NOEL = 1000 mg/kg/day
Maternal LOEL = 3500 mg/kg/day
Developmental Toxicity NOEL = 1000 mg/kg/day
Developmental Toxicity LOEL = 3500 mg/kg/day.

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/20/91
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Roger Gardner, Section Head Pamela M. Hurley 5/20/91
for Section I, Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: 83-3 - Teratology - Rabbit TOX Chem. No.: 661A

Accession No.: N/A MRID No.: 00046363

Test Material: Glyphosate, Technical; 98.7% Purity; White Powder; Lot XHJ-64

Synonyms: Roundup

Study No.: IRDC No. 401-056

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: IRDC, Mattawan, MI

Title of Report: Teratology Study in Rabbits.

Author: Dean E. Rodwell, M.S., Director of Teratology

Report Issued: February 29, 1980

Conclusions:

Glyphosate was tested in a developmental toxicity study in rabbits in which the animals received by gavage dosages of 0, 75, 175, and 350 mg/kg/day during days 6 to 27 of gestation.

The developmental toxicity NOEL was 350 mg/kg/day (HDT). The maternal toxicity NOEL was 175 mg/kg/day (mid-dose). The LEL was 350 mg/kg/day (HDT) and the effects were increased incidences of soft stool, diarrhea, nasal discharge, and death (10 does died on day 21).

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7): N/A

Testing Guideline Satisfied: 83-3 (Rabbit)

00250

009614

Review:

A Teratology Study in Pabbits (IRDC No. 401-056; February 29, 1980).

Test Material - Glyphosate, Technical; 98.7% purity; Lot No. XHJ-64; White Powder; Source: Monsanto Company.

A Quality Assurance Statement was signed by Barry W. Benson on February 21, 1980.

Animals - Virgin female Dutch Belted rabbits were purchased from Longshaw Farms, Augusta, Michigan at age 7 months. The animals were individually caged in controlled environment and received Purina Rabbit Chow Checkers 5301 and tap water ad libitum.

The female rabbits were artificially inseminated from semen from four proven male rabbit donors. Semen from one male was used to inseminate an equal number of females in each group. The day of insemination was designated as day 0 of gestation. The semen had been collected using an artificial vagina, evaluated for motility, and diluted with 0.9% sodium chloride solution prior to introduction into the anterior vagina of the female using an insemination pipette. Immediately after insemination, ovulation was induced by an injection of 100 units of chorionic gonadotropin into each female.

Methods:

Randomized groups of 16 rabbits were inseminated. Following insemination, single oral daily doses of test material were administered by gavage during days 6 to 27 of gestation at dosages of 0 (control: 0.5% aqueous methocel), 75, 175, and 350 mg/kg/day. A constant volume of 1 mL/kg was administered. No rationale for dose selection was given. The test article was suspended in vehicle daily. A magnetic stir bar and plate were used during administration to keep the material in suspension.

The does were observed daily for toxicity and mortality. Maternal body weights were determined on gestation days 0, 6, 12, 18, 24, and 28. Food consumption was not measured.

On gestation day 28, all surviving females were sacrificed. Does not surviving to the scheduled sacrifice were necropsied in an attempt to determine the cause of death. The uterus was examined, weighed, and the fetuses were removed. The number and location of viable fetuses, early and late resorptions, and the total number of implantations and corpora lutea were counted. The abdominal and thoracic cavities and viscera of the does were examined for gross lesions.

00031

All fetuses were individually weighed and examined for external malformations. Each fetus was dissected, internally sexed; and examined for visceral malformations, including the brain by a mid-coronal slice. The heart was dissected by Staples method. The eviscerated, skinned fetuses were fixed in alcohol, macerated in KOH, and stained with Alizarin Red S by the Dawson method for skeletal examination.

Statistical Analysis:

All statistical analyses compared the treatment groups to the control group, with a level of significance at $p < 0.05$.

The male to female fetal sex distribution and the number of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel to judge significance of differences.

The number of early and late resorptions and postimplantation loss were compared by the Mann-Whitney U-test as described by Siegal and Weil to judge significance of differences.

The mean number of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

Quality Assurance:

A signed quality assurance statement was provided. This study was conducted prior to the publication of the EPA GLP's. No GLP statement was provided.

Results:

1. Maternal Toxicity and Mortality - Soft stool or diarrhea was noted in all groups with a slight increase at 175 mg/kg/day and at least once in each rabbit of the 350 mg/kg/day group. A definite increase in nasal discharge was also noted in the 350 mg/kg/day group.

   As stated in the report:

   "Two rabbits in the control group aborted and were sacrificed, both on gestation day 22. One rabbit in the 75 mg/kg/day dosage group died on gestation day 26. In the 175 mg/kg/day dosage group, one rabbit aborted and was sacrificed on gestation day 27 and two rabbits died, one each on gestation days 22 and 25. One rabbit in the

350 mg/kg/day dosage group aborted and was sacrificed on gestation day 23 and 10 died by gestation day 21. One rabbit in this group died on gestation day 3. On the same day, a replacement female was selected and artificially inseminated.

"A cause of death was determined at necropsy for five rabbits only as indicated below:

Dam No.	Dosage Level (mg/kg/day)	Death attributed to:
2243	75	pneumonia
2267	175	gastroenteritis
2286	350	enteritis
2278	350	respiratory disease
2380	350	gasroenteritis and caecal ulcerations

"Causes of death for the other eight rabbits could not be determined at necropsy."

2. Maternal Body Weight - There were no toxicologically significant differences in mean body weight among control and treated groups as shown below:

Summary of Group Mean Maternal Body Weights and Body Weight Change

	Control (0 mg/kg/day)		Technical Glyphosate (mg/kg/day) 75		175		350	
Day of Gestation	Group Mean Maternal Body Weights (grams)							
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	2958	±146.6	2876	±176.3	2983	±157.5	2834	±196.9
6	2988	±177.5	2937	±187.0	3012	±206.9	2875	±232.1
12	3039	±165.6	2986	±191.5	3029	±216.1	2732	±330.5
18	3072	±166.4	3002	±213.4	2959	±276.6	2827	±317.2
24	3038	±182.3	3005	±219.9	2914	±321.2	2999	±315.1
28	3030	±231.7	3008	±142.7	2958	±307.5	2948	±238.7
Days of Gestation	Group Mean Maternal Body Weight Change (grams)							
0 to 6	30	-	61	-	29	-	41	-
6 to 12	51	-	49	-	17	-	-143	-
12 to 18	33	-	16	-	-70	-	95	-
18 to 24	-34	-	3	-	-45	-	172	-
24 to 28	- 8	-	3	-	44	-	-51	-
0 to 28	72	-	132	-	-25	-	114	-

3. Cesarean Section Data - There were no toxicologically significant differences between control and treated groups for the parameters evaluated. The summarized data are shown below:

Summary of Group Mean Maternal and Fetal Observations at Cesarean Section

	Control (0 mg/kg/day)			Technical (mg/kg/day) 75			175			350		
	No.	%	S.D.	No.	%	S.D.	No.	%	S.D.	No.	%	S.D.
Animals on study:	16	-	-	16	-	-	16	-	-	17	-	-
Animals that were gravid:	14	87.5	-	16	100.0	-	14	87.5	-	16	94.1	-
Animals that died:	0	0.0		1	6.3	-	2	12.5	-	10	58.8	-
Nongravid:	0	0.0	-	0	0.0	-	0	0.0	-	1	10.0	-
Gravid:	0	0.0	-	1	100.0	-	2	100.0	-	9	90.0	-
Animals that aborted:	2	14.3	-	0	0.0	-	1	7.1	-	1	6.3	-
Animals examined at cesarean section:	14	87.5	-	15	93.8	-	13	81.3	-	6	35.3	-
Nongravid:	2	14.3		0	0.0	-	2	15.4	-	0	0.0	-
Gravid:	12	85.7		15	100.0	-	11	84.6	-	6	100.0	-
Viable fetuses/dam:	5.3	-	±2.73	7.60	-	±1.86	5.9	-	±2.77	6.3	-	±2.35
Postimplantation loss/dam:	0.7	-	±0.99	0.4	-	±0.63	0.2	-	±0.40	0.8	-	±1.33
Total implantations/dam:	5.9	-	±2.30	8.0	-	±1.86	6.1	-	±2.84	7.2	-	±2.93
Corpora lutea/dam:	9.0	-	±2.13	10.1	-	±1.66	10.5	-	±3.65	8.5	-	±1.07
Fetal sex distribution - males:	28	44.4	-	53	46.5	-	32	49.2	-	17	44.7	-
females:	35	55.6	-	61	53.5	-	33	50.8	-	21	55.3	-
Mean fetal body weight (grams):	33.6	-	±7.77	30.9	-	±6.63	29.9	-	±7.28	29.3	-	±6.82
Resorptions/dam Early	0.4	-	±0.90	0.3	-	±0.59	0.1	-	±0.30	0.5	-	0.84
Late	0.3	-	±0.45	0.1	-	±0.35	0.1	-	±0.30	0.3	-	0.84

Nonviable fetuses were not present in any group.

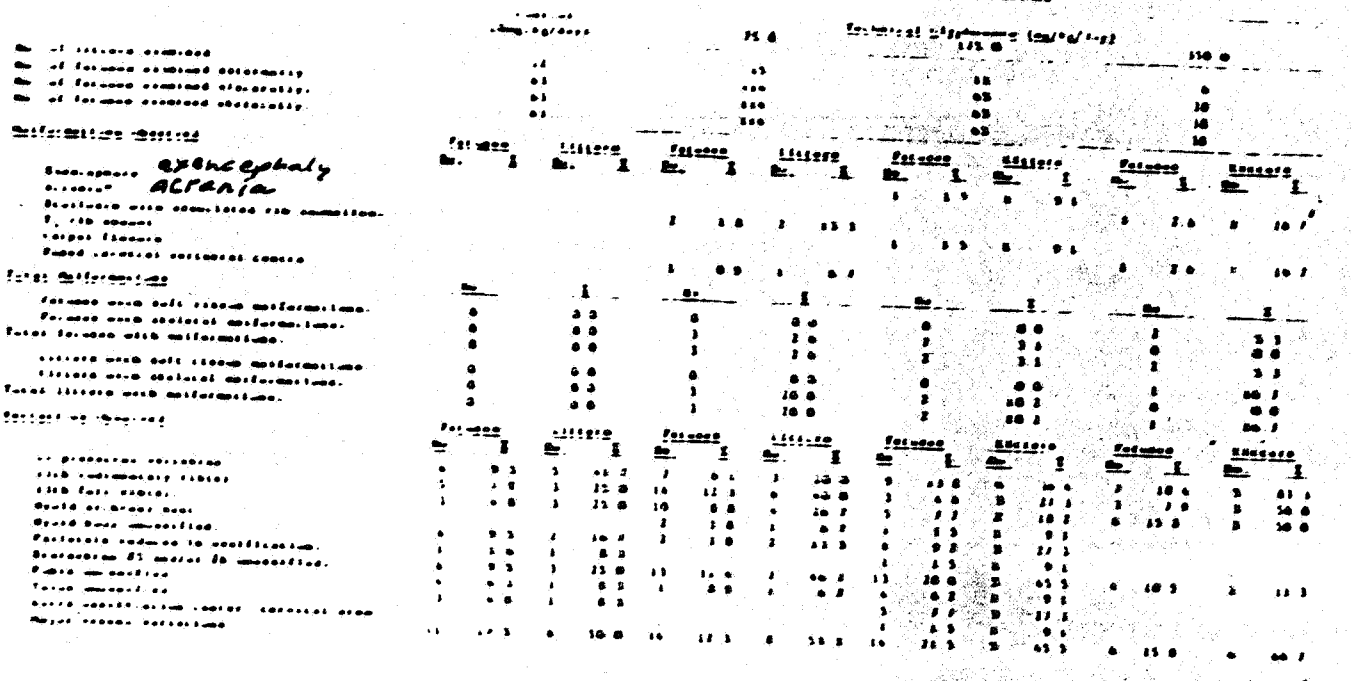
The statistically significant increase in viable fetuses/dam at 75 mg/kg/day in comparison to control (7.6 at 75 mg/kg vs. 5.3 in control) was not considered toxicologically significant since it was not dose-related.

The slightly decreased mean fetal body weight in all treated groups in comparison to the concurrent control was not considered toxicologically significant, since the historical control fetal body weight (30.9 grams for 160 fetuses) was comparable to the mean body weight in the treated groups.

4. Fetal Morphological Data - There were no compound-related malformations in fetuses from litters of treated rabbits in comparison to controls. Although there were no malformations in the controls, the malformations which were observed in the treatment groups did not occur in a dose-related pattern, were not similar in

009614

type, and the frequency did not exceed the historical controls. The data are shown below:



009614

Additionally, the incidences of percent litter and fetal variations were comparable between control and treated groups.

The following table gives historical control data from the same laboratory (dates not given).

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
HISTORICAL CONTROL DUTCH BELTED RABBITS

Summary of the Incidence of Malformations and of Developmental and Genetic Variations	
Number of litter examined:	23
Total number of fetuses examined externally:	161
Total number of fetuses examined skeletally:	161
Total number of fetuses examined for soft tissue:	161
	Number of Fetuses (No. of Litters)
Malformations Observed	
Scoliosis with or without associated rib anomalies:	1 (1)
Vertebral anomalies other than scoliosis:	1 (1)
Additional ossification of the sternum:	1 (1)
Carpal and/or tarsal flexures:	1 (1)
Kidney and/or ureter anomalies:	1 (1)
Hydrocephaly:	1 (1)
Heart anomalies:	1 (1)
Spleen and pancreas absent, stomach on right side:	1 (1)
Total number of fetuses (no. of litters) with malformations:	6 (6)
Variations-Developmental and Genetic Observed	
27 presacral vertebrae:	14 (7)
13th rudimentary rib(s):	6 (6)
13th full rib(s):	13 (5)
Sternebrae #5 and/or #6 unossified:	9 (7)
Sternebrae misaligned with or without fusion:	2 (2)
Reduced ossification of the skull:	1 (1)
Accessory skull bone(s):	1 (1)
Misshapen, misaligned vertebral centra:	2 (2)
Major vessel variations:	14 (7)
Gallbladder variations:	2 (2)

Discussion:

1. Maternal Toxicity: Maternal toxicity was evident at the highest dose level. The effects included soft stool or diarrhea, nasal discharge, and death.

2. Developmental Toxicity: There were no toxicologically significant signs of developmental toxicity at any dose level.

Study Deficiencies

The major deficiency in this study is that 10 does died in the high-dose group. Thus, only six survived to full term and only six litters were examined. This decreases the confidence in the results.

Core Classification: Core Minimum Data

Maternal NOEL = 175 mg/kg/day

Maternal LOEL = 350 mg/kg/day

Developmental Toxicity NOEL = 350 mg/kg/day (HDT)

009614

R:62820:Dykstra:LHED-05:KEVRIC:04/23/91:05/19/91:aw:WO:CL
R:62828:Dykstra:LHED-05:KEVRIC:05/17/91:06/13/91:aw:wo:aw

00358

009614

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/14/91
Section I, Toxicology Branch I - IRS (H7509C)
for Secondary Reviewer: Roger Gardner, Section Head Ronald M. Hurley 5/14/91
Section I, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 83-4, Two-Generation Reproduction - Rat

TOX Chem. No.: 661A

Accession No.: 245909

MRID No.: 00105995

Test Material: Glyphosate; technical; 98.7% purity; Lot No. XHJ-64

Synonyms: Roundup

Study Number: Bio/dynamics Project No. 77-2063 (BDN-77-417)

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Bio/dynamics, East Millstone, NJ

Title of Report: A Three-Generation Reproduction Study With Glyphosate in Rats.

Authors: Raymond E. Schroder, Study Director; March 31, 1981

Report Issued: July 31, 1981

Conclusions:

Glyphosate was tested in a three-generation reproduction study in the rat at the following dose levels: 0, 3, 10, and 30 mg/kg/day.

The NOEL reproductive is 10 mg/kg/day. The reproductive LEL is 30 mg/kg/day and the effect is increased incidence of focal tubular dilation of the kidney (both unilateral and bilateral combined) of male F_{3b} weanlings (pups). The incidence of this lesion in male pups was 2/10, 5/10, 3/10, and 8/10 in the control, low-, mid-, and high-dose groups, respectively. There were no other treatment-related effects on growth, fertility, gestation, lactation indices, pup survival, pup body weight, organ weights, or histopathology in adults and pups up to 30 mg/kg/day (HDT). The systemic NOEL is 30 mg/kg/day.

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7): N/A

00059

009614

Review:

A Three-Generation Reproduction Study With Glyphosate in Rats (Bio/dynamics Project No. 77-2063 (BDN-77-417); July 31, 1981).

Test Material - Technical Glyphosate; 98.7% purity; Lot No. XHJ-64; white powder.

Animals - Sprague-Dawley young rats, age 28 days, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA 01887. The rats were 43 days old at study initiation. They were individually caged (except during mating and lactation) and received Purina Lab Chow #5001 and tap water ad libitum. Nesting material - hardwood shavings - was added to cages on Day 19 of gestation and changed when wet or soiled through Day 14 of lactation.

Methods:

1. Mating - One male and two females of equivalent dose levels were caged together nightly until a sign of mating (sperm and/or copulation plug in the vagina) was observed or until 15 days had elapsed with no evidence of mating. Care was taken during matings of F_1 and F_2 generations to avoid brother-sister mating. The day on which evidence of mating was observed was defined as Day 0 of gestation.

2. Experimental Outline

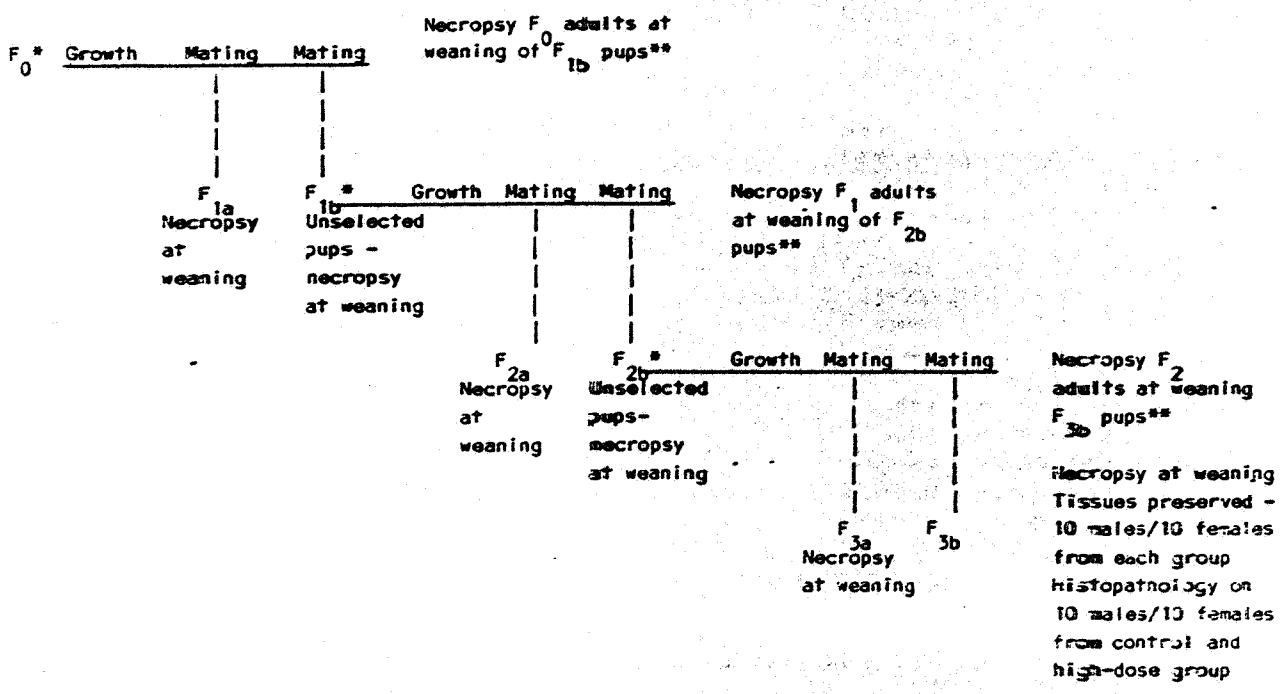
Group	Dose Level (mg/kg/day)	No. of Adults Initially Assigned to Mate (F_0, F_1, F_2) Males	Females	No. of Matings Per Generation (F_0, F_1, F_2)	Adults-Offspring Gross Post-mortem Examination	Histopathology (F_0, F_1, F_2, F_{3b}) Males	Females
I	0	12	24	2	All	10	10
II	3	12	24	2	All	-	-
III	10	12	24	2	All	-	-
IV	30	12	24	2	All	10	10

00360

009614

3. A schematic diagram of the reproduction study is shown below:

Schematic Diagram
3-Generation Reproduction Study



*All parental group contained 12 males and 24 females at the start of the growth period.
**Histopathology on 10 male/10 female control and high-dose group parents.

4. The test substance (glyphosate) was added in the basal diet, based on weekly measurements of body weight and food consumption, to achieve dietary levels of 3, 10, and 30 mg/kg/day. The control rats received basal diet only. Assays for stability, homogeneity, and concentration were acceptable.

5. Body weights and food consumption were measured during the growth period (63 days), and rest periods, Days 0, 6, 15, and 20 of gestation, and Days 0, 4, 14, and 21 of lactation. Animals were observed twice daily for toxicity and mortality.

6. Tissues listed below were taken from all parents (F_0, F_1, F_2) and from 10/sex/group (chosen randomly) of the F_{3b} weanlings. All tissues were preserved in 10 percent

neutral buffered formalin. (Eyes and testes were placed initially in Bouin's solution.)

Tissues Preserved:

Adrenal (2)	Mammary gland (right inguinal)
Aorta	Pancreas
Bone and bone marrow (sternal)	Pituitary
Brain (2 longtitudinal sections	Salivary gland
Eye (2) with optic nerve and Harderian gland	Skeletal muscle (biceps femoris with right sciatic nerve)
Gonads	Skin
Heart	Spinal cord (cervical and lumbar)
Intestine	Spleen
colon	Stomach
duodenum	Thyroid and Parathyroid (attached to trachea and esophagus)
ileum	Urinary bladder
Kidney (2)	Uterus/prostate
Liver (2 sections)	Gross lesions
Lung (section with mainstem bronchi)	Tissue masses
Lymph nodes (mesenteric)	Thymus

7. Organs Weighed - The following organs were weighed from all parents sacrificed after weaning of the second litters and from 80 F_{3b} weanlings (10 males and 10 females per group) with tissues preserved:

Adrenals	Spleen
Gonads	Liver
Kidneys	Heart
Brain	Pituitary

8. Histology and Histopathology - Section of all tissues listed above (Tissues Preserved) were prepared and examined microscopically from 10 male and 10 female animals from control and high-dose groups of the following:

Parents: F_0, F_1, and F_2
Offspring: F_{3b}

Any tissue masses observed in any animals were also examined.

9. Statistical Analysis - Litter examination data, growth and rest period body weight and food consumption data, and maternal body weight (gestation and lactation) data were compared to the control. Statistically significant differences from control are indicated in mean tables and appendices and were significant at $p < 0.05$. Statistical methods used in the study are attached.

Results:

A. Body Weight and Food Consumption

Parental Animals (F_0), (F_1), and (F_2)

Mean body weights of parental animals during the growth, rest, gestation, and lactation periods were comparable between control and treated groups for each generation throughout the study.

There were no compound-related effects on parental body weight data. Similarly mean food consumption data were considered comparable between control and treated groups of both sexes during the growth, rest, gestation, and lactation periods for each generation.

B. Mating, Pregnancy, and Fertility Indices

F_0 Generation

Mortality, Mating, Pregnancy, and Fertility Rates

Group mg/kg/day	Total Number Exposed Females	Males	Mortality[a] Females No. Dead	%	Males No. Dead	%	Mating Females Mated[b]/Total No.	%	Males Mated[c]/Total No.	%	Pregnancy Females Pregnant/No. Mated No.	%	Fertility Males Impregnating[d]/No. Mated No.	%
F_0 Mating (for F_{1a} generation)														
1 0	24	12	0	0.0	0	0.0	20/24	83.3	11/12	91.7	19/20	95.0	11/11	100.0
11 3	24	12	0	0.0	0	0.0	22/24	91.7	12/12	100.0	21/22	95.5	12/12	100.0
111 10	24	12	1	4.2	0	0.0	19/24*	79.2	10/12	83.3	16*/19	84.2	9/10	90.0
1V 30	24	12	0	0.0	0	0.0	21/24	87.5	11/12	91.7	19/21	90.5	11/11	100.0
F_0 Mating (for F_{1b} generation)														
1 0	24	12	0	0.0	0	0.0	20/24	83.3	11/12	91.7	19/20	95.0	11/11	100.0
11 3	24	12	0	0.0	0	0.0	23/24	95.8	12/12	100.0	19/23	82.6	12/12	100.0
111 10	24	12	1	4.3	0	0.0	17/23*	73.9	11/12	91.7	12*/17	70.6	10/11	90.9
1V 30	24	12	0	0.0	0	0.0	22/24	91.7	11/12	91.7	18/22	81.8	10/11	90.9

There were no dose-related effects in female mating or pregnancy ratios in the F_1a and F_1b generations, although the female mating and pregnancy ratios at 10 mg/kg/day were lower than control values. The findings, however, were not dose-related and are not compound-related.

009614

F_1 Generation

Mortality, Mating, Pregnancy, and Fertility Rates

Group mg/kg/day	Total Number Exposed Females	Males	Mortality[a] Females No. Dead	%	Males No. Dead	%	Mating Females Mated[b]/Total No.	%	Males Mated[c]/Total No.	%	Pregnancy Females Pregnant/No. Mated No.	%	Fertility Males Impregnating[d]/No. Mated No.	%
F_1 Mating (for F_{2a} generation)														
I 0	24	12	0	0.0	0	0.0	18/24	75.0	10/12	83.3	18/18	100.0	10/10	100.0
II 3	24	12	0	0.0	0	0.0	23/24	95.8	12/12	100.0	20/23	87.0	11/12	91.7
III 10	24	12	0	0.0	0	0.0	18/24	75.0	9/12	75.0	17/18	94.4	9/9	100.0
IV 30	23	12	1	4.2	0	0.0	19/23	82.6	11/12	91.7	18/19	94.7	10/11	90.9
F_1 Mating (for F_{2b} generation)														
I 0	24	12	0	0.0	0	0.0	17/24	70.8	9/12	75.0	15/17	88.2	9/9	100.0
II 3	24	12	0	0.0	0	0.0	19/24	79.2	10/12	83.3	15/19	78.9	9/10	90.0
III 10	24	12	0	0.0	0	0.0	17/24	70.8	10/12	83.3	14/17	82.4	10/10	100.0
IV 30	24	12	1	4.3	0	0.0	19/23	82.6	12/12	100.0	14/19	73.9	10/12	83.3

009614

F_2 Generation

Mortality, Mating, Pregnancy, and Fertility Rates

Group mg/kg/day	Total Number Exposed Females	Males	Mortality[a] Females No. Dead	%	Males No. Dead	%	Mating Females Mated[b]/Total No.	%	Males Mated[c]/Total No.	%	Pregnancy Females Pregnant/No. Mated No.	%	Fertility Males Impregnating[d]/No. Mated No.	%
F_2 Mating (for F_{3a} generation)														
I 0	24	12	0	0.0	0	0.0	24/24	100.0	12/12	100.0	23/24	95.8	12/12	100.0
II 3	24	12	1	4.2	0	0.0	20/24	83.3	10/12	83.3	20/20	100.0	10/10	100.0
III 10	24	12	0	0.0	0	0.0	20/24	83.3	10/12	83.3	16/20	80.0	8/10	80.0
IV 30	24	12	0	0.0	0	0.0	18/24	75.0*	10/12	83.3	17/18	94.4	10/10	100.0
F_2 Mating (for F_{3b} generation)														
I 0	24	12	0	0.0	0	0.0	23/24	95.8	12/12	100.0	22/23	95.7	11/12	91.7
II 3	23	12	0	0.0	0	0.0	19/23	82.6	10/12	83.3	16/19	84.2	10/12	83.3
III 10	24	11	0	0.0	1	8.3	20/24	83.3	10/11	98.9	16/20	90.0	9/10	90.0
IV 30	24	12	0	0.0	0	0.0	21/24	87.5	11/12	91.7	19/21	90.5	10/11	90.9

*$p < 0.05$

Over the three generations, there were no dose-related effects to indicate a compound-related effect on mating, pregnancy, and fertility indices for either sex. The statistically significant decrease in the high-dose females mating index (75.0% in high-dose vs. 100% in controls) for the F_{3a} litters was not shown to be a consistent finding, since the F_{3b} litters produced by the high-dose group females resulted from a mating index of 87.5 percent (high-dose) in comparison to 95.8 percent in controls. Additionally, pregnancy rates of the F_2 generation were unaffected by treatment. Also, the

00336

009614

mating indices of the F_0 and F_1 females were comparable between control and test groups.

C. Gestation Length, Offspring Viability, Survival, and Growth (Body Weight)

F_0 Generation

Group mg/kg/day	Mean Gestation Length Days	Mean No. Pups at Birth Live	Dead	Total	Pup Viability Index at Birth Live/Total Born No.	%	Mean No. Pups Weaned/ Litter	Days:	Postnatal Offspring Survival 0-4 No.	%	4-21 No.	%	Index of Litters Weaned[b] No.	%	Days:	Mean Weights of Live Offspring (grams) 0	4	21
									$F_0 \rightarrow F_{1a}$									
I 0	22.1	11.5	0.1	11.6	218/220	99.1	10.7		210/218	96.3	192/195[c]	98.5	19/19	100.0		6.0	9.9	41.
II 3	21.8	12.8	0.1	12.9	268/271	98.9	12.4		251/268	93.7	247/251	98.4	20/21	95.2		5.8	9.3	37.
III 10	21.8	12.3	0.3	12.5	196/200	98.0	11.9		194/196	99.0	192/194	99.0	16/16	100.0		5.9	9.4	39.
IV 30	21.8	11.6	0.1	11.7	221/222	99.5	11.3		217/221	98.7	215/217	99.1	19/19	100.0		6.0	9.6	39.
									$F_0 \rightarrow F_{1b}$									
I 0	22.0	11.7	0.2	11.9	223/226	98.7	11.3		218/223	97.8	215/218	98.6	19/19	100.0		6.1	9.9	40.9
II 3	21.8	12.2	0.6	12.8	232/243	95.5	11.4		223/232	96.1	206/223	92.4**	18/19	94.7		6.1	9.7	43.1
III 10	22.0	12.8	0.3	13.1	153/157	97.5	10.9		145/153	94.8	120/145	82.8**	11/12	91.7		5.8	9.0	37.1
IV 30	21.9	12.6	0.3	12.8	226/231	97.8	11.4		225/226	99.6	194/214[d]	90.7**	17/17[e]	100.0		6.2	9.9	36

Significantly different from control: - *p < 0.05; **p < 0.01

009614

F_1 Generation

Group mg/kg/day	Mean Gestation Length Days	Mean No. Pups at Birth Live	Dead	Total	Pup Viability Index at Birth Live/Total Born No.	%	Mean No. Pups Weaned/Litter	Days:	Postnatal Offspring Survival 0-4 No.	%	4-21 No.	%	Index of Litters Weaned[b] No.	%	Days:	Mean Weights of Live Offspring (grams) 0	4	21
									$F_1 \rightarrow F_{2a}$									
I 0	21.9	12.0	0.2	12.2	216/219	98.6	11.7		201/216	93.1	199/201	99.0	17/18	94.4		5.8	9.1	41.0
II 3	21.8	11.8	0.0	11.8	236/236	100.0	11.6		231/236	97.9*	231/231	100.0	20/20	100.0		6.0	9.7	43.4
III 10	21.9	12.7	0.0	12.7	216/216	100.0	12.4		214/216	99.1**	211/214	98.6	17/17	100.0		6.0	9.1	39.7
IV 30	22.0	11.5	0.4	11.9	207/214	96.7	11.1		206/207	99.5**	200/205	97.1	18/18	100.0		6.2	9.4	40.3
									$F_1 \rightarrow F_{2b}$									
I 0	21.9	12.4	0.4	12.8	186/192	96.9	11.9		178/186	95.7	178/178	100.0	15/15	100.0		5.9	9.4	41.1
II 3	21.9	12.5	0.4	12.9	187/193	96.9	12.7		166/187	88.8*	165/166	99.4	13/15	86.7		5.7	9.2	41.1
III 10	22.1	13.1	0.2	13.4	184/187	98.4	12.7		181/184	98.4	178/181	98.3	14/14	100.0		5.8	9.5	41.3
IV 30	22.1	11.3	0.3	11.6	147/151	97.4	11.1		144/147	98.0	144/144	100.0	13/13	100.0		6.4	10.3	41.3

Significantly different from control: *p < 0.05; **p < 0.01

F_2 Generation

Group mg/kg/day	Mean Gestation Length Days	Mean No. Pups at Birth Live	Dead	Total	Pup Viability Index at Birth Live/Total Born No.	%	Mean No. Pups Weaned/Litter	Days:	Postnatal Offspring Survival 0-4 No.	%	4-21 No.	%	Index of Litters Weaned No.	%	Mean Weights of Live Offspring (grams) Days: 0	4	21
								$F_1 \rightarrow F_{2a}$									
I 0	21.9	11.7	0.2	11.9	268/273	98.2	11.0		266/268	99.3	254/266	95.5	23/23	100.0	6.0	9.5	37.1
II 3	22.0	11.1	0.9	12.0	222/240	92.5**	10.6		219/222	98.6	100/219	86.6*	18/19	94.7	6.1	9.4	36.8
III 10	21.9	12.6	0.1	12.8	202/204	99.0	11.8		202/202	100.0	188/202	93.1	16/16	100.0	6.0	9.5	37.3
IV 30	21.9	11.8	0.3	12.1	200/205	97.6	11.0		198/200	99.0	187/198	94.4	17/17	100.0	6.3	9.8	36.7
								$F_1 \rightarrow F_{2b}$									
I 0	21.9	11.2	0.2	11.5	247/252	98.0	11.3		241/247	97.6	237/241	98.3	21/22	95.5	5.9	8.8	38.1
II 3	21.9	12.3	0.4	12.7	197/203	97.0	12.7		192/197	97.5	191/192	99.5	15/16	93.8	5.8	9.0	39.6
III 10	22.1	13.0	0.1	13.1	208/210	98.0	12.4		202/208	97.1	198/202	98.0	16/16	100.0	6.1	9.5	39.8
IV 30	21.9	9.8	0.5	10.4	187/197	94.9	9.9		183/187	97.9	178/183	97.3	18/19	94.7	6.3	8.1	38.5

Significantly different from control: *$p < 0.05$; **$p < 0.01$

The statistically significant decreases in Day 4 to 21 pup survival at all dose levels in the F_{1b} litter were attributed to high pup mortality within one or more litters at each dose level.

"As stated in the report, in the low-dose group the lower pup survival was attributed to one female (No. 1404) that experienced complete litter mortality (litter contained 14 live pups at Day 4). In the mid-dose group, one female (No. 617) died on Day 7 of lactation and all seven pups in her litter died during the Day 4 to 7 lactation interval. Additionally, three mid-dose litters (females No. 609, 610, and 620) lost five or more pups from their litters during the Day 4 to 21 lactation interval. In the high-dose group, female No. 815 lost 9 of 12 pups during the Day 4 to 21 lactation interval."

Pup survival between Day 4 and 21 in the F_1 and F_2 generations was comparable between control and treated groups. Therefore, the findings in the F_{1b} litters were not consistent and were not considered compound-related.

There were no compound-related effects on pup body weight or sex ratio for any of the litters of the F_0, F_1, and F_2 generations.

D. Pathology - With respect to the F_{3b} weanlings (pups), there were no compound-related effects in organ weights. The mean liver weight to body and brain weight ratios of the F_2 parental females of all treated groups were significantly lower than control values, but the differences were not dose-related. These findings were not considered compound-related, since similar effects in F_0 and F_1 parental animals were not observed and there were no histopathological findings associated with the lower liver weight results in F_2 adults. The incidence of tubular dilation of the kidney in F_{3b} male pups showed a significant increase at 30 mg/kg/day. The results are shown below:

	Control	3	10	30
Kidney				
Focal Tubular Dilatation				
- Unilateral	2/10	3/10	2/9	7/10
- Bilateral	0/10	2/10	1/9	1/10

The kidney microscopic finding in high-dose male F_{3b} pups is considered compound-related.

There were no other compound-related histopathological findings in parental animals or F_{3b} pups examined histologically.

The NOEL is 10 mg/kg/day.

The study is classified as core-minimum since there were three generations with two litters per generation, which exceeds the minimum requirements for a reproduction study. Although there often were less than 20 pregnant rats/dose, this deficiency is offset by the additional generation produced in this study. There also was a sufficient number of animals for statistical analyses to be conducted. Although only 10 animals/sex/dose were examined histopathologically, they included animals from the F_0, F_1, and F_2 adults (rather than just F_1 adults in current minimum studies) and also included 10/sex/dose of F_{3b} pups. For these reasons, the study is core-minimum.

Attachments

009614

62812:I:Dykstra:LHED-1:KEVRIC:04/12/91:PERM:TJK/CL:WO:EK:CL
R:62818:Dykstra:LHED-1:KEVRIC:4/19/91:PERM:EK
R:62827:Dykstra:LHED-1:KEVRIC:05/13/91:PERM:DD:WO:DD

GLYPHOSATE Sh # 103601

Page_____ is not included in this copy.

Pages 73 through 76 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/1/91
Toxicology Branch I - IRS (H7509C)
for Secondary Reviewer: Roger Gardner, Section Head Pamela M. Hurley 5/14/91
Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: Pharmacokinetics - Not a Mutagenicity Study

TOX Chem. No.: 661A

Accession No.: 251737

MRID No.: 00132685

Test Material: C^{14}-Glyphosate; Specific Activity 5 mCi/mmole

Synonyms: Roundup

Study Number: 830109; DMEH No. ML-83-218

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Environmental Health Lab, St. Louis, MO

Title of Report: A Study of the Plasma and Bone Marrow Levels of Glyphosate Following the Intraperitoneal Administration in the Rat.

Author: W.P. Ridley

Report Issued: October 24, 1983

Conclusions:

Thirty minutes following intraperitoneal (ip) administration of [^{14}C]-glyphosate to male and female Charles River Sprague-Dawley rats at 1150 mg/kg, the concentration of radiolabel present in bone marrow was 267 ± 31 and 413 ± 39 ppm, respectively (equivalent to 0.0044 and 0.0072 percent of the dose). Assuming first order kinetics, the decrease in radioactivity occurred with a half-life of 7.6 and 4.2 hours from the males and females, respectively. Similarly, the half-lives of radiolabel in the plasma were approximately 1 hour in both sexes.

Classification: Acceptable

Special Review Criteria (40 CFR 154.7): N/A

Note: The DER is based on parts of a Dynamac review.

00077

009614

Review:

Quality Assurance Statement - Present, signed, and dated October 20, 1983.

Test Material - The test material used was a mixture of [^{14}C-methyl] N-(phosphonomethyl) glycine sodium salt, and the protonated acid of the unlabeled test material. Radiolabeled glyphosate had a specific activity of 5 mCi/mmole and a radiochemical purity of 98 percent, whereas the purity of unlabeled glyphosate was 98.7 percent.

Protocol

1. Nine male and nine female Crl:CD Br rats were obtained from Charles River Laboratories, Wilmington, MA. The animals were acclimatized to laboratory conditions for a period of 7 days, then placed in stainless steel metabolism cages for 4 days prior to dosing, and for the duration of the study. Purina Rat Chow and water were available ad libitum. The rats were fasted for a period of 22 to 24 hours prior to dosing. The animals were 8 to 9 weeks old and the average weight of the male was 264 g and of females 186.0 g at dosing.

2. A dosing solution containing 12.25 g and 5.487 mg of the unlabeled and [^{14}C] labeled glyphosate, respectively, in Hank's Balanced Salt Solution was prepared. The final pH of this dosing solution was adjusted to 7.18 in the final volume of about 70 mL. The specific activity was determined to be 29.8 dpm/ug glyphosate based upon the protonated acid weight.

3. The rats were dosed by ip injection and the precise amount administered was calculated from the difference in weight of the syringe and needle before and after dosing. The males received 1150 $\pm$ 3.3 mg/kg containing 9.013 $\pm$ 0.09 x 10^6 dpm and the females received 1150 $\pm$ 7.5 mg/kg containing 6.394 $\pm$ 0.20 x 10^6 dpm of test material.

4. Blood samples were collected by orbital sinus puncture from six males and six females 15 minutes after dosing. Additional samples were collected from three animals of each sex at approximately 0.5, 1, 2, 4, 6, and 10 hours after dosing. No more than three blood samples were collected from any one rat during that period. The whole blood samples were centrifuged and 0.1 mL of plasma were radioassayed in 15 mL of Instagel.

At approximately 0.5, 4, and 10 hours after dosing, three males and three females were sacrificed by cervical dislocation, and the bone marrow from both the right and left femur of each animal collected. The bone marrows were weighed, digested in soluene-350 at 50 °C for 5 to 6 hours, then allowed to sit at room temperature overnight. The samples were decolorized, 15 mL of Dimilune-30 added, and then were allowed to equilibrate to temperature and light in the liquid - scintillation counter prior to counting. Counting efficiencies were determined by means of an external standard and corrections were made for quenching. The results were reported both in dpm/g tissue and ug glyphosate equivalents/g tissue (ppm).

Results:

A maximum concentration of radiolabeled material in male and female plasma was noted 30 minutes after ip administration. This corresponded to a level of 1867 ± 160 ppm and 2019 ± 83 ppm of glyphosate and/or its metabolites in males and females, respectively. The concentration of radiolabel in plasma decreased subsequently. Linear regression analysis of the data indicated that the decrease in radioactivity occurred with a half-life of approximately 0.99 and 1.0 hours in males and females, respectively.

The concentration of radiolabel measured in the bone marrow 30 minutes after administration was 267 ± 31 and 413 ± 39 ppm for males and females, respectively. Assuming first order kinetics, the decrease in radioactivity occurred with a half-life of 7.6 and 4.2 hours for the males and females, respectively.

Discussion:

The study was conducted in order to "confirm that glyphosate" reaches the bone marrow following ip injection. The amounts reaching the bone marrow were considered by the authors sufficient to justify cytogenetic evaluation. However, identification of the radiolabeled material in the bone marrow was not conducted, and only 0.0044 (13 ug/rat) and 0.0072 (15.4 ug/rat) percent of the dose administered ip reached the bone marrow in males and females, respectively.

Conclusion:

Thirty minutes following ip administration of [^{14}C]-glyphosate to male and female Charles River rats at 1150 mg/kg, the concentration of radiolabel present in the bone marrow was 267 ± 31 and 413 ± 39 ppm, respectively (equivalent to 0.0044 and 0.0072 percent of the dose). Assuming first order kinetics, the decrease in radioactivity occurred with a half-life of 7.6 and 4.2 hours for

the males and females, respectively. Similarly, the half-lives of radiolabel in the plasma were approximately 1 hour in both sexes.

Core Classification: Acceptable

009614

00030

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/1/91
Toxicology Branch I - IRS (H7509C)
for Secondary Reviewer: Roger Gardner, Section Head Pamela M Hurley 5/14/91
Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: 84-2a - Gene Mutation Mammalian Cell

TOX Chem. No.: 661A

Accession No.: 251737

MRID No.: 00132681

Test Material: Glyphosate Technical; 98.7% Purity; Lot No. XHJ-64

Synonyms: Sample No. T830044

Study Number: ML-83-155
EHL No. 830079

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Environmental Health Lab, St. Louis, MO

Title of Report: CHO/HGPRT Gene Mutation Assay With Glyphosate.

Author: A.P. Li

Report Issued: October 20, 1983

Conclusions:

Technical glyphosate did not induce a mutagenic response, with or without S9, up to limit of cytotoxicity (10 mg/mL) against standard reference mutagens. The range of glyphosate tested was 2 to 25 mg/mL.

Classification: Acceptable

Special Review Criteria (40 CFR 154.7): N/A

Note: Dr. Irving Mauer, geneticist, screened the mutagenicity assay for acceptability. The DER is based on parts of a Dynamac review.

00031

Review:

Quality Assurance Statement - Present, signed, and dated October 20, 1983.

Test Material - The test material was identified as Glyphosate, a white powder, Lot No. XHJ-64, submitted to Environmental Health Laboratory (EHL) and indicated to be 98.7 percent pure. It was assigned Sample No. I830044 by EHL and stored at room temperature.

Materials and Methods:

Preparation of the Test Material - Stock solutions of glyphosate were made in Ham's F12 V medium (K.C. Biological) and neutralized to pH 7.0 to 7.4 with 1N NaOH until a clear solution was obtained. Test solutions of different concentrations were made by diluting the stock with Ham's F12 V medium on the testing day.

Controls - The positive controls were benzo(a)pyrene (B(a)p) for S9 activation and ethyl methane sulfonate (EMS). Both were obtained from Sigma Chemical Company, St. Louis, MO.

Cell Line - The cell line was the Chinese hamster ovary line, K_1BH_4 originally obtained from A.W. Hsie* at Oak Ridge National Laboratory. Cultures of these cells were maintained in Ham's F12 medium supplemented with 10 percent newborn calf serum as logarithmically growing monolayers. Growth was at 37.5 $\pm$ 2 'C at a relative humidity of 95 percent under 5 percent CO_2.

Cytotoxicity - At 18 to 24 hours before dosing, 0.5×10^6 cells were seeded in 25 cm^2 plastic culture flasks; on the day of dosing the growth medium was replaced with 2.5 mL of Ham's F12 medium containing neither S9 nor serum. An equal volume of this medium containing 2X concentrations of the test material was added; the mixture was then incubated for 3 hours at 37.5 $\pm$ 2 'C, and then the dosing medium was removed and the cells washed with 5 mL of Hank's balanced salt solution. The cells were removed by trypsinization and scored (3 samples of 200 cells plated for assessment on cloning efficiency). All plates were then reincubated for 7 to 9 days, and colonies that developed were fixed with 70 percent methanol, stained by 10 percent Giemsa and hand-scored. To calculate cloning efficiency (CE) and relative survival (RS), the following expressions were used.

---

*Hsie, A.W., Li, A.P., and Machanoff, R. (1977) Mutant. Res. 45:333-342.

Mutagenesis Assays - The K_1BH_4 cells were plated the day before dosing with the test material, positive controls, or negative solvent controls. The procedure described for the cytotoxicity assay was followed, except that an additional 10^6 cells/10 mL were subcultured in hypoxanthine-free Ham's F12 medium, supplemented with 10 percent dialyzed newborn calf serum. Subculturing was carried out every 2 to 3 days, followed by the 7- to 9-day period allowed for phenotypic expression. After phenotypic expression, selective medium[1] (hypoxanthine-free Ham's F12 medium supplemented with 10 uM 6-thioguanine (6TG) and 5 percent dialyzed newborn calf serum) was used to select for the 6TG-resistant mutant clones. A total of 10^6 cells were assessed for mutant development, using five 100 mm plates, each containing 2×10^5 cells in 8 mL of selective medium. After incubation for 8 to 12 days, colonies were fixed, stained, and scored. The cloning efficiency was determined as previously described. Using the expression that follows, a mutation frequency (M.F.)[2] was calculated.

$$\text{C.E.} = \frac{\text{number of colonies developed}}{\text{number cells plated}}$$

$$\text{R.S.}^2 = \frac{\text{C.E. (dosed)}}{\text{C.E. (negative control)}}$$

Experimental Design - Two experiments were used to determine the mutagenicity of glyphosate. In Experiment A, three doses of test material (5, 17.5, and 22.5 mg/mL) estimated to yield 100, 50, and 10 percent survival were used in conjunction with S9 concentrations of 0, 1, 2, 5, and 10 percent. This test was to determine an initial estimate of mutagenic potential at an optimum S9 concentration. In Experiment B, five doses of test material (2, 5, 10, 10, and 20 mg/mL) estimated to yield 100, 70, 50, 20, and 10 percent survival were used. Since no mutagenicity was observed in Experiment A, no optimum S9 concentration was determined; therefore, a 5 percent S9 concentration was chosen as representative.

Metabolic Activation - The Aroclor 1254-induced rat liver S9 fraction was purchased from Litton Bionetics and was applied to cultures in varying amounts relative to the S9-cofactors. The S9-cofactor mix contained, in addition to different amounts of S9

---

[1]Li, A.P., Dahl, A.R., and Hill, J.O. (1982) Toxicol. Appl. Pharmacol. 64:482-485.

[2]R.S. was used to express cytotoxicity to the cell line;

$$\text{M.F.} = \frac{\text{Number mutant colonies}}{\text{Number cells plated}} \times \frac{1}{\text{CE}}$$

protein, 50 mM sodium phosphate (pH 7.5), 4 mM NADP, 5 mM glucose-6-phosphate, 30 mM KCl, 10 mg $MgCl_2$, and 10 mM $CaCl_2$. One mL of the S9-cofactor mix was added to 4 mL of medium for the cytotoxicity of mutagenicity assays.

Statistics - The method of Snee and Irr* was used to analyze the mutagenicity data; mutant frequency values were transformed using $Y = (X + 1)^{0.15}$ where Y is the transformed mutant frequency and X is the observed mutant frequency. Treatment data were compared to the solvent control data by the Student's t-test. Determination of dose-response relationships as linear, quadratic, or higher-order was possible by Snee/Irr analysis, and a program developed by Irr (DuPont) was incorporated into Monsanto's computer system.

Results:

Cytotoxicity Assay - Approximately 90 percent lethality occurred at glyphosate doses between 20 and 25 mg/mL. Hence, 22.5 mg/mL and 25 mg/mL were the high dose in Experiment A and Experiment B, respectively.

In the presence of varying S9 concentrations, mutant frequencies x 10^{-6} in the negative (medium) controls were 7.4 (0 percent), 5.9 (1 percent), 7.1 (2 percent), 4.4 (5 percent), and 9.1 (10 percent). At glyphosate doses of 5, 17.5, and 22.5 mg/mL, none of the mutant frequencies were significantly different from the control values. However, with 1 percent S9, the mutant frequencies (f) x 10^{-6} and p-values** at varying glyphosate doses were 5 mg/mL (f = 4.3, p = 0.6695), 17.9 mg/mL (f = 11.6, p = 0.3470), and 22.5 mg/mL (f = 19.1, p = 0.1796).

In the absence of S9 at various glyphosate doses, the mutant frequencies x 10^{-6} were 2 mg/mL (f = 3.5, p = 0.1789), 5 mg/mL (f = 11.3, p = 0.9994), 10 mg/mL (f = 10.8, p = 0.6314), 15 mg/mL (f = 20.8, p = 0.5318), and 20 mg/mL (f = 10.1, p = 0.8695).

At concentrations ranging from 5 to 25 mg glyphosate/mL in the presence of 5 percent S9, mutant frequencies x 10^{-6} varied from 5.7 (p = 0.8536) to 14.9 (p = 0.4811) compared to a control value of 7.7 x 10^{-6}.

The mutant frequency for treatment with 200 ug EMS/mL averaged 150 x 10^{-6} compared to the negative control values of 9.4 x 10^{-6}. Using 2 ug B(a)P/mL in varying amounts of S9 (expressed in percentage), the average mutant frequencies were (353 x 10^{-6}) (1 percent),

---

*Snee, R.D. and Irr, J.D. (1981) Mutat. Res. 85:77-93.
**Probability to be the same as control by the method of Snee and Irr (1981).

(186 x 10^{-6}) (2 percent), (99 x 10^{-6}) (5 percent), and (95 x 10^{-6}) (10 percent).

Discussion:

The authors concluded that glyphosate was cytotoxic to CHO cells at high concentrations, i.e., > 10 mg/mL, but that significant mutagenicity at the HGPRT locus was not produced.

Our assessment is that the authors have assayed the test material in an appropriate dose range without or with S9 activation at several concentrations, and their data showed no significant mutagenicity. Using 1 percent S9, however, a non-significant dose-related increase in the mutant frequency was seen in the glyphosate dose range of 5 to 22.5 mg/mL.

Conclusions:

The test material, 98.7 percent pure glyphosate, did not produce a significant mutagenic response either with or without S9 activation under the conditions of this study.

Classification: Acceptable

009614

62815:I:Draft:Dykstra:LHED-3:KEVRIC:04/19/91:05/17/91:CL:WO:EK:DD
R:62898:Dykstra:LHED-3:KEVRIC:04/25/91:05/25/91:CL

00336

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/13/91
Section I, Toxicology Branch I - IRS (H7509C)
for Secondary Reviewer: Roger Gardner, Section Head Pamela M. Hanley 5/14/91
Section I, Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: 84-2a, Gene Mutation TOX Chem. No.: 661A

Accession Number: N/A MRID No.: 00078620

Test Material: Glyphosate, technical; Sample No. 4

Synonym: Roundup

Study Number: LF-78-161

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Environmental Health Laboratory
St. Louis, MO

Title of Report: Final Report on Salmonella Mutagenicity Assay of Glyphosate.

Authors: L. Flowers and L.D. Kier

Report Issued: June 16, 1978

Conclusions:

Glyphosate was negative for mutagenicity when tested up to 1000 ug/plate (or toxicity), both with and without S-9, in Salmonella typhimurium strains (TA98, TA100, TA1535, and TA1537). Positive controls run concurrently produced the expected positive mutagenic results.

Classification: Acceptable

Special Review Criteria (40 CFR 154.7): N/A

Note: Dr. Irving Mauer, geneticist, screened this study for acceptability.

00087

009614

Methods:

Standard methods were employed.

Review:

The following compounds and amounts were used as positive controls:

Strain	S-9 Mix	Compounds	Amount/Plate
TA98	-	4-nitroquinoline-N-oxide	0.1 mcg
TA98	+	2-acetamidofluorene	30 mcg
TA100	-	4-nitroquinoline-N-oxide	0.5 mcg
TA100	+	benzo(a)pyrene	2 mcg
TA1535	-	$NaNO_2$	10 mg
TA1535	+	tris(2,3-dibromopropyl)-phosphate	30 mcg
TA1537	-	9-aminoacridine	30 mcg
TA1537	+	2-aminoanthracene	30 mcg

Results:

Reverse Mutation (Gene Mutation) Assay

1. TA98 and TA100

S-9 Ref: Litton IRL-148
Solvent H_2O

Without Microsomal Activation	Revertants per Plate						
Amount per Plate	TA98			TA100			TA1535	TA1537
0.1 ug	31	28	24	151	164	166		
1.0 ug	19	21	15	128	147	142		
10 ug	15	21	21	130	114	104		
30 ug	21	20	18	133	139	108		
100 ug	21	27	15	146	108	115		
1000 ug	TOX	TOX	TOX	77	92	77		
Solvent Controls	26	28	18	104	108	130		
Negative Control (H_2O)	25	25	9	102	103	93		
Positive Controls		380			1399			

00038

S-9 Ref: Litton IRL-148
Solvent H_2O (Cont'd)

With Microsomal Activation

Amount per Plate	Revertants per Plate TA98			TA100			TA1535			TA1537		
0.1 ug	56	73	56	143	104	127						
1.0 ug	52	50	65	152	145	108						
10 ug	72	53	64	92	133	135						
30 ug	78	76	40	111	132	115						
100 ug	64	56	68	145	126	138						
1000 ug	TOX	TOX	TOX	104	110	108						
Solvent Controls	54	55	63	108	98	108						
Negative Control (H_2O)	60	42	40	126	116	114						
Positive Controls	1234			369								

2. TA1535 and TA1537

S-9 Ref: Litton IRL-48
Solvent H_2O

Without Microsomal Activation

Amount per Plate	Revertants per Plate TA98			TA100			TA1535			TA1537		
0.4 ug	16	14	16	125	85	85	3	2	3	4	6	5
2.0 ug	9	9	16	98	86	84	1	2	3	7	2	4
10 ug							4	1	1	7	2	4
30 ug							2	1	1	6	2	4
100 ug							1	2	2	48	5	7
1000 ug							TOX	TOX	TOX	TOX	TOX	TOX
Solvent Controls	20	13	6	110	117	109	5	2	1	2	6	4
Negative Control (H_2O)	4	14	9	66	81	100	4	2	3	2	2	3
Positive Controls		139			963			48			19	

With Microsomal Activation

Amount per Plate	Revertants per Plate TA98			TA100			TA1535			TA1537		
0.4 ug	58	39	63	100	122	111	6	13	7	15	22	33
2.0 ug	40	52	54	108	137	135	9	7	3	26	5	11
10 ug							9	5	8	37	9	16
30 ug							8	6	5	27	10	26
100 ug							6	3	6	13	10	37
1000 ug							TOX	TOX	TOX	TOX	TOX	TOX
Solvent Controls	54	48	45	135	137	121	16	9	5	12	45	27
Negative Control (H_2O)	43	44	44	95	86	98	8	13	9	11	19	12
Positive Controls		1457		641				198			95	

009614

62819:I:Dykstra:LHED-07:LRAFT:KEVRIC:04/23/91:05/18/91:aw:wo:aw
R:62897:Dykstra:LHED-07:KEVRIC:05/01/91:06/01/91:DD

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/11/91
Section I, Toxicology Branch I - IRS (H7509C)
for Secondary Reviewer: Roger Gardiner, Section Head Pamela M. Hurley 5/14/91
Section I, Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: 84-2(b) - Structural Chromosomal Aberrations: Mouse Dominant Lethal Assay

TOX Chem. No.: 661A

Accession Number: N/A

HRID No.: 00046364

Test Material: Glyphosate, technical; 98.7% purity; Lot No. XHJ-64

Synonyms: Roundup

Study Nos.: IRDC No. 401-064; Monsanto No. IR-79-014

Sponsor: Monsanto Company
St. Louis, MO

Testing Facility: IRDC
Mattawan, MI

Title of Report: Dominant Lethal Study in Mice.

Author: Dean E. Rodwell

Report Issued: April 16, 1980

Conclusions:

Glyphosate was negative for a dominant lethal mutation in mice at dosages up to 2000 mg/kg, given as a single oral dose. All standard criteria were met except that there was no evidence of clinical or reproductive toxicity (hence, no evidence that glyphosate reached testes), and there were insufficient numbers of matings (fewer than 20 per week) for statistical constraints. Normally 30 to 50 pregnancies per week/dose are needed for statistical analysis.

Classification: Unacceptable

Special Review Criteria (40 CFR 154.7): N/A

Note: Dr. Irving Mauer, geneticist, screened the mutagenicity study for acceptability.

00091

Review:

Quality assurance was signed by Barry W. Benson and dated March 18, 1980.

Test Materials - glyphosate technical; 98.7% purity; Lot No. XMJ-64. Positive control: Cytoxan (cyclophosphamide), 240 mg/kg. Negative control: 0.5% methocel.

Animals - 90-day-old male CD-1 mice, obtained from Charles River Breeding Laboratories, Portage, MI, were used in the study. Eight hundred sexually mature, untreated virgin female mice of the same source and strain were used. The mice were individually housed, except during mating, and maintained in a controlled environment. The mice received Purina Rodent Chow #5002 and tap water ad libitum.

Methods:

Randomized groups of 10 male mice were divided into five groups. The groups received the following (I): Negative control; (II): Positive control (240 mg/kg); (III): Glyphosate, 200 mg/kg; (IV): Glyphosate, 800 mg/kg; (V): Glyphosate, 2000 mg/kg. The male mice were orally dosed on only the first day of the study at a 10 mL/kg volume, except for the positive control group, which was by intraperitoneal injection. Female mice received no treatment.

Immediately following treatment, each male was cohabitated with two virgin females for 7 consecutive days. Following the 7-day period, two new females were cohabitated with each male and the original females were returned to their individual cages. Females continued to be replaced in this manner for 3 weeks so that each male was mated with a total of 16 females.

Mice were observed daily for toxicity and mortality. Clinical observations and body weight were measured weekly for 9 weeks.

Thirteen days after mid-week of their caging and presumptive mating, each female was sacrificed and the uterus and ovaries were exposed by an abdominal incision. The number and location of viable and nonviable fetuses, early and late resorptions, total implantations and corpora lutea per dam were recorded. Gross necropsy examinations of thoracic and abdominal cavities and organs of the dams were performed.

Statistics:

Fetal deaths per dam and postimplantation loss were compared by the Mann-Whitney U-test as described by Siegel and Weil to judge significance of differences. The number of dams with fetal

deaths was compared using the Chi-square test criterion with Yate's correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel to judge significance of difference. The mean number of live fetuses and corpora lutea were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances, and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

Results:

There were no treatment-related toxic signs in the glyphosate groups. In the positive control group, yellow staining of the anogenital region was observed in 4 out of 10 males during various weeks of the study.

There were four unscheduled deaths: one male at 2000 mg/kg during week 6; one mated female at 2000 mg/kg during week 2; one female at 800 mg/kg at week 5; and one female at 200 mg/kg at week 3. The cause of death could not be determined.

There were no compound-related effects in body weight of male glyphosate-treated mice and positive control mice in comparison to the untreated negative control.

The principal criterion for determining a dominant lethal mutagenic effect is the increase in the number of early fetal deaths in treated groups in comparison to negative controls.

Cytoxan displayed a dominant lethal effect, as evidenced by an increase in the proportion of early fetal deaths in this group, when compared to the negative control during the first three weeks of mating. Glyphosate-treated males did not show a mutagenic effect by this criterion up to 2000 mg/kg over the entire 8-week mating cycle.

The following tables, as presented in the report, show these results.

Summary of Group Mean Maternal Observations at Uterine Examination

Dosage Level	Number Gravid	Number Nongravid	Fetuses: Viable Mean	Fetuses: Viable SD	Fetuses: Nonviable Mean	Fetuses: Nonviable SD	Resorptions: Late Mean	Resorptions: Late SD	Resorptions: Early Mean	Resorptions: Early SD	Postimplantation Loss Mean	Postimplantation Loss SD	Total Implantations Mean	Total Implantations SD	Corpora Lutea Mean	Corpora Lutea SD
Mating Week 1																
0 mg/kg (Vehicle Control):	17	3	11.8	1.08	0.0	0.00	0.0	0.00	0.4	0.93	0.6	0.93	12.5	1.28	12.9	1.68
240 mg/kg (Positive Control):	15	5	4.1**	3.77	0.0	0.00	0.1	0.35	4.9**	2.12	5.1**	2.12	9.1	2.95	11.5	2.80
200 mg/kg:	15	5	10.5	3.58	0.0	0.00	0.1	0.35	0.7	1.54	0.8	1.52	11.3	2.74	10.1	2.27
800 mg/kg:	16	4	10.5*	1.83	0.0	0.00	0.3	0.70	0.8	1.11	1.1	3.20	11.6	1.78	11.8	1.68
2000 mg/kg:	17	3	11.6	2.32	0.0	0.00	0.1	0.33	0.4	0.62	0.5	0.72	12.2	2.43	13.2	2.17
Mating Week 2																
0 mg/kg (Vehicle Control):	17	3	11.1	3.35	0.0	0.00	0.2	0.53	0.9	2.03	1.1	2.05	12.2	2.30	13.2	2.70
240 mg/kg (Positive Control):	16	4	3.3**	3.34	0.0	0.00	0.0	0.00	4.1**	1.89	4.1**	1.89	7.4	3.18	9.1**	0.44
200 mg/kg:	16	4	11.4	2.31	0.0	0.00	0.1	0.25	1.0	1.41	1.1	1.39	12.5	1.90	10.4	1.55
800 mg/kg:	18	2	10.1	3.26	0.0	0.00	0.8	2.81	0.7	1.46	1.5	5.22	11.6	1.89	10.1	2.49
2000 mg/kg:	18	2	12.1	1.89	0.0	0.00	0.2	0.44	0.8	0.90	1.0	1.06	13.1	1.25	10.4	1.42
Mating Week 3																
0 mg/kg (Vehicle Control):	14	6	12.0	1.30	0.0	0.00	0.1	0.27	0.5	0.75	0.6	0.76	12.6	1.22	10.0	1.38
240 mg/kg (Positive Control):	16	4	4.8**	3.1[illegible]	0.0	0.00	0.6	2.00	4.4**	0.18	4.9**	3.34	9.8	2.77	10.9**	2.25
200 mg/kg:	18	2	12.0	2.42	0.0	0.00	0.1	0.24	1.3	1.50	1.4	1.50	13.4	1.46	14.4	2.67
800 mg/kg:	18	2	11.4	2.15	0.0	0.00	0.1	0.24	0.8	1.00	0.8	0.99	12.2	1.83	??.1	1.??
2000 mg/kg:	17	3	10.3*	2.95	0.0	0.00	0.8	3.15	0.6	0.80	1.4	3.10	11.4	1.17	12.0	1.16
Mating Week 4																
0 mg/kg (Vehicle Control):	19	1	10.4	3.76	0.0	0.00	0.9	3.21	0.8	1.27	1.7	3.35	12.1	2.31	12.5	2.41
240 mg/kg (Positive Control):	13	7	10.9	2.87	0.0	0.00	0.2	0.60	1.5	2.07	1.8	2.42	12.7	1.44	13.5	1.05
200 mg/kg:	17	3	10.8	3.26	0.0	0.00	0.2	0.39	0.6	1.00	0.8	1.03	11.4	2.90	11.9	2.95
800 mg/kg:	17	3	11.3	3.10	0.0	0.00	0.0	0.00	1.2	1.67	1.2	1.67	12.5	2.48	11.2	2.35
2000 mg/kg:	14	6	9.9	4.44	0.0	0.00	0.0	0.00	0.9	0.86	0.9	0.86	10.8	3.83	12.0	4.28

SD = Standard Deviation

*Significantly different from control group $p < 0.05$.

**Significantly different from control group $p < 0.01$.

Summary of Group Mean Maternal Observations at Uterine Examination

Dosage Level	Number Gravid	Number Nongravid	Fetuses Viable Mean	Fetuses Viable SD	Fetuses Nonviable Mean	Fetuses Nonviable SD	Resorptions Late Mean	Resorptions Late SD	Resorptions Early Mean	Resorptions Early SD	Postimplantation Loss Mean	Postimplantation Loss SD	Total Implantations Mean	Total Implantations SD	Corpora Lutea Mean	Corpora Lutea SD
Mating Week 5																
0 mg/kg (Vehicle Control):	20	0	11.3	3.27	0.0	0.00	0.8	3.21	0.4	0.83	1.2	3.25	12.5	2.12	13.3	2.00
240 mg/kg (Positive Control):	12	8	11.7	2.77	0.0	0.00	0.1	0.29	0.5	0.52	0.6	0.51	12.3	2.09	13.1	1.44
200 mg/kg:	17	3	10.8	1.95	0.0	0.00	0.3	0.59	0.5	0.72	0.8	0.88	11.6	2.27	12.0	1.57
800 mg/kg:	18	2	10.4	4.09	0.0	0.00	0.1	0.33	1.5	3.39	1.6	3.47	12.0	2.26	12.2	2.13
2000 mg/kg:	18	2	10.4	2.94	0.0	0.00	0.4	0.78	0.9	1.28	1.3	1.88	11.8	1.90	12.6	1.54
Mating Week 6																
0 mg/kg (Vehicle Control):	18	2	11.8	2.43	0.0	0.00	0.2	0.55	0.9	1.48	1.0	1.56	12.8	2.36	13.8	1.77
240 mg/kg (Positive Control):	14	6	11.6	1.87	0.0	0.00	0.3	0.41	1.2	2.01	1.5	1.91	13.1	1.44	13.4	1.50
200 mg/kg:	19	1	12.6	1.42	0.0	0.00	0.2	0.57	0.6	0.77	0.7	0.99	13.4	1.39	13.9	1.20
800 mg/kg:	17	3	11.4	2.89	0.0	0.00	0.1	0.33	0.6	0.79	0.8	0.75	12.2	3.16	12.9	1.83
2000 mg/kg:	15	5	11.5	3.04	0.0	0.00	0.1	0.26	0.9	1.22	1.0	1.20	12.0	2.55	14.1	2.29
Mating Week 7																
0 mg/kg (Vehicle Control):	19	1	11.8	1.84	0.0	0.00	0.2	0.60	1.3	1.41	1.4	1.54	13.2	1.51	13.5	1.84
240 mg/kg (Positive Control):	12	8	10.3	3.08	0.0	0.00	0.2	0.39	1.0	1.21	1.2	1.19	11.4	2.91	11.8	2.44
200 mg/kg:	19	1	11.4	2.76	0.0	0.00	0.1	0.23	0.6	0.90	0.6	0.90	12.1	2.55	13.0	3.18
800 mg/kg:	18	2	11.1	3.05	0.0	0.00	0.3	0.75	0.4	0.61	0.7	0.97	11.8	3.20	13.2	2.07
2000 mg/kg:	15	5	12.2	1.98	0.0	0.00	0.3	0.59	0.5	0.64	0.7	0.70	13.0	1.96	13.4	1.80
Mating Week 8																
0 mg/kg (Vehicle Control):	18	2	11.4	2.20	0.0	0.00	0.1	0.32	0.5	0.99	0.6	0.98	12.0	2.06	12.7	1.45
240 mg/kg (Positive Control):	14	6	11.4	3.88	0.0	0.00	0.5	1.87	0.7	1.59	1.2	2.52	12.6	1.91	13.5	1.87
200 mg/kg:	18	2	10.7	4.25	0.0	0.00	1.3	3.56	0.9	1.15	2.2	3.47	12.9	2.91	14.1	1.88
800 mg/kg:	20	0	10.4	2.94	0.0	0.00	0.3	0.44	0.6	0.76	0.8	1.01	11.2	3.98	12.4	2.32
2000 mg/kg:	16	2	10.7	2.91	0.0	0.00	0.6	1.26	1.1	1.53	1.6	2.19	11.9	2.41	13.9	2.41

Values from the treated groups specified to be tested in the report did not differ significantly from the Control group.

$p < 0.05$.
SD = Standard Deviation

Discussion:

The authors concluded that glyphosate has no clastogenic effect on bone marrow cells under the conditions of the assay. Statistical analysis supported their results; however, there was a slight but nonsignificant increase in achromatic gaps (not considered aberrations) in the glyphosate treated group. Our assessment is that the authors' data support the conclusions. The assay sensitivity was supported by appropriate response from the positive control relative to the solvent control. The highest dose level of glyphosate used was limited to the test compound's solubility and by the volume that could be injected into a rat. A range-finding study (Study No. 830082) used to set the maximum dose presented data on cytotoxicity for levels of test compound up to 1000 mg/kg. However, there was no concurrent cytotoxicity data. Moreover, only a single concentration of test compound was tested.

Conclusions:

Glyphosate did not induce significant clastogenic effects in rats under conditions of the study which was limited to the assay of a single dose level of 1000 mg/kg. Cylophosphamide at 25 mg/kg caused a highly significant number of chromosomal aberrations demonstrating the sensitivity of the assay. Under the conditions of the study, glyphosate did not cause any fatalities or other signs of toxicity. Monsanto has addressed these issues in the letter of November 26, 1984 (memorandum of W. Dykstra of March 12, 1985, attached).

Classification: Acceptable

PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Glyphosate: EPA Reg. #: 524-308; Registrant's response to review of mutagenicity study
Caswell #: 661A

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767)

THRU: Robert P. Zendzian, Ph.D. 3/13/85
Acting Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: William Dykstra, Ph.D. William Dykstra 3/13/85
Toxicology Branch
Hazarad Evaluation Division (TS-769)

Recommendations:

1. The letter from Monsanto dated November 26, 1984 adequately addresses the Toxicology Branch review. The Toxicology Branch review of the in vivo bone marrow cytogenetics study of glyphosate in Sprague-Dawley rats stated that the study was unacceptable since dose-response data were not available (only a single dose) and concurrent cytotoxicity data were not available. The study number was ML-83-236.

Monsanto states in their letter that the range finding study was conducted at 200-1000 mg/kg. No cytotoxicity was produced in the range-finding study and, therefore, the single dose level of 1000 mg/kg was used.

Therefore, the study is acceptable since the previous basis of evaluation has been adequately addressed.

Review:

1. No new toxicity data were submitted.

2. A copy of the previous review is attached.

Attachment

171

009614

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12356)

EPA: 68-01-6561
TASK: 67
June 4, 1984

Subject File No. 6-614

DATA EVALUATION RECORD

GLYPHOSATE

Mutagenicity (Range-Finding Study)

STUDY: [illegible] Effects of glyphosate on rat bone marrow cells. An unpublished report (study no. ML-83-160) prepared for Monsanto Agricultural Products Company by Environmental Health Laboratory, Monsanto Co. St. Louis, MO. Dated October 21, 1983.

REVIEWED BY:

William McLellan, Ph.D.
Senior Scientist
Dynamac Corporation

Signature: William L. McLellan
Date: 6 June 1984

I. Cecil Felkner, Ph.D.
Mgr. Genetic Toxicology Dept.
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 6-6-84

Cipriano Cueto, Ph.D.
Department Director
Dynamac Corporation

Signature: [illegible]
Date: 6-6-84

APPROVED BY:

William Dykstra, Ph.D.
EPA Scientist

Signature: [illegible]
Date: 6-11-84

009614

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity (range-finding study).

CITATION: Li, A.P. Effects of glyphosate on rat bone marrow cells. An unpublished report (study no. ML-83-160) prepared for Monsanto Agricultural Products Company by Environmental Health Laboratory, Monsanto Co. St. Louis, MO. Dated October 21, 1983.

ACCESSION NUMBER: 251737.

LABORATORY: Environmental Health Laboratory, Monsanto Co. St. Louis, MO.

QUALITY ASSURANCE STATEMENT: Present, signed and dated October 21, 1984.

TEST MATERIAL: The test material was identified as glyphosate (EHL sample No. T830044) a white powder having a purity of 98.7 percent.

MATERIAL AND METHODS:

Preparation of Test Material: A stock solution of 100 mg/ml was prepared by suspending glyphosate in Hank's balanced salt solution (HBBS) and adjusting the pH to 7.5 with sodium hydroxide. Dilutions of the stock solution in HBBS were freshly prepared to yield solutions of 20, 40, 60, and 80 mg/ml.

Controls: Hank's buffered salt solution 10 ml/kg was used as the vehicle control.

Animals: The animals used in the study were male and female Sprague-Dawley rats [CD(SD)BR] from Charles River Breeding laboratories. The animals were approximately 10 weeks old at the time of test material administration; the males weighed 264-299 g and females weighed 179-202 g. Water and Purina Laboratory Chow were provided ad libitum except for a 14-24 hours fasting period just prior to dosing. Animals were maintained in individual cages in rooms maintained at 70-74 °F and a relative humidity of between 25 and 60 percent. The rooms had 12-hour light/dark cycles.

Experimental Design: Rats (4/sex/group) were fasted overnight and then injected intraperitoneally with 10 ml of HBSS containing glyphosate. The final doses in the groups were 0, 200, 400, 600, 800, and 1,000 mg/kg. Four hours after administration of glyphosate or vehicle control, 4 mg/kg colchicine were administered ip, and two hours later the animals were sacrificed by CO_2 asphyxiation and by severance of their spinal cords.

009614

Preparation of Bone Marrow Cells: Bone marrow was separated from each femur into a 5 ml plastic syringe containing 2 ml HBSS. The contents were added to plastic centrifuge tubes containing 5 ml HBSS and incubated at 37° C until they were prepared for analysis.

Cell Viability Determination: An aliquot of the cell suspension was stained with acridine orange and ethidium bromide (EPL SOP L-58081-6004). Slides were prepared, and approximately 100 cells/animal at each dose level were examined by fluorescent microscopy. Since viable cells take up acridine orange and appear green and non-viable cells take up ethidium bromide and appear orange, the viable cells could be quantitated.

Determination of Mitotic Index: The cell suspensions were centrifuged, the pellet suspended in 1 ml 0.075 M KCl at 37° , and an additional 5 ml of KCl added. After 30 min incubation at 37° C, 1 ml Carnoy's fixative was added (methanol-glacial acetic acid 3/1, v/v). The cells were then pelleted, 5 ml of fresh fixative added, and the cell suspension stored at 4° C. One to 2 drops of cell suspensions were fixed on slides and stained 15-20 min with 2 percent Geimsa solution. The slides were then rinsed and air dried.

Approximately 500 cells/slide were counted to quantitate metaphase and non-metaphase cells. The mitotic index (ratio of mitotic cells to the total number of cells counted) was calculated from this data.

RESULTS:

Viability: Viability ranged from 95.8 to 98.5 percent in males and from 93.2 to 97.8 percent in females groups. Solvent control values were 95.8 percent for males and 98.5 percent for females. Hence, the author assessed that glyphosate at doses up to 1000 mg/kg, had no effect on cell viability.

Mitotic Index: The mitotic index for control males was 0.028 and for control females 0.032 (average of 4 animals). The mitotic index for males dosed at 800 mg/kg was slightly but significantly ($p = 0.05$) increased over controls (0.049). In other dose groups of males the mitotic indices were similar to controls (0.030-0.037).

In glyphosate-treated females the mitotic index was slightly lower at 400 mg/kg (0.019, $p = 0.036$) than in controls, but there were no significant differences at other dose levels (mitotic indices ranged from 0.024-0.039).

DISCUSSION:

The authors concluded that doses up to 1,000 mg/kg glyphosate could be used to score the potential cytogenetic effect in vivo in rats since there was no significant reduction in the mitotic index. However, it was noted

that the highest dose used in the range finding study was the maximum dose that could be effectively administered based on solubility of the test compound and the volume that could be injected ip into rats.

This reviewer agrees the conclusions. The 4 percent reduction in mitotic index in 400 mg/kg females may not be compound related, since there was no dose-response relationship. Furthermore, such a slight lowering of the mitotic index would not affect the cytogenetic study. In selecting doses for in vivo cytogenicity testing, the limit should be based on solubility; the maximum dose will be inadequate if cytotoxic responses are the basis for selecting the maximum dose.

CONCLUSIONS:

Glyphosate (at dose levels between 200 - 1,000 mg/kg) did not cause any loss of viability in vivo in rat marrow cells. There was a slight decrease (4 percent) in mitotic index in females at 400 mg/kg but not at higher doses, and no effects in males. Therefore 1,000 mg/kg can be tolerated in an in vivo cytogenicity assay in rats.

CLASSIFICATION: Acceptable.

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/11/91
Toxicology Branch I - IRS (H7509C)
for Secondary Reviewer: Roger Gardner, Section Head Roger D. Gardner 5/11/91
Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: 84-4; Other Genotoxic Effects TOX Chem. No.: 661A

Accession No.: 251737 MRID No.: 00132686

Test Material: Glyphosate Technical, Lot No. XHJ-64, Purity 98.7%

Synonyms: Compound JJN-1020

Study Number: AH-83-181

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Naylor Dana Institute, Valhalla, NY 10595

Title of Report: The Hepatocyte Primary Culture/DNA Repair Assay on Compound JJN-1020 Using Rat Hepatocytes in Culture.

Authors: G.M. Williams and C. Tong

Report Issued: October 21, 1983

Conclusions:

Under the conditions of the assay, glyphosate did not induce DNA damage at concentrations between 1.25×10^{-5} and 1.25×10^{-1} mg/mL. All relevant study criteria were met except the following:

1. No preliminary toxicity testing reported,
2. No criteria for dose-selection, and
3. Stated to have been tested up to solubility limit, but no data or documentation presented.

Classification: Unacceptable

Special Review Criteria (40 CFR 154.7):

Note: Dr. Irving Mauer, geneticist, screened this mutagenicity study for acceptability. The DER is based on parts of a Dynamac review.

00135

Review:

Quality Assurance Statement - Although the report stated that a quality assurance review was prepared for the study, a signed and dated report was not present.

Test Material - The test material was identified as JJM-1020 of Lot No. XHJ-64, provided by Monsanto Company. Its purity was 98.7 percent and it was reported to be soluble in 0.1 N NaOH.

Materials and Methods:

Hepatocyte Primary Cultures (HPC) - The cells used in the study were freshly prepared hepatocytes from adult male F-344 rats. The hepatocytes were obtained by a modification of the procedure developed by Williams et al.* The rats were anesthetized with 50 mg/kg sodium nembutal and perfused with sterilized Solutions I and II by means of a sterile peristaltic pump. Solution I contained 0.5 mM ethyleneglycol-bis(B-amino-ethyl ether) N-N'-tetracetic acid (EGTA) in calcium and magnesium-free Hank's balanced salt solution, buffered with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes) adjusted to pH 7.35, using 1N NaOH. Solution II contained 100 unit/mL of type 1 collagenase in Williams' medium E (WME) buffered by 10 mM Hepes (pH 7.35).

Profusion was through the portal veir via a 21 gauge butterfly needle using a flow rate of 8 mL/min at 37 °C for Solution I. At the start of perfusion with Solution I, the process of ligating the infrahepatic vena cava was completed and the vein severed distally so that the perfusate ran to the waste container. When the liver was uniformly balanced, a cannula was inserted into the thoracic inferior vena cava so that the perfusate could be collected by means of this return cannula; then the flow rate was increased to 40 mL/min for 2.5 minutes. The perfusion with Solution I was followed by perfusion with sterile Solution II at a flow rate of 20 mL/min at 37 °C for 10 minutes (not recirculating the return perfusate). The liver was covered with sterile gauze warmed by a 40W light bulb.

The perfused liver was removed, trimmed of extraneous fat and connective tissue into a Petri dish with warm WME under sterile conditions. The tissue was then transferred to fresh Solution II. After opening the liver at numerous points on the inferior surface and removal of the capusule, the cells were detached by "gentle combing with a stainless steel comb and shaking off loose cells." After complete combing, the fibrous

---

*Williams, G.M., Bermutes, E., and Scaramuzzino, D. (1977) Vitro 13:809-817.

plug was discarded and 25 mL aliquots of the hepatocyte suspension were pipetted into 50 mL centrifuge tubes, adjusting the volume to 50 mL with WME, supplemented with 10 percent calf serum and 50 ug/mL gentamycin (WMES). The cell suspension was centrifuged for 2.5 minutes at 50 x g, and the cell pellet was resuspended in WMES. A 20-fold dilution of the cell suspension was prepared and 0.5 mL of this diluted suspension was added to 0.1 mL of 0.4 percent to trypan blue so that viability (differential staining) could be assessed using a hemocytometer. The author stated that cell yields of approximately 2.0×10^8 per 100 g body weight and hepatocytes viabilities of about 90 percent were usually obtained.

For the HPC/DNA studies, 5×10^5 cell/mL WMES were seeded immediately onto 25 mm round coverslips in 25 mm 6 well dishes under 5 percent CO_2, humidified in an incubator at 37 °C. The coverslips were washed with 1 mL WME 2 hours after seeding so that only the attached viable cells remained.

Preparation of Test Material - The test material, JJN-1020, was solubilized in 0.1 N NaOH at a maximum solubility of 12.5 mg/mL. Serial dilutions of the stock solution were made in 0.1 N NaOH and 20 uL of the stock solutions were add to 2 mL of assay medium so that the final test concentrations were 1.25×10^{-1}, 6.25×10^{-2}, 1.25×10^{-2}, 6.25×1^{-3}, 1.25×10^{-4} and 1.25×10^{-5} mg/mL.

Controls - The positive control chemical was benzo(a)pyrene at a final concentration of 5×10^{-5} M and the negative control was pyrene also at 5×10^{-5} M. Solvent controls included 1 percent dimethylsulfoxide (DMSO) and 1 percent of 0.1 N NaOH. An untreated negative control was also used.

Hepatocyte Primary Culture DNA Repair Assay - The HPC/DNA repair assay was performed using methods developed by Williams[1,2]. Immediately after washing with 1 mL WME, the test material and 20 uCi/mL tritiated thymidine ([^{3}H]-TdR) at 60 to 80 Ci/mM were added to 2 mL of the WME cell suspension. The test material was applied at five logarithmically decreasing concentrations on triplicate coverslips with the appropriate parallel positive and negative (untreated and solvent) controls.

After incubation for 18 to 24 hours in the presence of test material in [^{3}H]-TdR-WME, coverslips were removed from the wells and successively rinsed three times with 100 mL of WME. Each coverslip was then immersed for 10 minutes, cell surface up in 2

---

[1]Williams, G.M. (1977) Cancer Res. 37:1845-1851.
[2]Williams, G.M. (1980) In: Chemical Mutagens. Vol VI eds. de Serres, F.J. and Hollaender, A. Plenum Press, NY pages 61-79.

mL of 1 percent sodium citrate, in clean 6-well dishes, to cause nuclear swelling (permits better nuclear grain quantification), and finally fixed by three 30-minute changes of glacial acetic acid (3:1), air dried, and mounted on glass slides. Slides were dipped into NTA emulsion (Eastman Kodak) that had been prewarmed at 45 'C for 1 hour, removed, and dried in a light-tight box. Slides were wrapped in foil and stored at 4 'C in cardboard slide boxes.

Ten days after storage, autoradiographs were developed for 4 minutes in D19 (Eastman Kodak), placed in acidified tap water for 30 seconds, immersed in fixer (Eastman Kodak) for 10 minutes, and washed with tap water for 5 minutes. Next, slides were stained with Harris' alum hematoxylin, counterstained with eosin, dehydrated through 100 percent ethanol, air dried, and the coverslips sealed with Permount.

Slide Evaluation - Nuclear grains were scored with an Artek Model 880 electric counter equipped with a microscopic attachment, using the area mode (permits distinction between discrete grains, even in aggregates). The net increase in grains induced by the test chemical or the positive control relative to the solvent control was the method used for quantification. To avoid artifacts, only cells with swollen nuclei (viable cells at fixation) and those evenly coated with emulsion were scored. From each coverslip quadrant, between 5 and 20 randomly selected cells were scored (depending upon the nuclear/cytoplasmic grain ratio*). Background grain counts were assessed by counting three nuclear sized areas adjacent to the nucleus, and the net nuclear grain counts were calculated by subtracting the highest cytoplasmic count from the nuclear count.

Data Interpretation - By subtracting counts of the highest cytoplasmic background, false positive scores could be avoided. A minimum net grain count of five per nucleus, consistently observed in triplicate coverslips was the criteria for a positive sample, and if the minimum was consistently observed throughout the experiment, the compound was considered positive.

If S phase cells, identified by morphology and/or high grain density in the autoradiograph, were absent, then a cytotoxic response had occurred. A negative result was reported if less than five net nuclear grains counts were observed at the highest noncytotoxic dose.

---

*Rogers, A.W. (1973) In: Techniques of Autoradiography. Elsevier Sci. Pub. Co., p. 218.

Results:

The authors reported that cytotoxicity was not observed when the HPC cells were exposed to the highest concentrations of JJN-1020 used and that none of the net grain counts/nucleus exceeded a value of 5. The highest net grain value for the test material was 1.4 ± 0.5 (1.25×10^{-1} mg JJN-1020 per mL) while the negative control values were 0.3 ± 0.5, 0.3 ± 0.1, 0.2 ± 0.3, and 0.4 ± 0.4 for DMSO, 0.1N NaOH, untreated cell culture, and pyrene, respectively. The positive control, B(a)P, gave a net grain count of 22.9 ± 9.7. Hence, sensitivity of the assay was adequate.

Discussion:

The author concluded that under the conditions of the HPC/DNA repair assay, no genotoxicity was induced by treatment with JJN-1020 at concentrations from 1.25×10^{-5} to 1.25×10^{-1} mg/mL.

Conclusions:

Under the conditions of the assay and as reported, the test material (JJN-1020), glyphosate, did not induce DNA damage at concentrations between 1.25×10^{-5} and 1.25×10^{-1} mg/mL.

Classifications: Unacceptable

1. No preliminary toxicity testing,
2. No criteria for dose selection, and
3. Stated to have been tested up to solubility limit, but no data or documentation presented.

Reviewed By: William Dykstra, Ph.D. William Dykstra 5/13/91
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Roger Gardner, Section Head Pamela McHenley 5/14/91
Section I, Toxicology Branch I - IRS (H7509C)

009614

DATA EVALUATION REPORT

Study Type: 84-4, Other Genotoxic Effects, Rec-Assay in B. subtilis TOX Chem. No.: 661A

Accession Number: N/A MRID No.: 00078619

Test Material: Glyphosate, technical; 98.4% purity

Synonym: CP67573

Study Number: None

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Institute of Environmental Toxicology, Japan

Title of Report: Microbial Mutagenicity Testing on CP67573 (Glyphosate).

Authors: Y. Shirasu, M. Moriya, T. Ohta

Report Issued: July 20, 1978

Conclusions:

Glyphosate technical was negative for mutagenicity up to 2000 ug/disk in the rec-assay with Bacillus subtilis H17 (rec^+) and M45 (rec^-) and in the reverse mutation assays with and without S-9 up to 5000 ug/plates (or toxicity) employing Escherichia coli WP2 hcr and Salmonella typhimurium TA strains (TA1535, TA1537, TA1538, TA100, and TA98) as tester strains.

Classification: Acceptable

Special Review Criteria (40 CFR 154.7): N/A

Note: Dr. Irving Mauer, geneticist, screened these studies for acceptability.

00210

Methods:

Standard methods were employed.

Results:

1. Rec-Assay - As shown in the Table below, glyphosate did not show any inhibitory zone in H17 and M45 at all the tested doses of 20 to 2000 ug/disk.

   The positive control, Mitomycin C, caused an 11 mm difference in the length of the inhibitory zones of the two strains.

   The negative control, Kanamycin, included similar lengths of inhibitory zones in the two strains.

Rec-Assay With B. subtilis M45 and H17

Compound	ug/Disk	Inhibition Zone (mm) M45	H17	Difference (mm)
Control (DMSO)		0	0	0
CP67573	20	0	0	0
	100	0	0	0
	200	0	0	0
	500	0	0	0
	1000	0	0	0
	2000	0	0	0
Kanamycin	10	7	5	2
Mitomycin C	0.1	11	0	11

2. Reverse Mutation Assay (gene mutation) - As shown in the Table below, glyphosate did not induce any significant increase in the numbers of revertant colonies of any strains over the control values, either in the presence or absence of S-9.

009614

The positive controls, in contrast, induced reverse mutations in the tester strains.

Reverse Mutation Tests With and Without a Liver Metabolic Activation System

Compound	ug/Plate	S-9 Mix	Revertant Colonies/Plate					
			WP2 hcr	TA1535	TA100	TA1537	TA1538	TA98
Control (H_2O)		-	20	6	167	9	10	24
			24	14	129	10	13	23
CP67573	10	-	22	2	130	3	17	27
			21	5	160	7	24	28
	50	-	12	5	151	5	15	33
			25	5	159	6	15	40
	100	-	18	4	143	8	17	20
			20	5	160	8	24	20
	500	-	21	3	118	11	7	31
			26	1	143	9	15	24
	1000	-	15	9	87	10	18	21
			18	12	120	10	12	23
	5000	-	*	6	58	3	6	[illegible]
			*	6	87	3	7	3
Control (H_2O)		+	17	6	139	7	8	22
			22	5	140	5	11	16
CP67573	10	+	25	4	110	3	16	19
			18	1	135	3	11	23
	50	+	27	9	123	7	13	21
			22	5	131	9	17	26
	100	+	33	5	125	11	18	9
			17	7	115	6	14	20
	500	+	[illegible]	3	138	12	15	19
			30	3	111	5	7	26
	1000	+	29	11	97	11	20	15
			24	4	88	7	11	23
	5000	+	25	5	51	6	11	19
			34	7	36	3	15	22

00112

Reverse Mutation Tests With and Without a Liver Metabolic Activation System (cont'd)

Compound	ug/Plate	S-9 Mix	Revertant Colonies/Plate					
			WP2 hcr	TA1535	TA100	TA1537	TA1538	TA98
2-amino-anthracene	10	-	23	8	179	18	23	40
			16	11	201	13	21	48
	10	+	98	376	> 3000	370	> 3000	> 3000
			79	335	> 3000	388	> 3000	> 3000
		-	1672[a]	315[b]	1024[c]	10000[d]	> 3000[e]	326[f]
			2272	358	1150	10000	> 3000	296

[a] AF-2 0.25 ug/plate
[b] β-propiolactone 50 ug/plate
[c] AF-2 0.05 ug/plate
[d] 9-aminoacridine 200 ug/plate
[e] 2-nitrofluorene 50 ug/plate
[f] AF-2 0.1 ug/plate
*Toxic