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

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CASWELL FILE

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Glyphosate; AMPA Toxicology Studies; ID#: 285984; Miscellaneous Toxicology Data; Metabolite of Glyphosate; P.C. Code: 103601

Tox.Chem No.: 661A
MRID No.: several
DP Barcode No.: D207485
Submission No.: S473414

TO: Robert Taylor/Vickie Walters, PM Team #25
Fungicide/Herbicide Branch
Registration Division (H7505C)

FROM: William Dykstra, Ph.D., Toxicologist
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)
William Dykstra 12/21/95

THRU: Roger Gardner, Section Head, Toxicologist
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)
Roger Gardner 1/19/96
KR 1/26/96

ACTION REQUESTED: The Registrant, Monsanto Company, has submitted miscellaneous toxicology studies with AMPA, the plant metabolite of glyphosate. Toxicology Branch-I has been requested to review the studies and determine if any significant toxicological adverse effects are reported.

CONCLUSIONS: The studies are acceptable and support the decision of the HED Metabolism Committee to remove AMPA from the tolerance expression of glyphosate. The results of the studies are in general agreement with the Registrant's previously

conclusions and the conclusions by Monsanto are not appreciably different. There were no significant adverse effects. DER's for the studies are attached and brief summaries are presented below:

GUIDELINE 82-1 (b)

Randomized groups of 5/sex/dose outbred beagle dogs were orally administered by capsule doses of 0, 10, 30, 100, or 300 mg/kg/day of technical AMPA (based on the 88% a.i., the actual doses were **8.8, 26.4, 88, or 264 mg/kg/day**) for 92 days. All dogs were observed twice daily, weighed weekly, and food consumption was measured daily. Clinical pathology was performed at pretest, and weeks 5 and 12. All dogs were necropsied and organ weights were recorded and microscopic examination of the tissues was performed.

The NOEL is 264 mg/kg/day (HDT). There were no compound-related findings in clinical observations, body weight, food consumption, ophthalmological results, clinical pathology results, organ weights, and gross and microscopic tissue examination results.

Classification: **SUPPLEMENTARY - NO EFFECTS IN STUDY**

GUIDELINE 83-3 (a)

Randomized groups of 25 mated Sprague-Dawley rats were administered technical AMPA in corn oil, at a dosing volume of 10 ml/kg, at doses of 0, 150, 400, or 1000 mg/kg/day during days 6-15 of gestation. Dams were sacrificed at day 20 of gestation and necropsied. Weights of liver, kidneys and spleen were recorded together with the reproductive data. Fetuses were examined for external, visceral, and skeletal malformations and variations.

The developmental NOEL is 400 mg/kg/day. At the LEL of 1000 mg/kg/day, there was decreased fetal body weight (6%) which was statistically significant. There were no compound-related external, visceral, or skeletal malformation or variations.

The maternal NOEL is 150 mg/kg/day. At the LEL of 400 mg/kg/day, clinical signs of toxicity included hair loss, soft stool, and mucoid feces. Slight maternal toxicity was also seen as 21% decreased weight gain, which was statistically significant, at 1000 mg/kg/day during dosing days 12-16 of gestation.

Classification: **GUIDELINE**

GUIDELINE 84-2

AMPA was negative up to the limit doses of 2000 ug/disk in the rec-assay and up to 5000 ug/plate in the reverse mutation assay both with and without S-9 mix. Positive controls in both systems gave the expected results.

Classification: **ACCEPTABLE**

GUIDELINE 84-2

AMPA is negative for the induced in vivo mouse micronucleus test at doses of 100, 500, and 1000 mg/kg given intraperitoneally to randomized groups of both sexes of CD-1 mice. The positive control, cyclophosphamide, produced a statistically significant increase in micronuclei.

Classification: **ACCEPTABLE**

GUIDELINE 84-2

AMPA is negative in the in vitro rat hepatocyte DNA repair assay at concentrations up to 5000 ug/ml. The positive control, 2-AAF, produce a highly positive response in the same UDS assay at 3 ug/ml.

Classification: **ACCEPTABLE**

Reviewed by: William Dykstra Ph.D., Toxicologist William Dykstra 12/21/95
Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head, Toxicologist
Section I, Tox. Branch I Roger Gardner 1/19/96

DATA EVALUATION REPORT

STUDY TYPE: 82-1(B) 90 day dog oral capsule study

TOX. CHEM NO: 661A

ACCESSION NUMBER: n/a MRID NO.: 433347-02

TEST MATERIAL: AMPA, technical; purity, 87.8%

SYNONYMS: Glyphosate metabolite

STUDY NUMBER: WIL project No. WIL-50173

SPONSOR: Monsanto Co.

TESTING FACILITY: WIL research Labs, Ashland, OH

TITLE OF REPORT: 90-day oral (capsule) toxicity study in dogs with AMPA

AUTHOR(S): E.C. Tompkins

REPORT ISSUED: July 16, 1991

CONCLUSION: Randomized groups of 5/sex/dose outbred beagle dogs were orally administered by capsule doses of 0, 10, 30, 100, or 300 mg/kg/day of technical AMPA (based on the 88% a.i., the actual doses were **8.8, 26.4, 88, or 264 mg/kg/day**) for 92 days. All dogs were observed twice daily, weighed weekly, and food consumption was measured daily. Clinical pathology was performed at pretest, and weeks 5 and 12. All dogs were necropsied and organ weights were recorded and microscopic examination of the tissues was performed.

The NOEL is 264 mg/kg/day (HDT). There were no compound-related findings in clinical observations, body weight, food consumption, ophthalmological results, clinical pathology results, organ weights, and gross and microscopic tissue examination results.

Classification: **core-supplementary - no effects in study**

Special Review Criteria (40 CFR 154.7) n/a

A. MATERIALS:

1. Test compound: . Description - white solid, Batch # - PIT-9008-2407-T, Purity - 87.8%.

2. Test animals: Species: dog, Strain: outbred beagle, Age: 6 months, Weight: 8.1-11.9 ♂; 6.7-10.8 ♀ , Source: Ridglan Farms, Inc., Mt. Horeb, WI.

B. STUDY DESIGN:

1. Animal assignment

Animals were individually caged and assigned randomly to the following test groups:

Test Group	Dose in diet (mg/kg/d)	Main Study 3 months male	Main Study 3 months female	Interim Sac. months male	Interim Sac. months female
1 Cont	0	5	5		
2 Low (LDT)	10	5	5		
3 Mid (MDT)	30	5	5		
4 MidHigh (MHDT)	100	5	5		
5 High (HDT)	300	5	5		

2. Diet preparation

Diet was prepared daily and stored at room temperature. Approximately 400 grams of basal ration, Purina Certified Canine Chow #5007, was offered for 1-2 hours daily. Drinking water was offered ad libitum. Capsules containing weighed amounts of test substance were administered following the 1-2 hour feeding period. Samples of capsules were analyzed for concentration at the beginning and conclusion of the study. Doses were based on a one month dietary capsule study at doses of 0, 10, 30, 100, 300, and 1000 mg/kg/day in groups of 2/sex/dose beagle dogs. Hematologic changes in both sexes at the 1000 mg/kg/day dose included decreased RBC, increased reticulocytes, decreased hemoglobin and hematocrit. Hematologic changes at 300 mg/kg/day included decreased reticulocytes, hematocrit and hemoglobin in females.

Results - AMPA was stable throughout the study. Pre-test assay was 91.8% and post-test assay was 91.2%.

3. Animals received 400 g of food (Purina Certified Canine Chow #5007) for 1-2 hours and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Body weights, body weight changes, food consumption, clinical pathology data, and organ weights were analyzed by one-way analysis of variance followed by two-tailed Dunnett's test at 5% and 1% levels of significance when comparing the control and each treated group by sex.

5. A signed quality assurance statement was present.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected twice daily, once prior to dosing and once (1-2 hours) after dosing with the capsules, for signs of toxicity and mortality.

There were no deaths and all animals survived to terminal sacrifice. Numerous clinical signs were seen in control and treated dogs and consisted primarily of lacrimation, scabbed or reddened or swollen ears, soft stool, diarrhea, salivation and emesis. It should be noted that clinical observations were made prior to and after dosing and the results detailed in the DER are prior to dosing. After dosing, the incidences of scabbed, red, and swollen ears were greatly diminished in all treated groups in comparison to controls. For this reason, the findings prior to dosing were not considered treatment-related. Also, these findings occurred at similar frequency in control and treated groups and in a non dose-related manner when occasional disproportionate frequencies occurred. The table below shows the frequency of the most common findings prior to dosing. The results after dosing were different than the results shown below:

MALES (total occurrence/no. dogs)

	Control	10	30	100	300
Scab left ear	30/5	36/3	57/5	48/4	82/5
Scab R. ear	44/5	50/3	45/4	41/3	95/5
Red and swollen ears	11/3	27/4	35/3	21/4	59/4
soft stool	32/5	77/5	61/5	82/5	60/5
diarrhea	6/3	30/5	10/4	18/5	11/2
mucoid feces	2/1	11/1	4/3	0/0	6/3
salivation	51/3	7/2	10/2	101/4	16/4
emesis @ food	0/0	1/1	6/4	2/2	0/0

FEMALES (total occurrence/no. dogs)

	Control	10	30	100	300
Scab left ear	34/4	34/4	48/4	32/3	9/3
Scab R. ear	50/5	21/3	45/4	16/2	29/3
Red and swollen ears	17/3	11/4	77/4	42/3	6/2
soft stool	74/5	48/5	40/5	26/4	74/5
diarrhea	11/4	14/4	5/3	5/1	16/2
mucoid feces	2/2	0/0	1/1	2/1	8/4
salivation	0/0	1/1	4/2	8/3	1/1
emesis @ food	2/2	3/2	1/1	1/1	1/1

2. Body weight

Animals were weighed weekly for the entire study.

There were no compound-related effects in body weight between the controls and male and female treated groups. The percent increase in body weight from week 0 to week 12 in males was 18.5, 23.3, 30.5, 20.9, and 22.3% for the control, 10, 30, 100, and 300 mg/kg/day groups, respectively. The percent body weight increases in males are within normal limits for young dogs and the slightly elevated percentages in treated dogs in comparison to controls was not considered toxicologically significant. In females, the percent increase in body weight during this same period was 15.6, 13.0, 16.8, 9.2, and 14.3% for the control, 10, 30, 100, and 300 mg/kg/day groups, respectively. The variations in percentage between control and treated groups was not considered toxicologically significant.

MALES (GRAMS)

DOSE (MG/KG/DAY)

	0	10	30	100	300
Week					
0	9781	10064	9074	9861	9818
4	10128	10913	10143	10392	10399
8	11076	11859	11056	11278	11423
12	11597	12411	11849	11925	12017
% Inc.	18.5	23.3	30.5	20.9	22.3

FEMALES (GRAMS)

DOSE (MG/KG/DAY)

	0	10	30	100	300
Week					
0	8606	8683	8214	8443	8360
4	9052	8996	8604	8660	8678
8	9539	9509	9121	9206	9136
12	9956	9819	9597	9217	9562
% Inc.	15.6	13.0	16.8	9.2	14.3

3. Food consumption and compound intake

Food consumption was determined daily. There were no

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compound-related effects in food consumption between controls and treated male and female groups.

MALES (GRAMS/ANIMAL/DAY)

DOSE (MG/KG/DAY)

Week	0	10	30	100	300
0 - 1	257	281	271	268	250
4 - 5	309	352	312	310	308
8 - 9	318	335	365	339	341
12 - 13	335	347	337	365	361

FEMALES (GRAMS/ANIMAL/DAY)

DOSE (MG/KG/DAY)

Week	0	10	30	100	300
0 - 1	214	204	210	222	220
4 - 5	266	262	258	250	246
8 - 9	248	273	274	260	305
12 - 13	283	307	304	295	287

4. Ophthalmological examination

Performed at weeks -1 and 13 on all animals. There were no

compound-related ocular findings at weeks -1 and 13 in treated males and female dogs in comparison to controls.

5. Blood was collected before treatment and at weeks 5 and 12 for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood clotting measurements		
x	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic and chronic studies

Results - There were no statistically significant hematological results in males at pretest, and weeks 5 and 12. In females at the 10 mg/kg/day level, MCV was statistically significantly decreased at weeks 5 and 12, but not at pretest. There were no other statistically significant hematological findings suggestive of anemia in any of the treated female or control group females and the MCV findings at 10 mg/kg/day were not considered toxicologically significant, since the finding was not dose-related and not supported by associated hematological data.

b. Clinical Chemistry

X		X	
	Electrolytes:		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 (ALK)	x	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
	Creatinine phosphokinase*^		Serum protein electrophores

	Lactic acid dehydrogenase (LAD)
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*
x	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

Results - There were no statistically significant findings in females at pretest, and weeks 5 and 12 and in males at pretest and week 5. At week 12, in males, there were statistically significant increases in glucose at 30 and 100 mg/kg/day in comparison to controls. Additionally, although not statistically significant, the 300 mg/kg/day was elevated. The mean values of the control, 10, 30, 100, and 300 mg/kg/day groups for glucose were 91, 95, 101*, 101*, and 100 mg/dl, respectively. These mean treated values are not toxicologically significant in comparison to controls. Shown below are the individual values for each male dog on study demonstrating that all values, control and treated, are within the normal range:

GLUCOSE (MG/DL)

0	10	30	100	300
85	93	106	100	104
97	94	105	93	93
95	90	91	101	98
90	99	101	99	106
89	101	104	111	98

6. Urinalysis^

Urine was collected from fasted animals at 5 and 12 weeks. The CHECKED (X) parameters were examined.

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

^Not required for subchronic studies
* Required for chronic studies

Results - There were no statistically or toxicologically

significant urinalysis findings in male and female treated dogs in comparison to controls.

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*	x	Heart*	x	Periph. nerve*#
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*#
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen	x	Eyes (optic n.)*#
x	Jejunum*	x	Thymus*	Glandular	
x	Ileum*	Urogenital		xx	Adrenal gland*
x	Cecum*	xx	Kidneys*+		Lacrimal gland#
x	Colon*	x	Urinary bladder*	x	Mammary gland*#
x	Rectum*	xx	Testes*+	xx	Parathyroids*++
xx	Liver *+	xx	Epididymides	xx	Thyroids*++
x	Gall bladder*	x	Prostate	Other	
x	Pancreas*		Seminal vesicle	x	Bone*#
Respiratory		xx	Ovaries*+	x	Skeletal muscle*#
x	Trachea*	x	Uterus*	x	Skin*#
x	Lung*			x	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

a. Organ weight - There were no statistically significant differences between control absolute and relative organ weights and absolute and relative organ weights of male and female treated dogs.

b. Gross pathology - There were no compound-related gross necropsy findings in treated male and female dogs in comparison to controls.

c. Microscopic pathology - No compound-related findings

1) Non-neoplastic - There were no compound-related microscopic lesions in treated male and female dogs in comparison to controls.

2) Neoplastic - There was one oral papilloma in a high dose female dog on study

D. DISCUSSION: The results of this study agree with the summary information provided to HED by Monsanto prior to the HED Metabolism Committee decision to remove AMPA from the glyphosate tolerance expression. It should be noted that clinical observations were made prior to and after dosing and the results detailed in the DER are prior to dosing. After dosing, the incidences of scabbed, red, and swollen ears were greatly diminished in all treated groups in comparison to controls. For this reason, the findings prior to dosing were not considered treatment-related.

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 10/14/94
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head Roger Gardner 12/14/95
Review Section I, Tox Branch I

DATA EVALUATION REPORT

STUDY TYPE: 83-3 (a) Developmental Toxicity Study - Rats

TOX. CHEM NO: 661A

MRID NO.: 433347-05

TEST MATERIAL: AMPA, 94.38% purity

SYNONYMS: glyphosate metabolite

STUDY NUMBER: WIL Project No.: WIL-50159

SPONSOR: Monsanto Company

TESTING FACILITY: WIL Research Labs, Ashland, OH

TITLE OF REPORT: A Developmental Toxicity Study of AMPA in Rats

AUTHOR(S): Joseph F. Holson

REPORT ISSUED: August 6, 1991

CONCLUSION: Randomized groups of 25 mated Sprague-Dawley rats were administered technical AMPA in corn oil, at a dosing volume of 10 ml/kg, at doses of 0, 150, 400, or 1000 mg/kg/day during days 6-15 of gestation. Dams were sacrificed at day 20 of gestation and necropsied. Weights of liver, kidneys and spleen were recorded together with the reproductive data. Fetuses were examined for external, visceral, and skeletal malformations and variations.

The developmental NOEL is 400 mg/kg/day. At the LEL of 1000 mg/kg/day, there was decreased fetal body weight (6%) which was statistically significant. There were no compound-related external, visceral, or skeletal malformation or variations.

The maternal NOEL is 150 mg/kg/day. At the LEL of 400 mg/kg/day, clinical signs of toxicity included hair loss, soft stool, and mucoid fecess. Slight maternal toxicity was also seen as 21% decreased weight gain, which was statistically significant, at 1000 mg/kg/day during dosing days 12-16 of gestation.

Core Classification: **GUIDELINE**

A. Materials

Test Compound: AMPA

Purity: 94.38%
Description: white solid
Lot No.: HET-9001-1463T
Contaminant: list in CBI appendix No

Vehicle(s): Mazola corn oil, 100% purity

Test Animal(s): Species: Sprague-Dawley rats
Strain: Crl:CD™BR
Source: Charles River Breeding Labs, Portage, MI
Age: 71 days old on receipt
Weight: 213 to 261 on gestation day 0.

B. Study Design

This study was designed to assess the developmental toxicity potential of AMPA when administered by gavage to mated Sprague-Dawley rats on gestation days 6 through 15, inclusive.

Mating

Females were mated 1:1 with male rats of same strain and source. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or sperm in a vaginal smear

Group Arrangement: Randomly assigned by consecutive block design to groups of 25 mated rats each.

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	25
Low Dose	150	25
Mid Dose	400	25
High Dose	1000	25

Dosing: Constant volume of 10 ml/kg during dosing period.

The dosing solutions were analyzed for concentration and stability. Dosing was based on most recent gestation day body weight. Samples were analyzed at the Monsanto Company for concentration and stability and are reported.

Observations

The basal diet used in the study was Purina Certified Rodent Chow™ #5002. The animals were checked for mortality or abnormal condition twice daily. Maternal body weights and food consumption were recorded on gestation days 0, 6, 9, 12, 16, and 20. Dams were sacrificed on day 20 of gestation by carbon dioxide inhalation. Gross necropsy was performed and organs and tissues examined. Examinations at sacrifice consisted of: examination and weighing of uterus and examination of ovaries, number of corpora lutea were counted, individual uterine implantation sites were recorded, fetuses were examined externally, sexed and weighed, early and late resorptions and total number of implantation sites were recorded. Nonpregnant dams had uteri excised, opened and placed in 10% ammonium sulfide for detection of early implantation loss. The liver, kidneys and spleen from each dam were trimmed and weighed.

The fetuses were examined in the following manner: Each fetus was individually sexed, weighed, and examined for external anomalies. Crown-rump measurements were made for late resorptions and the tissues were discarded. Fetal sex was verified by internal examination. Each fetus was examined for visceral anomalies by fresh dissection technique to include heart and major vessels. Fetal kidneys were examined and graded for renal papillae development. Heads from one-half fetuses were placed in Bouin's fixative for subsequent soft tissue examination by the Wilson method. The heads from the remaining fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed, and stained with Alizarin Red S by Dawson method. Skeletal examination was carried out by low power stereomicroscope. External, visceral, and skeletal findings were recorded as developmental variations and malformations.

Historical control data were provided to allow comparison with concurrent controls.

Analysis of Dosing Solutions: Concentration analyses of corn oil solution containing AMPA ranged from 105 to 120% of target values for all day 0 and last day samples. Analytical recoveries of spiked samples were comparable to study sample results with analytical recoveries ranging from 87-111% of targeted doses.

Statistical analysis

The following statistical analysis methods were employed: All analyses were conducted using two-tailed tests at a 5% level of significance comparing each treated group to the control group. All statistical analyses were performed by a digital computer with appropriate programming. The following methods were used to analyze the data: Chi-square test with Yates correction factor for fetal sex ratios; Fisher's Exact Test for malformations and variations; Mann-Whitney U-test for early and late resorptions, dead fetuses, and postimplantation losses; one-way ANOVA with Dunnett's test for corpora lutea, total implantations, fetal body weight, viable fetuses, maternal body weights, body weight changes, food consumption, uterine weights, and organ weights; Kruskal-Wallis test for litter proportion of intrauterine data.

Compliance

A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided

A signed Quality Assurance Statement was provided.

Results

Maternal Toxicity: All dams survived until terminal sacrifice on gestation day 20. There were increased incidences of dams and numbers of days of hair loss, soft stool and mucoid feces in the 400 and 1000 mg/kg/day groups, predominantly. The following table shows the distribution of pregnancy rates and clinical signs:

	Control	Low	Mid	High
Hair Loss left forelimb	13/1	17/3	77/6	38/6
soft stool	49/15	89/19	102/22	135/23
Mucoid feces	0/0	3/1	14/7	48/18
Gravid	24	24	24	24
Nongravid	1	1	1	1

The effect of excreta-related findings was transitory and occurred only during the treatment period with an occasional incidence following the treatment period. These clinical findings are not considered highly significant, but only of marginal toxicological importance.

Mortality There were no mortalities during the treatment period and all animals survived to the planned sacrifice on gestation day 20.

Clinical Observations Described above

Body Weight

The investigators supplied the following data:

Table I: Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
Control	35	48	64	147		
LDT	33	49	64	146		
MDT	36	51	60	147		
HDT	34	43(11%)	59	136		

[1] = corrected body weight gain for dosing period = body weight gain for dosing period minus gravid uterus weight.
[2] = corrected body weight gain for entire gestation period = body

weight gain for entire gestation period minus gravid uterus weight.
a = Data extracted from Table 3 and 4 of report

During the initial six days of dosing (days 6-12 of gestation), body weight gains of the 1000 mg/kg/day group were comparable to other treated groups and the controls. During gestation days 12-16 of the dosing period, (not shown in the above table) mean weight gain in the 1000 mg/kg/day group was slightly, but statistically significantly, decreased by 21%. The weight gains for the control, low, mid, and high dose groups during the gestation days 12-16 were 33, 30, 32, and 26 grams, respectively. For the entire dosing period, the high dose effect was seen as only a 11% decrease in weight gain (shown in the above table). The affected high dose animals "rebounded" during the remainder of the gestation period (days 16-20), so that the high dose group had weight gains in the postdosing period which were comparable to controls.

Food Consumption

The investigators supplied the following data:

Table II: Food Consumption Data (gram/kg/day)[a]

Group:	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period
Control	82	56	80	68
LDT	83	57	80	68
MDT	85	60*	81	71*
HDT	82	56	81	68

[a] = Data extracted from Table 7 of report

Food consumption in the 1000 mg/kg/day group was slightly, but significantly, decreased during the first three days of dosing (gestation days 6-9) (not shown in above table) and was slightly, but significantly, increased during days 9-12 of gestation (not shown in above table). Food consumption was comparable to controls at the high dose during other times during dosing and post dosing and the entire gestation period. The significant increase in mid dose food consumption during the dosing period and the entire gestation period was not considered toxicologically significant in light of the absence of similar effects at the high dose.

Gross Pathological Observations

The investigators supplied the following data in Table 8:

ORGAN WEIGHTS (grams)

	Control	Low	Mid	High
No. Exam.	**24**	**24**	**24**	**24**
Kidneys	1.94	1.99	2.00	2.02
Liver	15.45	15.39	15.68	14.89
Spleen	0.67	0.60	0.64	0.61

There were no treatment-related effects in kidneys, spleen and liver between control and treated female rats.

Cesarean section Observations

Table III: Cesarean Section observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	25	25	25	25
#Animals Mated/Inseminated	24	24	24	24
Pregnancy Rate (%)	96	96	96	96
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	1	1	1	1
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	387	407	394	397
Corpora Lutea/dam	16.1	17.0	16.4	16.5
Total Implantation	361	366	360	363
Implantations/Dam	15.0	15.3	15.0	15.1
Total Live Fetuses	345	347	346	349
Live Fetuses/Dam	14.4	14.5	14.4	14.5
Total Resorptions	16	19	14	14
Early	15	19	13	14
Late	1	0	1	0
Resorptions/Dam	0.7	0.8	0.6	0.6
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.5	3.5	3.4	3.3*
Preimplantation Loss(%)	6.4	9.0	8.1	7.9
Postimplantation Loss(%)	4.4	5.3	3.9	3.8
Sex Ratio (% Male)	47.2	52.2	52.1	51.4

[a] = Data extracted from Tables 9 and 10

The only statistically significant finding in the uterine data is the statistically significant decrease in fetal weight (both sexes) at 1000 mg/kg/day. The 6% decrease in fetal body weight was essentially due to two litters (dams #72276 and #72284) with mean fetal weights of 2.4 and 2.8 grams, respectively. The study authors note that the fetal weight in the high dose group is

similar to the 3.3 gram minimum value of historical control data from WIL labs. The slight decrease in fetal weight at 400 mg/kg/day, 3.4 grams, has been observed in 24% of control studies (20/85) at the lab. The finding of slightly decreased fetal weight at 1000 mg/kg/day is considered a slight compound-related effect.

2. Developmental Toxicity

Table IV: External Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	345(24)	347(24)	346(24)	349(24)
#pups(litters) affected	1(1)	1(1)	0(0)	2(2)
Dizygotic conceptuses	0(0)[a]	1(1)	0(0)	0(0)
Sternoschisis	0(0)	0(0)	0(0)	1(1)
Localized fetal edema	0(0)	0(0)	0(0)	1(1)
Microphthalmia	1(1)	0(0)	0(0)	0(0)

(+) some observation may be grouped together
(a) fetal [litter] incidence

Table IV: Visceral Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	345(24)	347(24)	346(24)	349(24)
#pups(litters) affected	1(1)	2(2)	0(0)	0(0)
Interrupted aortic arch	0(0)[a]	1(1)	0(0)	0(0)
Situs Inversus	0(0)	1(1)	0(0)	0(0)
Hydrocephaly	1(1)	0(0)	0(0)	0(0)

(+) some observation may be grouped together
(a) fetal [litter] incidence

Table IV: Skeletal Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	345(24)	347(24)	346(24)	349(24)
#pups(litters) affected	0(0)	4(4)	0(0)	0(0)
Bent Limb Bones	0(0)[a]	1(1)	0(0)	0(0)
Ribs - hemispherical	0(0)	1(1)	0(0)	0(0)
Vertebral anomaly W/WO Rib Anomaly	0(0)	2(2)	0(0)	0(0)
Variations				
Unossified sternebrae #5 or #6	26(9)	25(10)	33(14)	25(10)
14th Rudimentary rib	7(6)	7(3)	3(2)	8(5)
Reduced ossification of 13th ribs	2(2)	6(5)	10(4)	9(3)

(+) some observation may be grouped together
(a) fetal [litter] incidence

D. Discussion/Conclusions

a. Maternal Toxicity: Slight maternal toxicity was seen at 1000 mg/kg/day as decreased weight gain during dosing days 12-16 of gestation. Additionally, clinical signs of toxicity included hair loss, soft stool, and mucoid feces at both 400 and 1000 mg/kg/day. Both findings are considered compound-related and toxicologically significant. Increases and decreases in food consumption at 400 and 1000 mg/kg/day are not considered toxicologically significant.

b. Developmental Toxicity: Slightly decreased (6%) fetal body weight at cesarean section at the high dose is considered compound-related.

i. Deaths/Resorptions: No compound-related effects

ii. Altered Growth: Slight decrease of 6% in fetal body weight

iii. Developmental Anomalies: No compound-related effects

iv. Malformations: No compound-related effects

D. Study Deficiencies: NONE

E. Core Classification: Core Guideline Data.

Maternal NOEL = 150 mg/kg/day
Maternal LOEL = 400 mg/kg/day
Developmental Toxicity NOEL = 400 mg/kg/day
Developmental Toxicity LOEL = 1000 mg/kg/day

F. Risk Assessment: Not required

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 10/12/94
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I Roger Gardner 1/19/96

DATA EVALUATION REPORT

STUDY TYPE: 84-2; Microbial Mutagenicity Study with AMPA

TOX. CHEM NO: 661A

MRID NO.: 43334707

TEST MATERIAL: AMPA, 99% purity; CP50435

SYNONYMS: Metabolite of Glyphosate

STUDY NUMBER: R.D. 1251

SPONSOR: Monsanto Company

TESTING FACILITY: The Institute of Environmental Toxicology, Tokyo, Japan

TITLE OF REPORT: CP50435: Microbial Mutagenicity Study

AUTHOR(S): Y. Shirasu; M. Moriya; T. Ohta

REPORT ISSUED: November, 1980

CONCLUSION: AMPA was negative up to the limit doses of 2000 ug/disk in the rec-assay and up to 5000 ug/plate in the reverse mutation assay both with and without S-9 mix. Positive controls in both systems gave the expected results.

Core Classification: **ACCEPTABLE**

REVIEW: Microbial Mutagenicity Study (The Institute of Environmental Toxicology; November, 1980)

Materials: CP50435; 99% AMPA (rather than 94.38% technical)

Assays: AMPA was tested in the repair test (rec-assay) with Bacillus subtilis H17 (rec+) and M45 (rec-) and in the reverse mutation tests with or without a rat liver metabolic activation system employing Escherichia coli WP2 hcr and Salmonella typhimurium TA series (TA1535, TA1537, TA1538, TA100 and TA98) as tester strains. In the rec-assay, AMPA was tested at 20, 100, 200, 500, 1000, or 2000 ug/disk. The positive control, Mitomycin C, was tested at 0.1 ug/disk and the negative control, Kanamycin, was tested at 10 ug/disk. In the reverse mutation assays, AMPA was tested at 10, 50, 100, 500, 1000, and 5000 ug/plate both in the presence and absence of S-9 metabolic activation. The positive control with S-9 was AF-2 and positive controls without S-9 were 2-nitrofluorene, AF-2, B-propiolactone, and 9-aminoacridine.

RESULTS: Rec-assay: AMPA did not induce any inhibitory zone in either strain in all the doses tested. The positive control, mitomycin C, caused a 7.5 mm length inhibitory zone in the two strains, whereas the negative control, kanamycin, induced similar lengths of inhibitory zones.

Reverse mutation assays: AMPA did not induce any significant increase in the number of revertant colonies of any strain, compared to controls, both with and without S-9 mix. In contrast, AF-2, B-propiolactone, 9-aminoacridine and 2-nitrofluorene induced reverse mutations in the absence of S-9 mix and 2-aminoanthracene was mutagenic for all the strains in the presence of S-9 mix.

Conclusions: AMPA was negative up to the limit doses of 2000 ug/disk in the rec-assay and up to 5000 ug/plate in the reverse mutation assay both with and without S-9 mix. Positive controls in both systems gave the expected results.

Quality Assurance Statement: There was no Quality Assurance Statement and the study was conducted before GLPs.

Table 1 Rec-assay with B. subtilis M45 and H17

Compound	µg/disk	Inhibitory zone (mm) M45 (recomb. deficient)	Inhibitory zone (mm) H17 (recomb. wild)	Difference (mm)
Control (H_2O)		0	0	0
CP50435	20	0	0	0
	100	0	0	0
	200	0	0	0
	500	0	0	0
	1000	0	0	0
	2000	0	0	0
- control Kanamycin	10	6	5	1
+ control Mitomycin C	0.1	9.5	2	7.5

Table 2 Reverse mutation tests with or without a liver metabolic activation system (S-9 mix)

Compound	µg/plate	S-9 Mix	No. of revertant colonies / plate					
			WP2 hcr	TA1535	TA100	TA1537	TA1538	TA98
Control (H_2O)		-	13	6	96	5	11	29
			10	8	98	6	6	31
CP50435	10	-	18	4	120	8	7	16
			18	6	124	7	3	30
	50	-	11	4	129	1	7	27
			14	2	141	1	9	30
	100	-	20	8	108	6	4	21
			9	3	137	7	4	17
	500	-	22	11	106	5	7	16
			14	4	124	4	9	30
	1000	-	20	6	84	4	7	24
			14	2	138	8	6	26
	5000	-	12	15	107	4	8	23
			13	6	81	8	5	31
Control (H_2O)		+	12	4	102	10	10	16
			6	5	107	4	13	22
CP50435	10	+	11	7	102	2	6	13
			11	9	105	9	14	19
	50	+	12	5	91	2	10	20
			8	2	91	5	4	12
	100	+	16	7	81	14	7	19
			10	5	83	1	7	19
	500	+	10	5	79	3	5	21
			21	4	103	7	13	16
	1000	+	11	3	97	9	9	14
			21	5	96	5	9	16
	5000	+	17	2	83	4	6	12
			11	4	99	4	7	17
Positive control (2-amino-anthracene)	10	-	9	10	117	22	8	38
			12	4	111	18	15	41
	10	+	52	164	>3000	128	>3000	>3000
			50	232	>3000	204	>3000	>3000
Positive control		-	1304[a]	694[b]	588[c]	>10000[d]	>3000[e]	212[f]
			1476	684	648	>10000	>3000	223

a) AF-2 0.25 µg/plate
b) β-propiolactone 50 µg/plate
c) AF-2 0.05 µg/plate
d) 9-aminoacridine 200 µg/plate
e) 2-nitrofluorene 50 µg/plate
f) AF-2 0.1 µg/plate

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 10/11/94
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I Roger Gardner 12/14/95

DATA EVALUATION REPORT

STUDY TYPE: 84-2; Mouse Micronucleus Study of AMPA

TOX. CHEM NO: 661A

MRID NO.: 43334708

TEST MATERIAL: AMPA, 94.38% purity; white solid

SYNONYMS: Metabolite of glyphosate

STUDY NUMBER: R.D. No. 1251

SPONSOR: Monsanto Company

TESTING FACILITY: Environmental Health Lab; Monsanto Company

TITLE OF REPORT: Mouse Micronucleus Study of AMPA

AUTHOR(S): L.D. Kier and S.D. Stegeman

REPORT ISSUED: 12/8/93

CONCLUSION: AMPA is negative for the induced *in vivo* mouse micronucleus test at doses of 100, 500, and 1000 mg/kg given intraperitoneally to randomized groups of both sexes of CD-1 mice. The positive control, cyclophosphamide, produced a statistically significant increase in micronuclei.

Core Classification: **ACCEPTABLE**

Review: Mouse Micronucleus Study of AMPA (Environmental Health Lab; Study No. 90170; Sponsor Project No. ML-90-404; 12/8/93)

Test Material: AMPA; 94.385 purity; white solid; Lot No. HET-9001-1463T

Animals: 7-10 week old male and female CD-1 mice (Source: Charles River Laboratories Inc., Portage, MI.) After quarantine, animals were randomly selected for test and control groups and fed Purina Certified Rodent Chow No. 5002 and tap water ad libitum.

Methods: Three dose levels for main study were based on range-finding data. The negative controls were treated with corn oil only (10 ml/kg) and positive controls were treated with cyclophosphamide (40 mg/kg). Test material was suspended in corn oil, the vehicle. Doses were administered intraperitoneally by injection for maximum absorption. Mouse bone marrow was sampled at 24, 48, and 72 hours after dosing for the vehicle and AMPA dosed groups and 24 hours after dosing with the positive control. Slides of bone marrow were prepared from five animals/sex/time point for each group and scored for the occurrence of micronucleated polychromatic erythrocytes (micronucleated PCEs) and PCE/total erythrocyte ratios.

Group	No. of Mice		Sacrifice Times					
			24 hrs		48 hrs		72 hrs	
	M	F	M	F	M	F	M	F
0(corn oil)	15	15	5	5	5	5	5	5
cyclophos-phamide	5	5	5	5				
100 mg/kg	15	15	5	5	5	5	5	5
500 mg/kg	15	15	5	5	5	5	5	5
1000 mg/kg	15	15	5	5	5	5	5	5

Results: Toxic signs were seen in the AMPA treated mice as decreased mean body weight change. These findings were seen at both 500 and 1000 mg/kg/day and were statistically significant. Micronucleus data are shown below:

Mean Micronucleated PCE/1000 PCE

Time	Sex	Dose (mg/kg) Control	100	500	1000	Positive Contr.
24 hr	M	0.2	0.2	0.1	0.8	18.3**
	F	1.0	0.8	2.0	0.8	12.0*
48 hr	M	0.6	0.0	0.6	0.2	
	F	0.4	0.2	0.2	0.0	
72 hr	M	0.2	0.0	0.0	0.0	
	F	0.0	1.6	0.8	0.4	

** = $p \leq 0.01$

* = $p \leq 0.05$

Conclusions: AMPA is negative for induced in vivo mouse micronucleus test at doses of 100, 500, and 1000 mg/kg given intraperitoneally to randomized groups of both sexes of CD-1 mice. The positive control, cyclophosphamide, produced a statistically significant increase in micronuclei.

Quality Assurance Statement: A GLP Compliance Statement and a Quality Assurance Statement were present and signed by the study authors and John L. Henshaw, Director of Quality and Compliance Assurance.

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 10/13/94
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I
Roger Gardner 12/14/95

DATA EVALUATION REPORT

STUDY TYPE: 84-2; UDS Mutagenicity Assay in Male Fischer 344 Rats

TOX. CHEM NO: 661A

MRID NO.: 43355801

TEST MATERIAL: AMPA, 94.38% purity; white solid

SYNONYMS: Metabolite of Glyphosate

STUDY NUMBER: 2495-V01-91

SPONSOR: Monsanto Company

TESTING FACILITY: SRI International, Menlo Park, CA

TITLE OF REPORT: Evaluation of the Potential of AMPA to Induce Unscheduled DNA Synthesis in the In Vitro Hepatocyte DNA Repair Assay Using the Male F-344 Rat

AUTHOR(S): J.P. Backke

REPORT ISSUED: 8/5/94

CONCLUSION: AMPA is negative in the in vitro rat hepatocyte DNA repair assay at concentrations up to 5000 ug/ml. The positive control, 2-AAF, produce a highly positive response in the same UDS assay at 3 ug/ml.

Core Classification: **ACCEPTABLE**

REVIEW: Evaluation of the Potential of AMPA to Induce Unscheduled DNA Synthesis in the in vitro Hepatocyte DNA Damage Repair Assay Using the Male F-344 Rat (SRI No. SR-91-234; SRI Study No. 2495-V01-91; 12/5/91)

Test Material: AMPA; 94.38% purity; white solid; Lot No. HET-9001-1463-T; Positive control, 2-AAF, >99%, purity.

Animals: 44 male Fischer-344 (F-344) rats, 15-19 weeks of age, weighing 315-346 grams, were used in the study. Only two rats, numbered 3 and 4, were used for the preliminary and replicate experiments, respectively. Purina Certified Rodent Chow #5002 and tap water were provided ad libitum.

Methods: AMPA was tested in the UDS assay in F-344 hepatocyte in vitro cell culture, according to established Guidelines, at concentrations of 5, 10, 50, 100, 250, 500, 1000, 2500, 3800 and 5000 ug/ml, with 5000 ug/ml being the high dose in both the preliminary (first) and replicate (second) experiments. Culture medium served as both the negative and vehicle control and the positive control was 2-AAF at a concentration of 3.0 ug/ml. After radiographic exposure, the slides were evaluated for cytotoxicity under a light microscope. Quantitative autoradiographic grain counting was done on at least 30 morphologically unaltered cells on a randomly selected area of the slide. Routinely, three slides per concentration were scored.

Criteria for Interpretation: The data from the assay is acceptable if the net grains/nucleus (NG) and the percentage of cells in repair (% IR) values of the solvent control group are within the normal historical ranges and if the positive controls produce a UDS response of greater than 5.0 NG.

Positive: The assay was positive if the mean net grain count for any dose group was greater than 5 NG.

Negative: The assay was negative if the mean net grain count was less than 0 NG and % IR was less than 10% for all dose groups.

RESULTS: The NG counts were negative for concentrations of the medium control in the preliminary and replicate experiments, yielding mean NG values of -15.3 NG with 3% IR and -12.6 with 2% IR, respectively, in contrast to the strong positive response produced by 2-AAF (9.4 NG with 63% IR in the preliminary test and 19.7 NG with 85% IR in the replicate study).

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AMPA was tested up to 5000 ug/ml in both experiments. Cytotoxicity was seen at 5000 ug/ml in the first experiment and 3800 and 5000 ug/ml in the second. Concentrations of 5, 10, 50, 100, 250, 1000, and 2500 ug/ml of AMPA in the first and second experiments yielded negative mean NG values, ranging from -18.6 to -12.9 NG with 0 to 6% IR and -13.0 to -8.8 NG with 1 to 2% IR, respectively.

Conclusion: AMPA is negative in the in vitro rat hepatocyte DNA repair assay at concentrations up to 5000 ug/ml. The positive control, 2-AAF, produce a highly positive response in the same UDS assay at 3 ug/ml.

Quality Assurance Statements: A Good Laboratory Practice Compliance Statement and Quality Assurance Statement was signed by the study director, James P. Backke, and Pam Pallakoff of the Quality Assurance Unit.

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Chemical:	**Isopropylamine glyphosate (N-(phosphono**
PC Code:	**103601**
HED File Code	**13000 Tox Reviews**
Memo Date:	**02/01/96**
File ID:	**TX011777**
Accession Number:	**412-02-0012**

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