I

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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

September 6, 2007
TXR # 0053314

SUBJECT: Glufosinate Ammonium - Review of 28-Day Inhalation Toxicity Study (MRID 47058101).

PC Code: 128850
DP Barcode: D337298

FROM: Lisa Austin, Ph.D.
Registration Action Branch I
Health Effects Division (7509P)

TO: Joanne Miller (RM 23)
Registration Division (7505P)

THROUGH: P.V. Shah, Ph.D., Acting Branch Chief
Registration Action Branch I
Health Effects Division (7509P)

I. CONCLUSIONS

The Registration Action Branch I (RAB 1) has reviewed the 28 day inhalation toxicity study (MRID 47058101) for glufosinate ammonium. The study was classified as **Acceptable/Non-guideline**. The submitted study did not meet the conditional requirement for a section 3 registration as set forth by HED because a NOAEL was not established, only two dose levels were tested and more importantly, glutamine synthetase activity was not measured in the brain, kidney, liver and lungs.

Recorded in RLC 9/26/07 D.B.

II. ACTION REQUESTED

The Registration Division (RD) requested Health Effects Division (HED) to perform a review of a 28 day inhalation toxicity study (MRID 47058101) for glufosinate ammonium. This study was identified as a data gap and as adverse data (6A2); therefore, it was required as part of the section 3 registration of glufosinate ammonium. The action was successfully completed. The data evaluation report (DER) is attached.

III. RESULTS/DISCUSSION

Study Type	MRID	Comments
870.3465 28-day inhalation rat	47058101	New DER

DATA EVALUATION RECORD
GLUFOSINATE
[Non-guideline]
STUDY TYPE: FOUR-WEEK INHALATION TOXICITY/RATS
MRID 47058101

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 173-2007

Primary Reviewer:
K. Clark Swentzel, B.S.

Signature: Robert H. Ross for K. Clark Swentzel
Date: MAY 17 2007

Secondary Reviewers:
Cheryl B. Bast, Ph.D., D.A.B.T.

Signature: Cheryl B Bast
Date: MAY 17 2007

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAY 17 2007

Quality Assurance:
Susan Chang, M.S.

Signature: S Chang
Date: MAY 17 2007

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Lisa Austin, Ph. D. **Signature:** Lisa Austin
Registration Action Branch I, Health Effects Division (7509C) **Date:** 9/6/07
EPA Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** [illegible]
Registration Action Branch I, Health Effects Division (7509C) **Date:** 9-26-07

Template version 02/06

TXR#: 0053314

DATA EVALUATION RECORD

STUDY TYPE: Subacute Inhalation Toxicity – rat (non-guideline)

PC CODE: 128850 **DP BARCODE:** 337298

TEST MATERIAL (PURITY): Glufosinate-Ammonium TK (Glufosinate) – 51.2%

SYNONYMS: Hoe 039866, SHA 128850, GA

CITATION: Blair, J. (2007) Glufosinate: Toxicity Study by Inhalation Administration to CD Rats for 4 Weeks. Huntingdon Life Sciences Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Report No. BAG 0408/063551, February 9, 2007. MRID 47058101. Unpublished.

SPONSOR: Bayer CropScience AG, Monheim, Germany

EXECUTIVE SUMMARY:

In a subacute inhalation study (MRID 47058101) Glufosinate-Ammonium (51.2% a.i., batch number 2005-001955) was administered to 5 Crl:CD® (SD)IGS BR rats/sex /concentration by dynamic (nose only) exposure at concentrations of 0, 0.056 or 0.105 mg a.i./L (equivalent to 0.109 or 0.205 mg a.i./L, respectively) for 6 hours per day, 5 days/week for a total of 28 days.

There were no toxicologically significant compound-related effects based on the assessment of body weight, food consumption, eyes, hematology and clinical chemistry. One female exposed to the 0.105 mg a.i./L level was sacrificed on humane grounds on Day 3 of exposure based on clinical signs which were piloerection, unsteady gait, partially closed eyes, hunched posture and pallor. Clinical signs, some indicative of neurological effects, were also seen in males at this exposure level as well as in females exposed to 0.056 mg a.i./L, however, they were typically observed in a small number of animals (1/5 females) and/or tended to be transient. Adjusted organ weight changes in animals exposed to 0.105 mg a.i./L were decreases in heart weight in males and liver weights in males and females as well as increased kidney weights in males and lungs/bronchi in females. At 0.056 mg a.i./L, increased kidney weight was noted in males and increased lungs/bronchi weight was seen in females ($p<0.05$). Gross observations in animals exposed to 0.105 mg a.i./L were dark area(s) in the liver of a male, congested lungs/bronchi in a male and female and pelvic dilation in the kidneys of a female; congested lungs/bronchi were also seen in 2 females exposed to 0.056 mg a.i./L. Tissues and organs were preserved for histopathologic assessments, however, none of the preserved samples were actually assessed

microscopically.

Under the conditions of this study, a NOAEL is not identified. The LOAEL is 0.109 mg/L based on lung/bronchial congestion in female rats.

This subacute inhalation toxicity study in the rat is **Acceptable/Non-guideline.**

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Glufosinate-Ammonium TK 50% (w/w)

Description:	Colorless liquid
Lot/batch #:	Batch no. 2005-001955
Purity:	51.2 %
Compound stability:	Used in study before expiration date (September 7, 2007); stored at 25 ± 5° C
CAS # of TGAI:	77182-82-2
Structure:	Not available

2. Vehicle and/or positive control: Not applicable

3. Test animals:

Species:	Rat
Strain:	Crl:CD® (SD)IGS BR
Age/weight at study initiation:	Group nos. 1 & 2: 61 to 74 days; Group no. 3: 75 to 88 days/Group nos. 1 & 2: males--337-376 g, females--197-252 g; Group no. 3: males--385-471 g, females--211-276g
Source:	Charles River (UK) Ltd., Margate, Kent, England
Housing:	Five/sex/cage unless reduced by mortality or isolation (all Group 3 males had bite marks after 2 days of exposure; they were housed individually thereafter)
Diet:	Rat and Mouse No. 1 Maintenance Diet, Special Diets Services Ltd. *ad libitum* (except during exposure)
Water:	Public water provided in bottles *ad libitum* (except during exposure)
Environmental conditions:	**Temperature:** 18-21EC **Humidity:** 40-70% **Air changes:** not reported **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	Group nos. 1 &2: 25 days; Group no. 3: 39 days

B. STUDY DESIGN:

1. **In life dates:** Start: Group nos. 1 & 2- July 3, 2006; Group no. 3- July 17, 2006 ; End: Group nos. 1 & 2- July 30, 2006; Group no. 3- August 13, 2006.

2. **Animal assignment:** Animals were assigned (*method of selection was not reported)* to the test groups noted in Table 1. The MMAD values, which were similar for both exposure groups, indicated a bimodal distribution of particles. The inhalable fraction was greater than 90% for each group, which is acceptable.

TABLE 1: Study design

Test group (number)[a]	Nominal conc. (mg/L)	Analytical conc. (mg a.i./L)[b]	MMAD (Φm)	GSD	Rats/sex
Control (1)					5
Low (2)	0.525	0.056	<0.26 and 2.71	2.15	5
High (3)	0.977	0.105	<0.26 and 2.57	2.29	5

[a] Animals were exposed to the test chamber atmospheres via nose-only, 6 hours/day, 5 days/week for 4 weeks; controls were exposed to air without test material. Exposure was initiated in Group 3 two weeks after exposure initiation in Groups 1 & 2.
[b] Concentrations of Glufosinate-ammonium; concentrations of test material were 0.109 and 0.205 mg/L for Groups 2 & 3, respectively.

3. **Dose selection rationale:** The selection of target exposure levels of GA in this study, 0, 0.05 or 0.1 mg/L, was based on a previous study with the solid form of this compound (A31612) in which rats were exposed to the powder at target concentrations of 0.012, 0.025 and 0.05 mg/L. Clinical effects were seen at the higher exposure levels and there were 4 mortalities (*exposure level(s) not reported*). The objective of the current study was to compare the relative toxicity of the liquid material to the solid material.

4. **Generation of the test atmosphere / chamber description:**

Time to equilibrium was not reported.

Test atmosphere concentration: Each sample of the exposure system atmosphere was drawn through a filter mounted in an open-face filter holder. The sample volume was measured using a wet-type gas meter placed in-line with the pump. The sampling rate was set daily using a tapered tube rotameter. Each filter was weighed before and after collection; the samples were analyzed using HPLC. Results are in table 1 above.

Particle size determination: Each sample of the chamber atmosphere was drawn through a Marple (Model 296) Personal Cascade Impactor with stainless steel collection substrates and a glass fiber filter. The sample volume was measured using a wet-type gas meter placed in-line with the pump. The sampling rate was set before use with a tapered tube rotameter. Each collection substrate and filter was weighed before and after use; the samples were analyzed using HPLC. Results are in table 1 above.

5. **Statistics:** All analyses were performed separately for each sex using the individual animal as the basic experimental unit. The following data types were analyzed separately at each time point: bodyweight (absolute and gain), clinical chemistry, hematology and organ weights (absolute and adjusted for terminal body weight). For continuous data, Bartlett's test was first applied to test the homogeneity of variance between the groups. Tests dependent on the outcome of Barlett's test were used to compare exposed and control groups, incorporating adjustments for multiple comparisons when necessary.

The following sequence of tests was used for body weight, organ weight and clinical pathology data. If 75% of the data (across all groups) were the same value (e.g., c), then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions and Fisher's Exact tests for each dose group against the control for

1) values <c versus values ≥ c and 2) values ≤ c versus values > c, as applicable. If Barlett's test for variance homogeneity was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response was not significant at the 1% level, William's test for a monotonic trend was applied. If the F1 test was significant, suggesting that the dose-response was not monotonic, Dunnett's test was performed instead. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H1 test was significant, suggesting that the dose-response was not montonic, Steel's test was performed instead. For organ weight data, analysis of covariance was initially performed using terminal bodyweight as a covariate. If the intra-group relationship between organ weight and body weight was significant at the 10 % level, then the treatment comparisons were made on adjusted group means in order to allow for differences in body weight which might influence the organ weights. Significant differences between control and exposed groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice daily for signs of toxicity and mortality.

1b. Clinical examinations: A detailed physical examination was performed weekly, however, the parameters that were examined were not reported.

1c. Neurological evaluations: A systematic neurological evaluation was not performed.

2. **Body weight:** Animals were weighed 1 week before exposure, on the initial day of exposure and weekly thereafter.

3. **Food consumption:** Food consumption for each cage was determined 1 week before the exposure period and weekly thereafter. Weekly food consumption per rat was calculated by

dividing cage consumption by the number of animals in the cage to derive g of food consumed/rat/week. Food efficiency was not determined.

4. **Ophthalmoscopic examination:** Eyes of all animals were examined before the exposure period and during Week 4 of exposure.

5. **Hematology and clinical chemistry:** Blood was collected during Week 4 of exposure (before dosing) from all animals, after an overnight withdrawal of food and chew blocks, for hematology and clinical analysis from all surviving animals. Blood was also collected from all animals immediately prior to necropsy to determine glutamine synthetase activity in a separate study. Blood was collected from one Group 3 female before the unscheduled sacrifice on Day 3 of exposure. The CHECKED (X) parameters were examined.

a. **Hematology:**

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	X	Reticulocyte count
	Blood clotting measurements	X	Abnormal morphology
X	(Activated partial thromboplastin time)		Bone marrow [a]
	(Clotting time)		
X	(Prothrombin time)		

[a] Samples were collected, but not examined

b. **Clinical chemistry:**

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
	Magnesium	X	Urea
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
X	**ENZYMES** (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase	X	Total serum protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase	X	Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)		
X	Aspartate aminotransferase (AST/also SGOT)		
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. **Urinalysis**: Urinalysis was not performed.

7. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for possible future histological examination; none of the collected tissues was examined. The (XX) organs were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta, thoracic	X	Brain
X	Salivary glands	XX	Heart	X	Peripheral nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus	X	GLANDULAR
X	Ileum			XX	Adrenal gland
X	Cecum	X	UROGENITAL	X	Lacrimal gland
X	Colon	XX	Kidneys	X	Parathyroid
X	Rectum	X	Urinary bladder	X	Thyroid
XX	Liver	XX	Testes	X	OTHER
	Gall bladder (not rat)	X	Epididymides	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate	X	Skeletal muscle
X	Pancreas	X	Seminal vesicles	X	Skin
X	RESPIRATORY	X	Ovaries	X	All gross lesions and masses
X	Trachea	X	Uterus	X	Harderian glands
XX	Lungs (with bronchi)	X	Mammary gland		
X	Nose	X	Vagina		
X	Pharynx				
X	Larynx				

II. RESULTS:

A. OBSERVATIONS :

1. **Clinical signs of toxicity**: Clinical signs of toxicity, which included those indicative of neurotoxicity, were observed in Group 2 females and Group 3 males and females; no signs were seen in Group 2 males. The clinical signs that were seen in these animals tended to be transient. All of the animals in these groups except one Group 3 female had ungroomed coats at several intervals during the study. Partially closed eyes (3/5) and fast breathing (2/5) were noted in Group 2 females at various intervals during the study; additionally, vocalization, underactive, salivation, aggressive, hunched posture, pallor and repetitive sideways movement of the head were noted for single animals in this group during the first 4 days of exposure. Signs in Group 3 females included nervous (3/5), underactivity (3/5), head moving left to right (2/5) and piloerection (2/5); most of these signs were seen only within the first 5 days of exposure. Additional signs observed in single animals from this group only during the first 3 exposure days included salivation, vocalization, fast breathing, closed eyelids, irritable, standing on hind limbs, shallow breathing, unsteady gait, hunched posture and pallor. Signs in Group 3 males included irritable (5/5), nervous (3/5) and underactive (2/5); these signs were seen most frequently during the first 5 days of the study. Left to right head movement

was seen in one male from this group during the first 18 days of exposure. Group 3 males became aggressive after 2 days of exposure; bite marks were seen on each animal. Consequently, these animals were housed singly after the third day of exposure. Clinical signs were not observed in Group 1.

2. **Mortality:** One Group 3 female was sacrificed on Day 3 of exposure due to clinical signs: piloerection, unsteady gait, partially closed eyelids, hunched posture and pallor.

3. **Neurological evaluations:** NA

B. **BODY WEIGHT AND WEIGHT GAIN:** There was no apparent compound-related effect on body weight gain (Table 2). One Group 3 male lost weight during the exposure period, however, the remaining animals in this group gained weight during the same period. Mean body weights in Group 3 males were higher than the control values at the beginning and end of the exposure period. Body weight gains in Group 2 and 3 females were statistically greater than the control value.

TABLE 2. Body weights and body weight gains

Group no.	Body weight (g±SD)						Total gain[a]
	Week -1	Week 0	Week 1	Week 2	Week 3	Week 4	g ± SD (% from control)[b]
Males							
1	340 ± 16.4	355 ± 10.9	380 ± 22.9	403 ± 24.3	420 ± 25.8	424 ± 33.0	69 ± 24.7
2	342 ± 4.4	365 ± 16.5	391 ± 15.9	408 ± 13.1	430 ± 16.5	435 ± 15.9	70 ± 9.9 (+1)
3	403 ± 31.7	417 ± 32.6	419 ± 38.4	452 ± 39.1	470 ± 44.1	463 ± 46.2	47 ± 26.5 (-32)
Females							
1	219 ± 14.2	229 ± 15.8	243 ± 14.4	251 ± 13.6	253 ± 12.6	250 ± 16.1	20 ± 13.0
2	202 ± 9.6	208 ± 7.6	229 ± 12.2	239 ± 13.2	251 ± 16.5	256 ± 19.9	48* ± 13.3 (+140)
3	248 ± 23.6	249 ± 26.7	261 ± 29.6	276 ± 34.1	275 ± 29.4	272 ± 27.4	29* ± 7.4 (+45)

Data obtained from pages 37 and 38 of MRID 47058101
[a] Body weight gain during Weeks 0-4
[b] Percent difference from control was calculated by the reviewer
* $p<0.05$

C. **FOOD CONSUMPTION:**

1. **Food consumption:** Food consumption, based on weekly consumption/cage ÷ the number of rats/cage, was comparable between control and exposed groups.

2. **Food efficiency:** NA

D. **OPHTHALMOSCOPIC EXAMINATION:** There were no compound-related effects on the eyes.

E. BLOOD ANALYSES:

1. **Hematology:** There were no toxicologically significant compound-related findings. Slight, but statistically significant (p<0.05 or 0.01) increases in hematocrit, hemoglobin concentration, mean corpuscular hemoglobin and mean corpuscular volume (females only) were seen in Group 3 males and females; there was not a concomitant increase in red blood cell count in either males or females. There were also statistically significant (p<0.05) increases in large unstained cell counts noted in these animals.

2. **Clinical chemistry:** No toxicologically significant compound-related findings were seen. There were some changes of small magnitude that were statistically significant (p<0.05 or 0.01). These included increased chloride and aspartate aminotransferase; and decreased phosphorous levels in Group 3 males, decreased bilirubin in Group 2 females and decreased alkaline phosphatase, bilirubin as well as urea in Group 3 females. Alanine aminotransferase and aspartate aminotransferase levels were elevated in the Group 3 female that was sacrificed on Day 3.

F. URINALYSIS: NA

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The adjusted organ weight data (Table 3) showed a slight decrease in heart weight in Group 3 males. Statistically significant (p<0.01) and dose dependent increases in kidneys weights were seen in males from Groups 2 and 3. Notable decreases in liver weights were found in Group 3 males and females (p<0.05). Slight increases in lung/bronchi weights were seen in Group 2 and 3 females. Statistically significant changes in absolute organ weights (unadjusted for bodyweight) included increased adrenal weight in Group 3 males and increased lung/bronchi weight in Group 2 and 3 males.

TABLE 3. Selected mean organ weights adjusted for body weight (g)

Organ	Group no.		
	1	2	3
Males			
Heart	1.635	1.615	1.511 (8%)
Kidneys	2.48	2.85**(15%)	3.01**(21%)
Liver	15.05	14.12 (6%)	13.52 (10%)
Females			
Kidneys	1.67	1.68	1.59
Liver	9.18	9.89 (8%)	8.01* (13%)
Lungs + bronchi	1.123	1.134	1.203 (7%)

Data obtained from pages 51 and 52 of MRID 47058101
Values in parentheses represent percent difference from control
* p<0.05; **p<0.01

2. **Gross pathology:** Observations included dark area(s) in the liver of a Group 3 male, congested lungs/bronchi in one Group 3 male, two Group 2 females and one Group 3 female as well as pelvic dilation in the kidneys of one Group 3 female versus 0 in Group 1 males and females.

3. **Microscopic pathology**: NA

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

Administration of technical liquid Glufosinate ammonium by inhalation at an exposure level of 0.205 mg/L (*0.105 mg a.i./L)* produced a number of clinical signs in male and female animals, including aggressive/irritable, nervous and underactive behavior, repetitive movements of the head, piloerection, hunched posture and eyes partially closed. One female given 0.205 mg/L was sacrificed on humane grounds following 2 exposures to the test article. The nature of the clinical signs was considered to be an adverse response. The no observed adverse effect level (NOAEL) was considered to be 0.109 mg/L (*0.056 mg a.i./L)* of the technical liquid, but due to the minor transient clinical signs seen at this level, the no observed effect level (NOEL) was not established.

B. REVIEWER COMMENTS:

The reviewer concurs that the nature of the clinical signs in the Group 3 female that was sacrificed should be considered adverse. Some of the other clinical signs in Group 2 females as well as in Group 3 males and females were indicative of neurologic effects from exposure to the test material, however, they were typically observed in a small number of animals and/or tended to be transient.

The reviewer does not concur with the NOAEL established by the study author. Instead, under the conditions of this study, a NOAEL is not identified. The LOAEL is 0.109 mg/L based on lung/bronchial congestion in female rats.

C. STUDY/REPORT DEFICIENCIES:

Study deficiencies: Since this was not a guideline study, flexibility in the test protocol should be permitted, however, the use of three test concentrations (rather than two) may have provided for an improved concentration-response assessment.

13544

R153421

Chemical: Glufosinate

PC Code:
128850
HED File Code: 13000 Tox Reviews
Memo Date: 9/6/2007
File ID: TX0053314
Accession #: 000-00-0122

HED Records Reference Center
10/22/2007