6-14-85

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

004469

JUN 14 1985

Caswell # 862B

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Triazolylalanine (THS 2212), Plant Metabolite of Bayleton; Review of Toxicity Data; Registrant: Mobay; EPA #3125-319; Action Code: 400; Accession #256058

TO: Henry Jacoby, PM-21
Registration Division (TS-767)

FROM: Alan C. Katz, D.A.B.T. 6/13/85
Toxicologist, Review Section IV
Toxicology Branch
HED (TS-769C)

6/15/85

THROUGH: Robert P. Zendzian, Ph.D.
Acting Head, Review Section IV
and
Theodore M. Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch

Action Requested

Review the following studies:

Report No.	Title
82662	THS 2212, Preliminary Subacute Toxicity Study on Male Rats, Administration in the Drinking Water
82727	THS 2212, Triazolylalanine, Salmonella/Microsome Test for Determination of Point Mutations
82738	THS 2212, Triazolylalanine, Study of DNA Damage Using the E. coli Pol A⁻ Test
84008	Triazolylalanine (THS 2212) Subacute Oral Toxicity Study on Rats
86476	Triazolylalanine (THS 2212) Study for Subchronic Toxicity to Rats (Three-Month Feeding Study)
86590	THS 2212 (Triazolylalanine) Subchronic Toxicity to Dogs on Oral Administration (13-Week Feeding Study)

Conclusions

Reviews of reports 82727, 82738 and 86590 are attached. Both of the in vitro tests (i.e., Report Nos. 82727 and 82738) are considered acceptable. Under the conditions of these assays, each conducted with and without microsomal activation, triazolylalanine elicited no apparent mutagenic effects. The subchronic dog study (Report No. 86590) is considered "CORE Supplementary" pending submission of additional information, as cited in the review.

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Conclusions (cont'd)

Deficiencies in the subchronic rat study (Mobay Report No. 86476; Bayer Report No. 12397) were noted in a previous memorandum (AK to HJ, 2/8/85). The requirement that the report be signed by responsible personnel is apparently not applicable in this case, since a translation copy was submitted; however, this renders a signed QAU statement all the more pertinent.

The two remaining studies, i.e., Report Nos. 82662 and 84008, are identical to those under review by Dr. George Ghali, Tox Branch Review Section IV. See the attached Data Review Record (Record No. 113141).

DATA EVALUATION REPORT

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A. Compound:

Triazolylalanine; 2-amino-3-(1,2,4-triazol-1-yl) propionic acid

B. Compound Number:

THS 2212; Batch E238099

C. Study Report Citation:

Title: "THS 2212; Triazolylalanine: Salmonella/Microsome Test for Point Mutagenic Effect."

Author: Dr. B. Herbold

Laboratory: Bayer AG Toxicological Institute

Report Number: 11388

Study Numbers: T 1006005; T 9007372

Date: 1/5/83

D. Reviewed By: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)
Alan C Katz (Signature)
6/13/85 (Date)

E. Secondary Review: Robert Zendzian, Ph.D.
Acting Head, Review Sec.IV
(Signature)
6/15/85 (Date)

F. Classification:

Acceptable.

G. Conclusion:

Under the conditions of this assay, the test compound showed no evidence of mutagenic effect at levels up to and including 12,500 ug/plate.

H. Materials and Methods:

An Ames test was conducted to investigate the potential of the test substance to induce point mutations in bacteria with and without rat liver microsomal (S-9) activation. The test substance (Lot No. E238099) was assayed at doses up to and including 12,500 ug per plate. Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. These strains were selected for detection of base pair substitutions and frame-shift mutations. The S-9 mix was prepared from the livers of adult male Sprague-Dawley rats which had been injected i.p. with Arochlor 1254 for enzyme induction. Endoxan (cyclophosphamide), tryptaflavine and/or 2-amino anthracene were used as positive controls. DMSO was used as the solvent for triazolylalanine (THS 2212), tryptaflavine, 2-amino anthracene, and the negative control, and demineralized water was used as the solvent for endoxan. Four plates were counted for each dose level and control in each test. The assays were performed twice with each strain.

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I. Results:

In a preliminary test, no cytotoxicity was found at levels up to and including 12,500 ug/plate. Results of the mutagenicity assays are presented in the following table:

MUTATION QUOTIENTS* (Mean Values of Duplicate Tests)

	Strain									
Dose	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
(ug/plate)	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
THS 2212										
20	0.9	1.1	0.8	0.8	1.2	0.6	0.8	0.8	1.4	0.8
100	1.3	1.1	0.9	0.9	1.3	0.7	0.7	1.2	1.5	0.8
500	0.9	1.5	0.9	1.1	1.0	0.9	0.8	1.2	1.1	0.9
2500	1.0	0.9	1.0	1.1	0.9	0.8	1.1	0.9	1.2	0.9
12500	1.4	1.2	1.2	0.7	1.3	0.9	0.6	1.1	1.0	0.8
Endoxan, 145	--	--	--	--	1.1	5.1	--	--	--	--
290	--	--	1.5	2.3	--	--	--	--	--	--
2-AA, 3	1.0	44.0	1.3	10.0	1.4	16.1	1.7	39.4	3.4	31.4
T.flavine, 50	2.9	25.9	--	--	--	--	13.0	41.0	2.3	59.0

*Mutation Quotient: $\frac{\text{Mean \# of mutants/plate in test group or positive controls}}{\text{Mean \# of mutants/plate in negative (DMSO) controls}}$

(These results were calculated from the mean values which were presented for the individual tests — see study report, Tables 1 through 10).

The above data show no evidence of THS 2212-induced mutation.

J. Discussion:

The negative (DMSO) controls used in this study do not provide an appropriate control for comparison with endoxan, which was dissolved in demineralized water. Therefore, the mutation quotients presented for this positive control are considered to be of questionable value.

DATA EVALUATION REPORT

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A. Compound:

Triazolylalanine

B. Compound Number:

THS 2212; Batch E238099

C. Study Report Citation:

Title: "THS 2212, Triazolylalanine, Study of DNA Damage Using the E. coli Pol A_1^- Test"

Author: Dr. B. Herbold

Laboratory: Bayer AG Toxicological Institute

Report Number: 11390

Date: 1/5/83

D. Reviewed By: Alan C. Katz, M.S., D.A.B.T. Alan C Katz (Signature)
Toxicologist
Toxicology Branch
Hazard Evaluation Division(TS-769C) 6/13/85 (Date)

E. Secondary Review: Robert Zendzian, Ph.D.
Acting Head, Review Sec. IV (Signature)
6/13/85 (Date)

F. Classification:

Acceptable.

G. Conclusion:

Under the conditions of this assay, triazolylalanine with and without S-9 mix did not elicit measurable DNA damage.

H. Materials and Methods:

This test was conducted to investigate the potential of the test substance to induce DNA damage in bacteria with and without rat liver microsomal (S9) activation. THS 2212 was assayed at levels of 0, 62.5, 125, 250, 500 and 1000 ug per plate. The maximum dose was applied as a suspension; solubility properties prevented testing at higher levels. Chloramphenicol was used as a negative control and methyl methane sulphonate (MMS) was used as a positive control. The solvent for THS 2212 and chloramphenicol was DMSO; this vehicle was used for a solvent control group. Two strains of E. coli were used, i.e., one deficient in DNA repair (pol A_1-) and one capable of repair (pol A+). A result was to be considered positive if the difference in the diameters of the inhibition areolae [(pol A_1-)-(pol A+)] exceeded +2mm.

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I. Results and Discussion:

No measurable inhibition areolae were found for pol A_1- or pol A+ strains in plates, with and without S-9 mix, treated at any level of THS-2212 or the solvent control. The validity of the positive and negative controls with S-9 mix was clearly demonstrated.

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DATA EVALUATION REPORT

A. Compound:

2-amino-3-(1,2,4-triazolyl-1-yl)-propionic acid; triazolylalanine

B. Compound Number:

THS 2212

C. Study Report Citation:

Title: "THS 2212 (Triazolylalanine) Subchronic Toxicity to Dogs on Oral Administration (13-Week Feeding Study)"

Laboratory: Bayer AG Institute of Toxicology
Wuppertal-Elberfeld

Report Numbers: 86590(EHR File Number 0124; Document Number AS 84-3807); 12562

Study Number: T7 015 713

Date: 3/26/84

EPA Accession Number: 256058

Submitted to EPA by: Mobay Chemical Corporation
Kansas City, MO 64120

Authors: Dr. E. von Keutz
Dr. P. Groning

D. Reviewed By: Alan C. Katz, M.S., D.A.B.T
Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Alan C Katz (Signature)

6/13/85 (Date)

E. Secondary Review By: Robert Zendzian, Ph.D.
Acting Head, Review Sec.IV

(Signature)

6/13/85 (Date)

F. Classification:

CORE Supplementary

G. Conclusion:

No final conclusion can be made regarding this study until additional data are provided. The CORE classification may be upgraded following review of the required data.

H. Materials:

Test compound: THS 2212, Batch No. TLB 1207; Purity: 97.5% (a.i.)
Test animals: Beagle dogs, 29-32 weeks of age, weighing 6.2-8.9 kg when assigned to study groups (week -1).

H. Materials (Cont'd):

The basal diet consisted of pulverized dry feed (Ssniff HH Sole Diet for Dogs, Double Ground), from Ssniff Versuchstierdiaeten GmbH, D-4770 Soest.

I. Methods:

The test compound was mixed in the diet, and given daily for 13 weeks (April-July, 1983). Storage conditions for the technical material and the blended diet were not specified in the study report. The diet was moistened (1:1) with warm tap water immediately prior to administration each day. The diet was rationed at 350 g per dog daily during weeks 1-4, and 380 g per day during weeks 5-13. Stability, homogeneity and concentration of the test substance in the diet were verified by HPLC analysis.

The dogs were assigned randomly to the following groups:

Group	Dietary conc.(ppm)	Males	Females
Control	0	4	4
I	3200	4	4
II	8000	4	4
III	20000	4	4

The animals were observed daily for signs of toxicity. Food consumption was determined daily and body weights were recorded weekly. Clinical examinations were performed prior to initiation of the study, and during weeks 2, 4, 7 and 13. Clinical pathology (hematology, clinical chemistry and urinalysis) tests were conducted at the same intervals.

Hematology

Hematocrit	Leukocyte count.
Hemoglobin	Thrombocyte count
MCV	Reticulocyte count
MCH	Thromboplastin time
MCHC	Sedimentation rate
Erythrocyte count	Differential leukocyte count

Clinical Chemistry

	Bilirubin
Blood sugar	Cholesterol
Urea	Serum protein electrophoresis
Creatinine	Sodium
Total protein	Potassium
Aspartate aminotransferase	Calcium
Alanine aminotransferase	Chloride
Alkaline phosphatase	Cytochrome P-450 (liver)
Glutamate dehydrogenase	N-demethylase (liver)

Urinalysis

Protein	Bilirubin
Glucose	Occult blood
Ketone	Microscopic

Ophthalmoscopic examinations were performed pretest and during weeks 7 and 13. The pretest and final examinations included fundus photography.

I. Methods (Cont'd):

At termination of the study, all animals were sacrificed by exsanguination under Evipan sodium anesthesia. Organs weighed at necropsy were: brain, heart, lung, liver, spleen, kidneys, adrenals, pancreas, testicles, prostate, ovaries and thyroid. The following tissues were collected for histopathologic evaluation:

Heart	Uterus	Colon
Lung	Ovaries	Rectum
Liver	Mammary tissue	Mesenteric lymph nodes
Spleen	Tonsils	Gallbladder
Kidneys	Parotid gland	Urinary bladder
Adrenals	Pancreas	Eyes
Pituitary	Thymus	Optic nerve
Brain	Esophagus	Sciatic nerve
Thyroid	Stomach	Aorta
Testicles	Duodenum	Skeletal muscle (m. quadriceps femoris)
Epididymides	Jejunum	Bones (femur, sternum)
Prostate	Ileum	Bone marrow (2 smears/animal)

Numerical data were analyzed for statistical significance using Wilcoxon's nonparametric rank sum test.

J. Results:

Based on data provided in the study report, mean levels of consumption of the test material over 13 weeks for low, mid and high dose males were calculated to be 144, 322 and 850 mg/kg/day, respectively. Consumption of test material for females was 150, 369 and 902 mg/kg/day. Mean food consumption and body weight gain of high dose females were reduced, but did not differ statistically from control values.

No deaths occurred during this study. The clinical examinations of reflexes, body temperature and pulse rate showed no apparent treatment-related effect. It was reported that no differences were found between groups with respect to appearance or behavior; however, because the daily clinical observations are not presented in the study report, this negative finding cannot be verified. Also, in the absence of supporting data, the validity of the statement in the report that ophthalmoscopic examinations "did not reveal any treatment-induced alterations in the transparent media...or in the eye fundus" is unsubstantiated.

No treatment-related effects were evident with respect to hematology, blood/liver chemistry or urinalysis test results, or in organ weight analysis or gross or histopathologic findings. It is not clear whether ALL gross findings at necropsy are presented in the report. Also, histopathologic observations were presented only for controls and high dose animals.

K. Discussion/Recommendations:

The registrant must submit individual and summary tables for observations of clinical signs, as well as individual ophthalmologic observations. Histopathologic evaluations are also required for all animals, including certain tissues (i.e., liver, kidneys and all tissues showing possible toxicity based on findings in high dose animals) from the low and mid dose groups. The registrant must also clarify whether all gross necropsy findings were included in the report. The CORE classification may be upgraded following submission and review of the additional required data.