CASWELL FILE

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

004562

JUL 16 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: CGA-64250; Tilt® Fungicide, Tolerance in Pecans, 0.1 ppm; Application for Amended Registration

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

FROM: Alan C. Katz, M.S., D.A.B.T. 7/15/85
Toxicologist, Review Section IV
Toxicology Branch, HED (TS-769C)

THROUGH: Robert P. Zendzian, Ph.D. 7/16/85
Acting Head, Review Section IV
and
Theodore M. Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch

Registration No. 4F3007
Accession Nos. 072206-8; 072218
Caswell # 323EE and 862B

Included in this review are detailed DER's for several studies with the plant metabolite, triazolylalanine, which were not previously evaluated by Tox Branch under petitions pertinent to the parent compound, CGA-64250 (see Appendix I). One-liners are also attached.

Action Requested:

Review petition for establishing a permanent tolerance for the combined residues of the fungicide CGA-64250 (Tilt) and its metabolites in or on pecans at 0.1 ppm.

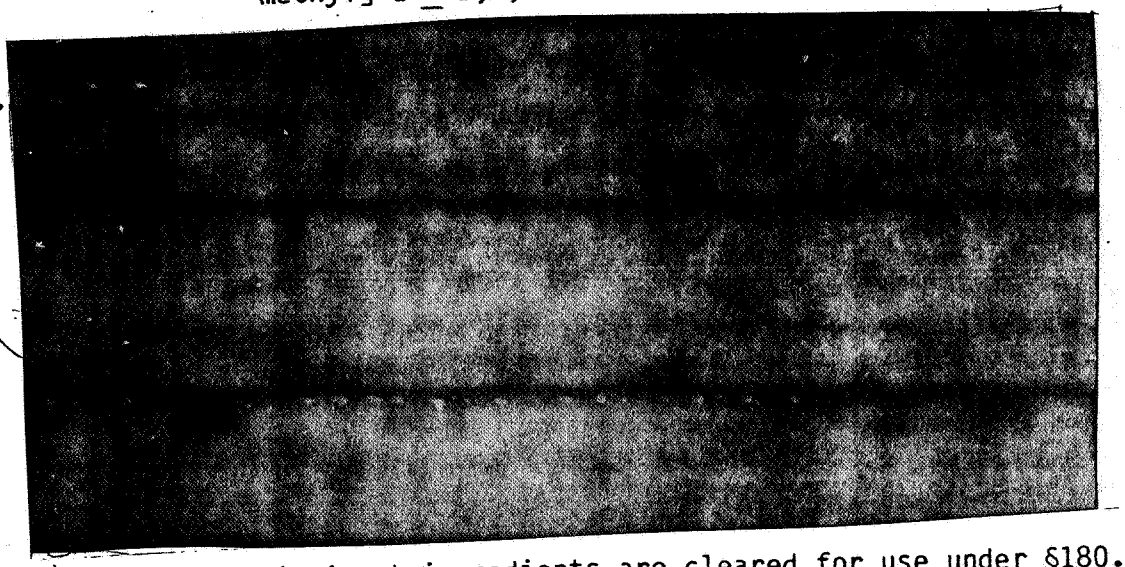
Recommendations:

Toxicology Branch recommends that the tolerance not be granted. CGA-64250 is oncogenic in the liver in male mice. Additional information has been requested with respect to a 2-year feeding study in rats. When this data is provided, oncogenicity will be evaluated by the Tox Branch ad hoc committee.

004562

Compound

1. CGA-64250 Technical; Common name: Propiconazole; Trade names: Tilt, Banner; Chemical name: 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl] methyl]-1-H-1,2,4-triazole

2. 

The formulation's inert ingredients are cleared for use under §180.1001.

3. Plant metabolite: Triazolylalanine (TA); triazole alanine; 2-amino-3-(1,2,4-triazol-1-yl) propanoic acid. Compound numbers: R152056; THS 2212.

Properties: crystalline; m.p.: 232°C; water solubility: 2.7% w/v at room temperature; n-octanol/water partition coefficient: log p< -2.

Summary of Data Submitted

1. Toxicology of CGA-64250 Technical

Studies of the distribution and degradation of CGA-64250 in plants and animals (mice, rats, and goats) have been reviewed by the Residue Chemistry Branch (see memorandum from A. Smith to H. Jacoby, 5/15/84).

a. Acute Studies

Results of acute studies with CGA-64250 are outlined in the attached one-liners (Appendix I), and may be summarized as follows:

Acute Study	Tox Category
Oral- rats, mice, Chinese hamsters, and rabbits	III
Inhalation- rats	III
Injection, ip- rats	II
Dermal Toxicity- rats	III

004562

Acute Study (contd)	Tox Category
Dermal Irritation- rabbits	IV
Eye Irritation- rabbits	II
Dermal Sensitization- guinea pigs	Negative

b. Short Term Testing

No systemic toxicity was reported in a 21-day dermal study in rabbits at the highest dose tested (1 g/kg). However, no NOEL for skin lesions was observed. The lowest dose tested was 3 mg/kg.

c. Subchronic Testing

A 90-day study was conducted in TIF:(RAIF)SPF rats with CGA-64250 technical at dietary concentrations of 0, 240, 1200 and 6000 ppm. A NOEL was apparent at the lowest dose tested. At 1200 ppm, body weight gain was reduced in females.

Beagle dogs were fed diet containing CGA-64250 at concentrations of 0, 50, 250 or 1250 ppm for 90 days. Treatment-related lesions involved lymphoid follicles in the mucous membranes of the pyloric region of the stomach, with an LEL of 250 ppm. No effect was apparent at 50 ppm. The in-life phase of a 1-year subchronic study in Beagle dogs with CGA-64250 at dietary levels of 0, 5, 50 and 250 ppm was scheduled to be completed October, 1984. The Toxicology Branch is awaiting the report on this study.

d. Chronic Testing and Oncogenicity

A 2-year feeding study in CD-1 mice was conducted with CGA-64250 at levels of 0, 100, 500 and 2500 ppm. A treatment-related increase in liver tumors was found at the high dose level (in males). No oncogenic effect was apparent at the mid dose. The NOEL for systemic toxicity was found to be 100 ppm. Based on the data from this study, the carcinogenic potency was calculated: $Q_1^* = 3 \times 10^{-2}$ $(mg/kg/day)^{-1}$.

In a 2-year feeding study in rats, no NOEL was established for several histological effects. Dermal fibromas in males and thyroid follicular tumors in females may be attributable to exposure to CGA-64250. Final conclusions on the results of this study will be made following submission and evaluation of pertinent historical control data.

e. Reproductive Effects/Teratogenicity

CGA-64250 did not demonstrate teratogenicity in the rat at doses up to and including 300 mg/kg. Maternal toxicity (decreased body weight gain and food consumption) was found at 300 mg/kg, while fetotoxicity (ossification retardation) occurred at 100 and 300 mg/kg. The NOEL's for maternal toxicity and fetotoxicity were 100 and 30 mg/kg, respectively. The compound was not teratogenic, fetotoxic or maternally toxic in the chinchilla rabbit at doses up to and including 180 mg/kg.

In a 2-generation study in Tif:RAIf[SPF] rats, CGA-64250 was administered at concentrations of 0, 400, 2000 or 5000 ppm in the diet. The compound

caused perinatal death in all pregnant high dose F_0 females. Mating ratios and implantation rates were also reduced in this group. No other reproductive parameters appeared to be affected. Dose-related reductions in body weight gain and increased relative liver weights were evident in all CGA-64250-treated groups. At a dietary concentration of 2000 ppm, the test substance caused hypertrophy of centrilobular hepatocytes in F_1 adults. This study is considered "Supplementary". It provides data which may be useful in evaluating the results of a repeat 2-generation study which was initiated in March, 1983.

f. Mutagenicity

The battery of mutagenicity assays listed below has been performed on CGA-64250 technical, with negative results:

1. An Ames-type assay (with and without activation); 4 strains of S. typhimurium
2. A dominant lethal study in the Tif:MAG F (SPF) mouse
3. A nucleus anomaly test in the Chinese hamster
4. A BALB/3T3 cell transformation assay

The tests listed below were also performed; however, these were considered unacceptable, and additional information has been requested from the sponsor:

1. L5178Y/$TK^{+/-}$ mouse lymphoma test (in vitro)
2. S. cerevisiae D^7/mammalian-microsome test
3. Point mutation assay with mouse lymphoma cells (host-mediated)
4. Chromosome studies in mouse spermatogonia
5. Chromosome studies in mouse spermatocytes
6. Autoradiographic DNA repair test on human fibroblasts
7. Autoradiographic DNA repair test on rat hepatocytes
8. Sister chromatid exchange study (Chinese hamster bone marrow cells, in vivo)

2. Toxicology of Tilt 3.6E

a. Acute Studies

The acute oral toxicity of Tilt 3.6E is low in rats (Tox Category III). All deaths occurred within 2 days after administration. This formulation appears to affect the central nervous system, although other target tissues may also be involved. Toxic signs included salivation, lacrimation, miosis, ptosis, exophthalmos, piloerection, polyuria, hematuria and ataxia. Necropsy observations included corneal opacity, and discoloration of the stomach, in-

testines, liver and spleen. Acute inhalation toxicity of this formulation in rats is also low (Tox Category III); symptoms noted were salivation, gasping, lacrimation, and nasal discharge.

The acute dermal toxicity of Tilt 3.6E is low (Tox Category III); application of the formulation did not cause death at a dose of 5010 mg/kg (the only level tested) in New Zealand White rabbits.

The Tilt 3.6E formulation is a moderate eye irritant (Tox Category II), and caused corneal opacity in 6/6 unwashed eyes and 2/3 washed eyes in New Zealand White rabbits. All opacities were reversed by day 7. The water rinse appears to have provided some protection, but the difference in irritation scores was not substantial (i.e., the maximum P.I.S. was 25.5/110 in unwashed eyes vs. 19.0/110 in washed eyes).

Tilt 3.6E is a moderate dermal irritant (Tox Category III: P.I.S. 4.6/8.0). The vehicle appears to contribute to the irritancy of this formulation, since CGA-64250 technical showed lower-grade potential (Tox Category IV; P.I.S. 1.4/8.0).

b. Dermal Sensitization

Skin sensitization to Tilt 3.6E was demonstrated in the guinea pig. As the technical material is not a sensitizer, this reaction can be attributed to the vehicle.

3. Toxicology of Triazolylalanine

The low n-octanol/water partition coefficient (log p<-2) suggests that TA has no significant potential for bioaccumulation.

TA levels in untreated grain crops were found to be 0.01 to 0.03 mg/kg, irrespective of the kind or variety of cereal (barley, wheat, rye) or the country in which the cereal was cultivated (Germany, Norway, Sweden, France, Yugoslavia, U.S.A.). Since TA levels in these investigated samples could not have originated from applications of triazole fungicides, they are regarded as ubiquitous and occurring naturally.

Because the formation of TA is not observed in animals, it is necessary to determine safety-related properties of this compound separately in order to assess the practical relevance of possible residues in edible crops.

a. Distribution, Degradation and Excretion of Triazole Alanine

Male and female rats were given a single dose of 0.5 or 50 mg/kg of ^{14}C- triazole alanine. At both dose levels, analyses indicated that 95-105% of the dose was excreted within 24 hours, with 85-103% in the urine. No sex- or dose-related differences were found with respect to the extent of absorption or the rate or route of excretion. After 7 days, no residues were found in tissues of rats dosed with 0.5 mg/kg. Residues of no more than 22 ppb were found in liver, kidney and blood of animals dosed at the 50 mg/kg level. The major radioactive residue components in urine and feces were unchanged TA (69-86%) and a less polar derivative, N-acetyl triazole alanine (8-19%).

b. Acute Studies

An acute oral toxicity study with TA in rats was considered invalid, since the study report did not sufficiently describe the materials or methods used. The following studies were regarded as supplementary, but may be upgraded if additional data are provided: acute oral toxicity study in fasted rats; acute oral toxicity study in unfasted rats; acute oral toxicity study in fasted mice; and acute intraperitoneal toxicity in rats.

c. Short Term Testing

A 2-week subacute toxicity study was conducted in male rats. TA was administered in the drinking water at levels of 0, 3000 or 10,000 ppm. No compound-related effects were apparent with respect to toxic signs, mortality, food consumption, body weight, organ weights or gross lesions at necropsy. In a follow-up, 4-week subacute oral toxicity study, male and female rats were given TA by gavage at doses of 0, 25, 100 and 400 mg/kg/day. No significant treatment-related effects were found.

d. Subchronic Studies

A 3-month feeding study in male and female rats with TA was classified as "Supplementary." The sponsor must address deficiencies in the report before the Toxicology Branch can complete its evaluation of the study.

e. Mutagenicity

Toxicology Branch data evaluation reports of the following studies with triazolylalanine are attached (see Appendix I):

1. Cell Transformation Test for Potential Carcinogenicity of R152056
2. Salmonella/Microsome Test for Point Mutagenic Effect
3. Micronucleus Test in CBC F1 Mice
4. Micronucleus Test for Mutagenic Effect on Mice
5. Pol A$^-$ Test on E. coli During Testing for Effects Harmful to DNA

The cell transformation test, as well as one of the two micronucleus tests, demonstrated apparent treatment-related mutagenic effects. In the Ames test, which used 5 strains of S. typhimurium, TA showed no mutagenic effect with or without S-9 activation. Also, in the E. coli pol A_1^- test, TA with and without S-9 mix did not cause measurable DNA damage.

004562

f. Reproductive Effects/ Teratogenicity

A 2-generation study with TA in the rat was initiated in 1983. The Toxicology Branch is awaiting submission of the final report for this study. The report on teratogenicity of TA in the rat was identical to that under review by Dr. George Ghali, Tox Branch Review Section IV (see attached Data Review Record, Record No. 113141, Appendix I).

Discussion:

CGA-64250 technical has a relatively low acute oral inhalation and dermal toxicity. Its action appears to be nonspecific. The compound has slight dermal irritation potential and is not a sensitizer, but causes a moderate level of eye irritation; no dermal toxicity was found at the highest dose tested (1 g/kg), except for local irritation.

Subchronic toxicity of CGA-64250 involved reduced weight gain in rats (NOEL, 240 ppm; LEL, 1200 ppm) and gastric lesions in dogs (NOEL, 50 ppm; LEL, 250 ppm). CGA-64250 caused liver tumors in mice in a 2-year feeding study; for systemic toxicity, the NOEL was 100 ppm and the LEL was 500 ppm. Based on data presented in a 2-year feeding study in rats, it is questionable whether CGA-64250 caused dermal fibromas in males and/or thyroid follicular tumors in females; historical control data will be used in making this determination.

The technical material is not teratogenic in the rat or rabbit. No conclusions have been made with respect to toxic effects on reproduction, pending review of a 2-generation study which was initiated in March, 1983.

The granting of a food tolerance for CGA-64250 requires establishing an ADI from a NOEL on an appropriate study. In the absence of additional data for the 2-year feeding study in rats, it is considered inappropriate to set an ADI at this time. B. Litt, Tox Branch Statistics Team Leader, has determined that, based on the mouse liver tumor data, the carcinogenic potency estimator $Q_1^* = 3 \times 10^{-2}$ (see Appendix II: memorandum, B. Litt to H. Jacoby, 1/22/85).

The Toxicology Branch has requested that the sponsor provide a mouse metabolism study with CGA 64250 to identify metabolites resulting from a 100 ppm feeding level and a 2500 ppm feeding level. These results may elucidate the mechanism of oncogenicity at 2500 ppm.

Conclusions:

The tolerance requested for CGA-64250 cannot be granted at this time, due to deficiencies in several key studies, as noted above and detailed in the separate data evaluation reports.

The toxicity classifications for the Tilt 3.6E formulation are:

Acute Oral	III
Acute Dermal	III
Dermal Irritation	III
Eye Irritation	II
Acute Inhalation	III
Dermal Sensitization	Positive

The question of potential toxicity of the plant metabolite, triazolylalanine, remains to be resolved. The significance of positive results in a cell transformation test and in one of two mouse micronucleus tests is questionable. Acute and subchronic oral toxicity tests must be validated.

APPENDIX I. DATA EVALUATION REPORTS

004562

DATA EVALUATION REPORT

A. Compound:

3-(1,2,4-triazol-1-yl)alanine; triazolylalanine

B. Compound Numbers:

R152056; Y01210/001/001

C. Study Report Citation:

Title: "Cell Transformation Test for Potential Carcinogenicity of R152056."

Authors: M. Richold
J. Allen
A. Williams
S. Ransome

Laboratory: Huntingdon Research Center
Huntingdon, Cambridgeshire, England

Sponsor: Central Toxicology Laboratories, ICI Ltd.

Report Numbers: ICI 394A/81153
CTL/C/1085

Date: 5/15/81

Accession Number: 072208

Submitted to EPA by: Ciba-Geigy Corporation

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

Alan C Katz (Signature)

7/15/85 (Date)

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

(Signature)

(Date)

F. Classification:

Acceptable.

G. Conclusion:

Under the conditions of this test, triazolylalanine (R152056) caused mammalian cell transformation in vitro with and without S-9 activation.

H. Materials and Methods:

The objective of this in vitro assay was to evaluate the test compound for its ability to induce morphological transformation of mammalian cells. The assay was performed with and without an S-9 mix. A preliminary cytotoxicity test was performed, using concentrations of R152056 up to and

10

including 1 mg/ml. Details of the toxicity test procedures are not presented in the study report.

For the transformation assay, R152056 (Batch No. P2 B2427-153) levels of 0.5, 1, 2, 4, and 8 mg/ml were tested in baby hamster kidney cells (BHK-21/C1-13) without S-9, and levels of 1, 2, 4, 8, and 16 mg/ml were tested with the S-9 mix. Positive controls were 4-nitroquinoline-N-oxide (0.00625, 0.0125, 0.025, 0.05 and 0.1 ug/ml) and p-dimethylaminoazobenzene (Butter Yellow; 25, 50, 100, 200 and 400 ug/ml); negative controls were untreated or vehicle (DMSO) treated. Transformation was assessed on the basis of colony growth in soft agar. The purity of the test compound was not reported. Although some information relating to the methods used are presented in the study report, sufficient details were not provided.

I. Results:

The results of this test are presented in Tables 1 through 6, and shown graphically in Figures 1 and 2, as excerpted from the study report. As noted in the report:

"In the absence of S-9 mix, an LD_{50} was not obtained with the dose levels used (Table 1). At the highest concentration (8 mg/ml), an LD_{39} was achieved (Table 1, Figure 1). At this concentration, the transformation frequency was 39 compared to a negative control value of 10 when 100% of cells were seeded into soft agar and 8 when the seeding level was reduced by 50% in order to simulate an LD_{50} (Tables 2 and 5). An increase in the number of transformed colonies was observed after treatment with R152056, although these were somewhat erratic. However, at the top concentration used, the number of transformed colonies observed was twice the 100% survival negative control number and six times the simulated 50% survival control number (Tables 2 and 5).

In the presence of S-9 mix, the LD_{50} occurred at 5.2 mg/ml (Figure 2). At this concentration, the transformation frequency was 28 (Figure 2) compared to negative control values of 0 when 100% of cells were seeded into soft agar and 12 when 50% were seeded (Table 5). There was a real increase in the number of transformed colonies observed after treatment with R152056 when compared to both the 100% survival negative control and to negative controls with similar viable cell densities (Tables 2 and 5)."

J. Discussion/Recommendations:

The study authors' findings that the compound "should be considered to be a potential carcinogen" are valid.

The Toxicology Branch requests additional details relating to the procedures used in this test. The registrant is required to submit a copy of HRC Protocol MCB 111. In addition, the registrant must provide information on the purity of the test compound and advise the Toxicology Branch of the significance of the reported "Alternative name" of Y01210/001/001, vs. Y01210/001/003 on the container (see study report, p.1), and whether this apparent discrepancy represents a potential misidentification of the test compound. Irrespective of these deficiencies, this study is considered to be "acceptable" at this time.

TILT CGA-64250 Reviews

PGS 12 - 16

The next 5 page(s) is/are not included in this copy of the TILT reviews.

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 product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

✓ Detailed methods and results of a registrant submission.

___ Duplicate pages.

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.

DATA EVALUATION REPORT

A. Compound:

Triazolylalanine

B. Compound Number:

THS 2212

C. Study Report Citation:

Title: "THS 2212; Triazolylalanine; Micronucleus Test for Mutagenic Effect on Mice"

Author: Dr. B. Herbold

Laboratory: Bayer AG Toxicological Institute

Study Number: T4011615; Report No. 11054

Date: 8/9/82

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

Alan C. Katz (Signature)

7/15/85 (Date)

E. Secondary Review: Robert Zendzian, Ph.D.
Acting Head, Review Sec.IV (Signature)

(Date)

F. Classification:

Acceptable.

G. Conclusion:

Under the conditions of this test, triazolylalanine (THS 2212) caused slight induction of micronuclei in bone marrow PCE stem cells, which was apparent 24 hours after an oral dose of 8000 mg/kg. No effect was seen at 48 or 72 hours. The data provided in this study are considered acceptable.

H. Materials and Methods:

A micronucleus test was performed in male and female mice in order to evaluate the test compound for clastogenic activity and interference with normal mitotic cell division in polychromatic erythrocyte (PCE) stem cells in mammalian bone marrow in vivo. A single dose of the test compound (Lot No. E238099) was administered by gavage (20 ml/kg) to Bor:NMRI (SPF) mice at 8000 mg/kg. The vehicle (0.5% cremophor, 20 ml/kg) was used as a negative control. Endoxan, 87 mg/kg (60 mg/kg cyclophosphamide; 10 ml/kg) served as a positive control. Five animals/sex/group were killed at 24, 48 and 72 hours. Bone marrow smears were prepared and PCEs were examined for induction of micronuclei. Two thousand PCEs were scored for each animal in the THS 2212-treated and negative control groups; 1000 PCEs were scored for each animal in the positive control group.

17

I. Results:

No clinical or cytotoxic effects were apparent in animals given the test compound. The results for males and females were evaluated on a combined basis, since no sex-related differences were found. These results may be summarized as follows:

Table 8. Micronucleus Test Results - THS 2212
Number of Cells Containing Micronuclei / 1000

Group	Normochromatic Erythrocytes	Polychromatic Erythrocytes
Negative Control (Vehicle)	1.2	1.4
THS 2212 24 hrs.	1.8	3.1*
48 hrs.	1.2	1.6
72 hrs.	1.0	1.2
Endoxan (Positive Control)	1.8	23.4**

* $p < 0.05$ (Wilcoxon signed rank test)
** $p < 0.01$ (Wilcoxon signed rank test)

J. Discussion:

As shown in Table 8, a slight but statistically significant increased micronuclei count was found in THS 2212 at 24 hours. The study report states that this finding is "biologically insignificant, since it lies within the biological fluctuation range of the system and thus represents no increase which might indicate evidence of any chromosome-breaking effect by THS 2212." However, this interpretation does not appear to be supported on the basis of data included in the report.

DATA EVALUATION REPORT

004562

A. Compound:

3-(1,2,4-triazol-1-yl) alanine

B. Compound Numbers:

R152056; ICI 156,342

C. Study Report Citation:

Title: "R152056: 3-(1,2,4-triazol-1-yl) alanine (ICI 156,342) Micronucleus Test in CBC F_1 Mice"

Study Director: P.A. Watkins

Laboratory: Imperial Chemical Industries PLC, Cheshire, England

Study Number: TQM4

Report Number: CTL/C/1164

Date: 9/14/82

D. Reviewed By: Alan C. Katz, M.S., D.A.B.T.
Toxicologist
Toxicology Branch
Hazard Evaluation Division(TS-769C)
Alan C Katz (Signature)
7/11/85 (Date)

E. Secondary Review: Robert Zendzian, Ph.D.
Acting Head, Review Sec.IV
(Signature)
7/11/85 (Date)

F. Classification:

Acceptable.

G. Conclusion:

Under the conditions of this test, R152056 did not cause induction of micronuclei in mouse bone marrow polychromatic erythrocyte stem cells when administered i.p. at doses up to and including 5000 mg/kg.

H. Materials and Methods:

A micronucleus test was performed in mice to evaluate the test compound for clastogenic activity and interference with normal mitotic cell division in polychromatic erythrocyte (PCE) stem cells in mammalian bone marrow in vivo. A single dose of the test compound (Batch No. 02199/49, Division Reference SC 3/81) was administered intraperitoneally (10 ml/kg) to male CBC F_1 mice at 2500 or 5000 mg/kg. The vehicle (0.5% aqueous Tween 80) was used as a negative control. Cyclophosphamide (40 mg/kg) served as a positive control. Five mice of each treated and control group were sacrificed at 24, 48 and 72 hours. Bone marrow smears were prepared and PCEs were examined for induction of micronuclei. One thousand PCEs were counted for each animal.

19

I. Results:

According to the study report, "5000 mg/kg was the maximum dose possible as determined by the physical properties of R152056. At concentrations above this, the suspension was too thick to be injected."

Results of this test are summarized in the following table:

Table 7. Number of Micronuclei/5000 PCEs

Group	Interval (hrs) 24	48	72
Vehicle control	8	3	3
R152056 (2500 mg/kg)	7	0	6
R152056 (5000 mg/kg)	4	4	6
Cyclophosphamide	58	66	6

J. Discussion:

Based on the results shown above, there was no evidence of R152056-induced cell transformation. The effects of the positive control were clearly demonstrated.

DATA EVALUATION REPORT

004562

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)

7/11/85 (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.

21

13

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)

Dose	Strain									
	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

004562

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
Toxicologist (Signature)
Toxicology Branch
Hazard Evaluation Division(TS-769C) 6/13/85 (Date)

E. Secondary Review: Robert Zendzian, Ph.D.
Acting Head, Review Sec. IV (Signature)

(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.

23

15

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.

7598

REGISTRATION DIVISION DATA REVIEW RECORD

Confidential Business Information — Does Not Contain National Security Information (E.O. 12065)

[redacted]562
1/16/84

CHEMICAL NAME Bayleton 109901-9

2. IDENTIFYING NUMBER	3. ACTION CODE	4. ACCESSION NUMBER	TO BE COMPLETED BY PM
3125-320	400	252132	5. RECORD NUMBER 113141
			6. REFERENCE NUMBER 15
			7. DATE RECEIVED (EPA) 12-27-83
			8. STATUTORY DUE DATE NA
			9. PRODUCT MANAGER (PM) Jacoby
			10. PM TEAM NUMBER 21

CHECK IF APPLICABLE

- ☐ Public Health/Quarantine
- ☐ Substitute Chemical
- ☐ Seasonal Concern
- ☐ Minor Use
- ☐ Part of IPM
- ☐ Review Requires Less Than 4 Hours

TO BE COMPLETED BY PCB
11. DATE SENT TO HED/TSS 1/16/84
12. PRIORITY NUMBER 50
13. PROJECTED RETURN DATE 5/8/84

INSTRUCTIONS TO REVIEWER

A. HED
- ☐ Total Assessment - 3(c)(5)
- ☒ Incremental Risk Assessment - 3(c)(7) and/or E.L. Johnson memo of May 12, 1977.

B. SPRD (Send Copy of Form to SPRD PM)
- ☐ Chemical Undergoing Active RPAR Review
- ☐ Chemical Undergoing Active Registration Standards Review

C. ☐ BFSD
D. ☐ TSS/RD
E. ☒ Other

F. INSTRUCTIONS
Rev Doc. "Properties and Safety Assessment of Triazolyl Alanine, C4 1983"

RELATED ACTIONS
Registration & Petitions with Bayleton products.

3(c)(1)(D)
- ☐ Use Any or All Available Information
- ☐ Use Only Attached Data
- ☐ Use Only the Attached Data for Formulation and Any or All Available Information on the Technical or Manufacturing Chemical.

18. REVIEWS SENT TO
- ☒ TB
- ☐ RCB
- ☐ EEB
- ☐ EFB
- ☐ EF
- ☐ CH
- ☐ PL
- ☐ BFSD

To	TYPE OF REVIEW	Registration	Petition	EUP	SLN	Sec. 18	Inert	MNR. USE	Other
✓	TOXICOLOGY	1							
	ECOLOGICAL EFFECTS								
	RESIDUE CHEMISTRY								
	ENVIRONMENTAL DATE								
	CHEMISTRY								
	EFFICACY								
	PRECAUTIONARY LABELING								
	ECONOMIC ANALYSIS								

Label Submitted | Confidential | Representative | 23. Date Returned to RD | 24. Include an Original and 4 (four) Copies of This Completed Form

25

APPENDIX II. PRELIMINARY RISK ASSESSMENT

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

004562

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

TO: Henry Jacoby, Product Manager, No. 21
Registration Division (TS 767)

FROM: Bertram Litt, Biostatistic Team Leader
Toxicology Branch, HED (TS 769C2)

Jan 18, 85

THROUGH: Reto Engler, Ph.D., Chief Mission Support Staff
Toxicology Branch, HED (TS 769C2)

SUBJECT: Preliminary Risk Assessment for Banner/Tilt Based on Mouse Liver Tumors: EPA Registration No. 100-AUR (Caswell Number 323 EE)
Ref. Accession Numbers 250783; 784; 786; 788; 789; 790

1/22/85

This assessment is considered to be preliminary in that there are unresolved issues in the rat study which will affect the weight of the carcinogenic potential of Banner. In the meanwhile, the early appearance of mouse liver carcinomas and adenomas indicates that all examined males were at risk and that the carcinogenic potency from the mouse liver data has a $Q_1^* = 3 \times 10^{-2}$.

The high background of liver tumors in the study controls indicates a need to compare the observed control data with historical controls from the same mouse strain studied at this same study laboratory, during the study interval (plus or minus 3 years.)

Background

A draft report by W. Dykstra reviewing the findings of the 105-week CD-1 mouse feeding study of Banner, Huntingdon Research Center # CBG 1196 (report dated November 4, 1982) identified differential survival, weight gain and food consumption among different dose groups in addition to benign and malignant liver tumors. The analysis was referred to us for more detailed statistical analysis of the surival, weight and tumor effects and interactions as well as a quantitative risk assessment. An additional 2-year study has been performed on rats - it was not available for review at that time. Subsequently, in January 1985, A. Katz has reviewed rat data which indicate a statistically significant dose related increase in lipid deposition in several livers of males and foci of enlarged cells in females plus statistically significant dose related

27

exocrine atrophy of the pancreas of females and of dermal fibromas in males. The latter require additional characterization by the registrant. No estimate of skin penetration of dermally applied Tilt has been made by EPA toxicologists; and 100% dermal penetration is therefore assumed below. Analytic runs of survival, food consumption and weight gain as well as risk modeling have been performed by Dynamac after they extracted the data from the Huntingdon reported data on mice.

Note: A problem which exists in the reporting of all chronic studies is that animal body weight is reported for each individual beginning at week 0 (after acclimitization and just before start of test compound intake) as opposed to food consumption which begins at the end of 1 week of study diet intake. Food consumption is determined by cage which is equivalent to individual rat but to 4 or 5 mice.

Qualitative Results

Results for weight gain, and food consumption and food efficency in the mouse study have been examined as percent change in weight gain during selected intervals, percent change in food consumption and the ratio between percent change in weight gain and food consumption.

When percent change in weight gain was examined per se.

$$\text{Percent Change} = \left[100X \frac{\text{Weight at end of interval} - \text{Weight at beginning of interval}}{\text{Weight at beginning of interval}} \right]$$

analysis of variance demonstrated that there was a statistically significant dose related decrease among males during the first 13 weeks ($P<.01$) which drifted to a borderline effect during weeks 13-26, and the order of the response pattern disappeared thereafter. Body weight became stabilized and gradually declined during the second year of study as is typical in normal rodents. When, however, weight was adjusted to remove within cage effects no meaningful effect could be interpreted for time intervals after the first 13 weeks. The same pattern was found for the other variables considered, ie food consumption and the ratio between percent change in weight gain and food consumption.

For females there was no dose related response at any time interval analysed which showed a consistent pattern across the control and 3 dose groups. As an example, this mean response measuring percent of weight gain during the first 13 weeks was +8.85 for the high dose group, +7.77 for controls,

+7.61 for the low dose and +5.0 for the mid-dose. Obviously, the statistical significance among these groups (F test for dose effect is significant at P<.01) is due to the difference between the mid-dose and any other dose level (Duncan's procedure). Following this early mid-dose effect, all patterns become essentially meaningless.

Dose-Response

Although the design of the original study called for 64 animals per dose per sex (52 for the full 2-year study and 12 additional satelite animals for 53 weeks interim kill,) the pathology reports for liver tissue include 64 control males, 64 low-dose males but only 62 each in the two higher dosed male groups. These 232 contributed liver pathology as shown in table 1, and the data are analyzed by Peto's test for trend on an interval prevalence basis (IARC Supplement 2 Addendum 1980.) When these data were analyzed using life table methods the findings shown below were obtained:

Study Concentration of Banner	0	100	500	2500
Animals Randomized to Sex-Group	64	64	64	64
Proportion of males with liver carcinoma	15/64	7/64	15/62*	26/62
Proportion of males with liver adenoma and/or carcinoma	28/64	14/64	25/62*	48/62

Unadjusted trend P<.0000, Cox P<.0000, Gehan-Breslow P<.0000
All pairwise comparisons against high dose P<.01

Proportion of females with liver carcinoma	1/64	1/64	1/64	2/64
Proportion of females with liver adenoma and/or carcinoma	5/64	4/64	1/64	7/64

No statistically significant effects.

---

* 2 mid-dose males were not examined Note: P <.0000 =<.0001

** 2 high dose males died during the second week and were not replaced.

(Unadjusted trend evaluates the dose-weighted trend omitting time of death; Cox utilizes time by assigning equal weight to all death times; Gehan-Breslow assigns greatest weight to early deaths and lesser weight as the animals age.)

004562

Table I.

Dose Weighted Liver Tumor Prevalence in Male Mice Analyzed at Design Intervals: Banner Dose in PPM

Dose Group	0-52 wk Deaths	53-week Kill	54-78wk Deaths	79-104 wk Deaths	Dead on Study	Final Kill	Total TBA Examined
Control	0/2	2/12	5/10	9/16	14/28	12/24	28/64
100	0/6	0/11	3/11	4/16	7/33	7/20	14/64
500	2/6	3/11	1/8	7/16	10/30	12/21	25/62
2500	1/8	4/9	14/15	15/16	30/39	14/14	48/62
Totals	3/22	9/43	23/44	35/64	61/130	45/79	115/252
% TBA	13.9%	20.9%	52.3%	54.7%	46.9%	57.0%	45.6%
T	281.8	5409.3	13521.8	14275	28557	13532	48609
V	3.24×10^6	6.68×10^6	1.39×10^7	1.65×10^7	3.38×10^7	1.39×10^7	5.65×10^7
Z	0.156	2.09	3.62	3.51	4.91	3.62	6.47
P	0.438	0.02	0.0001	0.0002	$<1 \times 10^{-6}$	0.0001	$<1 \times 10^{-10}$

TBA = Tumor Bearing Animals

T = The dose weight difference between the observed and expected TBA in the interval

V = The variance associated with the number of observed TBA in the internal

Z = The normalized statistic obtained when T is divided by the square root of V

P = The probability that this (Z) normalized relationship may be explained by chance

3-

These data are not random in behavior and some of the response patterns do not look as if they are the results of a real study; either some results have been altered, or there has been a misclassification of groups, or these are very unusual data. In any case, an audit of the mouse data seems to be indicated; and historical controls should be obtained.

Given the unique and highly significant results of the mouse study and the need for additional rat data, a worst case low-dose extrapolation has been performed on the mouse liver carcinoma and/or adenoma 2 year incidence in male mice.

The contractor, Dymanac, has conducted low dose extrapolations on: 28/64 control males

14/64 males administered 100 ppm

25/64 males administered 500 ppm

48/64 males administered 2500 ppm

for multistage, probit, logit, weibull and multihit models using both the independent and additive assumptions to explain background tumor rats. We have recalculated the multistage extrapolations using 62 mid and high dose males and have found that the differences did not materially alter the Dynamac estimate of potency given below for the multistage model.

Dynamac has converted the reported concentration of Banner in ppm of feed to mg/kg/day of mouse diet as mg/kg/day = ppm (0.13). The mouse dietary intake has then been converted to human dietary equivalents by the surface area conversion: mouse dose in

$$\text{mg/kg/day} \times \left(\frac{\text{mouse weight} = 25\text{grams}}{\text{human avg. wt.} = 60{,}000\text{g}}\right)^{1/3}$$

to obtain human equivalent doses of 0; 1.04; 5.20 and 26.00 mg/kg/day respectively.

Low Dose Extrapolation

Due to the high response rate in the control group, none of the conventional models fit the data at $P \geq .05$. In fact the multistage and multihit models fail to achieve convergence in estimating the maximum likelihood estimate of the dose at the attributable risk levels usually calculated (i.e., 1×10^{-1} to 1×10^{-8}), nevertheless, a reasonably accurate estimate of the potency estimator Q_1^* can be obtained. The value of Q_1^* associated with fitting the mouse liver tumors to the human equivalent doses, shown above, is 3×10^{-2}.

004562

Characterization of Risk

If the data in the Jacoby to Campt memo of March 16, 1984, are used as the basis for characterizing cancer risks associated with Banner, the following may be estimated:

Dietary TMRC

TMRC or Theoretical Maximal Residue Concentration = 2.87 mg/person/day

TMRC in mg/kg of Body wt (60 kg)/day = .0478

Associated Risk = $.0478 \times 3 \times 10^{-2} = .001435$

If the mixer-loader is exposed to "0.67% of 2.87 mg/per/day" this equates to 0.0067×0.0478 mg/kg/day = 3.2×10^{-4} mg/kg/day when this exposure is prorated for 1, 10 or 30 days of use for a 35 year work life the average daily exposure becomes

$$\frac{3.2\times10^{-4}}{365 \text{ days}} \times \frac{35 \text{ work years}}{70 \text{ year lifetime}} \times \begin{cases} (1 \text{ day} & = 4.4\times10^{-7} \\ (10 \text{ days} & = 4.4\times10^{-6} \\ (30 \text{ day} & = 1.3\times10^{-5} \end{cases}$$

The associated risks of cancer are $1.3\times^{-8}$, 1.3×10^{-7} and 4.10^{-7} respectively or when rounded as above 10^{-8}, 10^{-7} and 10^{-6} to 10^{-7} respectively.