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

004766

NOV 15 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTA

SUBJECT: EPA Reg. No. 3125-320; Bayleton Plant Metabolite (Triazolylalanine)
Caswell No. 862AA, Accession No. 252132.

TO: Henry Jacoby
Product Manager (21)
Registration Division (TS-769)

THRU: William Burnam
Deputy Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: George Z. Ghali, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

11/14/85

Registrant: Mobay Chemical Corporation
Kansas City, MO 64120

Action Requested:

Review and evaluation of toxicology data on triazolylalanine, a plant metabolite of the fungicide Bayleton.

Background Information:

According to the registrant "during continued research on the triazole group of fungicides a common metabolite, triazolylalanine, was identified in plant metabolism studies. Since this compound does not occur in animals data were developed to assess the toxicity of triazolylalanine. The registrant indicated that "in addition to these data, a subchronic study in the rat has been completed and once the report is translated into English, copies will be forwarded to the Agency".

Conclusions and Recommendations:

The toxicology data submitted by the registrant to assess the safety of triazolylalanine have been evaluated. A summary of the toxicology evaluation of all the studies is included and all data evaluation records are appended to this report.

The subject metabolite appears to be of a very low toxicity on acute basis. From the limited data available the test chemical did not appear to be teratogenic under the test conditions. However, the chemical induced a positive response in a cell transformation test, and a weakly positive response at high dose level in the micronucleus test in mice.

Additional mutagenicity testing is required before the impact of these findings on the regulatory position on the triazol pesticides can be assessed. In addition, Toxicology Branch will also require information on the percentage and magnitude of the subject metabolite in the plant residues.

APPENDIX I
Summary of Evaluation Data
"Toxicology One-Liner"

One Liner Summary Table of Studies with Triazolylalanine

Study / Lab / Study Date	Material	Accession No.	Results	Tox Category	Core Grade
1. Acute oral toxicity-dog; Institute fuer Toxikologie, FRG; Report #82663; 10/14/82	THS 2212 (Triazolyl-alanine) 99% purity	252132	Only 2 dogs used on study; both vomited a portion of the test material within 4 hours of dosing.	Not determined	Invalid
2. Acute oral toxicity-rats; Central Toxicology Laboratory, ICI Limited; Report #CTL/P/600; 1/18/81	R152056 (Triazolyl-alanine) purity unspecified	252132	No mortalities at 2000 mg/kg the only dose tested. The LD_{50} is greater than 2000 mg/kg.	III	Supplementary
3. Acute oral toxicity-rats and mice; Bayer AG, Institute for Toxicology; Report #82661; 10/19/82	THS 2212 (Triazolylalanine) purity unspecified ("analytically pure")	252132	Fasted male rats showed increased urinary output the day after dosing; no toxic signs in mice. The LD_{50} is greater than 5000 mg/kg for both sexes of rats and mice.	IV	Minimum
4. Acute intraperitoneal toxicity-rats; Bayer AG, Toxicology Institute; Report #82661; 10/19/82	THS 2212 (Triazolyalanine) purity unspecified ("analytically pure")	252132	At 5000 mg/kg, reversible CNS effects (spastic gait, lethargy, etc.) were observed within 1 hour of dosing. The lethal dose exceeds 5000 mg/kg.	NA	Acceptable
5. Two-week (range-finding) Oral loxicity-male rats; Bayer AG, Institut fur Toxikologie; Report #82662; 10/25/82	THS 2212 (Triazolyl-alanine ~ 100% purity	252132	Dose levels: 0, 3000, 10,000 ppm in drinking water. No. mortalities or clinical signs of toxicity.	NA	Supplementary

One Liner Summary Table of Studies with Triazolylalanine

Study / Lab / Study Date	Material	Accession No.	Results	Tox Category	Core Grade
6. Subchronic (28 day) oral toxicity-rats; Bayer AG, Institute of Toxicology; Report #11491; Study No. T6011644; 1/24/83.	THS 2212 (Triazolyl-alanine) "analytically pure"	252132	Dose levels: 0, 25, 100, 400 mg/kg. No mortalities or clinical signs of toxicity. Some changes in hematology, clinical chemistry, organ weights. 400 mg/kg produced no adverse effects.	NA	Supplementary
7. Teratogenicity-rats; Central Toxicology Laboratory, Imperial Chemical Industries PLC; Report #CTL/P/875; 10/13/83.	Triazole alanine 94.8% purity	252132	Dose levels: 100, 300, 1000 mg/kg. Not teratogenic. LOEL for fetotoxicity = 300 mg/kg, NOEL for fetotoxicity = 100 mg/kg.	NA	Minimum
8. Pilot reproduction study-rats; Central Toxicology Laboratory, Imperial Chemical Industries PLC; Report #CTL/L/470; 9/19/83.	Triazole alanine Batch 1 - 48% Batch 2 - unspecified purity	252132	Dose levels: 0, 150, 625, 2500, 10,000 ppm. No effects at 10,000 ppm.	NA	Supplementary
9. Two generation reproduction study (Progress Report)-rats; Central Toxicology Laboratory, Imperial Chemical Industries PLC; Report #RRO255/FD and RRO255/F1; 6/21/83.	Triazole alanine 97.8% purity	252132	Dose levels: 0, 500, 2000, 10,000 ppm. No effects noted in the first 3 weeks of the study.	NA	Reserved (Progress Report)
10. Micronucleus test-mice; Imperial Chemical Industries; Report #AC83-2413; 9/14/82.	R152056 (Triazolyl-alanine) purity unspecified.	252132	Dose levels: 2500, 5000 mg/kg. No toxicity, chromosomal damage, or erythropoietic effects; however, animals were dosed only once and only one sex tested.	NA	Unacceptable

One Liner Summary Table of Studies with Triazolylalanine

Study / Lab / Study Date	Material	Accession No.	Results	Tox Category	Core Grade
11. DNA Damage-*E. coli*; Bayer AG, Institut fuer Toxikologie; Report #82738; 1/5/83	THS 2212 (Triazolyl-alanine) purity unspecified	252132	Dose levels: 62.5, 125, 250, 500, 1000 µg/plate. Nonactivated-no DNA damage. S9 activated-inadequate assay.	NA	Nonactivated assay: Acceptable; S9 Activated assay: Unacceptable
12. Mutagenicity: cell transformation; Huntingdon Research Centre; Report #CTL/C/1085; 5/15/81	RIS2056 purity unspecified	252132	Induced cell transformation at doses from 500 to 8000 µg/ml in presence of S9, with dose response. Also positive in absence of S9 with no dose response.	NA	Acceptable
13. Mutagenicity: Micronucleus test-mice; Bayer AG, Institut fuer Toxikologie; Report #84005; 8/9/82	THS 2212 (Triazolyl-alanine) purity unspecified ("analytically pure")	252132	Weak positive response for 8000 mg/kg at 24-hr. Study unacceptable due to lack of critical data on positive and negative controls.	NA	Unacceptable
14. Mutagenicity: Bacterial Point Mutations; Bayer AG, Institute Fuer Toxikologie; Report #11388; 1/5/83	THS 2212 (Triazolyl-alanine) purity unspecified	252132	Dose levels of 20, 100, 500, 2500, 12,500 µg/plate did not induce reverse mutations in S9 activated *S. typhimurium* assay. Non-activated assay not evaluated due to lack of positive control.	NA	Nonactivated assay: Unacceptable; S9 Activated assay: Acceptable
15. Metabolism/Pharmacokinetic study-rats; Bayer AG; Report #11583; 2/24/83	[^{14}C] Tri-azolylalanine; radiochemical purity 99%	252132	Dose levels: 5 mg/kg (metabolism); 10 mg/kg (whole-body autoradiography). Rapid absorption and excretion in male rats:95 percent of administered dose was absorbed and 94.5 percent of the radioactivity measured in urine within 48 hours. None of the metabolites were identified.	NA	Acceptable
16. Metabolism study - rats; Agricultural Division CIBA-GEIGY Limited; Report #CGA 131013, 82/91-92/110; 3/2/83.	[^{14}C]D-L-triazolyl-alanine; radiochemical purity >99%	252132	Dose level - approx. 50 mg/kg. Almost entirely excreted within 24 hrs; primary route-urine, secondary route-feces. Metabolites: N-acetyl, and unaltered triazolylalanine in urine.	NA	Minimum

Metabolism in Rats. Ciba-Geigy Study No. 131013 Report No. 11/83 Date: Oct. 20,83	^{14}C-D,L- Triazolylalanine. Purity >99%	252132	In 24 hours, 69-86% of the dose was excreted unchanged in the urine, 8-19% was excreted as the acetyl derivative in the urine. about 3% of the dose was excreted in the urine as unkown metabolites. The total fecal radioactivity accounted for 3% of the total dose. The fecal metabolites were similar to those found in the urine except for one that could not be identified.	N/A	Acceptable

APPENDIX II
Data Evaluation Records
"Confidential Business Information"

DATA EVALUATION RECORD

Acute Oral Toxicity in Dogs

Hoffman, K. THS 2212 (Triazolylalanine), acute oral toxicity, dogs. Bayer Report No. 11213 (Mobay ACD Report No. 82663) prepared for Mobay Chemical Corporation by Institute fuer Toxikologie, Wuppertal- Elberfeld, Federal Republic of Germany, and dated October 14, 1982.

ACCESSION NUMBER: 252132.

LABORATORY: Institute fuer Toxikologie, Wuppertal-Elberfeld, Federal Republic of Germany.

TEST MATERIAL: The test material was identified as THS 2212 from batch E-238099, Lot No. 2839 with a purity of 99.0 percent.

PROTOCOL:

1. Two pure-bred beagles purchased from two sources (Winkelmann, Borchen 1, FRG and Marshall Research Animals, North Rose, NY) were used for these studies; one 11-month old male weighing 7.2 kg and one 21-month old female weighing 9.0 kg.

2. The dogs were individually housed in a temperature (21-22 C) and humdity (50-60 percent) controlled room with a 12-hour light/dark cycle. Each dog was fed approximately 350 g of "ssniff complete dog food" daily. Tapwater was provided ad libitum.

3. The test material was suspended in a 0.5 percent Tylose solution and was administered to fasted dogs via oral intubation at a dose of 5000 mg/kg body weight. The animals were observed for clinical signs of toxicity for 14 days after the administration of the test compound. Feed consumption was recorded daily and body weights were taken weekly.

RESULTS:

Both dogs vomited a portion of the test material within 4 hours after dosing. No other signs were observed in the male. "Slight salivation and a slightly unsteady gait" were reported for the female following dosing.

9

Throughout the remainder of the study the male consumed the entire daily feed offered; however, food consumption in the female was depressed. The male body weight increased slightly during the study while the female body weight was reduced.

DISCUSSION:

Only two animals were used in this study; an adequate test would require at least 5 animals per sex for acute oral toxicity studies. Since both animals vomitted a portion of the test material, it is not possible to determine the dose to which they were actually exposed.

CONCLUSIONS:

No valid conclusions can be made since only two animals were tested and both animals vomitted a portion of the test material.

CORE CLASSIFICATION: Invalid.

TOXICITY CATEGORY: Cannot be determined.

10

DATA EVALUATION RECORD

Acute Oral Toxicity in Rats

Henderson, C. and Parkinson, G.R. R152056: Acute oral toxicity to rats. Report No. CTL/P/600 (Study No. AR1893), prepared by Central Toxicology Laboratory for Imperial Chemical Industries Limited, dated January 18, 1981.

ACCESSION NUMBER: 252132.

LABORATORY: Central Toxicology Laboratory, Imperial Chemical Industries Limited, Alderley Park, Macclesfield Cheshire, U.K.

TEST MATERIAL: The test material was identified as R152056 [3-(1,2,4-triazol-1-y)alanine]. The material was described as a white solid from batch number P4. Purity was not specified.

PROTOCOL:

1. Five male and 5 female Alderley Park SPF albino rats, 38-50 days old, weighing between 205 and 230 g and between 145 and 165 g, respectively, were used in this study. The animals were selected at random and housed in suspended cages. BP Nutrition Porton Combined Diet and tapwater were supplied ad libitum. The animal room temperature was maintained between 20 - 22° C and 60 percent relative humidity with a 12-hour light/dark cycle.

2. The animals were fasted for 16-20 hours and then dosed with the test material at a dose volume of 10 ml/kg by stomach tube. The test material was administered as a 20 percent (w/v) suspension in distilled water at a dose of 2000 mg/kg body weight. Following dosing, animals were observed for a two-week period.

RESULTS:

The investigators reported that no deaths occurred during this study, and that toxic signs were not evident.

DISCUSSION:

The use of only one dose level is a major shortcoming of this study; EPA Subpart F Guidelines (1982) require at least 3 dose levels. Moreover, this study does not constitute a limit test since a dose level of 5000 mg/kg is required. The purity of the test compound was not reported.

CONCLUSIONS:

The acute oral LD_{50} of triazolylalanine in rats cannot be determined, since only one dose level (below that required for a limit test) was used and the purity of the test material was not reported. The acute oral LD_{50} is considered to be greater than 2000 mg/kg

TOXICITY CATEGORY: III

CORE CLASSIFICATION: Supplementary.

12

004765

DATA EVALUATION RECORD

Acute Oral Toxicity in Rats and Mice

Mihail, F. Acute oral toxicity of Triazolylalanine (THS 2212) in rats and mice. An unpublished report (Bayer Report No. 11229, Mobay ACD Report No. 82661) prepared by Bayer AG, Toxicology Institute, for Mobay Chemical Corporation, Stilwell, Kansas, dated October 19, 1982.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld, West Germany.

TEST MATERIAL: Triazolylalanine (THS 2212) [2-amino-3-(1,2,4-triazol-1-yl)-propanoic acid]. Sample No. PF-CF-SF. The purity of the test compound was not given in this report; however, in another report (unpublished report No. 11491) the same sample was reported to be "analytically pure."

PROTOCOL:

1. Male and female Wistar rats Bor.:WISW (SPF-Cpb) weighing 160 to 200 g, and male and female NMRI (SPF-Hah) mice weighing 18 to 23 g were used. The animals were housed in Makrolon cages under "standardized conditions" and acclimated to laboratory conditions for one week prior to dosing. Altromin R "feed for rats and mice", and water were available ad libitum. Acute toxicity was determined in fasted mice and groups of both fasted and non-fasted rats. Feed was witheld from fasted animals for 16 hours prior to , and for 2 hours, after dosing.

2. The test compound was "formulated in distilled water and Cremophor EL" and was administered orally at a dose volume of "1.0 ml/100 g body weight" to groups of 10 animals each. The reported dosage and test conditions used are shown in Table 1.

3. Animals were observed for 14 days following dosing, then sacrificed and necropsied. The LD_{50} values were to be determined using the method of Litchfield and Wilcoxon[1].

---

[1] J. Pharmacol. Exper. Therap. 96:99, 1949.

13

RESULTS:

None of the rats or mice died. Fasted male rats that received 5000 mg/kg showed increased urinary output the day after dosing. One male rat from the nonfasted group showed tachypnea, ataxia and piloerection on day 11 after dosing. No signs of toxicity were observed in mice. Gross examination showed "no changes in the internal organs...that deviated from the norm."

The 14-day LD_{50} was reported to be greater than 5000 mg/kg for both sexes of rats and mice.

DISCUSSION:

The study was adequately conducted and conforms with the EPA guidelines. However, the concentration of the test compound in the administered dose formulation was not reported. Consequently, the conclusions of this study are based on the assumption that the test animals received the reported dosage.

CONCLUSIONS:

Under the conditions of this study the acute oral LD_{50} of triazolyl-alanine in male and female NMRI mice and Wistar rats was over 5000 mg/kg.

TOXICITY CATEGORY: IV.

CORE CLASSIFICATION: Minimum.

14

TABLE 1. Study Design for Acute Oral Toxicity of Triazolylalanine

Study No.	Species	Test Condition	Dose (mg/kg)	Sex
T70-114-65	Rat	Fasted	1000	M
			2500	M
			5000	M
			5000	F
T80-114-66	Rat	Not fasted	2500	M
			5000	M
			5000	F
T90-114-67	Mouse	Fasted	5000	M
			5000	F

DATA EVALUATION RECORD

Acute Intraperitoneal Toxicity in Rats.

Mihail, F. Acute intraperitoneal toxicity of Triazolylalanine (THS 2212) in rats. An unpublished report (Bayer Report No. 11229, Mobay ACD report no. 82661) prepared by Bayer AG, Toxicology Institute, for Mobay Chemical Corporation, Stilwell, Kansas, dated October 19, 1982.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer AG, Institute for Toxicology Wuppertal-Elberfled, West Germany.

TEST MATERIAL: Triazolylalanine (THS 2212), [2-Amino-3-(1,2,4-triazol-1-yl)-propanoic acid]. Sample No. PF-CF-SF. The purity of the test compound was not given in this report; however, in another report (unpublished Report No. 11491) the same sample was reported to be "analytically pure."

PROTOCOL:

1. Five groups of male and four groups of female Wistar rats, Bor.:WISW (SPF-Cpb), each consisting of 10 animals were used. The test compound in physiological saline and Cremophor EL was injected "into the abdominal cavity" of each rat at a volume of 1.0 ml/100 g. Dose levels used were 250, 400, 1000, 2500 or 5000 mg/kg for males, and 630, 1000, 2500, or 5000 mg/kg for females.

2. Animals were observed for 14 days following dosing, then sacrificed and necropsied.

RESULTS:

No mortality occurred during the 14-day observation period. Within 20-60 minutes after dosing, animals that received the 5000 mg/kg dose level showed spastic gait, piloerection, lethargy and diarrhea. Signs subsided within 24 hours.

Necropsy showed that livers (group not specified) were partially or totally surrounded by a "hard, white, connective tissue-like capsule." No histopathologic examination was performed.

DISCUSSION:

The study was adequately conducted, although the intraperitioneal route of administration is not considered as relevant to safety testing and is not required by the EPA guidelines (1982). The concentration of the test compound in the administered formulation was not reported. Consequently, the conclusions of this study are based on the assumption that the test animals received the reported dosages.

CONCLUSIONS:

Under the conditions of this study the acute intraperitoneal lethal dosage of triazolylalanine to male and female Wistar rats was over 5000 mg/kg.

TOXICITY CATEGORY: NA.

CORE CLASSIFICATION: Acceptable.

17

DATA EVALUATION RECORD

Two-Week (Range-Finding) Oral Toxicity in Male Rats.

Bomhard E. THS 2212, preliminary subacute toxicity study on male rats, administration in the drinking water. Unpublished report no. 11253 (Mobay Report No. 82662), prepared by Institut Fur Toxikologie, Bayer AG, Wuppertal-Elberfeld, Germany. Dated October 25, 1982.

ACCESSION NUMBER: 252132.

LABORATORY: Institut fur Toxicologie, Bayer AG Wuppertal-Elberfeld, Germany.

TEST MATERIAL: The test material was identified as THS 2212, Triazolyl-alanine, from batch no. E 238 099, and approximately 100 percent pure.

PROTOCOL:

1. Male Wistar rats "strain BOR:WISW (SPF CPB)" were obtained from Winkelmann, Borchen, Germany. The animals were 5-6 weeks old and the average initial weight was 114 g. The rats were housed individually and the animal room maintained at 22 ± 1.5° C and 60 ± 10 percent relative humidity with a 12-hour light/dark cycle. Feed (Altromin 1324) and tapwater were available ad libitum.

2. Animals were randomly assigned to 3 groups of 10 animals each. The test material was dissolved in the drinking water at concentrations of 0, 3,000, and 10,000 ppm. The stability of the test material in water over a period of 8 days was determined in solutions containing 100 and 10,000 ppm.

3. The animals were observed for toxic signs twice daily. Individual body weight were determined initially and weekly thereafter. Food and water consumption were determined weekly. The animals were sacrificed after 2 weeks and necropsied. The weights of the thyroid, thymus, liver, spleen, kidneys, adrenals, and brain of each animal were determined at sacrifice.

4. The data were analyzed statistically by means of the U-test of Mann, Whitney, and Wilcoxon, at the significance levels of 5 and 1 percent.

RESULTS:

There were no mortalities nor compound-related signs reported. Similarly, there were no compound-related effects noted for body weight gain, and food and water consumption. The average volume of water ingested by the rats was about 22 ml of test water/animal/day. This volume provided an equivalent dosage of 0, 448, and 1491 mg/kg body weight of test material at test concentrations of 0, 3,000, and 10,000 ppm, respectively. The dosage calculations were based on body weights at 1 week.

There were no compound-related lesions noted during necropsy and mean organ weights were similar among control and treated animals.

DISCUSSION:

This 2-week oral toxicity study was a range-finding study performed to determine appropriate dosages for a subchronic study. The test material was administered in the drinking water because the analytical determination of the test material in the standard rat feed presented considerable problems. The U-test used to analyze the data statistically is a non-parametric test used for a single pair-wise comparison; a parametric test, such as the ANOVA would have been more appropriate to the results of this study. However, no statistical differences were noted by this reviewer using the ANOVA.

CONCLUSIONS:

Based on the results of this study, the author recommended that doses of 1,000, 3,000, and 10,000 ppm in drinking water be used in a proposed 90-day subchronic study. However, higher doses should be used, since no effects were noted at dosages up to 10,000 ppm.

CLASSIFICATION: Supplementary data.

19

DATA EVALUATION RECORD

28-Day Oral Toxicity Study in the Rat.

Mihail, F. and Vogel, O. Triazolylalanine (THS 2212)-Subacute oral toxicity study in the rat. Unpublished Report No. 11491 (Study No. T6011644) prepared by Bayer, AG, Wuppertal-Elberfeld, Germany. Dated 24 January 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany.

TEST MATERIAL: Triazolylalanine (THS 2212), chemically identified as 2-amino-3-(1,2,4-triazol-1-yl)-propionic acid. Sample PF-CF-SF from batch No. E238099 was received 10 May 1982 and was said to be "analytically pure."

PROTOCOL:

1. Eighty male and 80 female Wistar rats (Bor:WISW SPF/Cpb) were obtained from F. Winkelmann, Borchen, Germany. The initial body weights of the animals ranged from 120-140 g. The animals were accliminated to laboratory conditions for five days prior to study initiation.

2. Triazolylalanine was suspended in a Cremophor EL/distilled water vehicle (5 drops Cremophor EL/10 ml water) just prior to each dose to provide concentrations of 0.00, 0.25, 1.00, and 4.00 percent. The suspensions were administered daily for 28 days to the rats by oral gavage at a dose volume of 10 ml/kg body weight. The dose volumes were based on the weekly body weights and were equivalent to dosages of 0, 25, 100, and 400 mg/kg body weight.

   Homogeneity and stability analyses of each dose level were conducted prior to study initiation. Analyses for triazolylalanine concentration of one dosing suspension from each dose level were conducted once during the study.

3. The rats were housed in a temperature - (21-26° C) and relative humidity-controlled (65 percent) room with a 12 hour light/dark cycle. The animals were individual housed in Makrolon Type II cages.

Altromin 1324 rodent feed and tapwater were available ad libitum except during a 16-hour urine collection period.

4. Twenty rats per sex were randomly assigned by a weight stratified design to each of the four treatment groups. The animals were also randomly preselected into subgroups for clinical pathology and sacrifice schedule. The animals were observed daily for mortality, general appearance, and behavior. Individual body weights were recorded initially and weekly thereafter. Food and water consumption were recorded weekly on a sex/group basis.

   At the end of the 28-day dosing period five animals/sex/group were bled from the distal portion of the tail to determine blood sugar content and urine was collected for urinalysis. In addition, blood that was obtained by cardiac puncture just prior to necropsy was analyzed for the following hematology parameters: leukocyte count; RBC count; hemoglobin; mean corpuscular volume; thrombocyte count; reticulocyte count; Heinz inclusion bodies; hematocrit; mean cellular hemoglobin concentration; mean corpuscular hemoglobin; and a differential blood count. The clinical chemistry analysis of the blood measured the following parameters: SGOT; SGPT; alkaline phosphotase; BUN; glucose; creatinine, bilirubin; and cholesterol. The urine was analyzed for pH, urobilinogen, blood, protein, glucose, and sediment. Liver tissue was removed from the five animals/sex/group and analysed for aminopyrine N-demethylase, aminopyrine O-demethylase, and cytochrome P-450 activities, and triglycerides.

   Ten animals/sex/group, including the five selected for clinical pathology, were sacrificed at the end of the 28-day compound administration period and examined grossly. The weights of the brain, heart, gonads, liver, lungs, spleen, adrenals, kidneys, and thyroids were determined. Microscopic examinations were conducted on the intestines (3 sections), femur, skeletal muscle, lymph nodes, stomach, epididymis, esophagus, uterus, and the organs that were weighed.

   The remaining 10 animals/sex/group remained on study for a 28-day post-treatment period. At the end of this 28-day recovery period five animals/sex/group were examined for clinical pathology as described above, and 10 animals/sex/group were sacrificed and examined for gross and micropathology as described above.

5. The control group means were compared to the dosed group means by the Mann-Whitney U-test. Significance levels were set at probabilities of 5 and 1 percent. Unless otherwise stated, the use of the word "significant" in this report connotes statistical significance.

RESULTS:

Analytic Chemistry: Analytic chemistry results for homogeneity and stability were not presented in the report.

21

Clinical Observations: No deaths were reported during the study. The report stated that the clinical observations were similar among all groups. Specific clinical observations were not reported.

Body Weights and Food and Water Consumption: The mean body weights of the triazolylalanine-treated males and females were similar to the control males and females throughout the 28-day dosing and recovery periods.

The mean daily food and water consumptions for the males and females were similar among the four treatment groups throughout the dosing and recovery periods.

Hematology: Among the male rats sacrificed at the end of the dosing period, a significant decrease (approximately 5 percent) in the mean corpuscular hemoglobin at the 100 mg/kg dose level was the only difference detected between the treated and control groups. A significant decrease of 10-20 percent as compared to the controls was detected in the thrombocyte count of all female groups treated with triazolylalanine and sacrificed at the end of the dosing period. Mean cellular hemoglobin concentration among the 25 and 400 mg/kg females sacrificed after dosing was significantly greater (5 percent) than the controls. All remaining hematologic parameters that were examined were similar among the treated and control males and females sacrificed after the dosing period.

Among the male rats sacrificed at the end of the recovery period, leukocytes and mean cellular hemoglobin concentration were increased by 30-50 percent and 3 percent, respectively, at the 100 and 400 mg/kg dose levels when compared to the control males. Sporadic significant differences, compared to the controls, were observed in the differential blood count of the 25 and 100 mg/kg males sacrificed at the end of the recovery period.

There were no differences in the hematologic parameters of the females sacrificed at the end of the recovery period.

Clinical Chemistry: Significant decreases of 20 percent as compared to the controls were observed in BUN and creatinine concentrations of the high dose male rats sacrificed at the end of the dosing period. A significant decrease of 15 percent was observed in creatinine concentration when the high dose females sacrificed at the end of the dosing period were compared to the controls.

A significant decrease (17 percent) was detected in cholesterol concentration in the 25 mg/kg males when compared to the controls.

At the end of the recovery period significant decreases of less than 17 percent were detected in BUN concentrations among the 100 and 400 mg/kg males when compared to the controls. Among the 100 mg/kg females sacrificed at the end of the recovery period, the creatinine concentration was significantly greater (35 percent) and cholesterol concentration was

significantly less (18 percent) than the controls. All remaining clinical chemistry parameters were similar among the controls and treated males and females.

Enzyme Induction, Triglycerides, and Urinalysis: Triglyceride concentrations, and the enzyme activities of aminopyrine N-demethylase and aminopyrine O-demethylase, and cytochrome P-450 were similar in the triazolylalanine and control males and females sacrificed at the end of the dosing period. The determinations in the liver tissue, planned for the animals sacrificed after the recovery period, were not conducted because "no indications of a specific effect on the liver or damage" were found among the animals sacrificed after the dosing phase.

The urinalysis results in the triazolyalanine-dosed males and females sacrificed after the dosing or recovery periods were similar to their respective controls.

Pathology: Individual gross pathology observations were not presented in the report; the report stated that "no alterations deviating from the norm were noted" during the study. The microscopic examination of the tissues from the high dose and control male and female rats sacrificed after the dosing period did not produce any indications of treatment-related histologic changes. Because there were "no indications of organ changes, a corresponding examination of the restitution [recovery] groups was dispensed with."

The absolute and relative (mg/100 g body weight) organ weights of the triazolylalanine treated males were similar to the controls at both sacrifice intervals with the exceptions of a significant decrease of 6 percent in relative kidney weight among the 100 mg/kg males and a significant increase of 15 percent in relative spleen weight among the 25 mg/kg males sacrificed at the end of the recovery period.

Significant decreases, when compared to the controls, in the absolute (13 percent) and relative (10 percent) weights of the liver were noted among the 400 mg/kg females sacrificed at the end of the dosing period. This difference was not evident in the 400 mg/kg females sacrificed at the end of the recovery period.

In addition to the liver, the relative lung weight of the high dose and the relative adrenal weight of the mid dose females sacrificed after the dosing period were significantly different from the controls by 5 and 7 percent, respectively.

Among the females sacrificed after the recovery period the absolute and relative organ weights were similar among the four groups with the exception of a signficant increase of 5 percent in the absolute brain weight of 25 mg/kg females when compared to the controls.

DISCUSSION:

No overt adverse effects related to the administration of triazolylalanine were observed. Changes in some hematology and clinical chemistry parameters were detected; however, these did not appear to be indicative of toxic responses. Decreases is serum creatinine and urea concentrations generally do not have a clinical significance. The lower liver weights observed among the 400 mg/kg females were unlikely to have been treatment-related; histologic liver changes were not observed. Other observed organ weight changes also were not associated with histopathologic lesions.

The absence of overt toxicity among the high dose animals indicates that higher dosages could have been tolerated and that optimum test sensitivity may not have been achieved.

CONCLUSIONS:

Under the conditions of this study, the oral administration of up to 400 mg/kg triazolylalanine to Wistar rats for 28 consecutive days did not produce any adverse effects. The animals may have been able to tolerate higher test dosages.

CORE CLASSIFICATION: Supplementary data.

DATA EVALUATION RECORD

Teratology Study in Rats.

Clapp, M.J., et al. Triazole alanine: Teratogenicity study in the rat. Report No. CTL/P/875 (Study No. RR0240) prepared by Imperial Chemical Industries PLC. Dated October 13, 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Central Toxicology Laboratory, Imperial Chemical Industries PLC, Alderley Park, MacClesfield, Cheshire, UK.

TEST MATERIAL: Triazole Alanine, [2-amino-3-(1,2,4-triazol-1-yl) propionic acid], Bayer AG Batch No. TLB 12072 supplied by Bayer AG, Wuppertal, Germany. Described as a white crystalline solid with a purity of 94.8 percent.

PROTOCOL:

1. Alderley Park Alpk/AP rats were used in this study. All other information pertaining to the receipt and acclimation of the animals was unavailable to the reviewer because of the omission of page 4 from the ICI report.

2. Appropriate amounts of triazole alanine were weighed out and a sufficient quantity of distilled water added to provide 500 ml suspensions of 5, 15, and 50 mg/ml (w/v). The rats were administered 20 ml/kg of the suspensions daily from day 7 to day 16 of gestation to provide dose levels of 100, 300, and 1000 mg/kg body weight. The vehicle controls received distilled water. The dosing volumes were adjusted daily.

   The dosing suspensions were analyzed by HPLC for triazole alanine concentration prior to the initial dosing and after completion of the dosing phase. The analyses indicated that the actual triazole alanine concentrations of the suspensions were ± 11 percent of the nominal concentrations.

3. The rats were individually housed in a temperature - (20-25° C) and relative humidity - (33-44 percent) controlled room with a 12 hour light/dark cycle. Water was provided ad libitum.

4. Twenty-four female rats were randomly assigned to each of the four treatment groups on day 1 of gestation. A replicate design consisting of 24 replications each containing the four treatment groups, was used to facilitate the statistical analysis of the data. The rats appear to have arrived timed-pregnant on day 1 of gestation.

   Observations for mortality, behavior, and signs of toxicity were conducted daily. Individual body weights were recorded on gestation days 1, 4, 7-16, 19, and 22. Food consumption was measured for days 1-4, 4-7, 7-10, 10-13, 13-16, 16-19, and 19-22 of gestation.

   The females were sacrificed by an overdose of halothane BP vapor (Fluothane, Imperial Chemicals Industries, PLC) on day 22 of gestation and examined for gross lesions. The gravid uteri were weighted and the number of corpora lutea in the ovaries was recorded. The uteri were opened and the number and position of early and late resorptions and live fetuses recorded. The live fetuses were individually identified, weighted, and examined for external abnormalities.

   Approximately two-thirds of the fetuses from each litter were randomly selected, examined macroscopically for visceral abnormalities, eviscerated and prepared for skeletal examination according to the method of Staples and Schnell (Stain Technol 39:61-63, 1964). The remaining fetuses were immersed in Bouin's solution and examined for soft tissue abnormalities. The heads of the fetuses were examined using the Wilson's technique (Teratology: Principles and Techniques, University of Chicago Press, 1965). The thoracic and abdominal cavities were dissected with the kidneys sectioned transversely to reveal internal anatomy.

5. Normally distributed data were analyzed by an ANOVA. Percentages were analyzed by an ANOVA following an arcsine transformation of the percentages. A student's t-test (in which the experiment-wise error rate was controlled) was used to determine significant differences between means. Fisher's Exact Test was used to analyze proportions. With the exception of body weight gain, food consumption, and proportion of male fetuses, one-sided tests were used.

   Significance was determined at the 5 and 1 percent probability levels. Unless otherwise stated, the use of the word "significant" is intended to imply a statistical connotation.

RESULTS:

Clinical Observations: No deaths occurred prior to the sacrifice of the animals on day 22 of gestation. An examination of the dams at sacrifice showed slight hydronephrosis in two dams in each of the 0, 100, and 300 mg/kg groups.

The authors reported that "a few isolated occurrences such as hair loss were observed but none were treatment-related."

Body Weights and Food Consumption: The pre-dosing weight gain (days 1-7 of gestation) of the high dose dams was significantly less than the controls. The 100 mg/kg dams gained significantly more weight between days 16-22 of gestation than the control dams. The weight gains of the triazole alanine treated dams during the dosing period were similar to the controls.

The 300 mg/kg dams consumed significantly more feed per day than the controls from day 7 to day 22 of gestation. The food consumption of the 100 and 1000 mg/kg dams were similar to that of the control dams.

Reproduction Indices: There were no significant differences between the triazole alanine and control dams for the following reproduction indices: a) no. of pregnant females; b) mean no. of corpora lutea; c) mean no. of implantations; d) pre-implantation loss; e) post-implantation loss; f) mean no. of early and late resorptions; g) mean no. of live fetuses; h) mean gravid uterine weight; and i) mean fetal body weight.

Fetal Evaluation: A summary of fetal abnormalities that were major or occurred in a dose group at a significantly different incidence rate from the controls is presented in Table 1.

The individual abnormalities were not presented on a litters-affected basis, precluding a comparison of these data with the litter as the sample unit. A vestigial left testis was observed in one high dose fetus. A second high dose fetus was observed to be severely malformed. These malformations, described in Table 1, were clearly incompatible with life. It was not possible to ascertain from the data whether the two fetuses were from the same litter or with certainty that they were different fetuses; however, the data presentation suggests two fetuses.

With the exception of the malformations observed in the severely malformed high dose fetus, no major skeletal malformations were observed. An increase in the number of high dose fetuses observed to have delayed ossification was noted. A significantly greater number of high dose fetuses were observed to have non-ossified adontoid processes, partial ossification of the transverse processes of the 7th cervical vertebrae, partial ossification of the 13th thoracic centrum, and non-ossified 5th sternebrae. The total number of high dose fetuses with minor skeletal abnormalities was also significantly greater than the controls.

A significant increase in the number of mid-dose fetuses with non-ossified adontoid processes was also observed. There was a significant increase in the numbers of low-dose fetuses observed to have non-ossified calcanea; however, a similar increase in this hind-limb variation was not observed in the mid- and high-dose fetuses.

TABLE 1. Incidence of Selected Fetal Abnormalities[a] 004766

Observation	Dose Level (mg/kg) Control	100	300	1000
I. Soft Tissue				
No. of fetuses examined	279	301	264	293
No. of fetuses with abnormalities	13	5	12	7
Litters with abnormal fetuses	7/24	5/24	8/24	5/24
Vestigial left testis	0	0	0	1
Severe Malformations[b]	0	0	0	1
II. Skeletal				
No. of fetuses examined	185	201	178	195
No. of fetuses with abnormalities	95	95	92	122*
Litters with abnormal fetuses	23/24	23/24	23/24	23/24
Odontoid process not ossified	12	10	24*	29*
Partial ossification of transverse processes of 7th cervical vertebra-bilateral	1	3	2	12**
Partial ossification of transverse processes of 7th cervical ventebra-unilateral	17	9	11	24
Partial ossification 13th thoracic centrum	1	4	4	7*
5th sternebra not ossified	0	0	1	7**
Calcanea not ossified	157	187**	148	165

[a] Only major abnormalities or those showing statistical significance.

[b] The thoracic and abdominal organs were external to the abdomen. No diaphram was present. The lungs, liver, urogenitalia, and brain were only partially formed. Severe scoliosis was present. Very reduced ossification of the skull bones. Ribs were fused and the pubes were absent.

* Significantly different than the control group by Fisher's Exact Test ($p \leq 0.05$).

** Significantly different than the control group by Fisher's Exact Test ($p \leq 0.01$).

28

DISCUSSION:

One high-dose fetus was observed to have severe external, visceral, and skeletal malformations that are rarely observed. A second high-dose fetus was observed to have a vestigial testis. The absence of other visceral abnormalities occurring at a greater frequency of the highest dose level and the occurrence of the two abnormalities in single fetuses, suggests that these abnormalities were spontaneous in origin and not treatment related.

A delay in ossification was clearly established at the highest dose level. Delayed ossification is indicative of a toxic effect on the fetus rather than a teratogenic effect. An indication that the 300 mg/kg dose level was also fetotoxic was detected with the significant increase in the number of fetuses with non-ossified odontoid processes.

The increased number of low-dose fetuses with non-ossified calcanea does not appear to be treatment-related. Concurrent increases in this variation were not observed at the 300 and 1000 mg/kg dose levels.

The presentation of the data was deficient in the failure to report fetal abnormalities on the basis of the number of litters affected. This information is required to determine if a given abnormality is distributed among the litters and therefore indicating a general effect or if it is clustered into one or two litters and therefore indicating an effect peculiar to one or two dams. The absence of this information can result in effects that are litter-related being attributed to the treatment.

CONCLUSIONS:

Under the conditions of this study, the oral administration of triazole alanine to pregnant Alderley Park rats during the period of organogenesis produced fetotoxic effects with an LOEL of 300 mg/kg and a NOEL of 100 mg/kg. Triazole alanine was not found to be teratogenic.

CORE CLASSIFICATION: Minimum data.

DATA EVALUATION RECORD

Pilot Reproduction Study in the Rat.

Birtley, R.D.N., Triazole alanine: Preliminary reproduction study in the rat. An unpublished report (No. CTL/L/470; Study No. RRO230/FO) submitted by Imperial Chemical Industries PLC. Dated September 19, 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, MacClesfield, Cheshire, United Kingdom.

TEST MATERIAL: Triazole alanine, chemically identified as 3-[1,2,4 triazol-1-yl]alanine. Two batches of unspecified numbers were used. The first batch had a purity of 48 percent and the purity of the second batch was not reported.

PROTOCOL:

1. An unspecified number of 11-12 week old Alderley Park rats were received from an unspecified supplier. The length of the acclimation period if any, and the environmental conditions under which the study was conducted were not reported.

2. At the start of the study, the purity of the first batch was thought to be greater than 90 percent. Triazole alanine was mixed with a basal diet to produce nominal dietary concentrations of 0, 300, 1250, 5000, and 20,000 ppm. However, it was later determined that the purity of the test material was 48 percent. Consequently, the nominal dietary concentrations adjusted for purity were 0, 150, 625, 2500, and 10,000 ppm.

   The stability, homogeneity, and actual concentrations of the test material in the diets were analyzed.

3. Six male and 12 female rats were assigned by an unspecified method to each of the five experimental groups. The animals were fed the test diets for six weeks prior to mating. During this pre-mating phase, body weights and food consumption were recorded weekly, and the animals were observed for general appearance, health, and signs of toxicity.

30

After the six week pre-mating phase, the animals were mated on a two female to one male basis, and vaginal smears were examined for the presence of sperm. The length of the mating period was not reported.

The dams were weighed on the day of insemination and the last day of gestation. On the first and 29th days of lactation, the sex of the F_1 pups and the numbers of live and dead pups were determined. The F_1 litters were weighed on days 1, 5, 11, 22, and 29 of lactation. The animals were maintained on the test diets through the mating, gestation, and lactation phases of the study.

The F_0 males were sacrificed after mating and the F_0 females and F_1 pups were sacrificed on day 29 of lactation. All F_0 animals and "selected" F_1 pups were necropsied and "certain" tissues examined histologically.

5. Non-parametric data were analyzed by the Mann-Whitney U-test and normally distributed data were analyzed by a two-sided t-test that controlled the experiment-wise error rate. A probability level of 5 percent was selected as the level of significance. Unless otherwise stated, the word "significant" is intended to imply a statistical connotation with $p \leq 0.05$ as indicated in the final report.

RESULTS:

Analytical Chemistry: The diets were found to be homogeneous, stable for 11 weeks under unspecified environmental conditions, and within 14 percent of nominal levels with the exception of one 10,000 ppm diet determined to be 21 percent less than the target concentration.

Clinical Observations: One 10,000 ppm F_0 male was found dead on week 5 of the study. The necropsy and histological examination determined that obstructive urolithiasis and chronic inflammation of the urinary tract were the cause of death. No other deaths were reported.

Hair loss and scabs were observed on a "small", but unspecified number of females in the 625 and 2500 ppm groups. No other clinical observations were reported.

Body Weights and Food Consumption-Premating Phase: The weekly body weight gains of the F_0 males were statistically similar among all groups; however, the gains were slightly less among the 2500 and 10,000 ppm males when compared to the controls. The author stated that the smaller weight gains observed in the high dose group were partially attributable to the male that died during week 5. The large standard deviations in the male body weights for the 10,000 ppm group during the first four weeks of the study suggests that at least one animal was deviating greatly from the mean. The weekly body weight gains among the F_0 females were similar.

Food consumption by the F_0 males and females receiving triazole alanine was similar to their respective controls with the exception of a

significant, but less than 10 percent, increase in food consumption among the 10,000 ppm females during week 1.

A comparison of the ratio of body weight gain/100 g feed consumed between the treated animals and the controls did not produce consistent patterns. The ratio was significantly increased among the 10,000 ppm males during week 1 and significantly decreased among the 10,000 ppm females during week 2 and among the 625 ppm males during week 4.

Gestation Body Weights: The author stated that body weight gains during gestation were similar in all groups, but data were not presented.

Reproduction Indices: The number of mated females that became pregnant, the number of pregnant females delivering litters, the male fertility index, and mean gestation length were similar in all groups. The mean length of the mating period was significantly longer in the 10,000 ppm group (4.3 days) than in the controls (2.1 days). The author stated that the first day of insemination was missed on one 10,000 ppm female and a second insemination that was detected was not observed until 4-5 days later. Data for this animal were included in the means presented in the ICI report.

Postnatal Performance: With the exception of pup body weights, postnatal performance could not be evaluated because of inconsistencies (see Discussion) in the data presented in the ICI report.

The mean body weight of the 10,000 ppm male and female F_1 progeny were significantly less than their respective controls at birth. At day 5, the mean body weights of the high dose progeny were similar to their respective controls and remained similar throughout the remainder of the study.

Pathology: The incidences of abnormal lesions in the F_0 adults were similar among the experimental groups. Similar incidences of histopathologic lesions were observed among the triazole alanine and control F_1 progeny. A histologic examination of the livers was not conducted among the 150, 625, and 2500 ppm F_1 progeny. The final report stated that the histologic examination of the liver was not conducted because the livers of the high dose progeny had similar types and incidences of lesions as the controls.

DISCUSSION:

An evaluation of postnatal viability and litter sizes could not be performed because of inconsistencies in the data presented in the final report. These parameters could not be independently recalculated because of the lack of individual litter data in the ICI report.

One inconsistency involved the number of pups dying between birth and day 29. Table 7 of the ICI report stated that the 10,000 ppm litters averaged 12.8 pups on day 1 of lactation and 12.1 pups on day 29. Despite the

decrease of 7 pups [(12.8-12.1) x 10 litters] between days 1 and 29, Table 7 stated that the percentage of deaths was 0.0. The decrease of 7 pups is therefore unexplained by reported deaths. Similar inconsistencies were observed in the data for the 0, 150, and 2500 ppm groups.

The liveborn indices presented in Table 6 of the ICI report could not be reconstructed by our reviewers. Calculation of the liveborn index as the number of live births/total number of births, results in a liveborn index for the 150 ppm group (127/133 = 95.5) that does not agree with the live-born index presented in the final report (94.8). Similar inconsistencies were discovered with the liveborn indices for the 625 and 2500 ppm group.

The survival indices presented in Table 6 did not agree with either the number of live pups at day 1 or day 29 of lactation. If the number of live born pups in the control group (126) were multiplied by the control group survival index for day 29 (84.6), the calculated number of 107 live control pups on day 29 did not agree with the number of live control pups (118) on day 29 calculated from the data as it was presented in Table 7 of the ICI report (11.8 control pups per litter on day 29 x 10 control litters). Similar inconsistencies were found in the data for the 150, 2,500, and 10,000 ppm groups.

The definitions of the reproduction indices were not given by the investigator and the inconsistencies discussed may have resulted from differences between the investigator and this reviewer in defining the indices. For the purpose of this evaluation our reviewers defined the indices as follows:

1) Liveborn Index: Number of live births/total number born.

2) Survival Index Day 29: Number of pups alive on day 29/Number of live births.

3) Percent Deaths Day 29: (Number of deaths occurring between days 1 and 29/number of pups alive on day 1) x 100.

A deficiency in the study involved the purity of the first batch of the test material. This batch was only 48 percent triazole alanine instead of the intended greater than 90 percent purity. Therefore, during the first portion of the study, the animals received a test substance comprised of 52 percent unspecified impurities. The effects of the impurities, if any, are unknown.

A second question regarding the administration of the test material involves the method of analyses of the test diets. Triazole alanine was administered in the feed to the rats. The ICI report did not state that the determination of triazole alanine in the feed presented any analytical problems. However, Bayer AG Report No. 11253, Preliminary Subacute Toxicity Study on Male Rats, Administration in Drinking Water, stated that "the determination of the substance [triazole alanine] in standard rat feed presents considerable analytical problems, especially at the lower dose ranges (< 2000 ppm)." A description of the ICI analytical

techniques would be required to verfiy the applicability of those techniques.

CONCLUSIONS:

The absence of frank toxicity in adults from all dosage groups precluded the determination of LOELs and of the maximum tolerated dose levels for the male and female parents. The lack of effects, even at the highest dose tested, may have resulted from the initial presumption of the high purity of the test material. The actual purity was later determined to be 48% active ingredient. However, this range-finding study did serve to demonstrate that dietary dose levels of up to 10,000 ppm are insufficiently high, and that the potential reproductive toxicity of the test material should be tested at higher dose levels.

CORE CLASSIFICATION: Supplementary data.

DATA EVALUATION RECORD

Two Generation Reproduction Study in the Rat.
(Progress report)

Birtley, R.D.N. Triazole alanine: Two generation reproduction study in the rat. A progress report of Study No. RR 0255/FO and RR 0255/F1 prepared by Imperial Chemical Industries PLC, Dated June 21, 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

TEST MATERIAL: Triazole alanine, chemically identified as 2-amino-3-(1,2, 4-triazol-1-yl)propionic acid. CTC Reference No. YO1210/003/005 received from Bayer A.G., Wuppertal, Germany. Purity was 97.8 percent.

PROTOCOL:

The study protocol was included in the progress report. An evaluation of the procedures described in the protocol indicated that the protocol is consistent with the requirements for reproduction studies as suggested by the Pesticide Assessment Guidelines (USEPA, November 1982).

Based on the results of a pilot reproduction study (RR 0230/FO), dietary levels of 0, 500, 2000, and 10,000 ppm were selected.

RESULTS:

Body weights and body weight gains were presented for weeks 1-3 of the F_0 generation. The mean body weights and mean body weight gains of the triazole alanine treated F_0 males and females were similar to their respective controls.

DISCUSSION:

The presentation of the first three weeks of body weight data from a two-generation reproduction study provided minimal information for the evaluation of triazole alanine for reproductive toxicity. Therefore the core classification is reserved until completion of the study.

CONCLUSIONS:

Based on the body weight data presented for the first three weeks of the F_0 generation, triazole alanine did not affect the weight gain of the 10,000 ppm male and female rats during this time period.

CORE CLASSIFICATION: Reserved until completion of the study.

004766

DATA EVALUATION RECORD

Mutagenicity, Mouse Micronucleus Test

Watkins, P.A. R152056: 3-(1,2,4-triazol-1-yl)alanine (ICI 156,342) micronucleus Test in CBC F_1 mice - TQM/4. An unpublished report (AC 83-2413) submitted by Mobay Chemical Corp., Stillwel, KS. Dated September 14, 1982.

ACCESSION NUMBER: 252132.

LABORATORY: Imperial Chemical Industries (ICI) Alderley Park, Macclesfield, Chesire SK10 4TG.

TEST MATERIAL: 3-(1,2,4-triazol-1-yl)alanine (ICI 156,342), R152056, Batch number 02199/49, a metabolite of the fungicide diclobutrazol (vigil), was used for the test. The purity of the test material was not reported.

METHODS:

1. Test Animal: 60 male CBC F_1 mice were obtained from ICI breeding facilities.

2. Controls: A 0.5 percent aqueous solution of Tween 80 (Batch number ADM 785-80) served as the vehicle for the test compound, the positive control compound, and also as the negative control substance.

   Cyclophosphamide served as the positive control substance (Batch number 833145, supplied by Koch Light Laboratories, Inc.)

3. Procedure: The test compound was first screened for acute toxicity in male CBC F_1 mice 12-18 weeks of age, of unspecified body weight. Two animals per group received intraperitoneally 5,000, 1,000, 750, 500, 250, 100 or 0 mg/kg of the test material. All mice were dosed twice, the second administration following 24 hrs after the first, and the sampled for micronuclei during the next 4 days.

37

The micronucleus test was conducted with male CBC F_1 mice 12-18 weeks of age, weighing 15-35 g at receipt. Groups of fifteen animals received a single intraperitoneal injection of the following materials: test compound in 0.5 percent Tween 80 at 5,000, and 2,500 mg/kg, cyclophosphamide in 0.5 percent Tween 80 at 40 mg/kg; and the vehicle alone.

Test mice were housed 5 per cage (2 per group per cage during acute toxicity screen) in suspended galvanized mesh cages. The animal room was kept under a 12 hour light/dark cycle, but environmental conditions were not specified. Food (PCD selected diet, BP nutrition) and water (glass bottles) were available ad libitum.

Five animals from each of the 4 groups were sacrificed by cervical dislocation 24, 48, or 72 hours after dosing and bone marrow smears prepared. Bone marrow smears were prepared from the excised femur using a small paint brush moistened with saline. The brush was pushed into the exposed bone marrow, and 3-4 slides were smeared from each femur. Slides were air dried and stained with Wright's stain using an "Ames Haematek" automatic staining machine. The number of micronuclei per 1000 PCEs was counted, recorded and assessed blind. The number of normocytes in the fields assessed for the first 200 PCEs were also recorded in order to determine any disruption of bone marrow function.

4. Statistical Analysis: Micronuclei frequency data were transformed using the Freeman-Tukey poisson transformation analyzing each replicate separately. An analysis of variance was applied followed by a student's t-test to compare treatment groups to control. Fisher's exact test was used to confirm the results.

RESULTS:

No adverse clinical signs were produced in any animals during the acute toxicity screening test. Suspensions at concentrations above 5000 mg/kg were too thick to be injected.

Table 1 presents the total number of micronuclei observed per 1000 PCEs and the average number of normocytes/200 PCEs in the test groups at each sacrifice period. No increase in number of micronulcei, as compared to the vehicle control, was produced. No inhibition of erythropoiesis was indicated by the resultant PCE:NCE ratio.

DISCUSSION:

The author stated that bone marrow function was not impaired based on a comparison of the NCE to PCE count, and also, that the test compound did not cause an increase in the incidence of micronuclei under the conditions of the test. Given the solubility limitation of the compound and the conditions of the test, this assessment can be supported. The positive

TABLE 1

	24 hrs		48 hrs		72 hrs	
	Micronuclei 1000 PCEs	NCEs 200 PCEs	Micronuclei 1000 PCEs	NCEs 200 PCEs	Micronuclei 1000 PCEs	NCEs 200 PCEs
Vehicle control	8	223	3	211	3	202
Positive control	58*	255	66*	205	6	231
R 152056, 2500 mg/kg	7	221	0	233	6	224
R 152056, 5000 mg/kg	4	229	4	205	6	202

*Statistically significant at $p < 0.01$.
**Statistically significant at $p < 0.0001$.

control produced a statistically significant increase in micronuclei, thereby demonstrating the capability of the assay to show a positive response for chromosomal breakage. However, the following deficiencies were noted:

1. Only male animals were tested, and therefore sex related effect could not be detected by this testing protocol.

2. A change was made in the protocol, as documented by Appendix IV pg 14, to administer one dose instead of 2 doses, 24 hours apart. The most commonly accepted methods [1,2] require the administration of 2 doses if sacrifices are to be made more than 30 hours after dosing. No justification was given for this change. The preliminary acute toxicity assay included only 2 doses, neither of which produced toxicity effects.

3. The environmental conditions e.g., temperature and humdity under which the animals were maintained was not reported.

4. The number of independent reviewers examining the slides was not specified. Usually, two technicians/cytogeneticists examine 500 PCE's each from 2 separate slides prepared for one animal to eliminate bias.

5. Criteria for a positive response were not defined by the authors. Criteria considered for evaluation as an indication of positive mutagenicity ($p = 0.05$), were a 2.5-fold increase in spontaneous incidence of PCEs with micronuclei and no significant change in the PCE:NCE ratio as compared to vehicle control.

   Although dosage levels for the micronucleus test should ideally be 80 percent of the 7-day LD_{50}, there was no acute toxicity produced in the range finding experiment, and higher dosages were not used because of solubility. However, these upper dose levels were high, especially when compared to the dose level used for the positive control, and as such, are acceptable.

---

[1] Schmid, W. 1977. The micronucleus test: Handbook of Mutagenicity Test Procedures In: Kilbey et al. Elsevier Sci. Pub. Co. Amsterdam/NY/Oxford. pp. 235-242.

[2] Heddle, J.A. and Salamone, M.F. 1981. The micronucleus assay. I. In vivo in short-term tests for chemical carcinogens eds. Stich, H.F. and San RHC. Springer Verlag, NY/Herdeberg/Berlin pp. 244-250.

40

CONCLUSIONS:

Under the limited conditions of this micronucleus assay, the test compound produced no toxicity, chromosomal breakage or erythropoietic effects in male $CBCF_1$ mice.

CLASSIFICATION: Unacceptable. The animals were dosed only once and only one sex was tested.

41

DATA EVALUATION RECORD

DNA-Damage

Herbold, B. THS 2212 Triazolylanine: Study of DNA-Damage using the E. coli PolA⁻ test. An unpublished report (No. 82738) prepared by Bayer AG for Mobay Chemical Corporation. Dated January 5, 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer AG Institut fuer Toxikologie, Wuppertal, Federal Republic of Germany.

TEST MATERIAL: The test material used in this study was THS 2212, triazolylalanine, which is a breakdown product of triazole fungicides. Its empirical formula is $C_5H_8N_4O_2$ and it is chemically, 2-amino-3-(1,2,4-triazol-1-yl)-propionic acid. Its purity was not specified.

MATERIALS AND METHODS

Controls: The (antibiotic) negative control was chloramphenicol (Boehringer-Mannheim, Fed. Repub. Germany), batch no. 2124391080, an inhibitor of prokaryotic cell protein synthesis and hence bacteriotoxic [but not genotoxic]. The positive control compound was methylmethanesulfonate (MMS) from batch 117189, an alkylating agent and ultimate mutagen. The solvent control was dimethylsulfoxide (DMSO). Plate concentrations for chloramphenicol (CM) and MMS were 30 µg and 10 µl, respectively.

Bacterial strains: Escherichia coli K12, strains p3478 (polymerase deficient, pol A⁻), and E. coli W3110 (pol A⁺) were used in the assay[1] stock cultures stored were at -80° C and as needed, the cultures were thawed and 0.1 ml transferred to 5 ml of complete medium at 37° C.

---

[1] Rosenkranz, H.S. and Poirier, L.A. 1986. J. Nat'l Cancer Int 62:873-892.

Preparation of S9 Mix: Adult male Sprague-Dawley rats (minimum of six) weighing 200 to 300 g received a single ip injection of Aroclor 1254 in a peanut oil vehicle at a dosage of 500 mg/kg 5 days prior to sacrifice. Ten ml portions of S9 fractions[2] were stored at -80° C and as needed, were thawed in ice water. The S9 fraction used in this study was employed approximately 4 1/2 months after its preparation, and it constituted 30 percent of the S9 Mix.

Preparation of Test Material: The test material was solubilized or suspended in DMSO and diluted progressively by 1:2 so that the final concentrations were 1000, 500, 250, 125, and 62.5 µg/plate.

Procedure for DNA-Damaging Assay: The test compound was delivered to a "small, round filter paper disc" which was then placed on a previously prepared soft agar nutrient plate that was seeded with the bacterial tester strain, either with or without S9-mix. Quadruplet plates were used per dose level and for each strain. The same number of plates was used for the solvent control.

Data Evaluation: A reproducible zone of inhibition which differed between the mutant (pol A^-) and wild type (pol A^+) by +2 mm was considered to be positive.

RESULTS:

The mean zone of inhibition for both the pol A^+ and pol A^- strains was 0 mm at all dose levels of THS 2212 and for the solvent control. The negative (antibiotic) control caused a zone of inhibition of 16.4 mm and 25.2 mm in the pol A^- and pol A^+ strains, respectively (difference of -8.8 mm) in the absence of S9, and inhibition zones of 16.3 mm (pol A^-) versus 27.6 mm (pol A^+) in presence of S9 (difference of -11.3). The positive control inhibited pol A^- by 59.5 mm versus 45.5 mm inhibition in pol A^+ (difference of +15.8 mm)[3].

DISCUSSION:

The authors concluded that the test material, THS 2212 (triazolalanine), gave no positive responses at doses up to 1000 µg/plate either with or without S9-activation. However, the positive control response demonstrated that the system had "great sensitivity". Hence DNA-damaging activity did not occur following exposure to THS 2212 under the conditions of the experiment.

---

[2] Ames, B.N., Durston, W.E., Yamasaki E., and Lee, F.D. 1973. Proc. Nat'l. Acad. Sci. (USA) 70:2281.

[3] A negative number indicates that the wild type bacteria is more affected and a positive number indicates greater inhibition of the repair deficient, pol A^- mutant.

Our assessment is that the authors have correctly interpreted their data, but there are some problems with the conduct of the assay. First, a positive control requiring metabolic activation, e.g., Benzo(a)pyrene, was not included. Hence, sensitivity to the genotoxic S9 metabolite of a suitable positive control was not demonstrated. Furthermore, it is unlikely that a diffusible metabolite would reach the tester cells under the assays conditions. Since the S-9 mix was included in the soft agar rather than incubated in liquid with the test material or both S9 and test material included in the disc, it is doubtful if sufficient enzyme was in contact with the test material on the disc to convert the test material to an electrophilic metabolite, i.e., it would have been more appropriate to incubate the test material with S9 before application to the paper disc or to directly add S9 and test material to the disc. Therefore, the S9 activated system was inadequate.

CONCLUSIONS:

Under the conditions of the assay, the test material did not induce DNA damage in the nonactivated system at doses from 62.5 to 1000 µg/plate. The assay performed with S9 activation was inadequate for producing a valid response.

CLASSIFICATION: Acceptable nonactivated assay.
Unacceptable S9 activated assay.

DATA EVALUATION RECORD

Mutagenicity (Cell Transformation)

Richard, M., Allen J.A., Williams, A., and Ransome, S.J. Cell transformation test for potential carcinogenicity of R152056. An unpublished report (CTL/C/1085) prepared by Huntingdon Research Centre for Imperial Chemical Industries, Ltd. Dated May 15, 1981.

ACCESSION NUMBER: 252132.

LABORATORY: Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, U.K.

TEST MATERIAL: The test compound was only identified as R152056 or aY012010/001/001 (Y01210/001/003 on container). It was a white crystalline solid with no impurities identified and its batch number was P2 B2Y27-153.

MATERIALS AND METHODS:

Cell Culture: The cells used in this study were of the strain BHK 21 13 [accepted abbreviation for baby hamster kidney, but not stated in this report.]; source was unspecified.

Controls: The solvent control as "MEMS" and the positive controls were 4-nitroquinoline-N-oxide (0.1, 0.05, 0.025, 0.125, and 0.00625 µg/ml) and p-dimethylaminoazobenzene (DMAB 400, 200, 100, 50, and 25 µg/ml).

Preparation of The Test Material: The test material was solubilized in "MEMS" and used at final dosages of 1000, 100, 10, 1 and 0.1 µg/ml in the preliminary toxicity assay. In the transformation assay, dosages of 500, 1000, 2000, 4000, and 8000 µg/ml were used without S9; mix dosages of 1000, 2000, 4000, 8000, and 16000 µg/ml were used with S9 mix.[1]

[1] Ames, B.N., McCann, J. and Yamasaki, E. 1975. Mutation Res. 31:317.

DISCUSSION:

The authors concluded that their positive control chemicals gave proof of the assays' sensitivity to detect a positive response and the test material, "R152056 has produced a positive result in this in vitro test." They considered the test material to be a potential carcinogen, based on additional criteria[4] applied to this data and on a clear dose response with cell transformation of BHK 21 C13 cells.

Our assessment is that cell transformation was demonstrated in this assay, and that a carcinogenic potential exists for the test material. Although the authors used the term "malignant transformation in vitro" in their introduction, the ability to grow in soft agar is not evidence for malignant manifestation of transformed cells without further definitions and demonstrations. This terminology appears to be inappropriate, but does not alter the significance of the results.

CONCLUSIONS:

The test material, R152056 induced cell transformation at doses ranging from 500 to 8000 µg/ml in the presence of S9 mix with a definite compound dose response. In the absence of S9, the test material was also positive within this range but a definite dose response was not apparent. Therefore, a positive response was obtained in this in vitro assay.

CLASSIFICATION: Acceptable.

---

[4] Styles, J.A. 1980. Personal Communication [therefore not defined for the reviewer].

DATA EVALUATION RECORD

Mutagenicity (Micronucleus)

Herbold, B. THS 2212 Triazolylalanine: Micronucleus test for mutagenic effect on mice. An unpublished Report (Bayer No. 11054; Mobay Agchem No. 84005) prepared by Bayer AG for Mobay Chemical Corporation. Dated August 9, 1982.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer AG Institut fuer Toxikologie, Wuppertal, Federal Republic of Germany.

TEST MATERIAL: The test material was identified as THS 2212, batch no. E238099 (triazolylalanine), which is a degradation product of Bayleton^{R}. Chemically, it is described as 2-amino-3-(1,2,4-triazolyl-1-yl)-propanoic acid, and its empirical formula is $C_5H_8N_4O_2$. The purity of the test material was not stated in this report; however, in another report (report No. 11491) the same material with the same batch number was said to be "analytically pure."

METHODS:

Animal Test Species and Husbandry: Male and female Bor: NMRI (SPF Han) mice, supplied by F. Winkelman, Borchen, Federal Republic of Germany were used in the study. They were 8 to 12 weeks old and weighed 25 to 35 g when the assay was initiated. Animals were separated by sex and treatment group, caged in type II Makrolon cages (5 per cage), and identified by cage number and picric acid individual markings.

Animals were held at 23° C, average relative humidity of 68 to 79 percent with alternating 12 hour light/dark cycles. Food (Altromin 1324, Altromin GmbH, Lage, Federal Republic of Germany) and tapwater were supplied ad libitum.

Preparation of Test Material: The test material, THS 2212, was emulsified in 0.5 percent Cremophor and administered by oral intubation (p.o.) A single dose level of 8000 mg/kg was chosen because preliminary testing showed that this dosage could be "tolerated without symptoms".

Controls: The positive control, Endoxan[1], was dissolved in water and 10 ml/kg were administered p.o. at a dosage of 87 mg/kg. The vehicle (negative) control was also administered p.o. and was given at a volume of 20 ml/kg.

Bone Marrow and Slide Preparation: The method for preparing bone marrow slides was that of Schmid[2].

Evaluation of Slides: For each animal, 1000 polychromatic erythrocytes (PCEs) were counted, scoring the micronuclei (MN) and calculating their frequency of occurrence. Since this first evaluation did not yield conclusive results, an additional 1000 PCEs in the negative control and THS 2212 - treatment groups were evaluated. In addition, the PCE to normochronic erythrocyte (NCE) ratio was determined to identify animals with pathology leading to bone marrow depression that was unrelated to compound administration and to identify general activity of the test compound as it relates to erythropoiesis. If an individual animal had an NCE:PCE ratio of more than 3:1 without seeing or anticipating this response among other dosed animals, a pathological process unrelated to the test compound was recognized and these animals were excluded from further evaluation. If, however, the PCE:NCE ratio in dosed groups was substantially below the concurrent negative control group, the presence of a generalized erythropoietic effect could be inferred.

Statistics: The Wilcoxon rank sum test was applied only to the positive control and to the highest values obtained in THS2212-treatment groups. If the probability of error in the difference was less than 5 percent then the result was considered to be statistically significant.

RESULTS:

Animals Effects: At a p.o. dosage of 8,000 mg/kg THS 2212 caused no toxic symptoms in the mice. Their behavior, feeding habits, external appearance and motor activity did not show compound-related effects, and there were no test compound-related mortalities reported.

Slide Evaluation: In the negative control mice at 24 hr, the average ratio of PCE to NCE was 1.6 to 1 (2000/1246); MN per 1000 NCE was 1.2 and MN per 1000 PCE was 1.4. In the mice dosed at 8000 mg/kg at 24 hr, the average ratio of PCE to NCE was 1.16 to 1 (2000/1724); MN per 1000 NCE was 1.8 and MN per 1000 PCEs was 3.1. At 48 hr the mice dosed at 8000 mg/kg averaged 1.16 for PCE/NCE (2000:1724); MN per 1000 NCE was 1.2 and MN per 1000 PCE was 1.6. Seventy-two hours after treatment with 8,000 mg/kg of

---

[1] Cyclophosphamide, the a.i., was calculated to be a 60 mg/kg dose.

[2] Schmid, W. 1975. Mutation Res. 31:9-15; ibid 1975. Mitteilung III. pp. 53-61.

the test material, the average ratio of PCE to NCE was 1.14 to 1 (2000/1760). Twenty four hours post-treatment with 87 mg Endoxan/kg per os gave a PCE/NCE of 0.93 (1000/1079); 1.8 MN per 1000 NCE and 23.4 MN per 1000 PCE. By the Wilcoxin test, the number of NCEs/1000 PCEs and MN/ 1000 PCEs were statistically different from the negative control ($p < 0.01$) in the Endoxan treated mice; only the number of MN/1000 PCE at 24 hr after dosing with 8000 mg THS 2212/kg were elevated at a statistically significant level ($p < 0.05$).

The authors submitted a supplemental report in which mice were treated per os with 8,000 mg THS 2212 per kg because "the high value of one individual female resulted in a micronucleus frequency of 3.1/1000 for the THS 2212 group that had been sacrificed and prepared 24 hours after treatment." Hence, they increased the number of PCEs to be evaluated to 2,000 and the evaluation was performed with two different series using different slides. The summary of these results follows:

In the negative control animals MN per 1000 NCE and per 1000 PCE averaged 1.15 and 1.4, respectively, for 2 series; THS 2212-treated animals at 24 hr averaged 1.85 MN per 1000 NCE and 2.95 MN per 1000 PCE; THS 2212-treated animals at 48 hr averaged 1.15 MN per 1000 NCE and 1.5 MN per 1000 PCE; THS 2212-treated animals at 72 hr averaged 1.0 MN per 1000 NCE and 1.5 MN per 1000 PCE; THS 2212-treated animals at 72 hr averaged 1.0 MN per 1000 NCE and 1.3 MN per 1000 PCE. No positive control data, e.g., Endoxan-treated animals, were reported for this supplemental experiment. The authors stated this test compound did not produce significant differences any at sampling times using the Wilcoxon rank sum test.

DISCUSSION:

The authors concluded that there was "no indication of mutagenic effect of THS 2212 at a dose level of 8000 mg/kg per os in the micronucleus test on mice, i.e., in a somatic mutagenicity test in vivo" after the first assay. However, treatment at this dosage gave 3.1 MN/per 1000 PCE at the 24 hour sampling period (significant difference at $p < 0.05$ in the non-parametric rank sum test of Wilcoxon). In the supplementary assay, the THS 2212 treated groups averaged 3.1 and 2.8 MN per 1000 PCEs (2 series). This value was approximately twice that of the negative control group or other treatment groups sampled at different intervals, however, the standard deviation (1s) was equal or approximately equal to the mean value and the authors concluded that these results were of no biological relevance.

Our assessment is that micronuclei induction by THS 2212 did not exceed the negative control at 48 or 72 hr, if the 24 hour negative control value was used for comparison. However, negative control values were not reported at the 48 and 72 hours sampling periods in either the first assay or supplementary assays. Also, there were 3/5 males and 1/5 females with MN/1000 PCE values at 24 hours ranging from 3.5 to 7.5 in the first assay

where a statistically significant ($p < 0.05$) mean value was obtained at 24 hr. The positive control with Endoxan indicated that the assay was capable of a positive response.

In the supplemental assays, there were no negative control groups for the MN assay at 48 and 72 hr and no positive control group was included. The results of 24 hour sampling after treatment with 8000 mg THS 2212/kg appeared to be higher than the results presented for the negative control group and the other THS 2212 treatment groups assayed at 48 and 72 hr, although the differences were not found to be statistically significant ($p < 0.05$). We therefore assess that the supplemental assays were unacceptable because there was no positive control group and the required negative control groups at 48 and 72 hr were not included.

CONCLUSIONS:

Because the 24-hour-dosed mice had a statistically significant ($p < 0.05$) increase in the number of micronuclei per 1000 PCE when compared to the 24-hour-negative-control group in the first bioassay, a weak positive response was indicated for 8000 mg/kg THS 2212 (Triazolylaline) induction of micronuclei in polychromatic erythrocytes in NMRI (Han) male and female mice. However, the lack of negative controls at 48 and 72 hours precluded evaluation at these sampling times. Also the supplementary assays lacked a positive control for each sampling period and negative controls at 48 and 72 hr samplings, and hence cannot be evaluated.

CLASSIFICATION: Unacceptable. Critical data on negative and positive control groups were absent from the studies and the purity of the test compound was not stated.

DATA EVALUATION RECORD

Mutagenicity (Bacterial Point Mutations).

Herbold, B. THS 2212 triazoylalanine: Salmonella/Microsome test for determination of point mutations. Unpublished Report No. 11388 prepared by Bayer AG Institut Fuer Toxikologie for Mobay Chemical Corporation. Dated January 5, 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer AG Institut Fuer Toxikologie, Wuppertal, Federal Republic of Germany.

TEST MATERIAL: The test material was identified as THS 2212, batch E238099, a degradation product of triazole fungicides. It was defined chemically as 2-amino-3-(1,2,4-triazol-1-yl)-propionic acid with an empirical formula of $C_5H_8N_4O_2$.

METHODS:

Bacterial Strains: Five histidine-auxotrophic mutants of Salmonella typhimurium LT2 were used in this study. They included the Ames[1] strains TA1535, TA100, TA1537, TA1538, and T98.

Preparation of Test Material: The test material was solubilized in dimethylsulfoxide (DMSO) so that final concentrations delivered to the assay plates were 20, 100, 500, 2,500, and 12,500 µg per plate. The lower four concentrations were achieved by progressive 1:5 dilutions with the solvent.

Control: For the S9 activated assays, three promutagens were used for positive controls. These included Endoxan (Asta), batch 0378 (a.i. = cyclophosphamide); trypaflavin (Roth), batch 0282995 (a.i. in Panflavin and Rivanol); and 2-aminoanthracene (EGA-Chemie), batch 10630. Dimethyl-

---

[1] Ames, B.N. et al. 1975. Mutation Res. 31:347-364.

sulfoxide (DMSO) was the solvent for the test material, trypaflavin (Tflavin) and 2-aminoanthracene (2-AA); and water was the solvent for Exdoxan. There was no positive control which was mutagenic in the nonactivation system.

Preparation of S9 Mix: A single ip injection of 500 mg Aroclor 1254/kg (peanut oil vehicle) was used to induce mixed function oxidase activity in male Sprague-Dawley rats weighing 200 to 300 g. The livers of at least 6 rats were pooled and prepared according to Ames et al.[2] The S9 fractions were stored in 10 ml aliquots at -80° C and thawed in ice water as they were required.

Mutagenesis Assay: The assays were performed by plate incorporation procedure of Ames et al[2]. For mutation assessment, 4 agar plates per dose level of test material per strain were used for assays performed with and without S9 activation. The same number of plates were used for the negative (solvent control) and for the positive controls employed for each strain. The concentrations of each positive control chemical and the tester strain(s) for which it was used follows: 145 µg Endoxan[3]/plate (TA1535), 290 µg Endoxan[3]/plate (TA100); 50 µg Tflavin/plate (TA1537, TA1538, and TA98); and 3 µg 2-AAF/plate (TA1535, TA1537, TA1538, TA98, and TA100).

The viable counts on nutrient agar at 10^{-6} dilution was assessed for each 17 hour (O/N) culture used for the assay. The plates were scored after overnight incubation and revertants were scored after 48 hour incubation at 37° C.

Evaluation of Data: The authors stated that a dose-related increase in the revertant plate count with at least twice the number of revertants per plate as the solvent control in at least one mutant strain would be considered a positive result. No other evaluation criteria or statistical treatment was described.

RESULTS:

Table 1 is a summary of the results for all treatments in all strains in the nonactivated S. typhimurium assay.

---

[2] Ames, B.N., Lee, F.D., and Durston W.C. 1973. Proc. Nat. Acad. Sci. (USA) 70:782-786; Ames B.N, McCann, J., and Yamasaki, E. 1975. Mutat. Res. 31:347-364.

[3] Endoxan contains 69 percent of its a.i. (cyclophosphamide).

52

DISCUSSION:

Under the conditions of the study, the authors concluded that doses of THS 2212 from 20 to 12,500 µg/plate did not induce reverse mutations in the Salmonella/microsome test. They also concluded that the positive controls demonstrated that a positive result could be obtained and that the assay had adequate (or the expected) sensitivity.

Our assessment is that the authors have correctly interpreted their data from the S9 activated assays. However, there was no positive control compound for the nonactivated assay. Hence, the nonactivated assays cannot be properly evaluated.

CONCLUSIONS:

Under the conditions of the study, THS 2212 did not induce reverse mutations in the S9 activated S. typhimurium assay (Ames). No conclusions can be drawn from the nonactivated system because a direct acting positive control compound was not used.

CLASSIFICATION: Acceptable S9 activated assay
Unacceptable nonactivated assay

TABLE 1. His⁻ Revertants in Nonactivated S. typhimurium Mutagenicity Assay

Compound	Dose (µg/plate)	Average No. Revertants/Plate — STRAIN TA1535 -S9	TA1537 -S9	TA1538 -S9	TA98 -S9	TA100 -S9
DMSO	-	5,7 (6)[a]	3,4 (4)	2,2 (2)	6,70 (38)	23,48 (36)
THS 20	20	6,8 (7)	3,3 (3)	2,3 (3)	4,68 (36)	18,35 (27)
100	100	8,6 (7)	2,3 (3)	3,3 (3)	9,63 (36)	23,35 (29)
500	500	7,3 (5)	3,3 (3)	2,2 (2)	6,53 (30)	23,41 (32)
2,500	2,500	4,6 (5)	4,4 (4)	2,3 (3)	5,69 (37)	24,50 (37)
12,500	12,500	6,9 (8)	3,0 (2)	2,2 (2)	10,63 (37)	24,67 (46)
Endoxin	145	6,6 (6)				40,56 (48)
2AA	3	6,11 (9)	4,8 (6)	5,4 (5)	b,67 (67)	28,65 (47)
TFlavin	50	-	17,85 (50)	5,8 (7)	27,77 (52)	
		+S9	+S9	+S9	+S9	+S9
DMSO	-	8,11 (10)	4,8 (6)	16,14 (15)	12,55 (34)	51,105 (78)
THS 20	20	6,5 (6)	3,7 (5)	11,12 (12)	13,63 (38)	44,77 (61)
100	100	6,7 (7)	5,9 (7)	14,9 (12)	12,67 (40)	40,96 (68)
500	500	7,9 (8)	5,8 (7)	13,14 (14)	20,66 (43)	55,103 (79)
2,500	2,500	7,8 (8)	4,7 (6)	14,11 (13)	7,65 (36)	51,112 (82)
12,500	12,500	7,10 (9)	4,9 (7)	12,10 (11)	12,74 (43)	1,133 (67)
Endoxin	145	42,51 (47)				123,223 (173)
2AA	3	156,129 (143)	184,254 (219)	472,450 (461)	886,687 (787)	44,742 (393)
TFlavin	50	97,449 (273)	844,884 (864)	463,678 (571)		

[a] ( ) average of the results of the two assays performed.

[b] No data reported.

DATA EVALUATION RECORD

Metabolism/Pharmacokinetic Study in Rats.

Weber, H. and Suwelack, D. Preliminary biokinetic study on rats. Unpublished report No. 11583 (F) prepared by Bayer Pharmaceutical Division, Wuppertal, Germany. Dated February 24, 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Bayer Pharmaceutical Division, Bayer AG., Wuppertal, Germany.

TEST MATERIAL: [^{14}C]Triazolylalanine, D,L-alpha-amino-1H, 2,4-[3(5)-^{14}C]Triazole-propanoic acid. The test material was a racemic mixture (D:L ratio about 1:1) with a radiochemical purity of 99 percent.

PROTOCOL:

1. Sixteen male Sprague-Dawley rats (Mura SPRA, SPF 68 Han.) bred by "Mus Rattus," Brunnthal, Federal Republic of Germany were used. The rats were housed individually in metabolism cages and each received 15 g Altromin 1324 per day (Altrogee, Lage/Lippe, Germany) and water ad libitum. The animals received half the feed ration on the day prior to dosing.

2. The test material was dissolved in saline solution (concentration not specified) and administered by oral intubation or by intravenous injection at a dose volume of 10 ml/kg. Ten rats in the metabolism study received a dose of 5 mg/kg, whereas, 6 animals subjected to whole-body autoradiography received 10 mg/kg.

   In the metabolism study, one group of 5 animals was dosed orally and the other group dosed intravenously. The animals were held for 48 hours, and blood samples collected at regular intervals. The animals were then sacrificed by carbon dioxide asphyxiation. Tissue samples from the kidneys, liver, muscle, skin, brain, plasma, erythrocytes, testes, renal fat and body were collected. Tissue samples were freeze-dried and pulverized prior to oxidation and radioassay. Feces were prepared in saturation-thick layer and radioassayed with end window proportional counting tubes. Urine and plasma were radioassayed directly.

55

In the autoradiographic study, one animal was dosed intravenously and then sacrificed after 5 minutes; 4 animals were dosed orally and one animal sacrificed after 4, 8, 24, and 48 hours; one control animal was dosed with non-radioactive material and sacrificed after 4 hours. Whole-body autoradiography was conducted according to the Patzschke method. [Muench. Med. Wschr. 110:2043-2053, 1968].

3. The results were based on the total radioactivity measured in each sample and expressed as the relative (P) or equivalent (C) concentrations, where:

$$P = \frac{\text{activity measured/grams of plasma or tissue}}{\text{activity administered/grams of body weight}}$$

$C = P \times \text{dose}$ ($\mu g/g$ or $\mu g/ml$)

The half-life analyses and calculation of pharmacokinetic parameters were performed by the PHANAL program package with the DEC-20 computer.

RESULTS:

Absorption: During the 48-hour observation period a total of 94.5 percent of the radioactivity administered orally was excreted in the urine (Table 1). The absorption index was reported to be 95 percent of the administered dose. Absorption of radioactivity proceeded at a half-life of 8.4 minutes.

Kinetics of Radioactivity in Plasma: Following oral administration, the radioactivity increased in the plasma and a maximum was attained (P = 0.67) within approximately 40 minutes (Figure 1). The radioactivity declined during the 48 hour period in a biphasic manner with half-lives of approximately 3 and 8 hours.

Following intravenous injection the relative concentration in the plasma at five minutes was reported to have "decreased to P = 1.46 i.e., a value slightly above the theoretical equidistribution concentration of P = 1.0." The slope of the curve during the 48 hour period following dosing was triphasic with half-lives of 15 minutes, 4.4 hours and 12.4 hours.

The initial apparent volume of radioactivity distribution was calculated to be 63 percent of the body volume.

Distribution of Radioactivity in Tissues: The distribution of radioactivity, expressed as equivalent concentration, in the tissues and organs 48 hours after oral or intravenous dosing is presented in Table 2. The highest concentrations of radioactivity were found in the kidneys and liver.

Whole-Body Autoradiography: Whole-body sections of the rat 5 minutes following intravenous dosing at 10 mg/kg indicated that radioactivity was distributed in all tissues and organs of the body except the compacta of

the bone. Highest concentrations were detected in the cortical and medullary substances of the kidneys, the pancreas, the wall of the small intestine and the pituitary. The presence of high concentrations in the small intestine and lower concentrations in the lumen were associated with extrabiliary elimination of radioactivity. Four and eight hours after oral dosing, high concentrations of radioactivity were found in the medullary substance of the kidneys, the pancreas, the prostate and contents of the urinary bladder and the urinary tubes. Lower concentrations were detected in the liver, kidney papilla, epiphysis of the bones, the cartilage rings of the trachea, the eye, and the wall of the large intestine. The radioactivity was drastically reduced forty-eight hours after oral dosing. Only the contents of the large intestine, the intervertebral disks, the vitreous body of the eye, and the medullary substance of the kidneys had detectable radioactivity.

Excretion of Radioactivity in Urine and Feces: Excretion of radioactivity occurred mainly in the urine with 92.4 - 94.5 percent being excreted within 48 hours (Table 1). The feces contained only 2.2 - 3.6 percent of the radioactivity administered.

DISCUSSION:

The results from this study indicate rapid absorption and excretion of the radiolabelled material. The authors reported that the data also indicated that the test material is metabolized to a minimal extent. However, in the absence of chemical identification of the radioactive material detected in the biological samples from the rat, a definitive conclusion cannot be made on the nature of the radiolabel excreted.

The results presented for different parameters in the report (e.g., excretion, tissue distribution) were expressed in different units, and an accurate intercomparison of the data could not be made. Moreover, a P value of 1.46 was reported 5 minutes after intravenous injection, suggesting technical errors in the methodology used. Only the males were used in this study.

CONCLUSIONS:

The available data indicate rapid absorption and excretion of the radiolabel following administration of [^{14}C] triazolylalanine to male Sprague-Dawley rats. However, none of the metabolities were identified.

CORE CLASSIFICATION: Acceptable.

57

TABLE 1. Distribution of Radiolabel Following Administration of [^{14}C] Triazolylalanine to Rats at 5 mg/kg

Biological Sample	Percent of Radiolabel Administered	
	i.v.	p.o.
Urine	92.4	94.5
Feces	2.1	3.5
Body excluding GI[a]	0.18	0.19
Gastrointestinal Tract[a]	0.051	0.069
Total Recovery	94.7	98.3

[a] Gastrointestinal tract, see also Table 2.

TABLE 2. Distribution of Radiolabel in Organs Following Administration of [^{14}C] Triazolylalanine to Rats at 5 mg/kg

Biological Sample	Equivalent Concentration (ng/g)	
	i.v.	p.o.
Kidneys	33.0	31.0
Liver	16.0	20.0
Body excluding GI	10.0	11.0
Muscle	7.0	8.5
Skin	8.0	7.5
Brain	5.0	7.5
Plasma	5.5	7.0
Erythrocytes	5.0	6.5
Testes	6.5	5.5
Renal fat	ca 3.0	ca 4.5

Page 59 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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

____ The document is not responsive to the request.

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

DATA EVALUATION RECORD 004766

Metabolism Study in the Rat

Hamboeck, H. Distribution, degradation, and excretion of D,L,-2-amino-3-(1-H-1,2,4-Triazol-1-yl)-propanoic acid (D,L,-triazolyl- alanine) in the rat. Unpublished project report number 1/83 (Study No. CGA 131013, 82/91-82/110) prepared by Ciba-Geigy Limited, Basle, Switzerland. Dated 2 March, 1983.

ACCESSION NUMBER: 252132.

LABORATORY: Department of R and D Plant Protection, Agricultural Division Ciba-Geigy Limited, Basle, Switzerland.

TEST MATERIAL: [^{14}C]D-L-triazolylalanine, labeled with a specific activity of 80.3 µCi/mg in the triazole ring was used. The radiochemical purity was determined by TLC to exceed 99 percent. Unlabeled triazolyl-alanine with greater than 95 percent purity was also used.

PROTOCOL:

1. Ten male and 10 female rats [strain:Tif:RAI f (SPF)] obtained from the Ciba-Geigy animal farm (Stein, Switzerland) were used in the study. The initial body weights of the animals ranged from 164 to 204 g.

2. The test material was administered at two dose levels. ^{14}C-triazolylalanine was dissolved in tapwater at a concentration of 0.101 mg/ml with a specific activity of 80.3 µCi/mg. Both labeled and unlabeled triazolylalanine were dissolved in tapwater to yield a concentration of 9.71 mg/ml with a specific activity of 26.77 µCi/mg. Groups of four animals/sex each were administered 1.0 ml of the dosing solution by oral intubation after a 24 hour fast period. The mean ± SD dosages of triazolylalanine administered to the animals were 0.50 ± 0.01 mg/kg and 0.53 ± 0.01 mg/kg for the low-dose group males and females, respectively and 55.95 ± 1.95 mg/kg and 56.83 ± 1.75 mg/kg for the high dose males and females, respectively. One animal/sex was assigned to each of two control groups. One of the two controls/sex was kept in an open metabolism cage whereas all other animals were kept in closed cages. The initial individual body weights indicate that the selection of animals to treatment group was based on body weights. The five lightest males and females were

60

assigned to the high-dose group and a control group. The five heaviest males and females were assigned to the low dose group and a control group.

3. The animals were placed in Delmar-Roth metabolism chambers and housed in a temperature-(23° ± 3° C) and relative humidity-controlled (55 ± 5 percent) room with a 12 hour light/dark cycle. Nafag No. 890 feed and water were available ad libitum.

4. Urine, feces, and expired volatiles were collected at 24 hour intervals over a seven-day collection period. Individual body weights were determined prior to dosing and at the time of sacrifice. Acidic volatiles were trapped in a 1/2 (v/v) mixture of ethanol amine and ethylene glycol monomethyl ether. The basic volatiles were trapped in a 1/5 (v/v) mixture of 2N sulfuric acid/ethylene glycol monomethyl ether. The feces were freeze-dried, homogenized, and combusted. The report did not state how the urine was prepared for analysis.

   At the end of the seven day collection period, the animals were killed by cervical dislocation and samples of the liver, fat, kidneys, muscles, blood, spleen, heart, lungs, pancreas, small intestine, stomach, testes, ovaries, brain, and remaining carcass were prepared for analysis. The carcass was homogenized in a food blender while the organs were manually minced. All tissues were combusted.

   The samples were prepared in triplicate using the appropriate scintillation cocktails. A Packard Tri-Carb Scintillation Counter was used to measure the radioactivity. The measurements were corrected for quenching.

   The 0-24 hour urine samples were analysed by TLC, high voltage electrophoresis, and HPLC for triazolylalanine and its metabolites.

RESULTS:

^{14}C-Triazolylalanine Excretion: Actual data for excretion of ^{14}C-triazolylalanine in dpm were not presented. The data were presented as a percentage of the total dose. A summary of the excretion data for the 0-24 hour period and the 0-7 day period is presented in Table 1.

The results of tissue and excreta radioassays indicated that greater than 90 percent of the triazolylalanine or its metabolites were excreted in the urine with approximately 4 to 5 percent excreted in the feces. Less than 1 percent of the test material or its metabolites were excreted by respiration and flatulation. With the exception of one high dose female, almost all of the excretion occurred within the first 24 hours after dosing. This high-dose female was found to have excreted 5.21 percent of the ^{14}C-triazolylalanine in the feces between 24 and 48 hours after dosing compared to 0.66 percent during the first 24 hours.

Detectable levels of radioactivity were not found in the tissues of the animals receiving the low dose. Among the high-dose animals radioactivity equivalent to 0.002 to 0.02 ppm triazolylalanine was found in the liver, kidney, muscle, blood, stomach, small intestine, and carcass. The liver and kidneys had the highest concentrations.

The analysis of the 0-24 hour urine samples from the low and high dose animals detected two major fractions. Among the low dose males and females 72.0 and 80.3 percent, respectively, of the recovered radioactivity was determined to be ^{14}C-triazolylalanine. ^{14}C-N-acetyl-triazolylalanine was determined to contribute 19.4 and 12.7 percent of the recovered radioactivity in the male and female urines, respectively. Among the high-dose animals, ^{14}C-triazolylalanine accounted for 83.4 and 86.4 percent of the recovered radiation in the 0-24 hour urine of the males and females, respectively. ^{14}C-N-acetyl-triazolylalanine accounted for 10.6 and 7.7 percent of the recovered radioactivity in the 0-24 hour urine of the males and females, respectively. The remaining radiation was attributed to unidentified background sources.

DISCUSSION:

The data indicated that the test material was primarily excreted in the urine with the feces being a secondary route. The majority of the labelled material was excreted within 24 hours. The analysis of the urine metabolites indicated that N-acetylation of the triazolylalanine occurred and that the percentage of N-acetylation of the test material was decreased at the higher dose level.

The excretory samples were collected at 24-hour intervals during the seven-day study. Smaller time intervals should have been used during the first 24 hours after dosing, especially because most of the excretion occurred during this time period. Four or six hour intervals would have provided more information concerning when excretion of triazolylalanine began and when it began to decrease.

The USEPA Pesticide Assessment Guidelines (November, 1982) state that "the upper dose should produce toxic or pharmacologic signs." No indications of toxicity were detected at the highest dose level which suggests that higher doses could have been tolerated by the rats. Indeed, Bayer Report No. 11229, Triazolylalanine-Acute toxicity studies, reported that the oral administration of 2000 mg/kg triazolylalanine produced no signs of acute overt toxicity. Changes in the ratio of triazoylylalanine to N-acetyl-triazolylalanine between the low and high doses and the presence of labeled material in the tissues of only the high dose animals indicated that the metabolic fate of triazolylalanine changed with increasing doses. The decreased percentage of N-acetylation and the beginning of a body burden at the 48.55 mg/kg dose level suggest that dosages in the range of 2000 mg/kg may yield a different metabolic fate for triazolylalanine than that observed in this study.

Although the study design is deficient in that the highest dose level did not produce signs of toxicity, the study did meet the stated main purpose of metabolism studies "to produce data which fortify the understanding of the safety of the chemical in consideration of its intended uses and anticipated human exposure."

CONCLUSIONS:

Under the conditions of this study ^{14}C-triazolylalanine, orally administered at a dose level of approximately 50 mg/kg is almost entirely excreted by the rat within 24 hours. The major excretory route is the urine with the feces being a secondary route. The N-acetyl metabolite and unaltered triazolylalanine were the major metabolites detected in the urine excreted during the first 24 hours.

CORE CLASSIFICATION: Minimum data.

TABLE 1. Distribution and Excretion of ^{14}C-Triazolylalanine and Radiolabeled Metabolites [a]

	Sex and Dose Level (mg/ml)			
	0.101-Male	0.101-Female	9.71-Male	9.71-Female
Feces				
0-24 hours	3.64 ± 4.61	4.90 ± 3.76	2.64 ± 3.16	3.02 ± 2.60
0-7 days	4.26 ± 4.59	6.70 ± 4.02	3.28 ± 3.36	5.47 ± 3.16
Urine				
0-24 hours	96.06 ± 7.28	92.01 ± 3.96	97.67 ± 3.08	98.96 ± 3.34
0-7 days	98.25 ± 7.00	94.31 ± 3.81	104.62 ± 4.30	100.94 ± 3.54
Volatiles				
0-24 hours	0.01 ± 0.00	0.01 ± 0.005	0.01 ± 0.00	0.01 ± 0.00
0-7 days	0.01 ± 0.00	0.01 ± 0.005	0.01 ± 0.00	0.01 ± 0.00
Total excretion	102.52 ± 2.55	101.02 ± 0.63	107.92 ± 1.26	106.43 ± 0.40
Tissue residues	0.00	0.00	0.01 ± 0.01	0.00 ± 0.00[b]
Cage wash	0.06 ± 0.05	0.63 ± 0.76	0.25 ± 0.16	0.19 ± 0.16
Total recovery	102.58 ± 2.58	101.65 ± 1.33	108.17 ± 1.14	106.62 ± 0.26

[a]Presented as a percentage of the total dose ± SD.
[b]Present in detectable quantities, but less than 0.01 percent of the total dose.

DATE EVALUATION RECORD

The Metabolism of D,L-Triazolylalanine in the Rat

Hamboek, H. The metabolism of D,L,-2- amino -3 -(1H-1,2-4-triazol-1-yl)- propanoic acid (D,L-triazolylanine) in the rat. Unpublished project report number 11/83 (Study No. CGA 131013, 82/91-82/110) prepared by Ciba-Geigy Limited, Basle, Switzerland. Dated October 20, 1983.

G.G.

ACCESSION NUMBER: 252132

LABORATORY: Department of R and D, Plant Protection, Agricultural Division, Ciba-Geigy Limited, Basle, Switzeland.

TEST MATERIAL: [^{14}C]D-L-triazolylalanine, labeled with a specific activity of 80.3 uCi/mg in the triazole ring was used. The radichemical purity was determined by TLC to exceed 99 percent. Unlabeled triazolylalanine with greater than 95 percent purity was also used.

PROTOCOL:

For detailed protocol, see Report No. 1/83. This study is a continuation of the previous metabolism study. The present study is concerned with the elucidation of the major metabolic pathways of D,L-triazolylalanine in rats.

The excreta of rats obtained in the material balance study (1/83) after a single oral dose of 50 mg/kg ^{14}C-D,L-triazolylalanine were used for the isolation of the metabolites.

Isolation of the metabolites was performed on the pooled urine. The urine samples were lyphilized and kept at room temperature.

Radioactivity in the urine was measured directly using Packard Tri-Carb Scintillation spectrometer. Quenching was corrected using the Special Index External Standard or by channel ratio method. Radioactivity in the feces was determined by combustion in a Packard Tri-Carb sample oxidizer and then counting in Packard Tri-Carb Scintillation Counter.

65

Thin layer chromatography was used to separate metabolites. Precoated silica gel 60 F254 of 0.25 mm thickness was used. The following solvent systems were used: methylethylketone/ methanol/water/diethylamine (40:40:16:4), methylethy/ketone/ methanol/water (4:4:2), chloroform/methanol/ammonia 25% (50:50: 5), methanol/pyridine/formicacid/water (70% 9.6:0.4:20), and chloroform/methanol/ammonia 25% (50:50:1). Ninhydrine was used for the detection of aminoacid structure, and iodine vapour was used for all compounds. Authentic reference standards were used for co-chromatography. TLC plates were scanned for radioactivity using a thin layer scanner, or the pattern of radioactivity was visualized with a spark chamber AGA 2105 radiochromatogram camera. Quantification of radioactivity on TLC was performed by scraping off the radioactivity zones in a scintillation mixture and counted.

Column chromatography was used also in the separation of metabolites. Different types of stationary phases were used; Ambolite GG 120 I, Biorad AG 11 A8, or Sephadex LH20.

High pressure liquid chromatography was also used in the separation and detection of metabolites. The eluents were monitered continuously for radioactivity. The ^{14}C- signal, the UV signal and the gradient profile were synchroneously registered by three channel W + W recorder.

High voltage electrohoresis was generally run for 30 minutes on Camag high voltage electrophoresis apparatus using chromatography paper 2043 b as the immobile phase.

The identity of metabolites was comfirmed using NMR and mass spectrometric methods.

RESULTS:

Urinary metbolites: about 85 - 103% of the total radioactivity was recovered into the pooled urine of male and female rats in the first 24 hours. A major urinary metabolite that accounted for 69-86% of the dose was D, L-2-amino-3-(1H-1,2,4-triazol-1-ul) propanoic acid (u-1). The second major urinary metabolite was N-acetyl-D,L-triazolylalanine (u-2) and accounted for 8-19%. In addition 2-3 minor metabolite fractions were found by TLC in various solvent, but none of which exceeded 3% of the dose (Table 1).

Fecal metabolites: the total radioactivity in the feces from male and female rats in the first 24 hours, accounted for approximately 3% of the dose. The pattern of metabolites in feces extracts were similar to that found in the urine. About 50% of the total radioactivity recovered in the feces was D, L- triazolylalanine. A second metabolite found in the feces was D,L-2-acetyl amino-3-(1H-1,2,4-triazol-1-yl) propanoic acid, and accounted for about 16% of the total fecal radioactivity. A third metabolite that accounted for 30% of the fecal radioactivity could not be identified and did not occur in the urine (Table 1).

Table 1: Relative percentageof metabolite fractions in the 0-24 hours excreta of rats after a single oral dose of ^{14}C-D,L-triazolylalanie (in % of the dose)

Percent of metabolite fraction in 0 - 24 hours excreta				
	0.5		50	
Sex	male	female	male	female
Rat number	8292-95	8296-99	82102-105	82106-109
Urine fraction[1)]				
U-1	69.1	73.9	81.5	85.5
U-2	18.6	11.7	10.4	7.6
other	8.3	6.4	5.8	5.9
Subtotal urine	96.0	92.0	97.7	99.0
Faeces fraction	n.a.[1)]	n.a.		
FE-1	n.a.	n.a.	1.3	1.5
FE-2	n.a.	n.a.	0.5	0.6
FE-3	n.a.	n.a.	0.8	0.9
Subtotal faeces	n.a.	n.a.	2.6	3.0

1) n.a. = not analyzed

U-1 = FE - 1 = D, L-triazolylalanine
U-2 = FE - 2 = Acetylated D, L- triazolylalanine
FE - 3 = Unknown

The table above was taken as is from the registrant report.

DISCUSSION AND CONCLUSIONS:

The test chemical, when given orally, at doses of up to 50 mg/kg was completely absorbed and rapidly excreted. The major route of excretion is the renal route. Most of the dose (69 - 86%) was excreted as unchanged D,L-triazolylalanine, and 8-19% of its acetyl derivative. In addition some unknown metabolites were found in the urine and did not account for more than 3%. The total fecal radioactivity accounted for only 3% of the dose. The fecal metabolites were similar to those found in the urine, except for one that was never identified and did not occur in urine.

The close similarity of the test chemical to amino acids may result in interference in protien synthesis. However this can not be determined without experimental evidence. Because of the lack of experimental data in this regard, the toxicological significance of triazolylalanine can not be determined.

CORE CLASSIFICATION: Acceptable.

BIBLIOGRAHY

Mihail, F. Acute intraperitoneal toxicity of Triazolylalanine (THS 2212) in rats. An unpublished report (Bayer Report No. 11229, Mobay ACD report no. 82661) prepared by Bayer AG, Toxicology Institute, for Mobay Chemical Corporation, Stilwell, Kansas, dated October 19, 1982.

Mihail, F. Acute oral toxicity of Triazolylalanine (THS 2212) in rats and mice. An unpublished report (Bayer Report No. 11229, Mobay ACD Report No. 82661) prepared by Bayer AG, Toxicology Institute, for Mobay Chemical Corporation, Stilwell, Kansas, dated October 19, 1982.

Mihail, F. and Vogel, O. Triazolylalanine (THS 2212)-Subacute oral toxicity study in the rat. Unpublished Report No. 11491 (Study No. T6011644) prepared by Bayer, AG, Wuppertal-Elberfeld, Germany. Dated 24 January 1983.

Richard, M., Allen J.A., Williams, A., and Ransome, S.J. Cell transformation test for potential carcinogenicity of R152056. An unpublished report (CTL/C/1085) prepared by Huntingdon Research Centre for Imperial Chemical Industries, Ltd. Dated May 15, 1981.

Watkins, P.A. R152056: 3-(1,2,4-triazol-1-yl)alanine (ICI 156,342) micronucleus Test in CBC F_1 mice - TQM/4. An unpublished report (AC 83-2413) submitted by Mobay Chemical Corp., Stilwell, KS. Dated September 14, 1982.

Weber, H. and Suwelack, D. Preliminary biokinetic study on rats. Unpublished report No. 11583 (F) prepared by Bayer Pharmaceutical Division, Wuppertal, Germany. Dated February 24, 1983.

Hamboek, H. The metabolism of D,L,-2- amino -3 -(1H-1,2-4-triazol-1-yl)- propanoic acid (D,L-triazolylanine) in the rat. Unpublished project report number 11/83 (Study No. CGA 131013, 82/91-82/110) prepared by Ciba-Geigy Limited, Basle, Switzerland. Dated October 20, 1983.

REFERENCES

004766

Birtley, R.D.N., Triazole alanine: Preliminary reproduction study in the rat. An unpublished report (No. CTL/L/470; Study No. RR0230/FO) submitted by Imperial Chemical Industries PLC. Dated September 19, 1983.

Birtley, R.D.N. Triazole alanine: Two generation reproduction study in the rat. A progress report of Study No. RR 0255/FO and RR 0255/F1 repared by Imperial Chemical Industries PLC, Dated June 21, 1983.

Bomhard E. THS 2212, preliminary subacute toxicity study on male rats, administration in the drinking water. Unpublished report no. 11253 (Mobay Report No. 82662), prepared by Institut Fur Toxikologie, Bayer AG, Wuppertal-Elberfeld, Germany. Dated October 25, 1982.

Clapp, M.J., et al. Triazole alanine: Teratogenicity study in the rat. Report No. CTL/P/875 (Study No. RR0240) prepared by Imperial Chemical Industries PLC. Dated October 13, 1983.

Hamboeck, H. Distribution, degradation, and excretion of D,L,-2-amino-3-(1-H-1,2,4-Triazol-1-yl)-propanoic acid (D,L,-triazolylalanine) in the rat. Unpublished project report number 1/83 (Study No. CGA 131013, 82/91-82/110) prepared by Ciba-Geigy Limited, Basle, Switzerland. Dated 2 March, 1983.

Henderson, C. and Parkinson, G.R. R152056: Acute oral toxicity to rats. Report No. CTL/P/600 (Study No. AR1893), prepared by Central Toxicology Laboratory for Imperial Chemical Industries Limited, dated January 18, 1981.

Herbold, B. THS 2212 Triazolylanine: Study of DNA-Damage using the E. coli PolA$^-$ test. An unpublished report (No. 82738) prepared by Bayer AG for Mobay Chemical Corporation. Dated January 5, 1983.

Herbold, B. THS 2212 Triazolylalanine: Micronucleus test for mutagenic effect on mice. An unpublished Report (Bayer No. 11054; Mobay Agchem No. 84005) prepared by Bayer AG for Mobay Chemical Corporation. Dated August 9, 1982.

Herbold, B. THS 2212 triazoylalanine: Salmonella/Microsome test for determination of point mutations. Unpublished Report No. 11388 prepared by Bayer AG Institut Fuer Toxikologie for Mobay Chemical Corporation. Dated January 5, 1983.

Hoffman, K. THS 2212 (Triazolylalanine), acute oral toxicity, dogs. Bayer Report No. 11213 (Mobay ACD Report No. 82663) prepared for Mobay Chemical Corporation by Institute fuer Toxikologie, Wuppertal- Elberfeld, Federal Republic of Germany, and dated October 14, 1982.

Banner/ Appendices

Doc # 5094

✓ 1 – DER for rat chronic/onco
✓ 2 – (Table) Location of Dermal Fibromas
✓ 3 – Background elements of historical control studies
✓ 4 – Historical control data for incidence of dermal fibromas in male S-D rats
✓ 5 – Thyroid follicular adenomas / adenocarcinomas in female SD rats
✓ 6 – Incidence of liver lesions in SD rats
✓ 7 – Exocrine atrophy of pancreas
✓ 8 – Luminal dilatation of uterus
✓ 9 – DER, mouse study
✓ 10 – Memo, Katy → Jacoby, 9/10/85 (Diet analysis)

11 – Mutagenicity of TA
DER: Transformation/Liver Microsome Test
and Memo, A.K. → H.J., 5/20/86

12A – (Tox Doc # 4562)
Mouse micronucleus DER

12B – (Tox Doc # 4766)
Mouse micronucleus DER, Ekali

13 – DER – 2-generation Repro Study
14 – DER 1-Year Sulchron Dog Study
15 – DER Induction : o x o

M2