CASWELL FILE

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

MAY 20 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

SUBJECT: Triazolylalanine, Plant Metabolite of Bayleton; Review of Toxicity Data; Submitted by: Mobay Chemical Corporation; EPA ID# 3125-320; Action Code: 400; Accession # 257997; Caswell # 862B; Record # 153412;

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

FROM: Alan C. Katz
Toxicologist, Review Section III
Toxicology Branch
HED (TS-769C)
Alan C Katz 5/16/86

THROUGH: Marcia Van Gemert, Ph.D.
Head, Review Section III
Marcia Van Gemert 5.16.86
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
T. M. Farber 5/19/86

Action Requested:

Review toxicity data on Bayleton metabolite, triazolylalanine:

1) Killick, M.E.; Triazole Alanine: Teratogenicity Study in the Rat: Individual Data Supplement (1983). Central Toxicology Laboratory, Imperial Chemical Industries PLC, Alderley Park, Macclesfield, Cheshire, UK, Study No. RR0240; Report # CTL/P/8755.

2) Bielstein, P.; Transformation/Liver-Microsome Test (1984). Ciba-Geigy Ltd.;[CGA 64250 MET/A.I. CGA 131 013]; Report # 840324.

Background:

1) The Data Evaluation Record (DER) for this rat teratogenicity study was issued by G. Ghali (Tox Branch Document #004766, attached). In the DER, it is noted:

"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."

The Individual Data Supplement submitted for review included the following Appendices:

Appendix A. Individual maternal clinical observations and macroscopic findings at post mortem.

Appendix B. Individual maternal bodyweights.

Appendix C. Individual maternal food consumption.

Appendix D. Individual caesarian data.

Appendix E. Individual fetal data.

Appendix F. Description of fetus 80G: 1000 mg/kg/day triazole alanine.

2) A cell transformation assay was conducted, using mouse embryo fibroblasts BALB/3T3 with and without metabolic activation. The DER for this assay is attached.

Conclusions:

1) The supplemental data presented for the rat teratogenicity study do not include a tabulation of fetal abnormalities on the basis of the number of litters affected. This deficiency was noted in the attached DER (Ghali, Tox Branch Document #004766). Therefore, the CORE classification (i.e., Minimum Data) will not be upgraded. The conclusions expressed in the DER are also unchanged.

2) Results of the cell transformation assay with triazolylalanine in mouse embryo fibroblasts BALB/3T3 were negative without activation and inconclusive with activation. Deficiencies are discussed in the attached mutagenicity DER (see "Results/Discussion" section). The study is classified as unacceptable.

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.

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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."

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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.

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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 ($p \leq 0.01$).

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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 REPORT

A. Study Type: Mutagenicity; BALB/3T3 Cell Transformation

B. Compound: Triazolyl alanine; CGA 131 013 Technical, Batch no. TLB 1207 (Purity not disclosed)

C. Study Report Citation: Beilstein, P.(9/12/84);Transformation/Liver-Microsome Test (In vitro test for transformation-inducing properties in mammalian fibroblasts); Testing facility: Ciba-Geigy Limited, Basle, Switzerland; Test No. 840324; Submitted by Mobay Chemical Corporation, Kansas City, MO. EPA ID#: 3125-320; Action Code: 400; Caswell #862B; Accession #257997.

D. Reviewed by: Alan C. Katz, M.S., D.A.B.T.
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E. Secondary Review by: Irving Mauer, Ph.D.
Geneticist
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05/16/86
(Date)

F. Procedures:

See Appendix 1, attached (excerpt from study report).

G. Results/Discussion:

In the preliminary assay for cytotoxicity, the highest concentration of triazolyl alanine (1000 ug/ml) caused no reduction in the number of colonies formed with the non-activated system when compared with the negative solvent control. In the cytotoxicity test with activation (rat S9 mix), this concentration of test substance reportedly caused a reduction of approximately 20 percent; however, data substantiating this finding were not presented. Also, since it is noted in the study report (p.7) that "(t)he best suited concentration of the test substance as the highest for the transformation assay is that causing a 50% reduction in colony-forming ability in comparison with the negative (solvent) control," the rationale for selection of this concentration as the highest level for use in the transformation assay should be explained.

Results of the transformation assays, as excerpted from the study report, are presented in Tables 1 through 4. These data show that triazolyl alanine, at levels up to and including 1000 ug/ml, did not induce transformation of cells under the conditions of the assay without metabolic activation.

Data from an initial assay using the S9 activation system were not reported, due to "a high background of transformed colonies in the negative and positive controls and at the five concentrations of the test substance." Results of a repeat test with activation are shown in Table 3 and statistically analyzed in Table 4. Although the study report author stated that "(s)tatistical comparison of the results from the solvent control and all five treatment groups revealed no significant difference (confidence limit = 5 %)", this

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evaluation is subject to question. The statistical comparison which was applied to the data did not take into account the normalized values for the proportion of viable cells which were transformed. As shown in Table 3, the transformation frequency values for the test preparations, in order of increasing concentration, were: 0.49, 1.71, 1.83, 2.34 and 4.10, while values for the solvent and untreated controls were 1.15 and 1.09, respectively. These data suggest a slight treatment-related effect.

Mean viability control values for the solvent and untreated control groups were reported in the range of 20.7-26.1 percent. Individual or historical viability control values were not presented.

Cell transformation was clearly demonstrated in the positive control assays using methylcholanthrene (without activation) and 2-acetylaminofluorene (with activation), although cell viability levels were substantially reduced with both of these agents. Likewise, triazolyl alanine should have been tested at a higher concentration so as to cause greater cytotoxicity (or to a limit dose of 5000 ug/ml).

H. Conclusions:

Under the conditions of the assay without metabolic activation, triazolyl alanine at concentrations up to and including 1000 ug/ml did not appear to cause transformation of BALB/3T3 cells. In the presence of a metabolic (S9) activation system, however, there was evidence of a dose-related increase in transformation frequency.

I. Classification/Recommendations:

This study is classified as unacceptable. The assay should be repeated, and should include higher concentrations of the test substance.

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Pages 10 through 17 are not included.

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