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

009705

MEMORANDUM

AUG 27 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 9F03802; 003125-00347. Baytan. Review of Developmental Toxicity Study in the Rabbit

Tox. Chem. No. 074A
DP Barcodes: D180453, D180452

TO: James Stone, PM Team #22
Registration Division (H7505C)

FROM: Pamela M. Hurley, Toxicologist Pamela M Hurley 8/12/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner 8/14/92 KB 8/18/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

Submission: S421361, S421358

Background and Request:

Mobay Corporation had submitted developmental toxicity studies in the rat and rabbit in support of registration of Baytan. The Agency determined that both of the developmental toxicity studies did not meet the Agency's current standards and graded both studies as Core Supplementary. Mobay has conducted new developmental toxicity studies in both the rat and rabbit and has submitted the results to the Agency. The new rat study has already been reviewed and was again graded Core Supplementary because of several deficiencies. The Registrant has submitted additional data in order to address those deficiences. The Toxicology Branch (TB-I) has reviewed the additional data for the rat study and has commented on it in a separate memorandum. The rabbit study is reviewed in this memorandum.

Toxicology Branch Response:

The Toxicology Branch (TB-I) has reviewed the new rabbit study and has determined that it satisfies the regulatory requirement for a developmental toxicity study in rabbits for Baytan. The study is classified as Core Guideline. The following paragraph summarizes the results. (1)

Baytan was tested in a developmental toxicity study in New Zealand white rabbits in which the does were fed 0, 5, 25 or 125 mg/kg/day of the test chemical during gestation days 6-18. The maternal NOEL is 25 mg/kg/day and the maternal LEL is 125 mg/kg/day based on decreases in body weight gain and food consumption during the dosing period. The developmental NOEL is 125 mg/kg/day (HDT).

cc: N. Thoa

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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 8/12/92
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner, Head
Section I, Tox. Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Teratology - Developmental Toxicity (83-3)

SPECIES: Rabbit

SHAUGHNESSY NO./CASWELL NO.: 127201/074A

ACCESSION NUMBER/MRID NO.: 423650-01

DP BARCODE AND SUBMISSION NO.: D180452, D180453; S421358, S421361

TEST MATERIAL: Baytan Technical

SYNONYMS: Triadimenol

STUDY NUMBER(S): 102692

REPORT NUMBER: MTD0256

SPONSOR: Miles Inc., Agriculture Division, Kansas City, MO

TESTING FACILITY: Toxicology Department, Miles Inc. P.O. Box 40, Elkhart, IN 46515

TITLE OF REPORT: A Developmental Toxicity Study in Rabbits with Baytan Technical

AUTHOR(S): Clemens, G.R., Grosso, D.S., Anderson, E.J. and Hartnagel, R.E. Jr.

REPORT ISSUED: 6/9/92

CONCLUSION: Baytan was tested in a developmental toxicity study in New Zealand white rabbits in which the does were fed 0, 5, 25 or 125 mg/kg/day of the test chemical during gestation days 6-18. The maternal NOEL is 25 mg/kg/day and the maternal LEL is 125 mg/kg/day based on decreases in body weight gain and food consumption during the dosing period. The developmental NOEL is 125 mg/kg/day (HDT).

Classification: Core Guideline

Testing Guideline Satisfied: 83-3

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: β-(4-chlorophenoxy)-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol

Description: White to tan crystalline appearance
Batch #(s), Other #(s): PF8741
Purity: 96.0%
Source: Miles
Vehicle (if applicable): 0.5% carboxymethylcellulose sodium (CMC) and 0.4% polyoxyethylene sorbitan mono oleate (Tween 80 NF) in distilled water.

2. Test Animals):

Species and Strain (sexes): Male and female New Zealand White Rabbits

Age: ♂ > 1 year; ♀ 30 weeks.
Weight(s): ♂: 3.72 - 4.84 kg; ♀: 3.15 - 3.95 kg
Source(s): Hazleton Research Animals, Kalamazoo, MI

3. Study Design:

This study was designed to assess the developmental toxicity potential of Baytan Technical when administered by gavage to rabbits on gestation days 6 through 18, inclusive.

a. Mating:

Natural or artificial insemination? Artificial
Describe technique used: Semen was collected from proven bucks and evaluated for motility and spermatozoa counts (details on motility and spermatozoa counts were not provided in the report). It was then diluted in 0.9% sterile saline and administered intravaginally to the does. The does had been previously primed with HCG, administered intravenously (approximately 100 USP units).

b. Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	20
Low Dose	5	20
Mid Dose	25	20
High Dose	125	20

c. Dosing:

All doses were in a volume of 5 ml/kg (CMC vehicle) of body weight/day. Dosing was based on 6 gestation day body weight.

1) Basis For Selection of Dose Levels: Dose levels were based on the results of previously completed developmental toxicity studies with Baytan in rabbits.

2) Preparation: The test material was prepared as a suspension (0.1% (1.0 mg/ml), 0.5% (5.0 mg/ml), or 2.5% (25.0 mg/ml)) in the vehicle described above.

3) Frequency of Preparation: Once, at the beginning of the study.

4) Storage Conditions: Refrigerated.

5) Stability Analyses: Because of purity determination on day 0, the stability analyses were first conducted on day 1, not day 0. The stability study was conducted with 1.0 and 25.0 mg/ml concentrations in CMC. The suspensions were stored covered and refrigerated. On the day of analysis, once weekly, the suspensions were removed from the refrigerator and allowed to reach room temperature. A 1.0 ml aliquot was taken from the top, middle, and bottom of each concentration for analysis. The suspensions were removed from storage daily, stirred 15 minutes, then placed back in storage (2 weekends were inadvertently omitted from this procedure). Stability was measured on days 1, 7, 17, 21 and 28.

6) Homogeneity Analyses: A one ml aliquot was taken from the top, middle and bottom of each batch concentration (1.0, 5.0 and 25.0 mg/ml), diluted and analyzed by HPLC.

7) Concentration Analyses: After preparation, the suspensions were analyzed once to verify the concentrations of the active ingredient.

d. Maternal Examinations:

1) Clinical Observations and Mortality: All does were observed daily for clinical signs of toxicity and mortality.

2) Body Weight Determinations: Body weights were obtained on days 0, 6-19, 21 and 29 of gestation.

3) Food Consumption: Each rabbit was permitted approximately 130 grams of diet daily except during the time when food consumption was monitored (days 1, 6, 7, 12, 15, 19, 23 and 29 of gestation). Whenever food consumption was measured, exactly 130 grams of food was offered to each rabbit.

4) Measurement of liver enzymes: Blood samples were obtained prior to treatment (5-10 days prior to insemination) and on days 19 and 29 of gestation. Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were measured.

5) Gross Necropsy: Animals which died or were sacrificed in moribund condition prior to end of exposure period and were subjected to complete gross pathological examinations: The report had no statement on this, however, no does died prior to termination.

Animals sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All does. Only the abdominal and thoracic viscera were examined for gross abnormalities. Livers were removed and weighed.

6) Uterine Examinations: The following parameters were either measured or calculated.

Number of corpora lutea
Number of live fetuses
Number of dead fetuses
Early and late resorptions
Total implantations
Pre- and post implantation losses
Individual fetal weights
Uterine weights

7) Reproductive Indices: The following parameters were either measured or calculated.

Fertility index
Gestation index
Litter size
Sex distribution (fetal)

e. Fetal Examinations:

The fetuses were examined in the following manner: Each fetus was examined for viability and weighed. Individual placentas were also weighed. A complete external examination was conducted on each fetus. All fetuses were sacrificed by intracranial injection of barbituate and a complete internal examination was conducted (abdominal and thoracic viscera). The sex of each fetus was determined. Following these procedures, all fetuses were eviscerated and fixed in toto in 70% ethanol. The fetuses were then processed for clearing tissue (KOH Alizarin Red-S method, Staples and Schnell) and staining fetal bone and then evaluated for general skeletal development.

f. Historical Control Data:

Historical control data were provided to allow comparison with concurrent controls. The number of studies used to compile the historical control data base was 3 to 6 studies. The endpoints included body weights, food consumption, reproductive efficiency and fetal data, external and visceral findings and skeletal findings.

g. Statistical analysis:

Statistical analysis of the data consisted of application of one or more of the following tests: chi-square or Fisher's exact test for variables that are defined on does and can take only 1 of 2 values (such as pregnant, not pregnant or produced or did not produce a litter) if more than 20% of the expected cell frequencies are less than 5; Kruskal-Wallis one-way rank test for variables that are counts on does (such as number of corpora lutea, number of fetuses), indices based on counts on does (such as percent male fetuses or pre-implantation loss) or measurements on does (such as average weight of males fetuses); if the Kruskal-Wallis test is significant, pairwise comparisions between treatment groups will be made using the rank sum multiple comparison procedure of Dunn (ties will be handled by the method of average ranks); male, female and combined fetal weight data will also be analyzed using Dunnett's test; and Healy's test will be used for the fetal weight data in the event of a significant difference among groups in litter size. These procedures appear to be appropriate for this study.

h. Compliance:

A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

B. RESULTS:

1. Dosage Preparation: (see above under methods).

Homogeneity Analysis: At 1.0 mg/ml, the range of concentrations were from -7 to +6% of the nominal concentration for trial 1 and +5 to +11% for trial 2. At 5.0 mg/ml, the range of concentrations were from 0 to +4% of the nominal concentration for trial 1 and from -4 to +1% for trial 2. At 25.0 mg/ml, the range of concentrations were from +2 to +3% of the nominal concentration for trial 1 and from 0 to +7% for trial 2. It appears that the test material is fairly homogeneous at all concentrations.

Stability Analyses: At 1.0 mg/ml, the mean measured test material concentrations began at 14% above the nominal concentration and decreased and then increased again. The final mean measured concentration was 10% above the nominal by day 28. At 25.0 mg/ml, the mean measured test material concentrations began at 6% above the nominal concentration and generally increased to 11% above by day 21 and 9% by day 28. It does not appear that the test material is unstable under refrigeration.

2. Maternal Toxicity:

a. Clinical Observations and Mortality: No does died at any dose level during the course of the study. There was a statistically significant decrease in the number of does in the high dose group which were considered normal (i.e., did not have any clinical signs of toxicity). However, none of the individual clinical signs were statistically significantly different from controls in any dose group. It appears that the high dose does had a higher incidence of a small amount of stool than any other group; this was not statistically significantly increased. One doe in the 5 mg/kg group aborted 3 fetuses and 2 additional fetuses were found in utero. All 5 were normal for their stage of development. This doe had no clinical signs of toxicity preceding the abortion and had no gross pathological changes at necropsy. Two does had resorptions but no viable progeny. One was from the 25 mg/kg group and the other was from the 125 mg/kg group. The 25 mg/kg doe had a single resorption site. The doe had soft stool on one occasion and no gross pathological changes. The high dose doe had 2 resorptions. She had no clinical signs and her gross examination was normal. None of these incidences are considered to be related to treatment. The NOEL for clinical signs of toxicity is 125 mg/kg. The following table summarizes the clinical signs that were observed most frequently.

Summary of Gross Clinical Observations for Does

Observations	Control	5 mg/kg	25 mg/kg	125 mg/kg
Normal (%)	18 (90)	16 (80)	13 (65)	8 (40**)
Small amount of stool	1 (5)	0 (0)	0 (0)	8 (40)
Soft stool	1 (5)	2 (10)	3 (15)	3 (15)
No stool	0 (0)	0 (0)	0 (0)	1 (5)
Hairloss	0 (0)	1 (5)	1 (5)	2 (10)
Aborted	0 (0)	1 (5)	0 (0)	0 (0)

b. Body Weight Determinations: The high dose dams had a statistically significant decrease in mean body weight on gestation days 13 - 16 when compared to the control group. However, none of these values dropped below 94% of the control values. The body weight gain for the high dose dams was statistically significantly decreased when compared to the control group for days 6-19 and 0-29. In actuality, the does lost weight between days 6-19. The body weight gain for the high dose group for the entire gestation period was 71% of the control group. The NOEL for body weight gain is 25 mg/kg. The LEL is 125 mg/kg.

The investigators supplied the following data:

Table I: Body Weight Gains (Kg)[a]

Group:	Prior to Dosing Period[b]	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gain[1]
Control	0.17	0.12	0.17	0.45	-0.02
LDT	0.19	0.10	0.18	0.47	0.03
MDT	0.16	0.09	0.16	0.42	0.03
HDT	0.20	-0.07**	0.20	0.32*	-0.04

[1] = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.
a = Data extracted from (study 102692 or report number MTD 0256 and table II)
b = Days 0-6 for prior to dosing period, days 6-19 for dosing period and days 19-29 for post dosing period.
* Significantly different from control group at $p < 0.05$.
** Significantly different from control group at $p < 0.01$.

c. Food Consumption: Mean food consumption was statistically significantly increased for the mid- and high dose groups on day 1. Food consumption was statistically significantly decreased for the high dose group on days 7, 12 and 15. On day 7, food consumption was 94% of control values and on days 12 and 15, food consumption for this dose group was 24-26% less than controls. Since food consumption was not measured daily and cumulative food consumption was not measured, a comparison of food consumption values versus body weight gains for specified time periods could not be done.

The investigators supplied the following data:

Table II: Food Consumption Data (g)[a]

Group:	Day 1	Day 6	Day 7	Day 12	Day 15	Day 29
Control	129.3	129.7	129.7	129.3	129.4	125.9
LDT	129.4	129.4	129.6	129.3	128.6	123.2
MDT	130.0*	129.9	130.0	129.9	129.5	117.8
HDT	130.0*	129.8	122.2*	95.2*	98.2*	127.1

[a] = Data extracted from (study 102692 or report number MTD 0256 and table III)

d. Serum Liver Enzymes Activities: Aspartate aminotransferase and alkaline phosphatase were significantly decreased in the high dose group on gestation day 19. Decreases in these enzyme levels are not considered to be toxicologically significant. None of the parameters were statistically significant at any other time or with any other dose level.

e. Liver weights: No treatment-related differences were observed between treated and control does in either absolute or relative liver weights.

f. Gross Pathology: No treatment-related differences were observed between treated and control does. The following findings were randomly observed: non-gravid, stomach erosion, cyst in the fallopian tube, resorption sites only and aborted/sacrificed.

g. Cesarean section Observations: There was a statistically significant decrease in the median values of implantations for the mid- and high dose groups when compared to the control group. The mean values were decreased but not significantly

so. The median values were at the low end of the historical control range (6-9) and the mean value of the mid-dose group was slightly less than the historical control range (5.7 versus 5.9-8.8 in the historical controls). In addition, the control values were at the high end of the historical control range.

There was also a statistically significant decrease in the median litter size in the mid- and high dose groups when compared to controls, but not the mean litter size. The historical control range for median litter size was 6-9 and for the mean litter size was 5.6-8.5. The high dose group was at the low end of the historical control range (6) and the mid-dose group was just below the historical control range (5.0) for median litter size. For mean litter size, again the mid-dose group was less than the historical control range (5.2) and the high dose group was at the low end (5.6). Again, there was no dose-response. However, for both the mid- and the high dose group, although each dose level had one doe that had resorptions with no viable progeny, the individual litter data indicates that on the average, the litter sizes for these two dose groups appear to be less than the control group.

A further examinination of the data indicates that there was a decreased number of corpora lutea for the mid- and high dose groups, although not statistically significant. The number of litters were similar in all groups. In addition, the mean fetal weight as well as the placental weight were higher in the mid-dose group. The % post-implantation loss was equivalent in all groups, but the % pre-implantation loss was greater in the mid- and high dose groups. When considering all these data together, it does not appear that there are any toxicologically significant effects in the reproductive parameters at any dose level. There was no dose-response and the does in the mid- and high dose groups had fewer corpora lutea. Thus, the number of implantations, the litter size and the total number of fetuses in these two dose groups would be less than controls. In addition, the mean fetal weights were greater than controls for the mid-dose group, which is another manifestation of the smaller litter size. The following table summarizes the data for reproductive parameters.

Table III: Cesarean Section observations[a]

	Control	LDT	MDT	HDT
Dose:	0	5	25	125
#Animals Assigned	20	20	20	20
#Animals Mated/Inseminated	20	20	20	20
Pregnancy Rate (%)	18/20 (90)	17/20 (85)	17/20 (85)	18/20 (90)
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	2	3	3	2
#Aborted	0	1	0	0
#Premature Delivery	0	0	0	0
Corpora Lutea/Dam	8.9	8.3	7.2	7.1
Median	9.0	7.5	7.0	7.0
Range	(4-13)	(5-13)	(1-10)	(1-11)
Total Implantation	151	120	97	114
Implantations/Dam	8.4	7.5	5.7	6.3
Median	9.0	8.0	6.0**	6.0**
Range	(2-11)	(4-12)	(1-9)	(2-10)
Mean Litter Size	7.6	7.3	5.2	5.6
Median Litter Size	8.0	7.5	5.0*	6.0*
Range	(2-11)	(3-11)	(0-9)	(0-9)
Total No. Fetuses	137	117	88	101
No. Viable Fetuses	134	116	88	99
Live Fetuses/Dam	7.4	7.3	5.5	5.8
Mean Resorptions	0.8	0.2	0.5	0.7
Median	0.0	0.0	0.0	0.0
Range	(0-4)	(0-1)	(0-3)	(0-3)
Total No. Resorptions	14	3	9	13
# Does with > 1	3	0	3	4
Percent with Resorptions	44.4	18.8	29.4	44.4
Total Dead Fetuses	3	1	0	2
Mean Fetal Weight (gm)	43.9	43.8	48.6++	44.5
Preimplantation Loss (%)	10.7	12.0	23.4	14.7
Median	9.1	0.0	25.0	6.3
Range	(0-50.0)	(0-60.0)	(0-85.7)	(0-42.9)
Postimplantation Loss(%)	10.3	4.1	12.5	14.6
Median	5.0	0	0	0
Range	(0-44.4)	(0-25.0)	(0-100)	(0-100)
Sex Ratio (% Male)	47.2	50.0	56.3	42.9
Wt. of Placentas	5.4	5.5	6.0#	5.4

[a] = Data extracted from (study 102692 or report number MTD 0256 and table VII).

* = $p < 0.05$; ** = $p < 0.01$; ++ = $p < 0.01$ (Healy); # = $p < 0.05$ (Dunnett's).

3. Developmental Toxicity: There were no treatment-related external and visceral findings for either fetuses or litters. There were several statistically significant findings for skeletal effects when examined by the number of fetuses affected but the significance disappears when the litter data are examined. Some of these statistically significant findings were decreases when compared to controls. The decreases are not considered to be biologically meaningful and are not summarized in this evaluation. The following tables summarize the most frequent findings.

Table IV: External and Visceral Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	134 (18)	116 (16)	88 (16)	99 (17)
#pups(litters) affected	4 (4)	4 (4)	3 (3)	6 (5)
Major vessels, right subclavian, right and left carotid arteries branch at common juncture off innominate	1 (1)[a]	2 (2)	0 (0)	0 (0)
Major vessels, right subclavian retroesophageal	1 (1)	0 (0)	2 (2)	4 (4)
Gallbladder, small/rudimentary	0 (0)	1 (1)	0 (0)	1 (1)

(+) some observations may be grouped together. Data extracted from (study 102692 or report number MTD 0256 and table X).
(a) fetal [litter] incidence

Table V: Skeletal Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	134 (18)	116 (16)	88 (16)	99 (17)
Skull: bones incompletely ossified	45 (11)[a]	34 (12)	14* (9)	51* (14)
Fontanelle enlarged	39 (9)	34 (12)	13* (9)	49** (14)
Presence of calcified body	4 (4)	5 (4)	2 (2)	11* (7)
Scapula:				
Irregular spinous process	1 (1)	0 (0)	0 (0)	9** (7)
Appendages:				
Posterior - IO phalanges	2 (2)	2 (2)	0 (0)	9* (5)

(+) some observations may be grouped together. Data extracted from (study 102692 or report number MTD 0256 and tables XI and XII).
(a) fetal [litter] incidence
* Statistically significant when compared to controls $p < 0.05$.
** Statistically significant when compared to controls $p < 0.01$.

C. DISCUSSION:

1. Maternal Toxicity: Baytan induced decreases in maternal body weight gain and food consumption during gestation. The NOEL is 25 mg/kg and the LEL is 125 mg/kg (HDT). There were no other signs of maternal toxicity, except that there was a statistically significant decrease in the number of does which did not have any clinical signs when compared to the control group. There was a reduction in implantation and litter size in the mid- and high dose groups, however, this was not attributed to the administration of the chemical.

2. Developmental Toxicity:

   a. Deaths/Resorptions: There were no treatment-related increases in death and/or resorptions.

   b. Altered Growth: There was no treatment-related increase in altered growth.

c. Developmental Anomalies: There were no treatment-related increases in developmental anomalies.

d. Malformations: There were no treatment-related increases in malformations.

D. Study Deficiencies: There were no major study deficiencies; however, resorptions were not specified as either early or late.

E. Core Classification: Core Guideline Data.

Maternal NOEL = 25 mg/kg/day
Maternal LOEL = 125 mg/kg/day
Developmental Toxicity NOEL = 125 mg/kg/day (HDT)
Developmental Toxicity LOEL = N/A