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

JUL 24 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: MEB 6447 (Triadimefon; Bayleton) : Review of a Developmental Toxicity study (83-3) in Rabbits.

Project No: 0-1088
Tox. Chem. No.: 862AA
EPA ID No.: 3125-319
CHEM. No: 109901

TO: Cynthia Giles-Parker, Team #22
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Roger Gardner, Section Head
Review Section 1
Toxicology Branch
Health Effects Division (H7509C) Roger Gardner 7-26-91

FROM: Nguyen Bich Thoa, Ph.D Nthoa 07/16/91
Review Section 1
Toxicology Branch I
Health Effects Division (H7509C) KB 7/19/91

Registrant: Mobay Corp., Stilwell, KS.

ACTIONS REQUESTED

Review a developmental toxicity (83-3) study entitled: Teratology Study in the Rabbit with MEB 6447 (Triadimefon; MRID 414462-01).

CONCLUSIONS:

The developmental toxicity potential of MEB 6447 (Triadimefon; Bayleton) was studied by dosing artificially inseminated American Dutch rabbits, by oral gavage, with 0, 20, 50, or 120 mg/kg/day, from gestation day 6 to 18.

Based on the results of the study, the maternal NOEL was 50 mg/kg/day (MDT). The maternal LOEL was 120 mg/kg/day (HDT) based on a marginal body weight loss. The developmental NOEL was 20 mg/kg/day (LDT). The developmental LOEL was 50 mg/kg/day based on dose-related increases (MDT and HDT) in incidences of incomplete ossification of the pelvic pubes and the anterior and posterior phalanges, and of irregular spinous processes.

The following additional developmental effects were observed at the HDT: a statistically significant decrease in female fetal body weight (male fetal body weights were comparable with control weights), increases in incidences of external alterations (rudimentary/missing tails; 2 litters; 10 fetuses), and increases in incidences of skeletal alterations including incomplete ossification of the skull bones and enlargement of the fontanelles (fetal incidence increase only), and extra ribs, malformations of the caudal vertebrae arches and centra, incomplete or non ossification of the 1st, 2nd, 5th sternebrae, the posterior talus, and the posterior phalanges (fetal and litter incidences increases). The increases in incidence of each fetal alteration was statistically significant. Except for extra ribs, the litter incidences increases were not statistically analyzed.

Because the results described above indicate that Triadimefon causes developmental toxicity in rabbits, the study should be referred to the Health Effects Division's Peer Review Committee along with other similar studies for an assessment of Triadimefon's potential to cause developmental toxicity in humans.

CLASSIFICATION: Core Supplementary. Upgradable to Core Minimum upon satisfactory response to the following deficiencies:

1. The expiration date of the Triadimefon batch used in this study was 06/18/88 (pp 38 of report). The dates of artificial insemination (gestation day 0) were from 05/31/88 to 06/03/88 (5 rabbits/dose group/day; pp 153 of report) and the last dosing day (day 18) was from 06/18/88 to 06/21/88. According to these dates, only 5 animals/dose group would have received good test material for the entire dosing period (day 6-18). The remaining animals were given out of date test material, for 1-3 days. The registrant should clarify this matter since 1 to 3 days of mis-dosing might have severe impact on the integrity of a develop-mental toxicity study. The registrant may provide information demonstrating that the test material was stable up to 06/21/88.

2. Except for extra ribs, the litter incidences increases were not statistically evaluated. The registrant is requested to submit statistical evaluations of the following litter incidence increases: external alteration of the tail (HDT group), incomplete ossification of the pelvic pubes and the anterior and posterior phalanges, and irregular spinous process formation (MDT and HDT), and malformations of the arches and centra of the caudal vertebrae, incomplete/non ossification of the 1st, 2nd, 5th sternebrae, posterior talus, and posterior phalanges (HDT). Potential teratogenic effects should be evaluated on both a fetal and litter bases.

A DER on the above referenced study is attached.

GUIDELINE: 83-3

Primary Reviewer: Nguyen B. Thoa, Ph.D. Nthoa 7/16/91
Section 1, Toxicology Branch I/HED
Secondary Review: Roger Gardner, Section Head
Section I, Toxicology Branch I/HED 7-16-91

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species:
Guideline: 83-3

EPA Identification No.s: EPA MRID No.: 414462-01
EPA ID No.: 3125-319
EPA Record No.:
Chemical No: 109901
Caswell No.: 862 AA
HED Project No.: 0-1088

Test Material: MEB 6447 (Triadimefon)

Synonyms: Bayleton; 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone

Sponsor: Mobay Corp., Stilwell, KS.

Study Number: MTD 0149

Testing Facility: Corporate Tox. Department, Miles Inc., Elkhart, IN.

Title of Report: Teratology Study in the Rabbit with MEB 6447 (Triadimefon)

Author: G.R. Clemens and R.E. Hartnagel Jr.

Report Issued: 03/22/90

Bibliographic Citation: see attached

Conclusions: The developmental toxicity potential of MEB 6447 [Triadimefon; Bayleton; 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone] was studied by dosing artificially inseminated American Dutch rabbits, by oral gavage, with 0, 20, 50, or 120 mg/kg/day, from gestation day 6 to 18.

No treatment-related mortality was observed.

Maternal toxicity was not observed at either the LDT or the MDT and only a marginal body weight loss, unaccompanied by any body weight change, was observed at the HDT.

No treatment-related gross pathological alterations were observed. The pregnancy rates, gestation indexes, average numbers of corpora lutea, implantations, percentages of pre- and post-implantation losses, resorptions, fetuses/litter, viable number of fetuses/doe, male and combined fetal body weight, and percentage of male fetuses/doe, were comparable between groups.

Developmental Toxicity was observed at both the MDT and HDT.

Dose-related increases (MDT and HDT) in both the fetal and litter incidences of several skeletal alterations were observed at the MDT. These included incomplete ossification of the pelvic pubes and the anterior and posterior phalanges , and of irregular spinous processes. The increase in incidence of each fetal alteration was statistically significant. The litter incidences increases were not statistically analyzed.

The following additional developmental effects were observed at the HDT: 1) statistically significant decreases in placental weight and in female fetal body weight, 2) increases in incidences of external alterations (rudimentary/missing tails; 2 litters; 10 fetuses), and 3) increases in incidences of numerous skeletal alterations including incomplete ossification of the skull bones and enlargement of the fontanelles (fetal incidence increase only), and extra ribs, malformations of the caudal vertebrae arches and centra, incomplete or non ossification of the 1st, 2nd, 5th sternebrae, the posterior talus, and the posterior phalanges (fetal and litter incidences increases). The increases in incidence of each fetal alteration was statistically significant. Except for extra ribs, the litter incidences increases were not statistically analyzed.

Based on the results of the study, the maternal NOEL was 50 mg/kg/day (MDT). The maternal LOEL was 120 mg/kg/day (HDT) based on a marginal body weight loss. The developmental NOEL was 20 mg/kg/day (LDT). The developmental LOEL was 50 mg/kg/day based on dose-related increases (MDT and HDT) in incidences of incomplete ossification of the pelvic pubes and the anterior and posterior phalanges, and of irregular spinous processes.

This study meets or exceeds the criteria for potential teratogenic effect (40 CFR 158.34; criteria #5).

Classification: Core Supplementary. Upgradable to Core Minimum upon satisfactory response to the following deficiencies:

1. The expiration date of the Triadimefon batch used in this study was 06/18/88 (pp 38 of report). The dates of artificial insemination (gestation day 0) were from 05/31/88 to 06/03/88 (5 rabbits/dose group/day; pp 153 of report) and the last dosing day (day 18) was from

06/18/88 to 06/21/88. According to these dates, only 5 animals/dose group would have received good test material for the entire dosing period (day 6-18). The remaining animals were given out of date test material, for 1-3 days. The registrant is required to clarify this matter since 1-3 days of misdosing might have severe impact on the integrity of a developmental toxicity study. The registrant may provide information demonstrating that the test material was stable up to 06/21/88.

2. Except for extra ribs, the litter incidences increases were not statistically evaluated. The registrant is requested to submit statistical evaluations of the following litter incidence increases: external alteration of the tail (HDT group), incomplete ossification of the pelvic pubes and the anterior and posterior phalanges, and irregular spinous process formation (MDT and HDT), and malformations of the arches and centra of the caudal vertebrae, incomplete/non ossification of the 1st, 2nd, 5th sternebrae, posterior talus, and posterior phalanges (HDT). Potential teratogenic effects should be evaluated on both a fetal and litter bases.

A. MATERIALS:

Test Compound: Purity: 94.3% (technical grade).
Description: off-white powder, slightly soluble in water, freely soluble in organic solvents.
Lot No.: 203 780190; Expiration date 06/18/88.
Storage: At room temperature, in sealed container.

Vehicle(s): Carboxymethylcellulose (CMC) solution [0.5% CMC (Nutrit. Biochem. Corp., Cleveland, OH) and 0.4% tween 80 (polyoxyethylene sodium monosterate; Nutrit. Biochem. Corp., Cleveland, OH)] in distilled water (Miles Tox. Department still No. A 35836). The vehicle was stored refrigerated.

Test Animal(s): Species: Rabbit
strain: American Dutch
Source: Langshaw Farms, Augusta, MI
Age: 4 1/2 months old upon receipt.
Weight (kg): 2.66-4.25 (M); 2.23-3.59 (F)
Acclimation Period: ≥ 28 days.

B. STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of MB 6447 (Triadimefon; Bayleton; 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone), when administered by oral gavage to artificially inseminated female rabbits, from gestation day 6 through 18, at doses of 0, 20, 50,

or 120 mg/kg/day. Treatment was initiated from 06/06/88 to 06/09/88 (5 females/group/day) and the in-life phase of the study was completed on 07/01/88.

Mating

Mating was conducted once, through artificial insemination, from 5/31/88 to 6/3/88 (5/group/day). Semen samples collected from the male rabbits were evaluated for spermatozoa count and motility. They were then diluted with 0.9% sterile saline (Am. Mc Graw, Irvine CA; lot No. J8F 0108; expiration date May 1992), and were pipetted intravaginally (gestation day 0) into virgin rabbits. The females were primed 3 weeks earlier, with about 50 USP units of HCG injected intravenously (Sigma Chem. Co.; St Louis, Mo; Lot No. 86F-0010). They were again injected with about 100 USP HCG units, at insemination time.

Group Arrangement: Inseminated does were randomly assigned to the following experimental groups:

Test Group	Dose Level (mg/kg)	Number Assigned
Control (vehicle)	0	20
Low Dose (LDT)	20	20
Mid Dose(MDT)	50	20
High Dose (HDT)	120	20

Selection of the dose range was based on the results of a pilot study (4/13/88 - 5/11/88; 3 does/group; 0, 20, 60, 100, 140, or 180 mg/kg/day; day 6-18; results in APPENDIX H of report) in which the following were observed: 1) the doses 140 and 180 mg/kg/day each caused 1 death, 2) the doses 60 and 100 mg/kg/day caused some fetal alterations [2 fetuses affected at 60 mg/kg/day with digit I of anterior appendage missing or malrotation of posterior appendage; 3 fetuses affected at 100 mg/kg/day with hydrocephalus, digit I of anterior appendage missing, or malrotation of posterior appendage.

The inseminated does were housed individually (64-69°F; 40-70% relative humidity; 12-hr light/12-hr darkness cycle). They were fed Purina Certified Rabbit Chow # 5322 (about 130 g/animal/day) . Water (routinely monitored for contaminants and interfering substances; found to have iron levels "somewhat higher than EPA guidelines") was given ad libitum.

Dosing:

A stock suspension of the test vehicle was prepared once, and was stored refrigerated, in a sealed container. All doses

were in a volume of 5 ml/kg of body weight (controls received 5 ml vehicle/kg/day). Dosing suspensions were prepared daily from the stock suspension. Dosing was based on day 6 body weight.

Analyses of Test Material Homogeneity and Stability:

Samples collected from the top, middle, and bottom of all dosing suspensions prepared on 06/02/88 were tested the next day for homogeneity of mixing. Dosing suspensions containing the low and high dose, collected at unspecified time(s), were tested over a 3-week period for test material stability. According to the results (report dated 06/14/88) of the HPLC analyses, both the homogenity (97-109% of target concentrations) and stability (97-103% of target concentration) of the test material in the working suspensions were acceptable.

Observations:

All rabbits were checked daily for mortality and clinical signs. Individual body weights were recorded on days 0, 6, 10, 14, 18, 21, and 28. Feed consumption was recorded on days 1, 6, 7, 12, 15, 19, 23, and 28. All were sacrificed by overdosage with iv barbiturate on day 28, and were subjected to Caesarean section and subsequent gross necropsy. Does found dead prematurely were necropsied.

The following maternal examinations/observations were made: Inspection of ovaries, recording of individual intact gravid uterine weight, gross examination of thoracic and abdominal cavities, recording of the number of corpora lutea, the number/placement of implantations, early/late resorptions, and the number of live/dead fetuses. The placentas were blotted dry and weighed. Tissues/organs with gross lesions were preserved in 10% modified Millonigs buffered formalin for possible future histologic evaluation.

All fetuses were removed from gravid uteri and were subjected to the following external observations: recording of individual weight, sex, and gross abnormalities (frontal, dorsal, and lateral views of head; size/position of pinnae; bulging of eyes; irregular contour and visceral herniations on torso; limbs position; number of digits on fore/hind paws). This was followed by internal observations of the abdominal and thoracic viscera and of the sex. All fetuses were finally processed for skeletal examination (modified KOH Alizarin red S method of Staples, 1964). The skull, vertebrae, ribs, pelvis, appendages, scapulae, clavicles, and sternum were examined.

Historical control data, collected from 18 studies conducted between 1982 and 1988, were provided to allow comparison with concurrent controls.

Statistical analysis

The following were statistically analyzed:

1. Maternal body weight, body weight gain (day 6-18 and 0-28), actual weight, and actual weight gain using Dunnett's test (1955, 1964).
2. Feed consumption using Dunnett's test (1955, 1964).
3. Does Reproductive Parameters:
   a. fertility index, gestation index, % viable/dead fetuses using Fisher's exact test (1934).
   b. litter size, % male fetuses, numbers of resorption sites, viable fetuses, corpora lutea, implantations, pre-/post-implantation loss, and average placental weight using Kruskal-Wallis (1952) and Dunn's test (1964).
4. Average fetal weight (combined, male, and female), using Healy's test (1972).
5. Fetal Skeletal Analysis:
   a. All fetal skeletal structures alterations using Chi-square test, Fisher's exact test, Pair-wise.
   b. Fetal/litter incidence of malformations and select variations using Fisher's exact test.

Compliance

A Statement of Confidentiality Claim (dated 03/27/90), a Statement of compliance with EPA GLP's (dated 03/21/90), and a Statement of Quality Assurance (dated 03/27/90) were provided.

C. RESULTS:

I. MATERNAL TOXICITY:

Mortality: No mortality was observed in the study.

Clinical Observations:

Increased incidences of hair loss (11 HDT does; 1 control doe) and hyperactivity (2 HDT does; 0 control doe) were observed in the HDT group ."Reddish liquid discharge", an usual index of embryonic/fetal wastage, was observed in 1 MDT (# RS2112) and 1 HDT rabbits. The former showed complete litter resorption at necropsy. The latter was found not-pregnant.

Body Weight (see Table I):

Body weights were comparable between groups, during either the pre-dosing (day 0), dosing (day 6, 10, 14, 18) or post-dosing periods (day 28).

Table I: (Group) Mean (± SE) Body Weight (kg)[a]

Group	Control	LDT	MDT	HDT
No.Does	18	19	18	17
DAY				
0	2.9[illegible] 0.06	3.04± 0.07	3.05± 0.07	2.97± 0.06
6	3.0[illegible] 0.05	3.11± 0.07	3.14± 0.07	3.06± 0.06
10	3.08± 0.05	3.12± 0.07	3.14± 0.07	2.98± 0.07
14	3.14± 0.06	3.17± 0.07	3.22± 0.07	3.07± 0.07
18	3.18± 0.06	3.19± 0.07	3.25± 0.07	3.11± 0.07
21	3.21± 0.06	3.20± 0.07	3.27± 0.07	3.14± 0.07
28	3.20± 0.07	3.25± 0.07	3.33± 0.09	3.24± 0.07

[a] data excerpted from Table I of report.

Body Weight Gain (see Table II):

A marginal and transient (day 6-10) but statistically significant loss of body weight was observed in the HDT group (HDT loss = 0.08 kg ± 0.02; control gain = 0.02 ± 0.01; p≤ 0.01). Body weight gains were comparable between all other groups during this period and between every group during both the entire dosing (day 6-18) and gestation periods (day 1-28). Corrected body weight gains (CG) were also comparable between groups.

Table II: (Group) Mean (±SE) Body Weight Gains (kg)[a]

Group	Control	LDT	MDT	HDT
( )	18	19	18	19
DAY				
6-10	0.02± 0.01	0.01± 0.01	0.00± 0.01	-0.08± 0.02**
6-18	0.12± 0.02	0.07± 0.02	0.11± 0.03	0.05 ± 0.04
0-28	0.22± 0.05	0.21± 0.04	0.28± 0.05	0.27 ± 0.04
CG	-0.14± 0.05	0.15± 0.04	-0.08± 0.05	-0.08± 0.04

[a] Data extracted from Table I of report
** p< 0.01 treated group/control group (Dunnett's test)

Food Consumption (see Table III) :

Feed consumption of the HDT group was very transiently (day 7) but significantly decreased (40% decrease below control level; p< 0.05). No other significant changes were observed.

Table III: (Group) Mean (± SE) Food Consumption (g/day)[a]

Group No. Does	Control 18	LDT 19	MDT 18	HDT 17
DAY				
1	112.1± 4.2	110.4± 5.2	114.4± 5.0	113.9± 5.0
6	118.6± 2.6+	110.5± 4.8	120.6± 3.4	117.1± 5.5
7	116.9± 3.8	110.2± 4.8	116.5±4.9	68.7 ± 10.1*
12	107.0± 6.6	101.1± 6.4	108.0± 8.2+	101.4± 9.3
15	98.7 ± 8.2+	92.7 ± 9.1	103.9± 7.1	99.5 ± 9.0
19	107.4± 5.3	94.5 ± 7.9	103.8± 7.1	104.9± 8.8
23	80.8± 9.6	85.3 ± 7.7	87.1 ± 11.8+	109.3± 6.8
28	68.7 ± 9.4	71.2 ± 5.6	85.5 ± 9.6	90.5 ± 7.7

[a] Data extracted from Table II of report
* $p < 0.05$ treated group/control group (Dunnett's test)
\+ 17 rabbits/group (excluding rabbits with feed spillage).

Gross Pathological Observations:

No treatment-related gross pathological lesions were observed. A few non dose-related lesions were observed including trichobezoar (1 control, 1 LDT, and 1 MDT does), nodular thickenings in the ribs (1 control, 2 LDT, 1 MDT, and 2 HDT does), abcess of the lung (2 MDT does), ulcer of the stomach (1 HDT doe), and gray foci in the liver (1 HDT doe). These gross lesions were stated to be commonly observed in this strain of rabbits (data excerpted from APPENDIX D; Doe Necropsy Findings).

Cesarean Section Observations (see Table IV):

Cesarean observations were based on 20 rabbits per group.

A significant decrease in placental weight (14% below control; $p < 0.05$) and a slight but significant reduction in female fetal body weight were observed in the HDT group (7% below control; $p < 0.05$). All other Cesarean observations, including the pregnancy rates, gestation indexes, average numbers of corpora lutea, implantations, percentages of pre- and post-implantation losses, resorptions, fetuses/litter, viable number of fetuses/doe, male and combined fetal body weight, and percentage of male fetuses/doe, were comparable between groups.

Table IV: Cesarean Section observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	20	20	20	20
#Animals Inseminated	18	19	19	17
Pregnancy Rate (%)	90	95	95	85
Gestation index (%)	100	100	94.7	100
No. of Litters	18	19	18	17
No. with 100% resorption	0	0	1	0
No. Died/sacrificed	0	0	0	0
No. Aborted	0	0	0	0
Mean Corpora Lutea/dam	6.9	8.6	7.3	8.6
range	2-12	5-15	4-11	3-13
Total Implantation	136	148	132	126
Mean Implantations/Dam	7.6	7.8	6.9	7.4
range	2-12	2-13	2-13	2-11
Total Live Fetuses	120	129	121	118
(%)	(99.2)	(97)	(97.6)	(98.3)
Mean Litter size	6.7	7.0	6.5	7.1
range	2-12	1-13	0-12	1-11
Total Resorptions	15	15	6	6
No. with Resorptions	7	11	5	5
(%)	(38.9)	(57.9)	(26.3)	(29.4)
Total Dead Fetuses	1	4	3	2
(%)	(0.8)	(3)	(2.4)	(1.7)
Mean Fetal Weight (gm)				
Male	37.1	35.8	37.5	35.3
Female	36.1	34.6	35.9	33.7+
Combined	37	35.8	36.8	35.2
Preimplantation Loss(%)	5.4	12.6	12.4	15.5
Postimplantation Loss(%)	11.2	14.2	9.9	7.7
Sex Ratio (% Male)	47.2	57.1	53.6	44.4
Placental Weight(g)	5.8	5.0	5.5	5.0*

[a] = Data extracted from Table III and IV of report
+ p< 0.05 treated/control (Healy"s test)
* p< 0.05 treated/control (Kruskal-Wallis test)

II. DEVELOPMENTAL TOXICITY:

Fetal observations were based on 120, 129, 121, and 118 fetuses (18 control, 19 LDT, 19 MDT, and 17 HDT litters). All fetuses were examined for gross external, visceral and skeletal alterations.

External Alterations (see TABLE V):

The following alterations were observed in 2 HDT litters: rudimentary/missing tail [(9 fetuses/doe # RS2038; 1 fetus/doe RS2087), cleft palate (2 fetuses/doe # RS2038), and "anal imperforate and abnormal external genital pappilla" (1 fetus/doe # RS2038). The observed fetal incidences for tail rudimentary/missing were higher than historical control incidences. The incidences for cleft palate were not. Historical control incidences for "anal imperforate and abnormal external genital pappilla" were not reported. The statistical significance of the tail alterations was not evaluated.

One control and 1 HDT fetuses (from doe RS2072) showed downward and inward malrotation of anterior appendages.

Table V: External Examinations

Observations	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	120(18)	129(19)	121(19)	118(17)
#pups(litters) affected	1(1)	0(0)	0(0)	11(3)
Fetal (Litter) Incidences:				
Anterior/posterior Appendages Malrotation:	1(1)	0(1)	0(0)	1(1)
Tail Rudimentary/Missing	0(0)	0(0)	0(0)	10(2)
% Fetuses(Litters) affected				8(11.8)
Anal imperforate;abnormal external genital papilla				1(1)
Cleft Palate				2(1)
% Fetuses(Litters) affected				1.7(5.9)

Data excerpted from Table V of report.
Historical Control Incidences: Litter(%), Fetal(%), and study #:
(data excerpted from APPENDIX I):
Tail rudimentary/kinked: Litter: 2(13.3); Fetal: 2(1.8); #12 (90 fetuses; 15 litters).
Cleft Palate: Litter: 1(6.7) & 1(7.1); Fetal:1 (1.1) & 1(1.3); #1 (77 fetuses; 14 litters) & #9 (94 fetuses; 15 litters).

Visceral Alterations (see Table VI):

Several visceral alterations were observed in 1 control, 2 LDT, 4 MDT, and 4 HDT litters. These included 1 accessory spleen (control fetus/doe #RS2078), 2 accessory adrenals (LDT fetus/doe #RS2088 and MDT fetus/doe #RS2101), 1 displaced adrenal (HDT fetus/doe # RS2087), 1 rudimentary ovary (MDT fetus/doe #RS2036) 2 renal pelvic dilation (MDT fetus/doe #RS2096 and HDT fetus/doe # RS2038), 2 abnormal major blood vessels (MDT fetus/doe # RS2098 with subclavian retroesophageal deviation and HDT fetus/doe # RS2069 with acessory vessel off the left carotid), and 2 hydrocephaluses (LDT fetus/doe #RSRS2033 and HDT fetus/doe #RS2103). These alterations were stated to be commonly observed in the rabbit strain used.

Table VI: Visceral Examinations

Observations	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	120(18)	129(19)	121(19)	118(17)
#pups(litters) affected	1(1)	2(2)	4(4)	4(4)
Fetal Incidences:				
Spleen (Acessory)	1	0	0	0
Adrenal (Acessory)	0	1	1	0
Adrenal (Displaced)	0	0	0	1
Ovary (rudimentary)	0	0	1	0
Kidney (abnormal shape)	0	0	1	1
Major vessel (abnormal)	0	0	1	1
Hydrocephalus	0	1	0	1

Data excerpted from Table V of report.

Skeletal Alterations (see Table VII):

Skeletal alterations were widespread and affected 94, 95, 100, and 100% of the control, LDT, MDT, and HDT litters. The respective number of pups affected were 66 controls, 93 LDTs, 99 MDTs, and 115 HDTs.

Fetal incidences of the following alterations were significantly increased in the HDT group:

1. Skull bones incomplete ossification (63 HDT/34 control fetuses; $p \leq 0.01$; 14 HDT/14 control litters).
2. Enlarged fontanelles (63 HDT/33 control fetuses; $p \leq 0.01$; 14 HDT/14 control litters).

Both litter and fetal incidences of the following alterations were increased in the HDT group (fetal incidences increases were statistically significant; litter incidences

increases were not statistically analyzed):

1. Extra ribs: (26 HDT/11 control fetuses; p≤ 0.05; 11 HDT/9 control litters).
2. Vertebrae caudal:
   a) arch fusion (8 HDT/0 control fetuses; p≤ 0.01; 2 HDT litters),
   b) arch missing (12 HDT/0 control fetuses; p≤ 0.01; 4 HDT litters),
   c) abnormal arch (9 HDT/0 control fetuses; p≤ 0.01; 2 HDT litters),
   d) arch malpositioned (9 HDT/0 control fetuses; p≤ 0.01; 3 HDT litters),
   e) abnormal centra (12 HDT/0 control fetuses; p≤ 0.01; 3 HDT litters),
   f) centra malpositioned (9 HDT/0 control fetuses; p≤ 0.05; 3 HDT litters), and
   g) centra missing (10 HDT/0 control fetuses; p≤ 0.01; 4 HDT litters).
3. Incomplete ossification of:
   a) 1st sternebrae (9 HDT/1 control fetuses; p≤0.05; 8 HDT/ 1 control litter,
   b) 2nd sternebra (46 HDT/13 control fetuses; p≤0.01; 14 HDT/6 control litters), and
   c) posterior talus (28 HDT/9 control fetuses; p≤0.01; 6 HDT/5 control litters).
4. unossification of:
   a) 5th sternebra (21 HDT/8 control fetuses; p≤ 0.05; 8 HDT/6 control litters), and
   b) posterior phalanges (33 HDT/1 control fetuses; p≤ 0.01; 12 HDT/1 control litters).

Both litter and fetal incidences of the following alterations were increased in a dose-related fashion in the MDT and HDT groups (fetal incidences increases were statistically significant; litter incidences increases were not statistically analyzed):

1. Incomplete ossification of:
   a) pubis bone of pelvis (41 HDT/15 MDT/3 control fetuses; p≤ 0.01; 11 HDT/5 MDT/3 control litters),
   b) anterior phalanges (25 HDT/12 MDT/2 control fetuses; p≤ 0.01 and 0.05; 7 HDT/5 MDT/1 control litters), and
   c) posterior phalanges (52 HDT/22 MDT/9 control fetuses; p≤ 0.01 and 0.05; 14 HDT/9 MDT/6 control litters).

2. Irregular scapular spinous process (57 HDT/31 MDT/4 control fetuses; p≤ 0.01; 13 HDT/11 MDT/3 control litters).

Table VII: Skeletal Examinations

Observations[a]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	120(18)	129(19)	121(18)	118(17)
#pups(litters) affected	66(17)	93(18)	99(18)	115(17)
Fetal(litter) Incidences:				
SKULL:				
Bones incomp. ossif.:	34(14)	31(9)	47(15)	63**(14)
Fontanelle enlarged:	33(13)	29(8)	44(15)	63**(14)
EXTRA RIBS:	11(9)	26(12)	18(8)	26*(11)*
VERTEBRAE (CAUDAL):				
Arches fused:	0	0	0	8**(2)
Arches missing:	0	0	0	12**(4)
Arch abnormal:	0	0	0	9**(2)
Arch malposition:	0	0	0	9**(3)
Centra abnormal:	0	0	0	12**(3)
Centra malposition:	0	0	0	9*(3)
Centra missing:	0	0	0	10**(4)
PELVIS PUBES: Incomplete ossification (IO):	3(3)	7(4)	15**(5)	41**(11)
STERNEBRAE:				
1st IO:	1(1)	11*(6)	4(2)	9*(8)
2nd IO:	13(6)	19(9)	15(9)	46**(14)
5th unossified (UO):	8(6)	20(9)	11(7)	21*(8)
SCAPULAR:				
Irreg. spinous process:	4(3)	11(7)	31**(11)	57**(13)
APPENDAGES:				
IO Anterior metacarpals:	6(6)	19*(9)	10(5)	6(3)
IO Anterior phalanges:	2(1)	6(4)	12*(5)	25**(7)
IO Posterior talus:	9(5)	11(8)	19(8)	28**(6)
UO Posterior phalanges:	1(1)	3(3)	8(6)	33**(12)
IO Posterior phalanges:	9(6)	16(7)	22*(9)	52**(14)

Data excerpted from Table VI (Tabulation of groups mean skeletal findings with accompanying statistical evaluation), Table VII (Incidences summary of malformations and selected variations), and APPENDIX F (Individual skeletal findings) of report.

[a] alterations of comparable incidences between treated group(s) and control group are not included.

* p< 0.05 treated group/control group (Pair-wise comparisons)

** p< 0.01 treated group/control group (Pair-wise comparisons)

D. Author's Discussion/Conclusions:

The following are directly quoted from the Discussion and Conclusion section of the report:

"MEB 6447 was tested in the American Dutch Rabbit to assess the potential for the test article to promote embryotoxicity, fetotoxicity, and/or teratogenicity in this species".

"There was a statistically significant ($p \leq 0.01$) loss in body weight for the 120 mg/kg group during the first 4 days of treatment. Food consumption was significantly reduced for this group when measured on day 7 and returned to normal by day 12. Body weight and food consumption were unaffected at the lower doses".

Overall reproductive efficiency was considered normal for all treatment groups. In addition, there was no evidence of embryotoxicity (increase resorption) for any group. The test article, at 120 mg/kg, promoted a significant reduction in female fetal weights and in median placental weight. Corresponding to this apparent fetotoxic effect was a significant delay in the ossification of several skeletal elements. The 120 mg/kg dose also produce a significant increase in skeletal malformation involving the caudal vertebrae and significantly increased the occurence of a specific but relatively common skeletal variation, irregular spinous process of the scapula. This variation was also significantly increased for the 50 mk/kg group. Analyses of overall malformations revealed a statistically significant increase in the fetal incidence of malformations for the 120 mg/kg group.

In conclusion, Meb 6447, administered orally to gravid rabbits, produces clear evidence of maternal toxicity (reduced food consumption and body weight during treatment) at a dose of 120 mg/kg. All doses were free of any potential to promote embryotoxicity(increased resorption). Fetotoxicity, as evidenced by a significant increase in delayed ossification of skeletal elements linked to a decrease in fetal weight, as well as significantly increased malformations and skeletal variation, occured at a dose of 120 mg/kg and a significant increase in skeletal variation was observed at a dose of 50 mg/kg. Therefore, a dose of 50 mg/kg is considered the no effect level (NOEL) for maternal toxicity and a dose of 20 mg/kg is considered the NOEL for developmental toxicity).

Reviewer's Discussion/Conclusions:

The developmental toxicity potential of MEB 6447 [Triadimefon; Bayleton; 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone] was studied by dosing artificially inseminated American Dutch rabbits, by oral gavage,

with 0, 20, 50, or 120 mg/kg/day, from gestation day 8 to 18.

No treatment-related mortality was observed.

Maternal toxicity was not observed at either the LDT or the MDT. A marginal (80 g), transient (day 6-10), but statistically significant loss of body weight and a very transient (day 7) but statistically significant decrease in feed consumption (day 7) were observed in the HDT group. The biological significance of the former effect is equivocal since it only occured during the first four days of dosing and was unaccompanied by any concommittant decrease in body weight. The latter effect was too short lasting to have any real biological significance.

No treatment-related gross pathological alterations were observed.

The pregnancy rates, gestation indexes, average numbers of corpora lutea, implantations, percentages of pre- and post-implantation losses, resorptions, fetuses/litter, viable number of fetuses/doe, male and combined fetal body weight, and percentage of male fetuses/doe, were comparable between groups.

Developmental Toxicity was observed at both the MDT and HDT.

Dose-related increases (MDT and HDT) in both the fetal and litter incidences of several skeletal alterations were observed at the MDT. These included incomplete ossification of the pelvic pubes and the anterior and posterior phalanges, and irregular spinous process formation. The increases in incidence of each fetal alteration was statistically significant. The litter incidences increases were not statistically analyzed.

Statistically significant decreases in placental weight and female fetal body weight, and statistically significant increases in the incidences of many external and skeletal alterations were observed at the HDT. External alterations consisted of rudimentary or missing tails (2 litters; 10 fetuses). Skeletal alterations with only a fetal incidence increase included incomplete ossification of the skull bones and enlargement of the fontanelles. Those with increases in both fetal and litter incidences included extra ribs, malformations of the arches and centra of the caudal vertebrae, incomplete/non ossification of the 1st, 2nd, 5th sternebrae, posterior talus, and posterior phalanges. Those with dose-related increases in both fetal and litter incidences were mentioned earlier (MDT associated alterations). The increases in incidence of each fetal alteration was statistically significant. Except for extra ribs, the litter incidences increases were not statistically analyzed.

Based on the results of the study, the maternal NOEL was 50 mg/kg/day (MDT). The maternal LOEL was 120 mg/kg/day (HDT), based

on a marginal and transient body weight loss. The developmental NOEL was 20 mg/kg/day (LDT). The developmental LOEL was 50 mg/kg/day based on dose-related increases (MDT and HDT) in incidences of incomplete ossification of the pelvic pubes and the anterior and posterior phalanges, and of irregular spinous process formation.

This study meets or exceeds the criteria for potential teratogenic effect [40 CFR 158.34; criteria #5 (when compared with concurrent controls, treated animals show a dose-related increase in malformations (or deaths) on a litter basis in the absence of significant maternal toxicity at the same dose level).

D. Study Deficiencies:

1. The expiration date of the Triadimefon batch used in this study was 06/18/88 (pp 38 of report). The dates of artificial insemination (gestation day 0) were from 05/31/88 to 06/03/88 (5 rabbits/dose group/day; pp 153 of report) and the last dosing day (day 18) was from 06/18/88 to 06/21/88. According to these dates, only 5 animals/dose group would have received good test material for the entire dosing period (day 6-18). The remaining animals were given out of date test material, for 1-3 days. The registrant is required to clarify this matter since 1-3 days of misdosing might have severe impact on the integrity of a developmental toxicity study. The registrant may provide information demonstrating that the test material was stable up to 06/21/88.

2. The historical control data on fetal alterations is inadequate. Alterations incidences are mostly based on one or two studies. For example, tail malformation and missing vertebrae caudal centra were based on a single study (study #12; 111 fetuses; 15 litters).

3. Except for extra ribs, the litter incidences increases were not statistically evaluated. The registrant is requested to submit statistical evaluations of the following litter incidence increases: external alteration of the tail (HDT group), incomplete ossification of the pelvic pubes and the anterior and posterior phalanges, and irregular spinous process formation (MDT and HDT), and malformations of the arches and centra of the caudal vertebrae, incomplete/non ossification of the 1st, 2nd, 5th sternebrae, posterior talus, and posterior phalanges (HDT). Potential teratogenic effects should be evaluated on both a fetal and litter bases.

E. Core Classification: Core Supplementary Data. Upgradable to

Core Minimum upon satisfactory response to deficiencies Nos. 1 and 3.

F. Risk Assessment: A concern is suggested by the results of this study, and a margin of exposure should be estimated for both dietary and occupational exposures.

XI. REFERENCES

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