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

007200

MAY 23 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Terbuconazole (HWG-1608; Folicur) Fungicide - Review of Toxicology Data Submitted by the Registrant to Support Registration of this Chemical for Terrestrial, Nonfood Use

TOX Chem No.: 463P
Project Nos.: 8-1218;8-1043;8-1270
MRID Nos.: 407009-01 to 407009-55; 408215-00;408164-01

FROM: Yiannakis M. Ioannou, Ph.D., Acting Section Head
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: Susan Lewis, Acting PM 21
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Marcia van Gemert, Ph.D., Acting Chief
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

Registrant: Mobay Corporation, Kansas City, MO

Action Requested

Review toxicology data submitted in support of registration of terbuconazole for terrestrial, nonfood use.

Review of Data and Recommendations

Toxicology Branch II has completed the review of all toxicology studies submitted by the Registrant on terbucomazole (HWG 1608; Folicur). Studies reviewed (accompanied by major findings) were as follows:

Acute Oral Toxicity - Rats (MRID No. 407009-17)

HWG 1608, technical, 97.1% ai

$AOLD_{50}$ (Fasted) male rats > 5000 mg/kg

- Female rats - 3933 mg/kg (95% CL 3316.1-5665.2 mg/kg)

$AOLD_{50}$ (Unfasted) male rats - 4264 mg/kg (95% CL 3952.3-5330.2 mg/kg)

- Female rats - 3352 mg/kg (95% CL 2341.4-3977.5 mg/kg)

Toxicity Category IV (fasted males); III (fasted and unfasted females and unfasted males)

Classification - Core-Minimum

Acute Oral Toxicity - Mice (MRID No. 407009-17)

HWG 1608 technical, 97.1% ai

$AOLD_{50}$ - Male mice 1615 mg/kg (95% CL 1057.2-2179.6 mg/kg)

- Female mice 3023 mg/kg (95% CL 2127.4-5072.7 mg/kg)

Toxicity Category III

Classification - Core-Minimum

Acute Oral Toxicity - Rabbits (MRID No. 407009-17)

HWG 1608 technical, 97.1% ai

$AOLD_{50}$ - Males and females > 1000 mg/kg

Toxicity Category - Not assigned

Classification - Core-Supplementary

Acute Dermal Toxicity - Rats (MRID No. 407009-17)

HWG 1608 technical, 97.1% ai

$ADLD_{50}$ Males and females > 5000 mg/kg

Toxicity Category III

Classification - Core-Supplementary

Acute Inhalation Toxicity - Rats (MRID No. 407009-17)

HWG 1608 technical, 97.1% ai

$AILC_{50}$ - 4-hour exposure > 818 mg/m^3

- Five daily 6-hour exposures > 240 mg/m^3

Toxicity Category - Not established

Classification - Core-Supplementary

Acute Inhalation Toxicity - Rats (MRID No. 407009-22)

HWG 1608 technical, 96.2% ai

$AILC_{50}$ (aerosol) > 371 mg/m^3 for males and females

(dust) > 5093 mg/m^3

Toxicity Category - Aerosol, II; dust, III

Classification - Core-Guideline

Acute Intraperitoneal Toxicity - Rats (MRID No. 407009-17)

HWG 1608 technical, 97.1% ai

LD_{50} - Males: 751 mg/kg (95% CL 670.9-826.8 mg/kg)

Females: 395 mg/kg (95% CL 329.9-430.0 mg/kg)

Category of Toxicity - Not applicable

Classification - Not applicable (although study is acceptable)

Eye Irritation - Rabbits (MRID No. 407009-17)

HWG 1608 technical, 97.1% ai

Slight eye irritation was observed, reversible by 48 hours.

Toxicity Category III

Classification - Core-Minimum

Eye Irritation - Rabbits (MRID No. 407009-25)

HWG 1608 technical, 96.3% ai

Mild irritation was observed, reversible by day 7

Toxicity Category III

Classification - Core-Minimum

Skin Irritation - Rabbits (MRID No. 407009-17)

HWG 1608 technical, 97.1% ai

No skin irritation was seen - Nonirritant

Toxicity Category IV

Classification - Core-Minimum

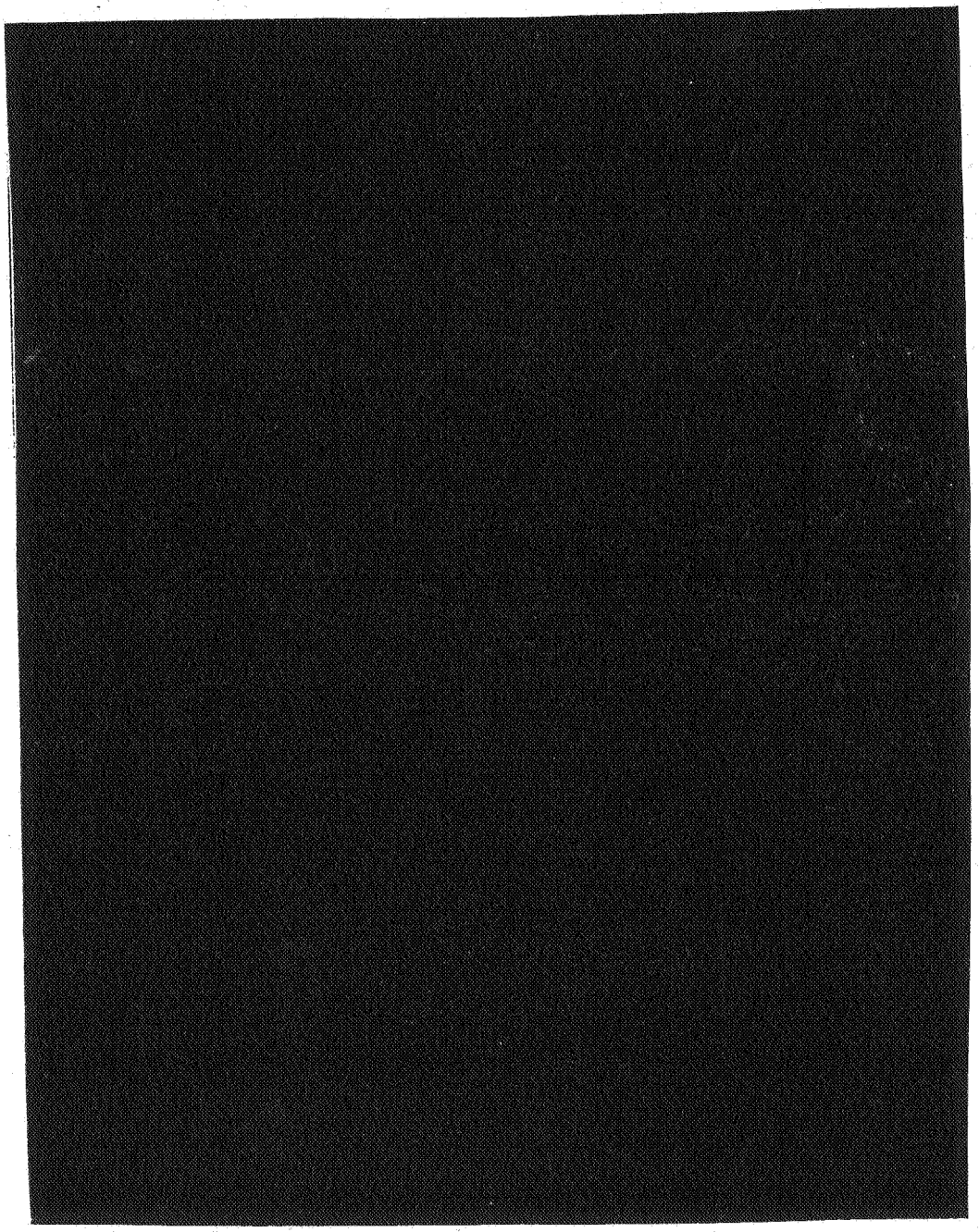
Skin Sensitization - Guinea Pigs (MRID No. 407009-28)

HWG 1608 technical, 97.4% ai

Not a sensitizer at dose levels tested. Higher dose levels need to be tested.

Classification - Core-Supplementary

[redacted]



[redacted]

Subacute Feeding Study - Rats (MRID No. 407009-32)

HWG 1608 technical, 97.0% ai

Rats were exposed (oral gavage) to terbuconazole (0, 30, 100, or 300 mg/kg/day) for 28 days followed by 28 days recovery period. LEL = 100 mg/kg/day (based on changes in hematology and clinical chemistry parameters). NOEL = 30 mg/kg/day.

Classification - Core-Supplementary

Subacute Inhalation Toxicity - Rats (MRID No. 407009-38)

HWG 1608 technical, 96.2% ai

Rats were exposed (head/nose only) to aerosol concentrations (analytical) of 0, 1.2, 10.6, or 155.8 mg/m^3 of terbuconazole, 6 hours/day for 15 days.

LEL = 155.8 mg/m^3 (based on piloerection and induction of liver enzymes, O-demethylase and N-demethylase).
NOEL = 10.6 mg/m^3

Classification - Core-Minimum

Range-Finding Study - Dogs (MRID No. 407009-35)

HWG 1608 technical, 93.4% ai

Dogs were fed diets containing 0, 500 or 5000 ppm of terbuconazole for 30 days.

LEL = 5000 ppm (based on increased alkaline phosphatase)

NOEL = 500 ppm

Classification - Core-Supplementary

21-Day Dermal Toxicity - Rabbits (MRID No. 407009-37)

EWG 1608 technical, 97.1% ai

Rabbits were exposed (dermally) to 0, 50, 250 or 1000 mg/kg/day of terbuconazole for 21 days.

No significant systemic effects were seen at any dose level.

NOEL > 1000 mg/kg/day (limit test)

Classification - Core-Guideline

[redacted]

90-Day Oral Toxicity - Dogs (MRID No. 407009-34)

EWG 1608 technical, 93.4% ai

Beagle dogs (4/sex) were fed diets containing terbuconazole at dose levels of 0, 200, 1000 or 5000 ppm for 13 weeks.

LEL = 1000 ppm (based on decreased body weight gains, decreased food consumption and increased N-demethylase activity).
NOEL = 200 ppm

Classification - Core-Minimum

90-Day Oral Toxicity - Rats (MRID No. 407009-30)

EWG 1608 technical, 93.4% ai

Rats (10/sex) were fed diets containing terbuconazole at dose levels of 0, 100, 400 or 1600 ppm for 13 weeks.

LEL = 1600 ppm (based on decreased body weight gains);
NOEL = 400 ppm

Classification - Core-Minimum

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12-Month Chronic Toxicity - Dogs (MRID No. 407009-40)

HWG 1608 technical, 96.9% ai

Dogs (4/sex) were fed diets containing terbuconazole at dose levels of 0, 40, 200 or 1000/2000 ppm for 52 weeks.

LEL = 200 ppm (based on ocular lesions - lenticular and corneal opacities - and hepatic toxicity - lobulation/swelling, increased iron-containing pigments, and lipids).
NOEL = 40 ppm

Classification - Core-Minimum

Chronic Toxicity/Oncogenicity - Rats (MRID No. 407009-39)

HWG 1608 technical, 95% ai

Rats (50/sex) were fed diets containing terbuconazole at dose levels of 0, 100, 300 or 1000 ppm for 2 years. Chronic toxicity LEL = 300 ppm (based on body weight depression, hematological changes, decreased hemoglobin, hematocrit, MCV and MCHC, and increase in liver enzymes). NOEL = 100 ppm.

An increased incidence of C-cell adenoma/carcinoma/hyperplasia was seen in the thyroid of male rats of the MDT and HDT. This increased incidence was within the historical control range and was not considered an oncogenic response.

Terbuconazole was not oncogenic to male or female rats at the dose levels tested.

Classification - Core-Minimum

Dose Range-Finding for Oncogenicity - Mice (MRID No. 407009-33)

HWG 1608 technical, 96.9% ai

Mice were fed diets containing terbuconazole at levels of 0, 500 or 2000 ppm for 8 weeks or at levels 0, 125, 500 or 2000 ppm for 5 days. Systemic toxicity was observed in the mid- and high-dose groups of the 8-week study and consisted of increased absolute and relative liver weight associated with increased liver

necrosis, vacuolization, degeneration, and lipidosis and increased bilirubin; increased pigment deposition in the spleen; increased heart relative weight; increased round cell infiltrates in kidneys; and increased lipid concentrations and sinus dilation in adrenals. In the 5-day study, terbuconazole induced microsomal enzymes at all dose levels tested.

Based on these data, the dose levels of 0, 20, 60, and 180 ppm were selected for the main oncogenicity study in mice.

Classification - Core-Supplementary

Oncogenicity Study - Mice (MRID No. 407009-41)
HWG 1608 technical, 95% ai

Mice (50/sex) were fed diets containing terbuconazole at dose levels of 0, 20, 60 or 180 ppm for 21 months.

Slight toxicity in the form of increased bilirubin and liver weight, associated with centrilobular and periportal vacuolation and lipid deposition, increased adrenal cortical cell size and hyperplasia and increased pancreatic interstitial edema, was observed in male and female mice of the high dose groups. Based on these findings, however it appears that the HDT (180 ppm) was not high enough to approximate the MTD for this study.

Terbuconazole was not oncogenic in mice at the dose levels tested.

Classification - Core-Supplementary

Dose Range-Finding Teratogenicity - Rabbit (MRID No. 407009-44)
HWG 1608 technical, 98.2% ai

Rabbits were exposed (oral gavage) to terbuconazole at dose levels of 0, 30, 100 or 300 mg/kg/day, on days 6 to 18 of gestation.

Maternal LEL = 300 mg/kg/day (based on reduced body weight gain and preimplantation losses). NOEL = 100
NOEL = 100 mg/kg/day.

Dose levels selected for the main teratology study in rabbits: 0, 10, 30 and 100 mg/kg/day

Classification - Core-Supplementary

Embryotoxicity (Teratogenicity) - Rabbit (MRID No. 407009-45)

HWG 1608 technical, 98.2% ai

Terbuconazole, at dose levels of 0, 10, 30 or 100 mg/kg/day, was administered (oral gavage) to rabbits on days 6 to 18 of gestation.

Maternal LEL = 100 mg/kg/day (based on depression of body weight gains on days 6 to 18 of gestation, and decrease in food consumption). NOEL = 30 mg/kg/day.

Developmental LEL = 100 mg/kg/day (based on increased post-implantation losses, early and late resorptions). NOEL = 30 mg/kg/day

Classification - Core-Minimum

Dose Range-Finding Teratogenicity - Rat (MRID No. 407009-42)

HWG 1608 technical, 98.2% ai

Rats were exposed orally to terbuconazole at dose levels of 0, 10, 30 or 90 mg/kg/day, on days 6 to 15 of gestation. Slight maternal toxicity was seen at the high dose (depression of body weight gains during dosing); developmental toxicity (increase in resorptions) was also seen at the high dose tested.

Dose levels selected for main teratology study in rats: 0, 30, 60, and 120 mg/kg/day.

Classification - Core-Supplementary

Embryotoxicity (Teratogenicity) - Rat (MRID No. 407009-43)

HWG 1608 technical, 98.3% ai

Rats were exposed (oral gavage) to terbuconazole at dose levels of 0, 30, 60 or 120 mg/kg/day on days 6 to 15 of

gestation. Maternal LEL = 60 mg/kg/day (based on elevated absolute and relative mean liver weights). NOEL = 30 mg/kg/day. Developmental LEL = 60 mg/kg/day (based on delayed ossification of thoracic, cervical, and sacral vertebrae, sternum, fore- and hind limbs and an increase in supernumerary ribs). NOEL = 30 mg/kg/day.

Classification - Core-Minimum

Special Study: Maternal Toxicity (Teratogenicity) - Mice (MRID No. 408215-00).

HWG 1608 technical, 97.4% ai

Mice were exposed to terbuconazole dose levels of 0, 10, 20, 30 or 100 mg/kg/day on days 6 to 15 of gestation.

Maternal toxicity LEL = 20 mg/kg/day (based on reduction of hematocrit); NOEL = 10 mg/kg/day. These data were used to establish the maternal toxicity LEL and NOEL in the main teratogenicity study with mice (Study No. T5021859).

Classification - Acceptable

Embryotoxicity (Teratogenicity) - Mice (MRID No. 408215-00)

HWG 1608 technical, 93.6% ai

Mice were exposed (by oral gavage) to terbuconazole at dose levels of 0, 10, 30 or 100 mg/kg/day on days 6 to 15 of gestation.

Maternal toxicity was not observed in this study. However, based on the results of another study titled "Maternal Toxicity" (Study No. T5025712 dated March 9, 1988) a NOEL of 10 mg/kg/day was established and a LEL of 20 mg/kg/day (reduction in hematocrit). Developmental LEL = 30 mg/kg/day (based on increased number of runts in this study); NOEL = 10 mg/kg/day.

Classification - Core-Minimum

Two-Generation Reproduction - Rat (MRID No. 407009-46)

HWG 1608 technical, 95.2% ai

Rats were exposed to terbuconazole at dietary concentrations of 0, 100, 300 or 1000 ppm.

Maternal systemic toxicity LEL = 1000 ppm (based on depressed body weights and food consumption, increased spleen hemosiderosis, and decreased liver and kidney weights); NOEL = 300 ppm. Reproductive LEL = 1000 ppm (based on neonatal birth weight depression); NOEL = 300 ppm.

Classification - Core-Minimum

Mutagenicity Studies

A. Gene Mutations

Salmonella/Microsome Point Mutation (MRID Nos. 407009-47 and 407009-48)

HWG 1608 technical, 96.6% ai

Terbuconazole was not mutagenic in S. typhimurium strains with or without activation at dose levels ranging from 37.5 to 2400 ug/plate.

Classification - Acceptable

CHO-HGPRT Forward Mutation (in vitro) (MRID No. 407009-49)

HWG 1608 technical, 96.6% ai

Terbuconazole was not mutagenic in CHO cells with or without activation, at dose levels ranging from 12.5 to 200 ug/mL. The highest dose tested did not produce a high level of cytotoxicity.

Classification - Unacceptable

B. Structural Chromosome Aberrations

Dominant Lethal Assay in Mice (MRID No. 407009-50)

HWG 1608 technical, 93.5% ai

Terbuconazole was found to be negative for dominant lethal mutations in mice at a dose level of 2000 mg/kg.

Classification - Unacceptable (only one dose level was used and no positive control).

Micronucleus Test in Mice (MRID No. 407009-51)

HWG 1608 technical, 95.3% ai

Terbuconazole at dose levels of 200, 500, or 2000 mg/kg, was not genotoxic in the mouse micronucleus test.

Classification - Acceptable

Sister Chromatid Exchange Assay in CHO Cells (MRID No. 407009-52)

HWG 1608 technical, 96.5% ai

Terbuconazole, at dose levels ranging from 4 to 30 ug/mL (without activation) or 15 to 120 ug/mL (with activation), did not increase sister chromatid exchange.

Classification - Acceptable

In Vitro Cytogenetics (Human Lymphocytes) MRID No. 407009-53)

HWG 1608 technical, 96.5% ai

Terbuconazole, at concentrations ranging from 30 to 300 ug/mL was not mutagenic in human lymphocytes, with or without activation.

Classification - Acceptable (with metabolic activation); Unacceptable (without metabolic activation-highest dose tested did not induce cytotoxicity-).

C. Other Cytotoxic Tests

DNA Damage and Repair (E. coli) (MRID No. 407009-55)

HWG 1608 technical, 97.1% ai

Terbuconazole, at concentrations ranging from 625 to 10000 ug/plate (with or without activation), did not produce an alteration in DNA and was not mutagenic under the conditions of the test.

Classification - Unacceptable (no growth inhibition zone was demonstrated in either strain (-/+); bacterial cell densities were not reported).

Unscheduled DNA Synthesis - Rat Primary Hepatocyte Assay (MRID No. 408164-02)
HWG 1608 technical, 96.5% ai

Terbuconazole at concentrations ranging from 0.504 to 25.2 ug/mL, did not increase unscheduled DNA synthesis in rat hepatocytes.

Classification - Acceptable

Based on the evaluation of all data submitted by the Registrant, the following Guideline toxicology studies have been identified as data gaps:

Technical (HWG 1608):

- Acute Dermal LD_{50} in Rats, and

- Skin Sensitization in Guinea Pigs.

- Mouse Oncogenicity

The above studies have been submitted by the Registrant but found to be of Core-Supplementary classification. Both acute studies can be upgraded however (to Core-Minimum) if the Registrant provides us with the requested additional data and/or clarifications. The Mouse Oncogenicity study should be repeated since the MTD was not reached in the submitted study.

- Metabolism (rat)

Label Considerations

Based on the review of the acute toxicity studies, the following changes should be made on the proposed labels:
Technical - The precautionary signal word "CAUTION" should be replaced with the signal word "WARNING" and the precautionary statement "May be fatal if. . ." should appear on the label.
Formulations - The word "Corrosive" should appear on the label.

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EPA: 68D80057
DYNAMAC No. 147-J1
April 6, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Heimann, K. G. T3015986 and T4215927 HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, conducted for Mobay Corp., Stilwell, KS; dated October 13, 1983.) Accession/MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Prieto for
Date: 4.6.89

007200

1. CHEMICAL: Terbuconazole; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-01; HWG 1608.

2. TEST MATERIAL: HWG 1608, from batch No. 16001/83 was not described; the purity was reported to be 97.1%.

3. STUDY/ACTION TYPE: Acute oral toxicity in rats.

T301596 and T401579

4. STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, conducted for Moba Corp., Stilwell, KS; dated October 13, 1983.) Accession/MRID No. 407009-17.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-6-89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: 4/6/89

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: Roman J. Pienta
Date: 4/6/89

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H-7509C)

Signature: J. M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head
Section I
Toxicology Branch II
(H-7509C)

Signature: _____
Date: _____

7. CONCLUSIONS:

CORE Classification: Minimum.

LD_{50} Values, Fasted Rats: Male rats: >5000 mg/kg. Female rats: 3933 mg/kg (3316.1-5665.2 mg/kg).

LD_{50} Values, Unfasted Rats: Male rats: 4264 mg/kg (3952.3-5330.2 mg/kg). Female rats: 3352 mg/kg (2341.4-3977.5 mg/kg).

Toxicity Category: IV (fasted males); III (fasted and unfasted females and unfasted males).

8. SUMMARY:

Groups of fasted (16 hours) and unfasted male and female Wistar rats (age not specified; Winkelmann, Borchen, FRG), weighing 160 to 200 g, received single oral gavage administrations of HWG 1608 as outlined below:

Dose Level (mg/kg)	No. of Males	No. of Females	No. of Males	No. of Females
500	--	--	5	5
1000	5	5	5	5
2500	5	5	--	5
3150	--	5	--	--
3550	--	5	10	5
3750	--	--	10	--
4000	--	--	5	--
4250	--	--	--	5
4500	5	--	--	5
5000	10	5	5	--

No explanation was given for the use of different doses or the increased number of males at selected doses.

The test material was prepared in distilled water containing Cremophor EL (5 drops Cremophor EL in 10 mL of water) and was administered in a dosing volume of 1 mL/100 g body weight. Animals were observed at unspecified intervals over a 14-day period for clinical signs and mortality; tabulated results suggest, however, that animals were observed, at minimum, 4 and 5 hours postdosing and daily thereafter. No information was provided on body weight determinations. Animals found dead and survivors at day 14 received gross necropsies. The LD_{50} values were calculated according to the A. P. Rosiello, J. M. Essigmann, and G. N. Wogan method.

Results for fasted animals are presented in Appendix A and indicated that 2 of 10 males died between days 7 and 11 and 4 of 5 females died between days 2 and 8 in the high-dose group. No other deaths were reported in the male rats; however, two of five and one of five females in the 3550 and 3150 mg/kg treatment groups died between days 4 and 8. No toxic signs were reported for either the males or females exposed to 1000 mg/kg HWG 1608. Toxic signs were reported for both sexes in the higher treatment groups and included disturbed behavior and motility, dyspnea, staggering, spastic gait, prostration, and moulting. Additional signs seen in males only were stiff posture, emaciation, and increased urination. Gross necropsies on the animals that died on study included patchy and distended lungs, and patchy livers and spleens for both sexes. Lentil-sized yellow areas on the liver were reported for males only and glandular reddened stomachs were reported only for females. No treatment-related lesions were seen in survivors at the 14-day necropsy. Based on graphic analyses presented in the report, the acute oral (fasted) LD_{50} values for males was > 5000 mg/kg and for females, 3933 mg/kg (3316.1 to 5665.2 mg/kg).

Results for unfasted rats are presented in Appendix B. As shown, percent mortality for both sexes was dose related; results further indicated that the test material was more toxic in the unfasted animals. In males, deaths occurred between days 4 and 7; percent mortality ranged from 30% in the group exposed to 3750 mg/kg of the test material to 80% in the high-dose group (5000 mg/kg). Percent mortality in females ranged from 20% at 2500 mg/kg to 100% at 4500 mg/kg and occurred between days 3 and 9. No deaths were observed in males administered 500, 1000, or 3550 mg/kg or in females receiving 500 or 1000 mg/kg. Toxic signs similar to those reported for fasted animals were observed in the males of all treatment groups with the exception of the low-dose group (500 mg/kg); weight loss, not previously reported for fasted males, was seen in unfasted males only. Toxic signs reported for females of all dosed groups, except the 500-mg/kg group, were also similar to those observed in fasted females; however, in this study, the unfasted females also exhibited reduced reflexes. In contrast to the earlier findings in fasted animals, the above

clinical signs were seen at doses ≥ 1000 mg/kg; in fasted animals, no clinical signs were reported for doses ≤ 1000 mg/kg.

Necropsy findings for unfasted rats that died on study were generally consistent with the gross lesions reported for the fasted rats. No treatment-related lesions were seen in the 14-day survivors. Based on these data, the study author calculated LD_{50} values of 4264 mg/kg (3952.3 to 5330.2 mg/kg) for the unfasted males and 3352 mg/kg (2341.4 to 3977.5 mg/kg) for the unfasted females. These LD_{50} values correspond to Toxicity Category IV for fasted males and Toxicity Category III for fasted and unfasted females and unfasted males.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that the reported results clearly demonstrated a dose-related increase in mortality and served as the basis for determination of the LD_{50} values.

The study author stated that "unfasted rats were more sensitive than fasted" and the data tend to support this statement. We conclude, however, that there is essentially no difference in the LD_{50} values for unfasted compared to fasted animals since the calculated LD_{50} values for fasted and unfasted animals fell within the respective LD_{50} ranges presented for both sexes.

A statement of compliance with Good Laboratory Practices was signed and dated June 9, 1988; no quality assurance statement was presented.

10. CBI APPENDIX: Appendix A, Results of the Acute Oral Toxicity Study in Fasted Rats, CBI p. 5; Appendix B, Results of the Acute Oral Toxicity Study in Unfasted Rats, CBI p. 6; Appendix C, Materials and Methods, CBI p. 4.

APPENDIX A

Results of the Acute Oral Toxicity Study in Fasted Rats

TEBUCONAZOLE Tox R 007200

Page ____ is not included in this copy.

Pages 21 through 25 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.

X 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.

007200

EPA: 68D80056
DYNAMAC No. 147-J2
April 5, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Acute Oral Toxicity Study in Mice

T5015928

STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished Study No. ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany for Mobay Corp., Stilwell, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Pienta Jr

Date: 4/4/89

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1. CHEMICAL: Terbuconazole; 1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol.

2. TEST MATERIAL: HWG 1608 from batch No. 16001/83 was not described; the purity was reported to be 97.1 percent.

3. STUDY/ACTION TYPE: Acute oral toxicity in mice.

4. STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished Study No. ~~94395~~ T5015928 conducted by Bayer AG, Wuppertal, Federal Republic of Germany for Mobay Corp., Stilwell, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-4-89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: margaret e. brower
Date: 4/4/89

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Control
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Signature: Thomas J. Fuenta for
Date: 4-4-89

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H-7509C)

Signature: J. M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head
Section I
Toxicology Branch II

Signature: _____
Date: _____

7. CONCLUSIONS:

Core Classification: Core Minimum

LD_{50} Values: Male mice: 1615 mg/kg (1057.2 to 2179.6 mg/kg)
Female mice: 3023 mg/kg (2127.4 to 5072.7 mg/kg)

Toxicity Category: III

8. SUMMARY: Groups of five male and five female NMRI mice (age not specified; Winkelmann, Borchen, FRG), weighing 17 to 23 g were fasted for 16 hours and given single gastric gavage administrations of 100, 500, 1000, 1800, 2500, 3150, or 3550 mg HWG 1608/kg (males) or 500, 1000, 1800, 2500, 3550, or 5000 mg HWG 1608/kg (females). The test material was prepared in distilled water containing Cremophor EL (5 drops Cremophor EL in 10 mL of water) and was administered in a dosing volume of 1 mL/100 g body weight. Animals were observed at unspecified intervals over a 14-day period for clinical signs and mortality; tabulated results suggest, however, that animals were observed during the first hour after dosing, at 2 and 4 hours, and daily thereafter. No information was provided on body weight determinations. Animals found dead and survivors at day 14 received gross necropsies.

As shown in Appendix A, deaths were observed in males between days 1 and 3, were dose-related, and ranged from 20 percent mortality at 1000 mg/kg to 100 percent mortality at 3550 mg/kg; no deaths occurred at the two low-dose groups (100 and 500 mg/kg). A wide range of toxic signs including disturbed behavior and motility, dyspnea, staggering, spastic gait, stiff posture, rolling, reduced reflexes, prostration, and weight loss were observed in males of all test groups except those in the 100-mg/kg group. Deaths in females were similarly dose-related and ranged from 20 percent at 1800 mg/kg to 100 percent at 5000 mg/kg and occurred between days 1 and 9. With the exception of weight loss, all clinical signs reported for males were noted in the females of all dosed groups but the lowest level (500 mg/kg). Necropsy findings for animals that died on study included patchy to dark red and distended lungs; pale, sometimes patchy liver, spleen, and kidneys; and glandular stomach that was sometimes reddened. No gross lesions were found in survivors at study termination. The LD_{50} values were calculated according to the method of A. P. Rosiello, J. M. Essigmann, and G. N. Wogan (1977). Based on graphic analyses presented in the report, the oral LD_{50} values were 1615 mg/kg (1057.2 to 2179.6 mg/kg) for males and 3023 mg/kg (2127.4 to 5072.7 mg/kg) for females.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: We assess that the intervals at which body weight determinations were performed were not reported and that no body weight data were presented to support the study author's comment that weight losses occurred in male mice. It was unclear from the reported results whether all clinical and necropsy findings were observed in all affected animals since only summarized results were presented. Nevertheless, the data were sufficient to produce a dose response curve and permit the determination of the LD_{50}. We conclude, therefore, in agreement with the study author, that the data adequately support the LD_{50} values reported for HWG 1608 (1615 mg/kg for males, 3023 mg/kg for females), which corresponds to Toxicity Category III.

A statement of compliance with Good Laboratory Practices was signed and dated June 9, 1988; no quality assurance statement was presented.

CBI APPENDIX: Appendix A, Results of the Acute Oral Toxicity Study in Mice, CBI p. 7; Appendix B, Materials and Methods, CBI p. 4.

APPENDIX A

Results of the Acute Oral Toxicity Study in Mice

(CBI p. 7)

TEBUCONAZOLE Tox R 007200

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Pages 31 through 33 are not included.

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007200

EPA: 68D80056
DYNAMAC No. 147-J3
April 5, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Acute Oral Toxicity Study in Rabbits

T8015813

STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp. Stilwell, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Prenta for
Date: 4/5/89

1. CHEMICAL: Terbuconazole; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol; Folicur; HWG 1608.

2. TEST MATERIAL: HWG 1608 from batch No. 16001/83 was not described; the purity was reported to be 97.1%.

3. STUDY/ACTION TYPE: Acute oral toxicity in rabbits.

4. STUDY IDENTIFICATION: Heimann, K. G. (HWG 1608. Study for acute toxicity. (Unpublished study No. ~~4395~~ T80158-13 conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwel, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-4-89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: margaret E Brower
Date: april 4, 1989

6. APPROVED BY:

Roman Pienta, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: Roman J Pienta
Date: 4-4-89

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H-7509C)

Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head,
Section I
Toxicology Branch II

Signature: _____
Date: _____

7. CONCLUSIONS:

CORE Classification: CORE Supplementary.

LD_{50} values: Male rabbits: >1000 mg/kg
Female rabbits: >1000 mg/kg.

Toxicity Category: Not assigned due to study deficiencies outlined in Section 9 (Reviewers' Comments and Quality Assurance Measures).

8. SUMMARY: Groups of three male and female albino HC:NZW rabbits (age and weight not specified; Hacking and Churchill, Ltd., UK) were fasted for 16 hours and given single gastric gavage administrations of 500 or 1000 mg/kg HWG 1608.

The test material was prepared in distilled water containing Cremophor EL (5 drops Cremophor EL in 10 mL water) and was administered in a dosing volume of 0.5 mL/100 g body weight. Animals were observed at unspecified intervals over a 14-day period for clinical signs and mortality; tabulated results suggest, however, that animals were observed at least daily. No information was provided on body weight determinations. All animals received gross necropsies at day 14.

As shown in Appendix A, no animals died during the study. All animals in the high-dose groups showed signs of appetite loss, which was noted between days 1 and 3 in males and on day 6 in the females. Necropsy findings at study termination included slightly distended and patchy lungs, and slightly patchy kidneys. The report did not indicate if these necropsy findings were observed in both dose groups of both sexes. The study author concluded that the oral LD_{50} in rabbits was >1000 mg/kg of HWG 1608.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES: We assess that the study is insufficient to establish acute oral LD_{50} values in rabbits and does not comply with EPA Pesticide Assessment Guidelines for the following reasons:

1. No deaths were observed at the high dose (1000 mg/kg), which was lower than the dose suggested for a limit test (5000 mg/kg).

2. The number of animals per sex per group (3) was lower than that recommended by guidelines (5).

3. Age and weight of animals at initiation were not reported.

4. Body weights were either not determined or not reported.

5. It was unclear from the reported results whether all clinical and necropsy findings were observed in all affected animals since only summarized results were presented.

We assess, therefore, that while the limited data support the study author's conclusion that the oral LD_{50} of HWG 1608 in rabbits is >1000 mg/kg, a toxicity category can not be assigned because of the identified study deficiencies.

A statement of compliance with Good Laboratory Practices was signed and dated June 9, 1988; no quality assurance statement was presented.

CBI APPENDIX: Appendix A, Results of the Acute Oral Toxicity Study in Rabbits, CBI p. 8. Appendix B, Materials and Methods, CBI p. 4.

APPENDIX A

Results of the Acute Oral Toxicity Study in Rabbits

TEBUCONAZOLE Tox R 007200

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007200

EPA: 68D80056
DYNAMAC No. 147-J5
April 6, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Acute Dermal Toxicity Study in Rats

STUDY IDENTIFICATION: Heimann, K. G. T3015809 HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated October 13, 1983.) Accession/MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Puenta for

Date: 4/5/89

007200

1. CHEMICAL: Terbuconazole; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol; Folicur; HWG 1608.

2. TEST MATERIAL: HWG 1608, from batch 16001/83 was not described; the purity was reported to be 97.1%.

3. STUDY/ACTION TYPE: Acute dermal toxicity in rats.

4. STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ T3015809 conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated October 13, 1983.) Accession/MRID No. 407009-17.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-4-89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: margaret E. Brower
Date: April 4, 1989

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Assurance
Dynamac Corporation

Signature: Roman J Prieto
Date: 4/4/89

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H-7509C)

Signature: J.M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head
Section I
Toxicology Branch II
(H-7509C)

Signature: _____
Date: _____

7. CONCLUSIONS:

A. CORE Classification: Supplementary.

Dermal LD^{50} values for HWG 1608 in rats: >5000 mg/kg for males and females.

Toxicity Category: III.

B. Recommendations:

The study can be upgraded if the study author can provide information on test site dimensions and individual body weight data showing that the majority of rats weighed at least 200 g at initiation of treatment. This information, if available, would alleviate concerns regarding the adequacy of test sites to accommodate the test material and insure that conditions were appropriate for test material penetration, if any, of the skin.

8. SUMMARY:

Groups of five male and five female Wistar rats (age not specified; Winkelmann, Borchen, FRG), weighing 160 to 200 g, were shaved and treated topically with 1000, 2500, or 5000 mg/kg HWG 1608 for males and 5000 mg/kg HWG 1608 for females. Test material doses were prepared as pastes by mixing the appropriate quantity of HWG 1608 with 10-15 drops of physiological saline. Test areas were covered with occlusive dressings and aluminum foil; the dimensions of the treatment site were not reported. Five males and five females were similarly treated with saline and served as the negative control group. Twenty-four hours postapplication, dressings were removed and the treated sites were washed with soap and water. Animals were observed for mortality and signs of toxicity at unspecified intervals for 14 days. The report did not indicate that body weights were determined during the course of study or at study termination. Necropsies were performed on all animals at study termination and skin sections were examined histiologically.

No animals died on study and the report stated that "the treatment was tolerated without ill effects; no local irritant effects were noted." Neither the necropsy nor histological findings indicated a compound-related effect. The only microscopic findings were very slight fibrosis (one male), hyperkeratosis (one male), and cellular or inflammatory infiltrates (one females) in control animals. Based on these findings, the study author concluded that the acute dermal LD_{50} for HWG 1608 was >5000 mg/kg for male and female rats, which corresponds to Toxicity Category III.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that no acute toxicity resulted from dermal application of the test material up to a level (5000 mg/kg) that exceeded the dose suggested by guidelines for an acute dermal toxicity test (2000 mg/kg). However, the weight range of rats used in this study (160 to 200 g) was below the suggested range of 200 to 300 g. It is conceivable, therefore, that using smaller rats interfered with topical application and test material penetration of the skin. Additionally, the test site dimensions were not reported, and body weight determinations, if performed during the study or at study termination, were not reported. A statement of compliance with Good Laboratory Practices was signed and dated June 9, 1988; no quality assurance statement was presented.

10. CBI APPENDIX: Appendix A, Materials and Methods, CBI p. 4 and 10.

APPENDIX A

Methods and Materials
(CBI pp. 4 and 10)

TEBUCONAZOLE Tox R 007200

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[redacted]

EPA: 68D80056
DYNAMAC No. 147-J6
April 4, 1989

007200

DATA EVALUATION RECORD

TERBUCONAZOLE (FOLICUR)

Acute Inhalation Toxicity--Rats

STUDY IDENTIFICATION: Pauluhn, J. HWG 1608. Study for acute toxicity to the rat to OECD guideline No. 403. (Unpublished study Nos. T2015844 and T3015845 conducted by Toxicology Division Bayer AG, Wuppertal, FRG, for Mobay Corporation, Kansas City, KS; dated June 26, 1987). MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Puntenney Jr.

Date: April 4, 1989

007200

1. CHEMICAL: HWG 1608; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol; Folicur; terbuconazole.

2. TEST MATERIAL: HWG 1608 technical, colorless crystals, was 97.1% pure.

3. STUDY/ACTION TYPE: Acute inhalation toxicity--rats.

4. STUDY IDENTIFICATION: Pauluhn, J. HWG 1608. Study for acute toxicity to the rat to OECD guideline No. 403. (Unpublished study Nos. T2015844 and T3015845 conducted by Toxicology Division Bayer AG, Wuppertal, FRG, for Mobay Corporation, Kansas City, KS; dated June 26, 1987). MRID No. 407009-17.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: April 5, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 5, 1989

6. APPROVED BY:

Roman Pienta, Ph.D.
Technical Quality Assurance
Dynamac Corporation

Signature: Roman J Pienta
Date: April 4, 1989

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II

Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head
Section I
Toxicology Branch II

Signature: _____
Date: _____

7. CONCLUSIONS:

CORE Classification: CORE supplementary; aerosol particle size, temperature, and humidity were not measured in the 4-hour exposure.

LC^{50} -- 4-hour exposure: > 818 mg/m^3.
LC^{50} -- Five daily 6-hour exposures: >240 mg/m^3.

Toxicity Category: Not established since no mortality was seen at the highest dose tested.

8. SUMMARY:

A. Materials and Methods: Groups of five male and five female Wistar Bor:WISW strain rats were exposed for 4 hours to 100 mg/m^3 (nominal) HWG 1608 and groups of 10/sex were similarly exposed to 250, 2500, and 5000 mg/m^3 HWG 1608. Rats were observed for 14 days. Groups of 10 males and 10 females were exposed for 5 days, 6 hours/day, to nominal concentration of 100, 300, or 1000 mg/m^3 and observed for 14 days. Signs of toxicity were recorded. and body weights were determined prior to exposure after days 5, 12, and 19. The rats were sacrificed 14 days after exposure and given a gross pathological examination. At initiation of the study, the rats were 8 to 12 weeks old and weighed between 160 and 320 g.

The test compound was formulated with Lutrol (polyethylene glycol 400:ethanol, 1:1). Rats were exposed dynamically by head/nose only exposures using a 40-L chamber with a baffled antechamber. A volume of 200 µL/minute formulation was sprayed into the atmosphere of 10 L/minute air. Air flow was monitored continually with a rotameter, and temperature and humidity were monitored during exposure. Air samples in the breathing zone area were withdrawn and analyzed for HWG 1608 by HPLC. The particle size distribution of the aerosol was determined with a cascade impactor and the MMAD (mass medium aerosol diameter) and geometric standard deviation (sigma) were calculated and the percent respirable aerosol was estimated. Detailed materials and methods are given in Appendix A.

B. Results:

a. Four-hour exposure:

The analytical concentrations were 16, 49, 387, or 818 mg/m^3 at nominal concentrations of 100, 250, 2000, or 5000 mg/m^3. Particles less than 5 µm were estimated to

be 50% on a mass basis (not measured). There were no deaths.

There were no toxic signs at 16 mg/m^3; motility was reduced slightly (for 4 hours) at 49 mg/m^3 and moderately at 387 or 818 mg/m^3. There were no signs in the vehicle controls. There were no important effects on weight gains or any increase in gross lesions in exposed rats. The LC_{50} for a single 4-hour exposure is >818 mg/m^3.

b. Five 6-hour exposures:

The analytical concentrations and particle size analyses are summarized in the table below:

Concentration (mg/m^3) Nominal	Analytical	MMAD[a]	Sigma (μm)	Mass of Particles $\leq 5\mu$ (%)
100	24	7.1, 10	2.0, 2.4	27
300	60	5.0, 4.7	1.8, 2.0	53
1000	240	4.6, 4.2	1.8, 2.0	58

[a]Abbreviations:

MMAD = mass median aerodynamic diameter.
Sigma = Geometric standard deviation.

There were no deaths. Reduced motility (slight to moderate and lasting 6 to 7 hours) was noted in all exposed groups without a correlation with concentration. No gross findings in the lung or other organs were indicated. Body weights decreased in all exposed groups of males and they recovered after exposure; no dose trends were seen. In females, weight loss in the exposure period was less at 100 mg/m^3 than at higher doses; 2 weeks after exposure, weight gains occurred and mean weights were similar in all dosed groups.

The LC_{50} for 5 daily 6-hour exposures is >240 mg/m^3.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study was a repeat of a previous report (No. 12168). Some confusion was experienced by the reviewers when data on CBI p. 41 (Table 12) was compared with that on CBI p. 79. The

tabular data were identical, yet the derived values of MMAD, and percent respirability differed. This was the result of using the natural logarithm in calculations rather than Brigg's log (see footnote, CBI p. 62). In the 4-hour exposure, the temperature and humidity of the chamber were not measured nor were the particle sizes determined. Adequate acute and subacute studies with technical active ingredient have been subsequently performed (DER Nos. 147-B and 147-H).

A quality assurance statement was not available.

10. CBI APPENDIX: Appendix A, Materials and Methods, CBI p. 53-66.

APPENDIX A

Methods and Materials
(CBI pp. 53-66)

TEBUCONAZOLE Tox R 007200

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007200

EPA: 68D80056
DYNAMAC No. 147-H
April 4, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE (FOLICUR)

Acute Inhalation Toxicity--Rats

STUDY IDENTIFICATION: Pauluhn, J. HWG 1608. Study for acute inhalation toxicity to the rat. (Unpublished study No. ~~96754~~ T5C25GU and T9GR5) conducted by Bayer AG Toxicology Division, Wuppertal, FRG, for Mobay Corp., Agricultural Chemicals Division, Kansas City, MO; dated January 7, 1988). MRID No. 407009-22.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir for

Date: 4/4/89

69

~~03~~

1. CHEMICAL: HWG 1608; Folicur; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol; terbuconazole.

2. TEST MATERIAL: HWG 1608 technical, was a beige to colorless powder, from batch No. 132, with a 96.2% purity.

3. STUDY/ACTION TYPE: Acute inhalation toxicity study in rats.

4. STUDY IDENTIFICATION: Pauluhn, J. HWG 1608. Study for acute inhalation toxicity to the rat. (Unpublished study No. ~~96754~~ T902564! and T902564? conducted by Bayer AG Toxicology Division, Wuppertal, FRG, for Mobay Corp., Agricultural Chemicals Division, Kansas City, MO; dated January 7, 1988). MRID No. 407009-22.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: April 5, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 5, 1989

6. APPROVED BY:

Roman Pienta, Ph.D.
Technical Quality Assurance
Dynamac Corporation

Signature: Roman J Pienta
Date: 4/4/89

Mike Iannou, Ph D.
EPA Reviewer, Section I
Toxicology Branch II

Signature: M. Iannou
Date: 5-10-89

Mike Iannou, Ph.D.
EPA Acting Section Head
Section I
Toxicology Branch II

Signature: _____
Date: _____

7. CONCLUSIONS:

LC_{50} (aerosol): Greater than 371 mg/m^3 air for male and female Wistar-Bor rats exposed 1 x 4 hours and observed for 14 days (particle mass $\leq$ 5 μm: 100%).

LC_{50} (dust): Greater than 5093 mg/m^3 air particle mass $\leq$ 5 μm: 8%. The rats showed a temporary reduced weight gain on day 3.

Toxicity Category II for aerosol: III for dust.

CORE Classification: Guideline.

8. SUMMARY:

A. Materials and Methods: Groups of five male and five female Wistar Bor:WSW strain rats (Winkelman, Borchen, FRG), weighing 150-200 g, were exposed (nose only) to HWG 1608 aerosol or dust for 4 hours and observed for 14 days. Appearance and behavior were assessed after exposure and rats were observed daily. Body weights were recorded at 0, 3, 7, and 14 days. Rats were autopsied at 14 days and the respiratory tract was evaluated. Groups were exposed to air only, vehicle, vehicle and a spray solution of test compound (20% g/v), or dust.

For aerosol exposure, the test compound was nebulized with a polyethylene glycol E400 ethanol mixture (as vehicle). The HWG 1608-vehicle aerosol was sprayed into the baffled 30L chamber under dynamic conditions (200 μL vehicle/10 L /minute air) There were 30 air changes/minute. See Appendix A for a diagram of the inhalation chamber. Air flow was monitored continually with a rotometer, and temperature and humidity were also monitored (every 10 minutes). Air concentrations in the vicinity of the breathing zone were analyzed for three samples (start, middle, and end of exposure period). The air samples were collected in glass tubes containing Florisil. Air (20 L) was drawn through analyses tubes at a rate of 4 L/minute. Analysis was by high-pressure liquid chromatography. Aerosol particle distribution was analyzed in the breathing area with a TSI-laser velocimeter with two dilution stages. Mass media aerodynamic diameter (MMAD) and geometric standard deviation were calculated. Stability of the aerosol was determined continuously with an aerosol photometer.

Dust was generated by spraying pulverized active ingredient into the inhalation chamber with an Exactomet 4200 under dynamic conditions. The air supply was about 26 L/minute

which resulted in about 78 air changes per hour. The dust concentration in the breathing area was determined gravimetrically on 5-L samples of air drawn at 4-L/minute over Sartorius acetate filters with a 0.45 μm pore size. Particle size analysis was with an Anderson cascade impactor.

B. Results: The nominal aerosol concentration was 4000 mg/m^3 air and the analytical concentration was 371 mg/m^3. The lower analytical concentrations are attributable to precipitation of larger particles in the baffle chamber. The MMAD was 1.40 μm and 100% of the aerosol particles were of respirable size (<5 μm). For dusts, the analyzed concentration was 5093 mg/m^3. The MMAD was 12.8 μm and the geometric standard deviation was 1.9 μm. Eight percent of the particles were ≤ 5 μm (respirable).

There were no deaths in exposed or control groups. There were no clinical signs of toxicity. Weight gain was transiently decreased (day 3) in the dust-exposed group. No important gross findings were reported in the lungs or other organs of exposed rats. Foci of "hepatoid" appearance were reported in the lungs of one male and female exposed by aerosol and one male exposed to dust.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study was conducted in the appropriate manner. Variations in humidity in the aerosol exposure group do not impact the study findings. The hepatoid (leberartig-german) foci in the lungs are probably not of toxicological importance. We are not sure what the term "leberartig" means. The nominal concentration (4000 mg/m^3) during aerosol exposure was much higher than the analytical concentration (371 mg/m^3). This probably is the result of saturation of solubility of HWG 1608 in the polyethylene glycol after volatilization of ethanol. It was reported that vaporization of larger quantities of vehicle per time unit was not practicable. No data on efficiency of aerosol generation versus quantity of vehicle were presented. However, increasing vehicle may have resulted in solvent toxicity. If the dust concentration is corrected for respirable concentration, it would be about 8% of the analyzed or about 400 mg/m^3, which is close to the respirable aerosol concentration.

A quality assurance statement was signed and dated December 22, 1987, and a GLP compliance statement signed and dated November 25, 1987.

10. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 11-35.

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APPENDIX A

Materials and Methods

TEBUCONAZOLE Tox R 007200

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007200

EPA: 68D80056
DYNAMAC No. 147-J4
April 5, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Acute Intraperitoneal Toxicity Study in Rats

STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. T2015808 ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Ramon J. Prieto for

Date: 4/5/89

93

007200

1. CHEMICAL: Terbuconazole; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol; Folicur; HWG 1608.

2. TEST MATERIAL: HWG 1608 from batch No. 16001/83 was not described; the purity was reported to be 97.1%.

3. STUDY/ACTION TYPE: Acute interperitoneal toxicity in rats.

4. STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. T201580 ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

5. REVIEWED BY:

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Principal Reviewer
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Date: 4-4-89

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I. Cecil Felkner, Ph.D.
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Signature: Roman J Prento
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Mike Ioannou, Ph.D.
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Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head,
Section I
Toxicology Branch II

Signature: _____
Date: _____

94

7. CONCLUSIONS:

CORE Classification: Not applicable; test material administration by intraperitoneal injection is not required by guidelines.

LD_{50} Values: Male rats: 751 mg/kg (670.9 to 826.8 mg/kg)
Female rats: 395 mg/kg (329.9 to 430.0 mg/kg).

Toxicity Category: Not applicable, see above.

8. SUMMARY: Groups of five male and five female Wistar rats (age not specified; Winkelmann, Borchen, FRG), weighing 160 to 200 g, received single intraperitoneal injections of 50, 100, 500, 630, 710, 900, or 1,000 mg/kg HWG 1608 for the males and 50, 100, 355, 400, or 560 mg/kg HWG 1608 for the females; 10 males and 10 females were similarly administered 800 or 450 mg/kg of the test material, respectively. The report did not indicate whether animals were fasted prior to dosing. The test material was prepared in distilled water containing Cremophor EL (5 drops Cremophor EL in 10 mL water) and was administered in a dosing volume of 1 mL/100 g body weight. Animals were observed at unspecified intervals over a 14-day period for clinical signs and mortality; tabulated results suggest, however, that animals were observed frequently during the first hour after dosing and daily thereafter. No information was provided on body weight determinations. Animals found dead and survivors at day 14 received gross necropsies.

As shown in Appendix A, deaths observed in males 2 to 3 hours postdosing and between days 1 and 3 were dose-related and ranged from 20% mortality at 630 mg/kg to 100% mortality at 1000 mg/kg; no deaths occurred in the three lowest dosed groups (50, 100, and 500 mg/kg). A wide range of toxic signs, including disturbed behavior and motility, dyspnea, staggering, spastic gait, uncoordinated movements, reduced reflexes, prostration, and an anesthesia-like state, were observed in males of all test groups except the low-dose group. Deaths in females were similarly dose-related and ranged from 20% at 355 mg/kg to 100% at 560 mg/kg and occurred between days 1 and 4. No deaths occurred in groups receiving 50 or 100 mg/kg of HWG 1608. All clinical signs reported for males were noted in the females of all dose groups but the lowest level (50 mg/kg). Necropsy findings of animals that died on study were reported as follows: lungs patchy to dark red and distended; spleen and kidneys patchy, sometimes pale; livers patchy; animals dosed at 500 mg/kg and above exhibited slightly swollen livers, with the individual lobes adherent to each other and to the pancreas, diaphragm, stomach and fatty tissue; reddened glandular stomach; walls of stomach thin and unpatterned; clear fluid in the abdomen; and whitish deposits on all abdominal organs. Necropsy findings on survivors included swollen liver,

liver lobes adherent to each other, and spleen covered with a white deposit.

The LD_{50} values were calculated according to the A. P. Rosiello, J. M. Essigmann, G. N. Wogan method (1977). Based on graphic analyses presented in the report, the intraperitoneal LD_{50} values were 751 mg/kg (670.9 to 826.8 mg/kg) for males and 395 mg/kg (329.9 to 430.0 mg/kg) for females.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that acute toxicology evaluation of the test material administered by intraperitoneal injection is not required under FIFRA Guideline Subdivision F: Pesticide Assessment Guidelines: Hazard Evaluation--Human and Domestic Animals, dated 11-30-82, to support registration of pesticide products. The study does, however, provide useful information and the data support the LD_{50} values calculated by the study author for HWG 1608. As expected, intraperitoneal LD_{50} values in rats were appreciably lower than oral LD_{50} values (see Data Evaluation Record D99147J1). No Toxicity Category is assigned for HWG 1608 administered via this route.

A statement of compliance with Good Laboratory Practices was signed and dated June 9, 1988; no quality assurance statement was presented.

CBI APPENDIX: Appendix A, Results of the Acute Intraperitoneal Toxicity Study in Rats, CBI p. 9; Appendix B, Materials and Methods, CBI p. 4.

APPENDIX A

Results of the Acute Intraperitoneal Toxicity Study in Rats

APPENDIX B

Materials and Methods

APPENDIX A

Results of the Acute Intraperitoneal Toxicity Study in Rats

TEBUCONAZOLE Tox R 007200

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Pages 100 through 102 are not included.

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007200

EPA: 68D80056
DYNAMAC No. 147-J8
February 17, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Eye Irritation Study in Rabbits

T5015849

STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp. Stilwell, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Puenta (for)

Date: 2-17-89

103

007200

1. CHEMICAL: Terbuconazole; 1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-01; HWG 1608.

2. TEST MATERIAL: HWG 1608 from batch No. 16001/83 was not described; the purity was reported to be 97.1%.

3. STUDY/ACTION TYPE: Eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Heimann, K. G. T5015847 HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp. Stilwell, KS; dated October 13, 1983). Accession/MRID No. 407009-17.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
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Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L McLellan (for)
Date: 2-17-89

6. APPROVED BY:

Roman Pienta, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: Roman J Pienta
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Mike Ioannou, Ph.D.
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Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
Acting EPA Section Head,
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Date: _____

7. CONCLUSION: CORE Minimum.
Primary Eye Irritation Rating: Nonirritating.

Toxicity Category: III. No corneal opacity; irritation was reversible within 7 days.

8. SUMMARY: Three adult albino HC:ZNW rabbits (age and sex not specified; weight range: 2.9 to 3.2 g; Hacking and Churchill, Ltd., UK) were used in this study. A single dose of 100 µL (50 mg) of the test material was placed in the conjunctival sac of one eye of each animal and the eyelids of the treated eyes were held together for 1 second; nontreated eyes served as the negative control. Twenty-four hours after exposure, treated eyes were washed with physiological saline. Treated and untreated eyes were examined for ocular and nonocular lesions at 1, 24, 48, and 72 hours and at 7, 14, and 21 days posttreatment.

Cornea (opacity and area), iris, and conjunctiva (redness, swelling, and tear flow) were graded for ocular lesions using the Draize scoring system. To facilitate corneal examinations, one drop of 1% fluorescein was placed on the eyes immediately prior to the 24-hour reading and prior to the other scoring times, if positive results were seen at 24 hours.

Individual data for treated eyes of each rabbit were presented. As shown in Appendix A, corneas and irises were unaffected by treatment. One-hour posttreatment, conjunctival irritation, which included redness (grade 2 in all animals), swelling (grade 1 in all animals), and dacryorrhea (grade 1 in one animal only), were recorded. Redness was less intense (grade 1) in one animal at 24 hours and was reversed at 48 hours. All other signs of eye irritation were resolved by 24 hours in the three test animals. Based on these findings, the study author concluded that HWG 1608 was neither irritating nor corrosive to rabbit eyes.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that the number of rabbits selected for study (3) was lower than the number recommended by guidelines (at least 6). However, we assume that the rationale used by the study author to justify the use of fewer rabbits in the primary skin irritation study of HWG 1608 (Data Evaluation Record D99147J7) also applies to this eye irritation study. Since all signs of ocular irritation were reversible in < 72 hours and the test was performed with an adequate test material dose, we conclude that the data support the study author's conclusion that HWG 1608 is not an ocular irritant. This assessment corresponds to Toxicity Category III.

A statement of compliance with Good Laboratory Practices was signed and dated June 9, 1988; no quality assurance statement was presented.

CBI APPENDIX: Appendix A, Results of the Eye Irritation Study in Rabbits, CBI p. 17. Appendix B, Methods CBI pp. 4 and 16.

Appendix A

Results of the Eye Irritation Study in Rabbits

TEBUCONAZOLE Tox R 007200

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EPA: 68D80056
DYNAMAC No. 147-C
April 27, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Sheets, L. P. Primary eye irritation of Folicur (HWG 1608) technical in albino rabbits. (Unpublished study No. ~~96704~~ conducted and submitted by Mobay Corp., Stilwell, KA; dated May 12, 1988.) Accession/MRID No. 407009-25.

87-333-03

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Lantz for

Date: April 27, 1989

112

007200

1. CHEMICAL: Terbuconazole; α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol.

2. TEST MATERIAL: Folicur technical from batch No. 86R0082I was described as a tan crystalline powder with a purity of 96.3 pecent.

3. STUDY/ACTION TYPE: Eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Sheets, L. P. Primary eye irritation of Folicur (HWG 1608) technical in albino rabbits. (Unpublished study No., ~~96704~~ 89-333-03 conducted and submitted by Mobay Corp., Stilwell, KA; dated May 12, 1988.) Accession/MRID No. 407009-25.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-27-89

Margaret E. Brower, Ph.D.
Independent Reviewer
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Date: 4/27/89

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Control
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Date: 4-27-89

Mike Ioannou, Ph.D.
EPA Reviewer and
Section Head, Section I
Toxicology Branch II
(H-7509C)

Signature: M. Ioannou
Date: 5-10-89

7. CONCLUSIONS: Core Guideline.

Eye Irritation Rating: Mildly irritating.

Toxicity Category: III--No corneal opacity; irritation reversible within 7 days.

8. SUMMARY:

Three male and three female young adult New Zealand White rabbits (age not specified; Small Stock Industries AK) were given pretest ocular examinations (24 hours prior to treatment) and received a single 100-mg dose of the test material in the conjunctival sac of the left eye. The eyelids of treated eyes were held together for 1 second; right eyes served as the negative control. Treated and untreated eyes were examined for ocular or nonocular lesions 1, 24, 48, and 72 hours postdosing and on days 7, 8, 14 and 21 or as long as irritation persisted. Cornea (opacity and area), iris, and conjunctiva (redness, chemosis, and the extent of discharge) were graded using a standard scoring system. Individual data for each rabbit were presented.

No effects were observed on the cornea or iris of the six rabbits; however, all treated conjunctivae showed signs of redness (grade 1), chemosis (grade 1 for all males; grade 1 or 2 for females), and discharge (grade 2 or 3) at 1 to 24 hours posttreatment. By 72 hours, eye irritation was reversed in the males. Redness was apparent in two females at 72 hours; redness persisted to day 7 in one female but was resolved by day 8. Chemosis and discharge were generally reversed after 48 hours; a grade 1 chemosis was scored for one female at 72 hours but was not apparent 7 days posttreatment. Based on these findings the author concluded that the test material was mildly irritating to the eye with effects confined to the conjunctiva.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

Although a detailed protocol was not provided, Standard Protocol and Procedure numbers were furnished and were reported to be based on guideline procedures. We conclude, therefore, that the study was properly conducted and the author interpreted the data correctly. The data are adequate to support the study author's conclusion that Policur technical is a mild eye irritant which corresponds to Toxicity Category III.

A quality assurance statement was signed and dated April 22, 1988.

10. CBI APPENDIX: **Appendix A, Methods, CBI pp. 9-10.**

APPENDIX A

Methods

TEBUCONAZOLE Tox R 007200

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CONFIDENTIAL BUSINESS INFORMATION
NATIONAL SECURITY INFORMATION (EO 12065)

007200

EPA: 68D80056
DYNAMAC No. 147-J7
April 6, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Primary Skin Irritation Study in Rabbits

STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. 94395 5015849 conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated October 13, 1983.) Accession/MRID No. 407009-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Prente Jr

Date: 4/6/89

1. CHEMICAL: Terbuconazole; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol; Folicur; HWG 1608.

2. TEST MATERIAL: HWG 1608, from batch 16001/83 was not described; the purity was reported to be 97.1%.

3. STUDY/ACTION TYPE: Primary skin irritation study in rabbits.

4. STUDY IDENTIFICATION: Heimann, K. G. HWG 1608. Study for acute toxicity. (Unpublished study No. ~~94395~~ 5015247 conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated October 13, 1983.) Accession/MRID No. 407009-17.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
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Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-4-89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: 4/4/89

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Roman Pienta, Ph.D.
Technical Quality Control
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Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H-7509C)

Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head
Section I
Toxicology Branch II
(H-7509C)

Signature: _____
Date: _____

7. CONCLUSIONS:

CORE Classification: CORE Minimum.

Primary Skin Irritation Rating: Nonirritant.

Toxicity Category: IV.

8. SUMMARY:

Three adult albino HC:NZW rabbits (age and sex not specified; weight range, 2.7 to 3.8 kg; Hacking and Churchill, Ltd., UK) were shaved (6 x 6-cm area) and were dermally treated with a single application of the test material. The test material was prepared as a paste by mixing 500 mg with H_2O on a 2.5 x 2.5-cm cellulose square. The cellulose material was applied to the intact skin and secured with tape. Similar squares, moistened with H_2O, were placed on the opposite flank. Four hours posttreatment, dressings were removed; test areas were washed an scored for erythema, eschar, and edema in accordance with the Draize scale. Sites treated with H_2O served as the negative controls. Irritation was scored 1, 24, 48, and 72 hours after patch removal and 7 and 14 days after exposure.

Individual results and irritation grades were presented and indicated that the test material did not induce a primary skin irritation response in the three test animals at any of the scoring intervals up to day 7 posttreatment.

The study author concluded that HWG 1608 was not an irritant.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that although the study was conducted with less than the number of rabbits recommended by guideline (at least six), the study author provided justification for using fewer animals (see Appendix A). Since there was no indication that 500 mg of HWG 1608 elicited a dermal response throughout the course of study, we agree with the study author's rationale and conclude that the number of rabbits used in this study was sufficient to support the assessment that HWG is nonirritating to rabbit skin, which corresponds to Toxicity Category IV. This conclusion is further supported by the findings of an acute dermal toxicity study in rats, which indicated that 5000 mg/kg HWG 1608 did not induce gross or microscopic skin irritation (Data Evaluation Record D99147J5).

A statement of compliance with Good Laboratory Practices was signed and dated June 9, 1988; no quality assurance statement was presented.

10. CBI APPENDIX: Appendix A, Justification for Use of Three Rabbits in the Dermal Irritation Study of Technical-Grade Folicur, CBI P. 105. Appendix B, Materials and Methods, CBI p. 4 and 15.

APPENDIX A

Justification for Use of Three Rabbits in the Dermal Irritation Study of Technical-Grade Folicur

APPENDIX A

Justification For Use of Three Rabbits in the Dermal Irritation Study of Technical-Grade Folicur

TEBUCONAZOLE Tox R 007200

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EPA: 68D80056
DYNAMAC No. 147-I
April 5, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Skin Sensitization Study in Guinea Pigs

STUDY IDENTIFICATION: Heimann, K. G. HWG 1608 technical study of skin sensitization effect on guinea pigs, Buehler patch test. (Unpublished study No. ~~95695~~ conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated November 19, 1987.) Accession/MRID No. 407009-28.
T2025339

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir for
Date: 4/5/89

129

129

1. CHEMICAL: Terbuconazole; α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; HWG 1608.

2. TEST MATERIAL: HWG 1608 technical from batch No. 16012/86 was described as a lumpy yellowish-white powder with a purity of 97.4% and was stored in the dark at 22 to 24°C.

3. STUDY/ACTION TYPE: Skin sensitization study in guinea pigs.

4. STUDY IDENTIFICATION: Heimann, K. G. HWG 1608 technical study of skin sensitization effect on guinea pigs, Buehler patch test. (Unpublished study No. ~~95695~~ T2025339 conducted by Bayer AG, Wuppertal, Federal Republic of Germany, for Mobay Corp., Stilwell, KS; dated November 19, 1987.) Accession/MRID No. 407009-28.

5. REVIEWED BY:

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Date: 4-4-89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 4, 1989

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: Roman J. Prenta for
Date: 4/4/89

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(H7509C)

Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head,
Section I
Toxicology Branch II

Signature: _____
Date: _____

7. CONCLUSIONS:

a. Core Classification: Core Supplementary.

Skin Sensitization Potential: HWG 1608 technical (25%) is not considered a dermal sensitizer (Buehler test) under the conditions of the study; however, the potential of higher concentrations to elicit an acute dermal or skin sensitization reaction was not established.

b. Recommendations: It is recommended that the study author supply further information regarding the test material dose selection to justify use of a 25% solution or repeat the study with higher concentrations. If the study is repeated, a positive control should be included.

8. SUMMARY:

Thirty-six male DHPW guinea pigs (5 to 7 weeks old; weight range: 290-363 g; Winkelmann, Borchen, FRG) were used in this study. Strain sensitivity was verified using formaldehyde in the maximization test of Magnusson-Klingman. Dose selection for the induction and challenge phase was based on the results of a range-finding test, which indicated that 3, 6, 12, and 25% of the test material did not elicit a skin reaction, and the study author's comment that higher concentrations were not possible due to unspecified technical reasons. Twenty-four hours prior to treatment, the backs and flanks of the animals were shaved. Patches saturated with 0.5 mL of the 25% test solution (formulated in a 2% aqueous solution of Cremophor EL) were placed on the left flank of 12 animals, secured with a bandage, and removed after 6 hours; dermal application was performed once weekly for 3 weeks. The remaining 24 animals were treated as described with patches saturated with the vehicle control. Two weeks following the last induction dose, animals in the treatment group were challenged with the 25% test material solution and 12 control animals received the challenge dose of the vehicle. Left flanks were used as test sites and right flanks were covered with patches saturated with the vehicle. The remaining 12 vehicle control animals were held in reserve if a second challenge was required to confirm first challenge results. A concurrent positive control group was not included in the study; however, historical control data from delayed dermal hypersensitivity tests with formaldehyde (0.5 and 2%) were included as an appendix to the report.

Animals were examined daily for signs of toxicity; body weights were determined prior to study initiation, weekly thereafter, and at study termination. Skin reactions were recorded at 48

criteria. Results were evaluated by subtracting the number of animals with skin reactions on the control site from the number of animals with an irritation reaction on the test site. Individual data were presented for the measured parameters.

All animals gained weight while on study; weight gains at study termination were slightly higher in the test group when compared to the control group. No deaths or clinical signs were observed over the course of study. Similarly, no skin reactions were seen in either treatment or control animals following induction or challenge. The study author stated that because the results of the first challenge were conclusive, a second challenge was not conducted and concluded that HWG 1608 technical (25%) did not have a skin sensitizing potential.

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that using only male guinea pigs did not affect the outcome of the study or compromise the study results and conclusions. However, the dose selected for evaluation of the skin sensitization potential of HWG 1608 technical (25%) may not have been adequate; this concentration in the range-finding test did not elicit a dermal response. The study author indicated that, for technical reasons, evaluation of higher percent solutions was not possible; however, without specific information justifying dose selection, we are unable to fully assess the skin sensitization potential of the test material. We conclude, therefore, that 25% HWG 1608 technical is not a skin sensitizer but the dermal hypersensitivity potential of higher test material concentrations cannot be established.

Additionally, it was noted that skin reactions were not recorded at 24 hours following challenge, as suggested by guidelines.

A quality assurance statement was dated October 23, 1987, but not signed.

CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 10-19.

APPENDIX A

Materials and Methods

TEBUCONAZOLE Tox R 007200

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EPA: 68D8C056
DYNAMAC No. 147-A
April 5, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Sheets, L. P. Acute oral toxicity of Folicur (HWG 1608) 1.2 emulsifiable concentrate in albino rats. (Unpublished Study No. ~~96752~~ 80-011-09 conducted and submitted by Mobay Corp., Stilwell, KS; dated June 1, 1988.) Accession/MRID No. 407009-20.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Ronnen J. Pineta for
Date: 4/5/89

007200

1. CHEMICAL: Terbuconazole; α-[2-(4-chlorophenyl)ethyl-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; Folicur; HWG 1608.

2. TEST MATERIAL: Folicur 1.2 emulsifiable concentrate from batch No. 5030124 was described as a clear, amber-colored liquid, which contained 16.6% active ingredient in the test formulation (See Appendix A).

3. STUDY/ACTION TYPE: Acute oral toxicity in rats.

4. STUDY IDENTIFICATION: Sheets, L. P. Acute oral toxicity of Folicur (HWG 1608) 1.2 emulsifiable concentrate in albino rats. (Unpublished study No. ~~96752~~ 87-011-09 conducted and submitted by Mobay Corp., Stilwell, KS; dated June 1, 1988.) Accession/MRID No. 407009-20.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-4-89

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 4, 1989

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Control
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Signature: Roman J. Puerta for
Date: 4/4/89

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
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(H-7509C)

Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Acting Section Head,
Section I
Toxicology Branch II

Signature: _____
Date: _____

7. CONCLUSIONS:

CORE Classification: Guideline.

LD_{50} Values: Male rats: 1790 mg/kg
Female rats: 1292 mg/kg.

Toxicity Category: III.

8. SUMMARY:

Groups of five male and five female young adult Sprague-Dawley rats (age not specified; Sasco, Inc., Omaha, NE) weighing 200 to 237 g for males and 167 to 203 g for females were fasted overnight and given single oral gavage administrations of 800, 1200, 1600, or 2000 mg/kg Folicur, prepared in deionized water (dosing volume was 5 mL/kg). Animals were observed twice daily during the week and once daily over weekends for signs of toxicity and mortality. Weights were recorded on the day of treatment and on days 7 and 14 posttreatment.

Deaths in both sexes were observed between days 1 and 3, were dose-related, and occurred as follows: in males, 1 of 5 and 4 of 5 in the 1600- and 2000-mg/kg groups, respectively; in females, 2 of 5, 5 of 5, and 4 of 5 in the 1200-, 1600- and 2000-mg/kg groups, respectively (See Appendix B). Dose-related toxic signs (ataxia, decreased activity, salivation, lacrimation, and urine stains) were noted in males and females of all treatment groups on days 0 through 1 but were reversed by day 5. The frequency of toxic signs was more pronounced in females and extended over a wider dose range (1200 to 2000 mg/kg) as compared to the incidence of toxic signs in males.

Body weights were increased for surviving males and females on day 7; however, the high-dose groups had the lowest body weights. By day 14, male body weights were generally comparable among groups and body weight gain was highest for the remaining males administered 2000 mg/kg. Body weight gains for surviving females were lower than the weight gains reported for corresponding groups of males. Gross observations for males and females found dead included red-to-brown fluid in the urinary bladder, discolored staining or fluid around the urogenital area, nasal stains, salivation, and fluid-filled stomach and small intestines. No gross lesions were found in survivors at necropsy on day 14. The oral LD_{50} values, as reported by the author, were 1790 mg/kg (1224 to 3024 mg/kg) for males and 1292 mg/kg (923 to 1641 mg/kg) for females (Table 1).

9. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

We assess that the study was conducted properly and the study author interpreted the test results correctly. We conclude, therefore, that the data adequately support the LD_{50} values reported by the author for Folicur (1790 mg/kg for males and 1292 mg/kg for females), which corresponds to Toxicity Category III.

Standard Protocol and Procedure numbers were furnished and were reported to be based on referenced guideline procedures.

A quality assurance statement was signed and dated 5/25/88.

10. CBI APPENDIX: Appendix A, General Test Material Formulation, CBI p. 22. Appendix B, Summary Results of the Acute Oral Toxicity Study in Rats Administered Folicur 1.2 EC. Appendix C, Methods, CBI pp. 9-10.

TABLE 1. Acute Oral Toxicity Evaluation of Folicur in Rats

Sex	LD_{50} Value (mg/kg)
Male	1790 (1224-3024)[a]
Female	1292 (923-1641)

[a]Numbers in parentheses represent 95% confidence limits as calculated by probit analysis.

APPENDIX A

General Test Material Formulation

TEBUCONAZOLE Tox R 007200

Page_____ is not included in this copy.

Pages 150 through 155 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.

X 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.

Tox review 007200

Page_____ is not included in this copy.

Pages 156 through 212 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.

__X__ Information about a pending registration action.

____ FIFRA registration data.

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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.

007200

EPA: 68D80056
DYNAMAC No.: 147-M
Task No.: 1-47
March 30, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Subacute Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 3/30/89

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007200

EPA: 68D80056
DYNAMAC No.: 147-M
Task No.: 1-47
March 30, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Subacute Feeding Study in Rats

REVIEWED BY:

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Principal Reviewer
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Signature: Claire Kruger-McDermott
Date: March 30, 1989

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Date: March 30, 1989

APPROVED BY:

Roman Pienta, Ph.D.
Technical Quality Control
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Date: March 30, 1989

Mike Ioannou, Ph.D.
EPA Reviewer and
Acting Section Head,
Section I
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(H7509C)

Signature: M. Ioannou
Date: 5-10-89

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007200

DATA EVALUATION RECORD

STUDY TYPE: Subacute feeding study in rats.

ACCESSION/MRID NUMBER: 407009-32.

TEST MATERIAL: HWG 1608.

SYNONYM(S): Terbuconazole; Folicur; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)-pentane-3-ol.

STUDY NUMBER(S): T0015905.

SPONSOR: Mobay Corporation, Stillwell, Kansas.

TESTING FACILITY: Bayer AG, Wuppertal-Elberfeld, Federal Republic of Germany.

TITLE OF REPORT: HWG 1608. Study of the Subacute Oral Toxicity to Rats.

AUTHOR(S): Heimann, K.G., and Kaliner, G.

REPORT ISSUED: November 12, 1984.

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CONCLUSIONS: HWG 1608 was administered to rats, by oral gavage, at levels of 0, 30, 100, or 300 mg/kg/day for 28 days followed by a 28-day recovery period. Body weights were decreased in both sexes at the high dose and returned to normal after dosing. Hemoglobin and hematocrit were slightly decreased in both males and females receiving 100 and 300 mg/kg/day, but erythrocyte counts were not affected. Serum transaminases were increased in high-dose males and females and alkaline phosphatase was increased in high-dose males. There was a dose-related increase in liver microsomal enzymes (N- and O-demethylases) and activities were significantly ($p < 0.05$) greater than controls in males at the mid and high doses and females at the high dose ($p<0.01$). Cytochrome P450 was significantly increased in both sexes ($p < 0.01$) at the high dose. Liver triglyceride was also increased ($p < 0.01$) in high-dose males. The induction of the liver monooxygenases was accompanied by an increase in liver weights. The effects on hematology, clinical chemistry, and liver were reversed after a 4-week recovery period. There were histologic liver changes at the high dose in both sexes as well as histologic changes in the spleen and adrenals. All effects on the liver were reversed after a 4-week recovery phase. The LOEL is 100 mg/kg/day and the NOEL is 30 mg/kg/day.

Classification: CORE Supplementary

A. MATERIALS:

1. Test Compound: HWG 1608; description: not provided; batch No.: 16001/83; purity: 97.0%.

2. Test Animals: Species: rats; strain: Wistar, Bor; age: not provided; weight: 146-177 g; source: F. Winkelmann, Borchen, FRG.

B. STUDY DESIGN:

1. Animal Assignment: Following 8 days of acclimation, animals were randomly assigned to the following test groups:

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Test group	Dose in diet (mg/kg body weight)	# of animals/group: Treatment Period (4 weeks) Males	Treatment Period (4 weeks) Females	Recovery Period (4-8 weeks) Males	Recovery Period (4-8 weeks) Females
1 Control	0	20	20	10	10
2 Low (LDT)	30	20	20	10	10
3 Mid (MDT)	100	20	20	10	10
4 High (HDT)	300	20	20	10	10

2. Dosage Preparation: The test compound was formulated prior to each treatment as a suspension in Cremophor EL and deionized water (0.2 mL Cremophor diluted with water to 10 mL), so that a constant volume of 10 mL/kg body weight was administered at all dose levels. The dose and volume administered were adjusted weekly on the basis of body weights. Prior to the start of the study, the homogeneity and stability of the test compound formulations at the highest and lowest concentrations over a storage period of 6 hours were analyzed. All formulated dose levels of the compound were analyzed once during the study. The test compound was stored at 23 to 27°C.

   Results: Data on homogeneity and stability of the dosing formulations were not reported.

3. Food and Water Consumption: Animals received food (Altromin 1324) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Arithmetic group means, standard deviations, and 95 and 99% confidence limits were calculated. Values from the dose groups were compared to the appropriate control population using the significance test (U test of Mann, Whitney, and Wilcoxin) at $p < 0.05$ and $p < 0.01$.

5. Quality Assurance: A quality assurance statement was signed and dated October 26, 1984.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of morbidity and mortality.

Results: One control female and one high-dose female died during the treatment period. No conclusive cause of death could be established for either animal. One high-dose female died during the recovery period; the cause of death was not reported.

There was no effect of dosing on clinical observations; individual data were not reported.

2. Body Weight: All rats were weighed at the beginning of each week of the study and prior to necropsy.

Results: During the treatment period, the mean body weights of low- and mid-dose males and females were comparable to controls. Body weights of high-dose males and females were found to be significantly ($p < 0.01$) decreased during study weeks 1 to 4 but were similar to concurrent controls during the recovery period (Table 1).

3. Food Consumption and Compound Intake: The test compound was administered once daily to the rats for 28 days via oral intubation at 0, 30, 100, and 300 mg/kg body weight. The control group received the same volume of formulation vehicle per kg body weight as the treatment groups, but without test compound. The dose and volume administered were adjusted weekly on the basis of body weights.

Results: Food consumption data were not provided.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from five animals/sex/group at the end of the 28-day dosing period and at the end of the 4-week recovery period. Blood for glucose determinations was obtained prior to necropsy from the distal caudal vessels. For other tests, blood was obtained at necropsy by cardiac puncture. The checked (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)+	X	Leukocyte differential count
X	Hemoglobin (HGB)+	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)+	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)+	X	Mean corpuscular volume (MCV)
X	Platelet count+		Coagulation: thromboplastin time (PT)
X	Reticulocyte count (RETIC)		
	Red cell morphology		

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 1. Body Weights of Rats Administered HWG 1608 Orally for 4 weeks

Dose (mg/kg/day)	Mean Body Weights (g) at Week: 0[a]	1[a]	2[a]	3[a]	4[a]	5[b]	6[b]	7[b]	8[b]
Males									
0	164 ± 6	188 ± 9	215 ± 12	237 ± 15	253 ± 18	267 ± 26	278 ± 26	287 ± 29	298 ± 32
30	163 ± 6	184 ± 9	208 ± 11	229 ± 13	245 ± 16	259 ± 20	271 ± 20	282 ± 22	288 ± 22
100	163 ± 7	184 ± 10	208 ± 12	226 ± 14	246 ± 19	260 ± 17	274 ± 19	285 ± 21	294 ± 21
300	162 ± 6	172 ± 7*	193 ± 11*	213 ± 14*	227 ± 17*	256 ± 18	271 ± 20	283 ± 21	289 ± 22
Females									
0	157 ± 6	167 ± 5	176 ± 6	179 ± 9	184 ± 8	190 ± 6	197 ± 8	198 ± 8	198 ± 6
30	159 ± 7	170 ± 7	178 ± 8	182 ± 8	188 ± 9	198 ± 7	202 ± 8	205 ± 8	204 ± 8
100	156 ± 5	164 ± 9	172 ± 9	177 ± 7	184 ± 8	187 ± 11	190 ± 10	192 ± 11	195 ± 12
300	160 ± 5	150 ± 13*	160 ± 8*	164 ± 12*	174 ± 11*	188 ± 9	189 ± 13	193 ± 12	194 ± 13

[a]Based on 20 rats/group.

[b]Based on 10 rats/group.

*Significantly different from control value ($p < 0.01$).

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Results: Table 2 summarizes hematologic data at the end of the dosing period. There was a dose-related decrease in hemoglobin level and hematocrit value in males and females. The decreases were slight in males but were both significant ($p < 0.05$, HGB; $p < 0.01$, HCT) in the group receiving 300 mg/kg/day; in females, the values were significantly ($p < 0.01$) decreased in the mid-and high-dose groups and this was accompanied by a decrease ($p < 0.01$) in mean corpuscular volume and mean corpuscular hemoglobin content at the high dose. Erythrocyte counts were not markedly decreased; the significant decreased mean in high-dose males ($p<0.05$) was attributed to a low value for one rat. At the end of the recovery period no significant effects were seen in any hematologic parameter in either sex.

b. Clinical Chemistry:

	Electrolytes
X	Calcium[+]
	Chloride[+]
	Magnesium[+]
	Phosphorus
	Potassium[+]
	Sodium[+]

	Enzymes
X	Alkaline phosphatase (ALP)
	Cholinesterase
	Creatinine phosphokinase[+]
	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (SGPT)[+]
X	Serum aspartate aminotransferase (SGOT)[+]
	Gamma glutamyltransferase (GGT)

	Other
X	Albumin[+]
	Albumin/globulin ratio
X	Blood creatinine[+]
	Blood urea nitrogen[+]
	Cholesterol[+]
	Globulins
X	Glucose[+]
	Total bilirubin[+]
	Direct bilirubin
	Total protein[+]
X	Urea

	Liver tissue
X	N-Demethylase
X	O-Demethylase
X	Cytochrome P-450
X	Triglycerides

Results: The clinical chemistry findings in blood plasma at the end of the administration period were comparable to controls for the rats in the 30- and 100-mg/kg groups. In the 300 mg/kg group, the SGOT, SGPT and ALP activities were significantly ($p < 0.01$) higher in females. The significant increase in glucose concentration in males of the 100-mg/kg group, the significantly lower glucose concentration in females of the 300-mg/kg group, and the

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 2. Hematological Findings of Rats Administered MNG 1608 Orally for 4 Weeks[a,b]

Dose (mg/kg/day)	Parameter						
	Leu	Ery	Hgb	MCV	Hct	MCH	Reti
				Males			
0	8.0 ± 2.2	8.5 ± 0.23	165 ± 6	59 ± 1	0.50 ± 0.01	19.5 ± 0.7	16 ± 2
30	7.8 ± 2.0	8.16 ± 0.33	163 ± 4	60 ± 2	0.49 ± 0.01	20.0 ± 1.0	14 ± 7
100	6.8 ± 1.1	8.15 ± 0.25	159 ± 1	59 ± 2	0.48 ± 0.01*	19.6 ± 0.6	14 ± 2
300	9.4 ± 1.5	7.93 ± 0.58*	153 ± 4*	59 ± 3	0.46 ± 0.01**	19.4 ± 1.2	17 ± 3
				Females			
0	6.0 ± 0.7	8.02 ± 0.29	157 ± 2	58 ± 2	0.47 ± 0.01	19.6 ± 0.8	14 ± 1
30	5.6 ± 0.3	7.77 ± 0.21	152 ± 4	59 ± 2	0.46 ± 0.01	19.6 ± 0.7	13 ± 2
100	6.0 ± 1.5	7.88 ± 0.38	147 ± 6**	56 ± 1*	0.44 ± 0.02**	18.7 ± 0.4	19 ± 5
300	10.4 ± 2.1**	7.94 ± 0.47	136 ± 9**	52 ± 2**	0.41 ± 0.03**	17.1 ± 0.6**	21 ± 7

[a]Hematological findings at the end of the dosing period.

[b]Abbreviations:

Leu = Leucocytes ($10^3/mm^3$)
Ery = Erythrocytes ($10^6/mm^3$)
Hgb = Hemoglobin (g/L)
MCV = Mean corpuscular volume (FL)
Hct = Hematocrit (%)
MCH = Mean corpuscular hemoglobin (pg)
Reti = Reticulocytes (% RBC)

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

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significantly increased urea concentration in females of the 30-mg/kg group were considered random occurrences since no correlating values in other groups or dose relationship could be established (Table 3). The clinical chemistry values in blood plasma measured at the end of the recovery period revealed no toxicologically relevant differences between the dose groups and controls.

At the end of the dosing period, N-demethylase ($p < 0.05$) and O-demethylase ($p < 0.01$) activities of liver were significantly increased in males that received 100 or 300 mg/kg/day and in females that received 300 mg/kg/day ($p < 0.01$); cytochrome P450 was significantly ($p < 0.01$) increased in both sexes at the highest dose. Males receiving 300 mg/kg/day exhibited a significantly ($p < 0.01$) higher triglyceride concentration in liver (Table 4). At the end of the recovery period, there were no significant changes at any of the dose levels in microsomal enzymes or triglycerides.

6. Urinalysis: Urine was collected from fasted animals (five/sex/group) at the end of the 28-day treatment period and at the end of the 4-week recovery period. The checked (X) parameters were examined:

X	Appearance*	X	Glucose*
	Volume*		Ketones*
	Specific gravity*		Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

Results: Blood was detected in the urine of two high-dose females at the end of the dosing period and in one mid-dose female at the end of the recovery period. This occurred spontaneously and was not considered a toxicologically relevant finding. No other treatment-related differences were observed either during dosing or recovery.

*Recommended by Subdivision F (October 1982) Guidelines.

TABLE 3. Representative Clinical Chemistry Values of Rats Administered HWG 1608 Orally for 4 Weeks

Dose (mg/kg/day)	Parameter SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Urea (mmol/L)	Glucose (mmol/L)
			Males		
0	61.7 ± 20.9	55.4 ± 6.1	435 ± 78	7.49 ± 0.37	5.45 ± 0.28
30	54.8 ± 6.6	52.8 ± 7.4	368 ± 34	7.49 ± 0.90	5.69 ± 0.36
100	56.0 ± 7.7	49.4 ± 3.6	371 ± 30	7.01 ± 0.90	6.51 ± 0.86**
300	74.5 ± 9.6	74.4 ± 13.2	394 ± 94	7.04 ± 0.73	6.05 ± 0.40
			Females		
0	50.6 ± 8.8	35.7 ± 4.2	192 ± 43	6.61 ± 0.91	6.24 ± 0.32
30	56.9 ± 22.5	39.9 ± 4.1	191 ± 34	9.44 ± 0.[illegible]**	5.87 ± 0.45
100	65.5 ± 15.2	42.4 ± 4.8	178 ± 29	7.46 ± 0.80	5.91 ± 0.28
300	144.6 ± 74.6**	120.1 ± 44.8**	544 ± 144**	8.78 ± 2.14	5.42 ± 0.5[illegible]

**Significantly different from control value ($p < 0.01$).

TABLE 4. Representative Clinical Chemistry Determinations in Liver Tissue of Rats Administered HWG 1608 Orally for 4 Weeks

Dose (mg/kg/day)	N-DEM[a] (nmol/g/min)	O-DEM[b] (nmol/g/min)	P-450[c] (µmol/g)	TRIGL[d] (µmol/g)
		Males		
0	152.6 ± 20.4	9.1 ± 1.0	29.8 ± 7.2	5.22 ± 0.56
30	170.8 ± 27.9	11.6 ± 2.1	40.0 ± 6.7	4.52 ± 0.58
100	220.9 ± 42.7*	14.3 ± 2.8**	35.3 ± 9.4	6.49 ± 1.32
300	205.5 ± 42.5*	19.4 ± 2.0**	46.9 ± 10.4**	8.33 ± 1.52**
		Females		
0	60.0 ± 18.8	8.8 ± 1.1	29.6 ± 5.3	6.52 ± 1.10
30	70.7 ± 7.0	10.2 ± 1.2	33.1 ± 5.2	6.29 ± 1.34
100	81.5 ± 15.1	11.0 ± 2.4	33.5 ± 8.2	6.15 ± 0.76
300	128.2 ± 37.1**	14.1 ± 2.2**	55.3 ± 5.8**	6.53 ± 1.66

[a]N-demethylase.

[b]O-demethylase.

[c]Cytochrome P450.

[d]Triglycerides.

*Significantly different from control value $p < 0.05$.

**Significantly different from control value $p < 0.01$.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination from five animals/sex of the high-dose group and control group. The other five/sex/group were used for clinical studies, liver enzymes and triglyceride analysis. At the end of the recovery period, liver, lungs, spleen, bone/bone marrow, and adrenal glands were examined from the 100-mg/kg group and the spleen was examined from the 30-mg/kg group. At the end of the observation period, liver, lungs, spleen, bone/bone marrow, and adrenal glands were examined from the control and 300-mg/kg groups and the liver, spleen, and adrenal glands were examined from the 100-mg/kg group. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta		Brain[+]
X	Salivary glands[+]	XX	Heart		Peripheral nerve
	Esophagus[+]	X	Bone marrow[+]		(sciatic nerve)[+]
X	Stomach[+]	X	Lymph nodes[+]		Spinal cord (3 levels)
X	Duodenum[+]	XX	Spleen[+]		Pituitary
X	Jejunum[+]		Thymus[+]		Eyes (optic nerve)[+]
X	Ileum[+]				
	Cecum[+]		Urogenital		Glandular
	Colon	XX	Kidneys	XX	Adrenals[+]
	Rectum		Urinary bladder[+]		Lacrimal gland
XX	Liver[+]	XX	Testes[‡]		Mammary gland[+]
	Gallbladder[+]	X	Epididymides		Parathyroids[+]
	Pancreas[+]		Prostate	XX	Thyroids[+]
			Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
	Trachea[+]	X	Uterus[+]		Other
XX	Lung[+]			X	Bone (femur)[+]
				X	Skeletal muscle[+]
					Skin
					All gross lesions and masses

Histopathological examinations were performed on control, mid-and high-dose males and females; the spleens of low-dose females were also examined following the dosing period.

[‡]Recommended by Subdivision F (October 1982) Guidelines.

1. Results:

a. Organ Weights: At the end of the dosing period, the relative mean liver weights were significantly higher in males and females from the 100- and 300-mg/kg groups when compared to controls (Table 5). Relative spleen weights for males in the 300-mg/kg group and absolute and relative spleen weights for females in the 100- and 300-mg/kg groups were also significantly increased. In addition, there was a significant increase in relative kidney weights in females of the 100- and 300-mg/kg groups. At the end of the recovery period, no toxicologically relevant treatment related changes in absolute or relative organ weights were noted.

b. Gross Pathology: No compound-related changes were found on gross examination of the animals at the end of the treatment and recovery periods.

c. Microscopic Pathology:

1) Nonneoplastic: Table 6 summarizes the histologic findings at the end of the dosing period. Histologic findings were primarily in the males, and females receiving 300 mg/kg/day. There was an increased occurrence of fat in the livers of both sexes, an enlargement of centrilobular hepatocytes in males, and an increase in periportal stroma associated with bile duct proliferation in females, for the 300-mg/kg/day dose groups. In the spleen, there was sclerosis (increased connective tissue) in the red pulp in all females receiving 300 mg/kg/day and a decrease in iron pigment in high-dose males and mid- and high-dose females. In the adrenal cortex, there was an increase in fat vacuoles in the zona glomerulosa of high-dose females and an enlargement of the zona glomerulosa in high-dose males. Two high-dose females showed increased fat cells in the bone marrow and two had hyperplastic changes of the endothelial cells of the lungs.

After the 4-week recovery period, there were no remarkable histologic changes except a persistence of sclerotic changes in the spleen and an increased periportal stroma in the high-dose females.

TABLE 5. Mean Organ Weights (± S.D.) and Organ-to-Body Weight Ratios of Rats Administered HWG 1608 Orally for 4 Weeks

Dose (mg/kg/day)	Liver		Spleen		Kidney	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Males						
0	9.9 ± 0.74	3.9 ± 0.13	0.50 ± 0.055	0.20 ± 0.016	1.6 ± 0.10	0.64 ± 0.033
30	9.8 ± 0.73	4.0 ± 0.18	0.46 ± 0.038	0.19 ± 0.012	1.5 ± 0.13	0.62 ± 0.035
100	10.4 ± 1.51	4.2 ± 0.29*	0.48 ± 0.068	0.20 ± 0.015	1.6 ± 0.20	0.64 ± 0.032
300	11.1 ± 1.57	5.0 ± 0.54**	0.51 ± 0.095	0.23 ± 0.031*	1.4 ± 0.11	0.62 ± 0.031
Females						
0	6.5 ± 0.76	3.6 ± 0.35	0.37 ± 0.051	0.20 ± 0.018	1.1 ± 0.11	0.61 ± 0.037
30	6.8 ± 0.62	3.7 ± 0.22	0.39 ± 0.035	0.21 ± 0.016	1.1 ± 0.072	0.61 ± 0.034
100	7.3 ± 0.74	3.9 ± 0.30*	0.46 ± 0.042**	0.25 ± 0.021**	1.2 ± 0.071	0.65 ± 0.034*
300	9.6 ± 1.44**	5.4 ± 0.69**	0.56 ± 0.092**	0.31 ± 0.032**	1.3 ± 0.10**	0.72 ± 0.041**

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

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TABLE 6. Representative Histopathological Findings in Rats at the End of the 28-Day Administration Period

Organ/Finding	Dose Group (mg/kg) Males 0	30	100	300	Females 0	30	100	300
Spleen	(5)[a]	--[b]	(5)	(5)	(4)	(5)	(5)	(5)
Reduced occurrence of iron pigment	0	--	0	5	0	0	5	5
Increased occurrence of reticular fibers in the red pulp	0	--	0	0	0	0	0	5
Liver	(5)	--	(5)	(5)	(4)	--	(5)	(5)
Increased hepatocellular mitosis	0	--	0	0	0	--	0	2
Fatty change in hepatocytes	0	--	0	5	1	--	0	3
Increase in periportal stroma	0	--	0	0	0	--	0	5
Enlarged centrilobular hepatocytes	0	--	0	5	0	--	0	0
Lungs	(5)	--	(5)	(5)	(4)	--	(5)	(5)
Proliferated endothelium, rich in cytoplasm, in the blood vessels	0	--	0	0	0	--	0	2
Foreign body granuloma	0	--	1	0	0	--	0	0
Adrenals	(5)	--	(5)	(5)	(4)	--	(5)	(5)
Hypertrophy of the zona glomerulosa of the adrenal cortex	0	--	0	4	0	--	0	0
Irregularly arranged fasciculata cells with increased variable-sized fat vacuoles	0	--	0	0	0	--	0	5
Bone marrow	(5)	--	--	(5)	(4)	--	(5)	(5)
Increased occurrence of yellow marrow	0	--	--	0	0	--	0	2

[a]Numbers in parentheses are number of tissues examined.

[b]-- indicates tissue not examined.

D. STUDY AUTHORS' CONCLUSIONS:

The 4-week oral treatment of male and female rats dosed 30 or 100 mg/kg HWG 1608 did not produce any clinical signs of toxicity at the end of the treatment and observation periods. Weight gains were comparable to controls. Rats in the 300-mg/kg dose group exhibited mild lethargy during the treatment period but were normal during the observation period. The mean weight gain of the rats in the high-dose group showed a delay in weight gain during the treatment period; weight gains were comparable to controls at the end of the observation period. Three rats died during the course of the study; there was no clear explanation on the basis of the necropsy findings.

The hematological findings (decreased hemoglobin concentration and hematocrit values for males and females, reduced corpuscular hemoglobin, and lower corpuscular volume of the individual erythrocytes in female rats) in addition to a slight compensatory increase in reticulocytes in the female rats of the 100- and 300-mg/kg dose groups indicate a slight but compound-related effect on the red blood profile. No effect compared to controls was seen at 30 mg/kg. An increased leukocyte count for the high-dose group was explained by the activation of the hematopoietic system.

The 300-mg/kg dosed rats exhibited a significant increase in transaminase activity, an induction of the microsomal enzyme system, and an increased triglyceride concentration in liver tissue. Rats dosed with 100 mg/kg HWG 1608 also had an induction of the liver microsomal enzyme systems. Correlated with these findings was an increase in absolute and relative liver weight of rats dosed with 100 and 300 mg/kg. These results indicate a compound-related effect on liver function. Supportive of these findings were the histopathological results of fat in the liver, increased periportal stroma with bile duct proliferations, and enlarged centrilobular hepatocytes of rats dosed with 300 mg/kg HWG 1608. Histopathological examinations at the end of treatment also revealed changes in the adrenal cortices of rats dosed with 300 mg/kg, indicating increased activity. Changes in the spleen of the 100- and 300-mg/kg dose groups included sclerosis of the red pulp, sideropenia, and increased spleen weights. These changes were associated with effects on the blood and the increased metabolic efficiency of the spleen. No histopathological compound-related effects were seen at the lowest dose.

No biologically relevant changes were seen at the end of the recovery period with the exception of female rats at the highest dose, which exhibited increased fiber content of the red pulp of the spleen and the periportal fields of the liver. There were also fat vacuoles and mild reactions of the sinus

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There were also fat vacuoles and mild reactions of the sinus endothelial cells in the zona fasciculata. This indicates incomplete recovery of the organs involved.

Overall, the female rats were more sensitive to HWG 1608 than male rats. A dose level of 30 mg/kg was tolerated by both male and female rats with no observable effect when administered orally for 28 days.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The oral administration of HWG 1608 to male and female rats for 4 weeks followed by a 4-week recovery period resulted in the treatment being tolerated without clinical signs or changes in body weight gain in the 30- and 100-mg/kg dose groups. The mild lethargy and delay in body weight gain seen at the 300-mg/kg dose was seen during treatment but the rats were comparable to controls during the recovery period. The 100- and 300-mg/kg doses resulted in effects on the red blood cell profile. Impairment of liver function at 300 mg/kg, induction of the microsomal enzyme system at 100 and 300 mg/kg, and increased triglyceride concentration at 300 mg/kg was correlated with an increase in absolute and relative liver weights. Histopathological data also indicated effects in the liver. Compound-related changes in the adrenal cortex and spleen were noted. The effects on the spleen were associated with the changes in red blood cells. The rats from the 100-mg/kg dose group recovered completely and had no biologically relevant changes at the end of the observation period. The female rats in the 300-mg/kg dose group, however, still exhibited microscopic organ changes of the liver, spleen, and adrenals, indicating an incomplete recovery. The 30-mg/kg dose was tolerated by male and female rats with no observable effect during either the treatment or recovery periods.

The conduct and reporting of the study were, in general, adequate. There was no summary tabulation of histopathology and only five animals/sex/group were used (five animals were used for clinical chemistry and liver enzyme analyses). A limited number of tissues were examined in the treatment groups; an even more limited examination was performed in the recovery groups. However, the target organs were examined. We agree with the study authors' conclusions. The LOEL is 100 mg/kg/day and the NOEL is 30 mg/kg/day.

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007200

EPA No.: 68D80056
DYNAMAC No.: 147-Q
TASK No.: 1-47Q
April 4, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE (FOLICUR)

Subacute Inhalation Toxicity in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Puente for

Date: 4/4/89

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007200

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April 4, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE (FOLICUR)

Subacute Inhalation Toxicity in Rats

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

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Date: April 5, 1989

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007200

DATA EVALUATION RECORD

GUIDELINE § NA

STUDY TYPE: Subacute inhalation in rats.

ACCESSION/MRID NUMBER: 407009-38.

TEST MATERIAL: HWG 1608.

SYNONYM(S): Terbuconazole; Folicur; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazole-1-yl-methyl)-pentane-3-ol.

STUDY NUMBER(S): 94559.

SPONSOR: Mobay Corporation, Kansas City, MO.

TESTING FACILITY: Bayer AG Institute for Toxicology, Wuppertal, FRG.

TITLE OF REPORT: Study for Subacute Inhalation Toxicity to the Rat for Three Weeks (Exposure 15 x 6 hours).

AUTHOR(S): Pauluhn, J.

REPORT ISSUED: February 22, 1985.

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CONCLUSIONS: Wistar rats tolerated aerosol exposure to HWG 1608 at 1.2 or 10.6 mg/m^3 for fifteen daily 6-hour exposures without toxic signs or any effects on in-life parameters or on organ weights and on gross or histologic findings. Exposure at 155.8 mg/m^3 caused piloerection. There was also a moderate induction of liver O-demethylase in liver at the highest exposure level and a sig- nificant induction of liver N-demethylase ($p < 0.01$) in both sexes. There were no effects of toxicologic importance on clinical laboratory parameters or organ weights nor was there an increase in histologic lesions. The LOEL is 155.8 mg/m^3 and the NOEL is 10.6 mg/m^3.

Classification: CORE-Minimum

A. MATERIALS:

1. Test Compound: HWG 1608; batch No.: 16001/83; purity: 96.2%.

2. Test Animals: Species: rat; strain: Wistar Bor; age: approximately 6 weeks old; weight: 160-200 g; source: Winkelmann, Borchen, FRG.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test group:

Test Group	Concentration (mg/m^3)	Main Study Males	Main Study Females
1 Control Air	0	10	10
2 Control Vehicle	0	10	10
3 Low (LDT)	5	10	10
4 Mid (MDT)	50	10	10
5 High (HDT)	500	10	10

2. Inhalation Exposure Conditions: The animals were exposed by head/nose only in a 20-L chamber that had a 20-L antechamber. The test material for spraying was dissolved

in lutrol-ethanol (1:1 polyethylene glycol E40/ethanol) at 0.025, 0.25, or 2.5%. Using a binary jet nebulizer, 200 µL of the compound in vehicle was sprayed per minute into the airflow (10 L/minute air) while simultaneously 8 L/minute air exited from the chamber. There were about 15 air changes/minute. The temperature of the chamber and flow rates were checked. The atmosphere was analyzed for sampling times of 250, 50, and 12.5 minutes at the low-, mid-, and high-exposure levels, respectively. One analysis was performed for the low level and three analyses for the mid- and high-exposure levels. Samples were taken near the breathing area to determine particle count and mass distribution with a cascade impactor.

Results: The stock solutions were prepared weekly and stored at 4°C and analyzed twice weekly for stability by high-performance liquid chromatography. Table 1 summarizes data on analytical concentrations in the inhalation chamber, particle size, and the percent of mass that was of respirable size. The air samples were analyzed at 10 intervals during the study; three samples/interval were taken at the mid and high doses. The range of mean values for the interval was 0.7 to 1.79 mg/m^3 at the low dose, 7.49 to 14.05 mg/m^3 at the mid dose, and 115 to 209 mg/m^3 at the highest dose. Data (tabular and graphic) were presented for the particle size and distribution of aerosol at 50 and 500 mg/m^3 at one interval.

3. Food and Water Consumption: Animals received food (Altromin 1324) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Means, standard deviations and confidence limits at 95 and 99% were calculated. The Mann-Whitney test and Wilcoxon U test were employed for statistical comparisons. All exposure groups were compared with the air control and not the vehicle control.

5. Quality Assurance: A quality assurance statement was dated February 20, 1985, but unsigned.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected several times on exposure days for signs of toxicity and mortality, but not during the exposure.

TABLE 1. Analysis of HWG 1608 in the Air and Particle Analysis

Nominal Concentration (mg/m^3)	Analytical Concentration ± SD (mg/m^3)	MMAD[a] ($\bar{X}$) (μm)	MAV (%)
0 (Vehicle)	0	2.0 ± 2.1	90
5	1.2 ± 0.41	2.1 ± 2.0	90
50	10.6 ± 2.33	2.0 ± 2.0	92
500	155.8 ± 36.9	2.1 ± 2.1	89

[a]Abbreviations:

MMAD - Mass median aerodynamic diameter.

($\bar{X}$) - Geometric standard deviation.

MAV - Respirable mass (particles with MMAD below 5 μm).

Results: One sham-exposed female was accidentally killed when the exposure tube; all other rats survived to study termination. High-exposure group rats had bristling coats after each exposure. There were no adverse effects in any other groups.

2. Body Weight: Rats were weighed before the first exposure and then weekly.

Results: There were no toxicologically important effects on body weights. The mean weights in males exposed at 500 mg/m^3 were significantly lower than controls at 1 and 3 weeks. However, the mean body weight for this group was 7 g lower than controls at study initiation and the weight gain over the 3 weeks of the study was similar to that in control males. Table 2 presents body weight data.

3. Food Consumption: Food consumption was not measured.

4. Hematology and Clinical Chemistry: Blood was collected at study termination for hematology and clinical chemistry analysis. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)		
X	Platelet count	X	Mean corpuscular volume (MCV)
	Reticulocyte count (RETIC)	X	Coagulation: thromboplastin time (PT)
	Red cell morphology		

Results: There was no effect of exposure on any erythroid parameter. Differential leukocyte counts and thromboplastin time were similar in dosed and control groups. Likewise, there was no effect of vehicle on any parameter.

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TABLE 2. Mean Body Weight in Rats Exposed to HWG 1608

Exposure Group (mg/m^3)	Mean Weight (g ± SD) at Week 0	1	2	3
		Males		
(Air) 0	197 ± 5	200 ± 6	211 ± 9	218 ± 12
(Vehicle) 0	195 ± 8	202 ± 10	214 ± 12	221 ± 14
5	198 ± 5	202 ± 10	215 ± 10	225 ± 11
50	191 ± 6	198 ± 7	204 ± 6	215 ± 7
500	190 ± 6	192 ± 8*	200 ± 9	209 ± 10*
		Females		
(Air) 0	169 ± 6	166 ± 6	168 ± 8	170 ± 8
(Vehicle) 0	173 ± 7	172 ± 6	174 ± 9	178 ± 8
5	169 ± 5	168 ± 5	170 ± 5	173 ± 5
50	171 ± 6	174 ± 4	170 ± 5	174 ± 4
500	169 ± 5	172 ± 5	173 ± 6	177 ± 6

*Significantly different from control value, $p<0.05$.

b. Clinical Chemistry:

	Enzymes		Other
X	Alkaline phosphatase (ALP)		Total protein
	Cholinesterase		Albumin
X	Creatinine phosphokinase		Albumin/globulin ratio
X	Lactic acid dehydrogenase	X	Blood creatinine
X	Serum alanine aminotransferase (SGPT)	X	Blood urea nitrogen
			Cholesterol
X	Serum aspartate aminotransferase (SGOT)		Globulins
		X	Glucose
	Gamma glutamyltransferase (GGT)		Total bilirubin
X	Glutamic dehydrogenase (GLDH)		Direct bilirubin
	Mixed function oxidases (Liver)		Triglycerides
X	Cytochrome P-450		
X	N-Demethylase		
X	O-Demethylase		

Results: It was reported that there were no clinical chemistry changes that were of toxicologic importance. Table 3 presents data for glutamic dehydrogenase, creatinine, and urea. The laboratory control range for glutamic dehydrogenase (two standard deviations) was 6 to 29 U/L. For creatinine the corresponding range was 24 to 89 µmol/L. All values that were indicated as significant in Table 3 were within the normal range. The vehicle may have caused the significant decreases in urea seen in exposed groups.

There was a significant induction of N-demethylase in both males and females exposed at 500 mg/m^3 and a marginal increase in O-demethylase at the high exposure level in males but not females (Table 4). Cytochrome P450 was not affected by exposure.

5. Urinalysis: Urine was collected from fasted animals prior to termination. Protein, blood pH, and glucose were determined semi-quantitatively and sediment was examined microscopically.

Results: There were no effects of exposure on urinary parameters.

TABLE 3. Selected Mean Clinical Chemistry Parameters in Rats Exposed to HWG 1608 for 21 Days

Nominal Air Level (mg/m^3)		Glutamic Dehydrogenase (U/L)		Creatinine (µmol/L)		Urea (mmol/L)	
		Males	Females	Males	Females	Males	Females
(Air)	0	1.1 ± 0.7	1.2 ± 2.5	74 ± 12	67 ± 9	9.19 ± 0.87	8.65 ± 0.62
(Vehicle)	0	0.7 ± 0.5	7.4 ± 19.0[a]	71 ± 18	65 ± 17	7.80 ± 0.75**	7.70 ± 0.83**
	5	1.8 ± 0.9	0.8 ± 1.2	60 ± 5*	56 ± 8*	7.46 ± 0.65**	6.66 ± 0.69**
	50	3.7 ± 1.3**	2.5 ± 0.9	52 ± 2*	53 ± 7*	7.45 ± 1.25**	7.27 ± 0.80**
	500	3.9 ± 1.4**	1.8 ± 0.9	51 ± 4*	52 ± 7**	7.37 ± 0.65**	7.19 ± 0.63**

[a]A value of 63 U/L was included in calculating the mean.

*Significantly different from control level, $p<0.05$.

**Significantly different from control level, $p<0.01$.

TABLE 4. Inducible Liver Enzymes in Rats Exposed to HWG 1608

Nominal Air Level (mg/m^3)	N-Demethylase (mmol/g/min)		O-Demethylase (mmol/g/min)	
	Males	Females	Males	Females
(Air) 0	104.2 ± 13.9	45.2 ± 6.6	10.6 ± 2.2	11.1 ± 1.3
(Vehicle) 0	96.8 ± 21.0	44.0 ± 9.8	9.2 ± 1.6	10.7 ± 1.6
5	113.2 ± 11.9	46.5 ± 6.9	9.4 ± 1.4	11.0 ± 3.1
50	117.4 ± 16.1	46.9 ± 8.3	9.8 ± 1.7	11.0 ± 2.1
500	154.4 ± 27.5**	63.9 ± 6.7**	12.3 ± 1.9*	11.6 ± 1.6

*Significantly different from control level, $p<0.05$.

**Significantly different from control level, $p<0.01$.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination from five animals/sex of the high-dose group and control group. The other five/sex/group were used for clinical studies, liver enzymes and triglyceride analysis. At the end of the recovery period, liver, lungs, spleen, bone/bone marrow, and adrenal glands were examined from the 100-mg/kg group and the spleen was examined from the 30-mg/kg group. At the end of the observation period, liver, lungs, spleen, bone/bone marrow, and adrenal glands were examined from the control and 300-mg/kg groups and the liver, spleen, and adrenal glands were examined from the 100-mg/kg group. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta		Brain+
X	Salivary glands+	XX	Heart		Peripheral nerve
	Esophagus+	X	Bone marrow+		(sciatic nerve)+
X	Stomach+	X	Lymph nodes+		Spinal cord (3 levels)
X	Duodenum+	XX	Spleen+		Pituitary
X	Jejunum+		Thymus+		Eyes (optic nerve)+
X	Ileum+				
	Cecum+		Urogenital		Glandular
	Colon	XX	Kidneys	XX	Adrenals+
	Rectum		Urinary bladder+		Lacrimal gland
XX	Liver+	XX	Testes+		Mammary gland+
	Gallbladder+	X	Epididymides		Parathyroids+
	Pancreas+		Prostate	XX	Thyroids+
			Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
	Trachea+	X	Uterus+		Other
XX	Lung+			X	Bone (femur)+
				X	Skeletal muscle+
					Skin
					All gross lesions and masses

Histopathological examinations were performed on control, mid-and high-dose males and females; the spleens of low-dose females were also examined following the dosing period.

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 5. Liver Weight Data in Males Exposed to HWG 1608 for 3 Weeks

Nominal Exposure Level (mg/m^3)	Mean Values ± S.D. (g)	(% ±)
(Air) 0	8.7 ± 0.94	4.0 ± 0.30
(Vehicle) 0	8.1 ± 0.56	3.7 ± 0.17*
5	8.2 ± 0.53	.7 ± 0.17*
50	8.1 ± 0.51	3.8 ± 0.21
500	7.4 ± 0.85*	3.6 ± 0.26**

*Significantly different from control value, $p < 0.05$.

**Significantly different from control value, $p < 0.01$.

b. Gross Findings: It was reported that there were no exposure related organ changes "induced by the active ingredients". Gross findings were not tabulated.

c. Microscopic Pathology: There were no neoplastic findings. Table 6 summarizes nonneoplastic findings. There were no alterations caused by test compound in lung, liver, or any other organs examined. The vehicle caused some irritative changes in the respiratory system; these were not severe and did not increase in HWG 1608 exposed groups.

D. STUDY AUTHORS' CONCLUSIONS:

Groups of 10 male and 10 female rats were exposed (head-nose) to an HWG 1608 aerosol at concentrations of 1.2, 10.6, or 155.8 mg/m^3 for fifteen 6-hour exposures over 3 weeks. Controls were exposed to air or the vehicle (lubrol-ethanol aerosol). There were no toxicologically important effects on body weights, hematology, clinical chemistry, or urinary parameters, or on gross or microscopic findings. The only toxic signs were piloerection (hair bristling) in the highest exposed group. There was also a slight induction of N-demethylases in the liver at the highest exposed group. Organ weight data and histologic examination did not indicate any specific alterations in the liver (or other organs). Based on enzyme induction, the NOEL is 10.6 mg/m^3.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate. The respirable mass was approximately 90% for vehicle controls and for all three exposure concentrations and the particle size was not affected by the concentration. We agree with the study author's conclusions that there was no effect of toxicologic importance on body weights, hematology, or urinary parameters. It is our assessment that the significant increases in glutamic dehydrogenase and creatinine were not of toxicologic importance. The significant values for glutamic dehydrogenase were well within normal variations found for the assay and occurred in only one sex. Decreased creatinine values do not indicate an adverse effect and the values in the dose groups were within one standard deviation of the vehicle control. The urea concentrations were affected primarily by the vehicle and not the test compound. We assess that the induction of liver N-demethylase in males and females at 166 mg/m^3 was related to exposure; this is not considered an adverse effect but an adaptive effect. The decrease in liver weight in males exposed at 166 mg/m^3 was minimal and related to vehicle rather than test compound. Decreased liver weights are usually not

TABLE 6. Histologic Lesions in Rats Exposed to HWG 1608 for 21 Days

jan/Lesion	Nominal Exposure Level (mg/m^3)									
	Males					Females				
	0	0(v)[a]	5	50	500	0	0(v)	5	50	500
il/paranasal cavities	(10)[b]	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
peremia, slight	10	5	6	8	7	7	5	9	9	7
peremia, moderate	0	4	2	1	1	2	9	0	0	3
menal debris, light/moderate	1	0	1	3	2	0	0	2	0	0
chea	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
minal cellular ebris, slight	6	3	4	2	2	2	1	1	0	1
ood in lumen	1	2	0	1	1	2	1	0	1	2
gs	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
rginal emphysema, light to moderate	7	5	7	7	7	8	6	6	7	7
creased macrophages, light	0	3	0	0	0	1	0	0	0	0
cal hemorrhage, light	0	0	1	1	0	0	3	0	1	0
g-Associated Lymph Nodes	(9)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
nus catarrh	3	6	6	3	4	1	5	4	4	3
er	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
stiocytic modules	1	0	1	0	0	1	1	2	2	0

v = vehicle control.

he figures in parentheses are the number of animals from which the tissue was examined microscopically.

indicative of toxicity whereas increases are. The histologic changes in the respiratory system were very slight and probably related to the vehicle.

007200

EPA No.: 68D80056
DYNAMAC No.: 147-P
Task No.: 1-47P
March 29, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Range-Finding Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J. Pinto (for)

Date: 3-29-89

247 247

007200

EPA No.: 68D80056
DYNAMAC No.: 147-P
Task No.: 1-47P
March 29, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Range-Finding Study in Dogs

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EPA Reviewer and
Section Head
Review Section I
Toxicology Branch II
(TS-769C)

Signature: M Ioannou
Date: 5-10-89

007200

DATA EVALUATION RECORD

STUDY TYPE: Range-finding study in dogs.

ACCESSION/MRID NUMBER: 407009-35.

TEST MATERIAL: HWG 1608.

SYNONYM(S): Terbuconazole; Ethyltrianol; Folicur; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)-pentane-3-ol.

STUDY NUMBER(S): 94573.

SPONSOR: Mobay Corporation, Stilwell, KS.

TESTING FACILITY: Bayer AG Institute of Toxicology, Pharmaceutical, Wuppertal-Elberfeld, Federal Republic of Germany.

TITLE OF REPORT: Range-Finding Toxicological Study to Establish Dosage for a Subchronic Study of Toxicity to Beagle Dogs.

AUTHOR(S): Keutz, E. Von

REPORT ISSUED: July 1, 1986.

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CONCLUSIONS: When 500 or 5000 ppm HWG 1608 was fed to Beagle dogs for 30 days in a range-finding study, there were no overt signs of toxicity or dose-related effects on mortality, body weight, food consumption, hematology, or urinalyses. Alkaline phosphatase was increased in high-dose males and females (combined) throughout the study. Organ weights and histopathological examinations were not performed. Based on increased alkaline phosphatase at 5000 ppm, the NOEL is 500 ppm HWG 1608.

Classification: CORE Supplementary

A. MATERIALS:

1. Test Compound: HWG 1608; description: light yellow crystals; batch No.: 16007/83; purity: 93.4%.

2. Tes Animals: Species: dog; strain: Beagle; age: 37 to 53 weeks at study initiation; weight: 9.2 to 11.8 kg at study initiation; source: F. Winkelmann, D-4799 Borchen.

B. STUDY DESIGN:

1. Animal Assignment: Following a preliminary examination, animals were randomly assigned to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study (30 days) Males	Females
1 Control	0	2	2
2 Low (LDT)	500	2	2
3 High (HDT)	5000	2	2

2. Diet Preparation: The test compound was uniformly mixed with the basal diet at the appropriate test concentrations. Test diet concentration (reported under the category of homogeneity) and stability analyses were performed with 10-and 5000-ppm nominal concentration test diets. Analyses of the homogeneity of the test diets were not reported. Control animals received the basal diet only.

Results: Recovery values of the diets were within acceptable limits, e.g., 96-100% of the nominal 10-ppm diets and 102-106% of the nominal 5000-ppm diets. Recovery of the test material in dry feed was found to be 99% over 7 days of storage and 106% over 14 days of storage for a 10-ppm diet. Recovery in the 5000-ppm diet was found to be 105% over 7 days of storage and 100% over 14 days of storage. Recovery of the test material in wet feed varied from 78 to 83% over a 24-hour period for the 10-ppm diet and 104 to 95% over this same time interval for the 5000-ppm diet.

3. Food and Water Consumption: Animals received food (approximately 400 g daily of Ssniff HH Sole diet for dogs, double ground) and water ad libitum.

4. Statistics: No statistical analyses were utilized in analyzing the numerical data.

5. Quality Assurance: A quality assurance statement was not presented.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of morbidity and mortality.

Results: There were no deaths reported during the study. There were no clinical observations found that were considered to be attributable to dosing.

2. Body Weight: Dogs were weighed prior to study initiation, at study initiation, and weekly thereafter.

Results: There was no effect of dosing on mean body weights. All dosed animals gained weight during the study with the exception of one high-dose female. Representative weight data are summarized in Table 1.

3. Food Consumption and Compound Intake: Food consumption and drinking water intake were determined daily.

Results: Food consumption was decreased in three of four high-dose males and females during study week 1; however, the food consumption of these dogs was comparable to that of controls from study weeks 2 to 5. Quantitative tabulation of food consumption was not provided. Water intake was not affected.

TABLE 1. Representative Body Weights and Total Weight Gain of Dogs Fed HWG 1608 for 30 Days[a]

Dose (ppm)	Mean Body Weight (kg ± SD) at Week 0	Mean Body Weight (kg ± SD) at Week 5	Weight Gain From Weeks 0 to 5
		Males	
0	11.0 ± 0.63	11.5 ± 0.28	+ 0.5
500	11.0 ± 1.20	11.4 ± 1.20	+ 0.4
5000	10.2 ± 0.57	11.0 ± 0	+ 0.8
		Females	
0	9.9 ± 0.99	10.2 ± 1.20	+ 0.3
500	10.5 ± 0.64	10.8 ± 0.57	+ 0.3
5000	10.6 ± 0.64	10.6 ± 0	+ 0.0

[a]Standard deviations were calculated by reviewers.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from the jugular vein prior to study initiation and weekly from week 2 to study termination for hematology and clinical analyses from all animals. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count	X	Coagulation: thromboplastin time (PT)
X	Reticulocyte count (RETIC)	X	Blood sedimentation rate
	Red cell morphology		

Results: It was reported that there were no changes of toxicological significance in hematology parameters. Analyses of these parameters were performed using combined values of males and females. Slight decreases in the erythrocyte count, hemoglobin concentration, and hematocrit of high-dose dogs at weeks 4 and 5 were considered within the range of normal biological variation.

b. Clinical Chemistry:

	Electrolytes
	Calcium
X	Chloride
	Magnesium
	Phosphorus
	Potassium
X	Sodium

	Enzymes
X	Alkaline phosphatase (ALP)
	Cholinesterase
X	Creatinine phosphokinase
	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (SGPT)
X	Serum aspartate aminotransferase (SGOT)
	Gamma glutamyltransferase (GGT)
X	Glutamate dehydrogenase (GLDH)

	Other
X	Albumin
	Albumin/globulin ratio
	Blood creatinine
	Blood urea nitrogen
X	Cholesterol
	Globulins
X	Glucose
X	Total bilirubin
	Direct bilirubin
X	Total protein
	Triglycerides
X	Urea

Results: Alkaline phosphatase was found to be increased in high-dose males and females (combined) from study weeks 2 to 5; this increase was especially high in one high-dose male (animal No. 275) and one high-dose female (animal No. 530). This change was considered to be compound related and of toxicological significance. Urea was found to be increased in males and females (combined) fed 500 ppm HWG 1608 at week 5; however, high-dose males and females were unaffected. This change was not considered to be of any toxicological significance. All other clinical chemistry parameters were similar in control and dosed males and females.

6. Urinalysis: Urine was collected from fasted animals at study initiation and weekly to study termination. Prior to urine collection, animals were administered 250 mL tapwater by stomach tube. The CHECKED (X) parameters were examined:

	Appearance		
X	Volume	X	Glucose
X	Specific gravity	X	Ketones
X	pH	X	Bilirubin
X	Sediment (microscopic)	X	Blood
X	Protein		Nitrate
			Urobilinogen

Results: Urinalysis results were similar for dosed and control animals.

D. STUDY AUTHOR'S CONCLUSIONS:

In a range-finding study to establish dosage for a subchronic toxicity study, the author concluded that a daily dose of up to 500 ppm HWG 1608 administered to Beagle dogs did not produce any signs of toxicity. The only effect attributable to treatment with the test substance was an isolated rise in alkaline phosphatase activity in two of four dogs treated with 5000 ppm HWG 1608, which was considered a possible manifestation of a compound-related liver effect. A subchronic toxicity study using the doses of 0, 200, 1000, and 5000 ppm was proposed.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate although there were some deficiencies in the conduct of the study and in data reporting. Test diet concentration was incorrectly reported under the category of homogeneity. Analyses of the homogeneity of the test diets were not reported. Doses of 10 and 5000 ppm were used to test diet concentration and stability rather than the

actual test diets of 500 and 5000 ppm. No explanation was provided for this substitution. Standard deviations were not provided for body weight data even though the study protocol indicates that these analyses would be reported. Hematological and clinical chemistry results were reported as combined for males and females. The reviewers do not consider this grouping to be valid.

We agree with the study author that the only toxicologically significant compound-related effect is an increase in alkaline phosphatase in high-dose dogs. Based on this parameter, the NOEL is 500 ppm HWG 1608.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO [illegible])

EPA: 68D80056
DYNAMAC No.: 147-N
April 4, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

21-Day Dermal Toxicity Study in Rabbits

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 4/4/89

007200

EPA: 68D80056
DYNAMAC No.: 147-N
April 4, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

21-Day Dermal Toxicity Study in Rabbits

REVIEWED BY:

Claire Kruger-McDermott, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Claire Kruger-McDermott
Date: April 4, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 4, 1989

APPROVED BY:

Roman Pienta, Ph.D.
Technical Quality Control
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Signature: Roman J Pienta
Date: April 4, 1989

Mike Ioannou, Ph.D.
EPA Reviewer, Section I
Toxicology Branch II
(TS-769C)

Signature: M. Ioannou
Date: 5-10-89

Mike Ioannou, Ph.D.
EPA Section Head, Section I
Toxicology Branch II
(TS-769C)

Signature: _____
Date: _____

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007200

DATA EVALUATION RECORD

STUDY TYPE: 21-Day dermal toxicity study in rabbits.

ACCESSION/MRID NUMBER: 407009-37.

TEST MATERIAL: HWG 1608.

SYNONYM(S): Terbuconazole; Folicur; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-tri-azol-1-yl-methyl)-pentane-3-ol.

STUDY NUMBER(S): 93093.

SPONSOR: Mobay Chemical Corporation, Stillwell, KS.

TESTING FACILITY: Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, Federal Republic of Germany.

TITLE OF REPORT: HWG 1608. Subacute Study of Dermal Toxicity to Rabbits.

AUTHOR(S): K. G. Heimann and B. Schilde.

REPORT ISSUED: May 8, 1984.

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NCLUSIONS: Under the conditions of the study, no biologically gnificant systemic or local effects were found when HWG 1608 was lministered on rabbit skin at 0, 50, 250, or 1000 mg/kg/body ight for 21 days. The minimal skin effects seen at the high dose re attributed to mechanical irritation of the skin and not an fect of the compound.

assification: CORE Guideline.

MATERIALS:

1. Test Compound: HWG 1608; description: yellowish white powder with clumps; batch No.: 16001/83; purity: 97.1%. Test compound was formulated before each treatment with Cremophor EL/Lewatit water (five drops to 10-mL formulation).

2. Test Animals: Species: rabbit; strain: New Zealand White; age: 10-17 weeks for rabbits in the 0- and 1000-mg/kg groups (no age given for the other groups); weight: 2.4 to 3.0 kg for 0-, 50-, and 250-mg/kg groups and mean of 3.23 kg for males and mean of 3.15 kg for females of the 0- and 1000-mg/kg groups; source: Breeder Hacking and Churchill Ltd., Huntington, England.

STUDY DESIGN:

1. Animal Assignment: After 5 days of acclimation for the 50- and 250-mg/kg and concurrent control groups and 43 days for the 1000-mg/kg and concurrent control groups, animals were randomly assigned to the following test groups:

Test group	Dermal Dose (mg/kg)	Average Volume Applied (mL/kg) Males	Females	Main Study (21 days) Males	Females
1 Control	0	0.5	0.5	6(5*)	6(5*)
2 Low (LDT)	50	0.5	0.5	6	6
3 Mid (MDT)	250	0.5	0.5	6	6
4 High (HDT)	1000	0.5	0.5	5*	5*

*Second control group and high-dose group were tested in a separate experiment.

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2. Preparation of Animal Skin: Fourty-eight hours prior to dosing, the fur was shorn from the back and flanks of all rabbits. In addition, the skin areas to be treated of three males and three females per group (0, 50, and 250 mg/kg) were abraded with sandpaper 24 hours before the start of treatment.[1] New hair growth was reshorn twice weekly over the treatment areas.

   The test material was applied to the skin on back and flank once daily on 15 consecutive workdays (5 days/week) and left uncovered for 6 hours. The skin area, to which the dose was applied, measured approximately 5 x 8 cm. During the 6-hour exposure periods, the rabbits were restrained and food and water were withheld. At the end of each exposure, the treated skin areas were cleaned with soap and water. Control rabbits were treated with the vehicle control formulation (without active ingredients) at the same volume per kilogram body weight as treated animals.

3. Food and Water Consumption: Animals received food (Ssniff K Sole Diet for rabbits) and water ad libitum. Food consumption was not determined.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Arithmetic group means, standard deviation, and upper and lower confidence limits ($p \leq 0.05$ and $p \leq 0.01$) were calculated for body weights, skin findings, medical laboratory examinations, and organ weights. Collective figures for treated and control animals were compared using the Mann-Whitney and Wilcoxon significance test (two-tailed U-test) at the significance levels of $p \leq 0.05$ and $p \leq 0.01$.

5. Quality Assurance: A quality assurance statement was signed and dated February 25, 1988.

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[1] Rabbits in the 1000-mg/kg test group and their respective controls did not have the skin areas abraded.

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C. METHODS AND RESULTS:

1. Observations: Animals were inspected once daily for appearance and behavior. Treated skin areas were examined for signs of inflammation before the start of the study and 24 hours after each treatment.

   Results: Redness as a sign of inflammation of the skin occurred in the animals with abraded skin during the first 3 days of treatment but not thereafter. This effect was present before the start of treatment and was comparable in treated groups and controls; thus, it was attributed to the abrading and not the HWG 1608. A slight and transient redness occurred in the female rabbits (unabraded skin) dosed 50 and 25 mg/kg between days 4 and 7. This did not occur in the males with intact abraded skin or in the females with abraded skin and was therefore considered biologically insignificant. Skin fold measurements did not reveal any significant alterations. Examination of the skin from the rabbits in the 1000-mg/kg group revealed a slight redness between days 5 and 14 in males. One female produced a comparable finding between days 6 and 11; an additional female had a slight skin redness on day 14 of treatment. At the end of treatment, no alterations were noted. Controls did not exhibit any redness. Skin fold measurements revealed slightly thicker skin folds in males on day 4 of treatment. This was not seen in the females (Table 1).

2. Body Weight: Animals were weighed weekly at the end of each study week.

   Results: No significant changes in body weight occurred in treated animals compared to controls. There were no significant variations between treatment groups (Table 2).

3. Food Consumption and Compound Intake: Food consumption was not determined.

4. Ophthalmology: Ophthalmological examinations were not performed.

TABLE 1. Topical Skin Effects in Rabbits Treated with HWG 1608 for 21 Days

Dosage Level (mg/kg/day)	Days					
	Erythema[a]			Skin Thickness[b]		
	0	8	15	0	8	15
Males						
0	(0)0[c]	(0)0	(0)0	(3.1)3.1	(3.0)2.6	(3.1)2.6
50	0	0	0	2.8	2.8	3.4
250	0	0	0	2.8	2.8	3.4
1000	0	1**	0	3.1	3.5	3.3
Females						
0	(0)0	(0)0	(0)0	(2.8)2.4	(2.4)2.0	(2.4)2.2
50	0	0	0	2.7	2.3	2.6
250	0	0	0	2.2	2.3	3.1
1000	0	0	0	2.5	2.4	2.4

[a]Erythema was graded on a basis of 0 to 4 according to Draize.

[b]No units were given for skin thickness.

[c]Values in parentheses are the controls, which were run concurrently with the 1000-mg/kg group.

**Significantly different from control values, $p \leq 0.01$.

TABLE 2. Mean Body Weights (± S.D.) of Rabbits with Abraded Skin Treated With HWG 1608 for 21 Days

Dosage Level (mg/kg/day)	Mean Body Weights (kg) at Week 0	1	2	3
		Males		
0	2.67 ± 0.07	2.71 ± 0.11	2.74 ± 0.12	2.91 ± 0.06
	3.29 ± 0.25[a]	3.26 ± 0.29	3.21 ± 0.22	3.18 ± 0.22
50	2.74 ± 0.07	2.82 ± 0.02	2.77 ± 0.11	3.02 ± 0.12
250	2.66 ± 0.12	2.74 ± 0.09	2.73 ± 0.10	2.98 ± 0.10
1000	3.17 ± 0.19[a]	3.18 ± 0.14	3.10 ± 0.13	3.04 ± 0.17
		Females		
0	2.64 ± 0.23	2.64 ± 0.17	2.65 ± 0.13	2.76 ± 0.06
	3.21 ± 0.13[a]	3.10 ± 0.20	3.04 ± 0.19	2.93 ± 0.22
50	2.74 ± 0.15	2.69 ± 0.18	2.77 ± 0.17	2.93 ± 0.13
250	2.90 ± 0.14	2.90 ± 0.12	2.93 ± 0.10	2.97 ± 0.06
1000	3.09 ± 0.14[a]	2.99 ± 0.18	2.93 ± 0.21	2.89 ± 0.16

[a]Nonabraded skin; experiment was performed separately from the 0-, 50-, and 250-mg/kg dose groups.

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5. Hematology and Clinical Chemistry: Blood was collected from the ear vein of all animals prior to study initiation and after the 3 weeks of treatment. The CHECKED (X) parameters were examined:

a. Hematology

X	Hematocrit (HCT)+	X	Leukocyte differential
	count		
X	Hemoglobin (HGB)+	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)+	X	Mean corpuscular HGB
X	Erythrocyte count (RBC)+		concentration (MCHC)
X	Platelet count+	X	Mean corpuscular volume
	Reticulocyte count (RETIC)		(MCV)
	Red cell morphology		Coagulation:thromboplastin
			time (PT)

Results: There were no significant variations from the controls in the hematology parameters.

b. Clinical Chemistry:

	Electrolytes		Other
	Calcium+		Albumin+
	Chloride+		Albumin/globulin ratio
	Magnesium+	X	Blood creatinine+
	Phosphorus		Blood urea nitrogen+
	Potassium+		Cholesterol+
	Sodium+		Globulins
		X	Glucose+
	Enzymes		Total bilirubin+
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase		Total protein+
	Creatinine phosphokinase+		Triglycerides
	Lactic acid dehydrogenase	X	N-demethylase
X	Serum alanine aminotransferase	X	O-demethylase
	(SGPT)+	X	Cytochrome P-450
X	Serum aspartate aminotransferase		
	(SGOT)+		
	Gamma glutamyltransferase (GGT)		
X	Urea		

+Recommended by Subdivision F (October 1982) Guidelines.

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Results: It was reported that there were no significant changes in the clinical chemistry parameters compared to controls. The liver homogenate examination showed that treatment did not result in induction of the microsomal enzyme systems examined and there were no biologically relevant variations between treatment and control animals.

6. Urinalysis: Urine was collected from fasted animals prior to initiation and at termination. The CHECKED (X) parameters were examined:

X	Appearance+	X	Glucose+
	Volume+		Ketones+
	Specific gravity+		Bilirubin+
	pH	X	Blood+
	Sediment (microscopic)+		Nitrate
X	Protein+	X	Urobilinogen

Sediment:

X	Bacteria	X	Erythrocytes
X	Amorphous salts	X	Calcium oxalates
X	Epithelia	X	Leukocytes
X	Triple phosphates		

Results: There were no significant deviations from control in the results of the analysis of the urine or the urine sediment.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

+Recommended by Subdivision F (October 1982) Guidelines.

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta		Brain
	Salivary glands	XX	Heart		Peripheral nerve (sciatic nerve)
	Esophagus		Bone marrow		
	Stomach		Lymph nodes		Spinal cord (3 level
	Duodenum	XX	Spleen		Pituitary
	Jejunum		Thymus		Eyes (optic nerve)
	Ileum				
	Cecum		Urogenital		Glandular
	Colon	XX	Kidneys	XX	Adrenals
	Rectum		Urinary bladder		Lacrimal gland
XX	Liver	XX	Testes		Mammary gland
	Gallbladder	X	Epididymides		Parathyroids
	Pancreas		Prostate	XX	Thyroids
			Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
	Trachea	X	Uterus		Other
XX	Lung				Bone (femur)
					Skeletal muscle
				X	Skin (treated and untreated)
					All gross lesions and masses

Results:

a. Organ Weights: The absolute and relative spleen and kidney weights were increased in the male rabbits dosed 250 mg/kg. This was attributed to very high organ weights in the animals whose histopathological examination revealed an infestation with Encephalitozoon. The female animals dosed 1000 mg/kg showed a significant increase in relative liver weight; however, due to the absence of gross pathological, histopathological, or clinical chemistry correlations, this was not considered to be a treatment-related effect (Table 3).

b. Gross Pathology: At autopsy at the end of treatment, no compound-related alterations were observed.

c. Microscopic Pathology:

1) Nonneoplastic: The histopathological findings for the internal organs revealed spontaneous alterations unrelated to treatment. No compound-induced skin alterations were noted at 50 or 250 mg/kg. Histopathological examination of the skin from the 1000-mg/kg group revealed a minimal thickening of the epidermis, in comparison to untreated skin, in two males and four females. In the four females, minimal hyperkeratosis was also noted.

TABLE 5. Selected Relative Organ Weights (g/kg) of Rabbits Treated with HWG 1608 for 21 Days

Organ	Intact Skin				Abraded Skin			
	0	50	250	1000[a]	0	50	250	1000[a]
				Males				
Spleen	0.333 ± 0.06 (0.515 ± 0.14)	0.453 ± 0.08	0.800 ± 0.32	0.438 ± 0.14	0.253 ± 0.06	0.297 ± 0.03	0.323 ± 0.08	---
Kidney	5.55 ± 0.73 (5.59 ± 0.35)	6.38 ± 0.31	8.67 ± 1.59	5.86 ± 0.81	6.16 ± 0.70	5.55 ± 0.29	5.28 ± 0.69	---
Liver	30.05 ± 4.72 (26.55 ± 2.63)	34.29 ± 2.52	34.33 ± 4.29	28.35 ± 0.69	35.74 ± 3.06	34.81 ± 5.01	30.94 ± 5.51	---
				Females				
Spleen	0.430 ± 0.08 (0.521 ± 0.08)	0.320 ± 0.07	0.473 ± 0.22	0.558 ± 0.15	0.360 ± 0.04	0.417 ± 0.12	0.377 ± 0.03	---
Kidney	5.98 ± 0.20 (5.26 ± 0.39)	5.98 ± 0.84	5.84 ± 0.33	6.04 ± 0.06	6.29 ± 0.57	6.37 ± 0.29	5.89 ± 0.42	---
Liver	28.78 ± 1.02 (23.89 ± 1.73)	27.54 ± 1.53	29.59 ± 2.39	27.52 ± 1.52*	29.58 ± 4.74	26.01 ± 2.33	27.69 ± 3.31	---

[a]The relative organ weights of animals dosed 1000 g/kg are compared to the control values in parentheses.

*Significantly different from control values, $p \leq 0.05$.

D. STUDY AUTHORS' CONCLUSIONS:

Dermal treatment for 21 days with HWG 1608 did not produce any biologically significant systemic effects or local effects at 50 or 250 mg/kg. No significant systemic effects were noted at 1000 mg/kg; slight alterations in the skin revealed by clinical and histopathological examinations were presumed to be caused by mechanical irritation of the skin from the combination of the viscous formulation and the pressure of the occlusive dressing.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

There were no biologically significant effects from dermal treatment of rabbits with HWG 1608 at 50, 250, or 1000 mg/kg for 21 days on the mean body weight, hematology, clinical chemistry, urinalysis, relative and absolute organ weights, gross pathology, or histopathology parameters. Minimal thickening of the epidermis and hyperkeratosis, which was noticed at 1000 mg/kg, was attributed to mechanical irritation and was not an effect of the compound.

Tox review 007200

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EPA No.: 68D80056
DYNAMAC No.: 147-R
TASK No.: 1-47R
May 9, 1989

DATA EVALUATION RECORD

HWG 1608

Subchronic Feeding Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 5/9/89

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007200

EPA No.: 68D80056
DYNAMAC No.: 147-R
TASK No.: 1-47R
May 9, 1989

DATA EVALUATION RECORD

HWG 1608

Subchronic Feeding Study in Dogs

REVIEWED BY:

Margaret E. Brower, Ph.D.
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Dynamac Corporation
Signature: Margaret E. Brower
Date: May 9, 1989

William L. McLellan, Ph.D.
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Date: may 9, 1989

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EPA Reviewer and Section
Head, Section II
Toxicology Branch I (H-7509C)
Signature: M. Ioannou
Date: 5-10-89

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic feeding study in dogs. GUIDELINE §82-1

MRID NUMBER: 407009-34.

TEST MATERIAL: HWG 1608.

SYNONYM(S): Folicur; terbuconazole; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)pentane-3-ol.

STUDY NUMBER(S): T 6 016 919; Report No. 94984.

SPONSOR: Mobay Corporation, Agricultural Chemicals Division, Kansas City, MO.

TESTING FACILITY: Bayer AG, Institute of Toxicology, Federal Republic of Germany.

TITLE OF REPORT: HWG 1608. Subchronic Study of Toxicity To Dogs With Oral Administration (Thirteen-Week Feeding Study).

AUTHOR(S): E. von Keutz.

REPORT ISSUED: May 6, 1987.

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CONCLUSIONS:

When 200, 1000, or 5000 ppm HWG 1608 was fed to beagle dogs for 13 weeks, mean body weights, body weight gains, and food consumption were decreased at the mid- and high-dose. One high-dose female died on study day 2. Compound-induced lens opacity of the eyes was found in high-dose animals. Anisocytosis and a change in erythrocyte morphology were found at study termination and were accompanied by a histological increase in siderosis of the liver and spleen in high-dose animals. Increased platelet counts and increased spleen weights were also exhibited in these animals. Effects on the liver were indicated by increased alkaline phosphatase, decreased albumin, increased globulins, and increased cytochrome P-450 activity at the high-dose as well as a dose-related increase in N-demethylase activity. Degeneration of the lens of the eyes and cataracts were found histologically in high-dose animals. An astrocytoma of the brain was found in one control female, causing blindness. The increased incidence of vacuole formation in the adrenals of dosed females was indicated to be an adaptive response to dosing. Based on decreases in mean body weights, body weight gains, and food consumption and an increase in N-demethylase activity at 1000 ppm, the Lowest-Observed Effect Level (LOEL) is 1000 ppm, and the No-Observed-Effect Level (NOEL) is 200 ppm HWG 1608.

Classification: Core Minimum.

A. MATERIALS:

1. Test Compound: HWG 1608; description: colorless crystals (solid); batch No.: 16007/83; purity: 93.4%.

2. Test Animals: Species: Canis familiaris; strain: beagle; age: 24 to 27 weeks; weight: 6.3 to 9.0 kg; source: F. Winkelmann, D-4799 Borchen, Federal Republic of Germany.

   The dogs had been vaccinated for distemper, hepatitis, leptospirosis, and parasites by the supplier. Dogs showing signs of conjunctivitis and rhinitis during quarantine were treated with the appropriate eye ointment or antibiotic; the quarantine period for these animals was extended beyond 14 days. Dogs were also wormed and treated with antibiotics and eye ointments by the study laboratory during the acclimitization period.

B. STUDY DESIGN:

1. Animal Assignment: Following acclimatization, animals were assigned to the following test groups using a computerized randomization procedure:

Test[a] Group	Dose in Diet[b] (ppm)	Main Study (13 weeks) Males	Females
Control	0	4	4
Low (LDT)	200	4	4
Mid (MDT)	1000	4	4
High (HDT)	5000	4	4[c]

[a]The study report indicated that the untreated control group also served as a control for a concurrent study with a different test material.

[b]Doses were based on results of a range-finding study (Bayer Report No. 14784; results of this study were not provided).

[c]One high-dose female (animal No. 0656) was found dead on study day 2 and replaced with animal No. 0648.

Dogs were housed individually in an environmentally controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: The test diets were prepared on a weekly basis. Warm tap water was added to the test and control diets at a 1:1 ratio immediately prior to feeding. The study author reported that analyses of test diet concentration were conducted at scheduled intervals throughout the study. Prior to study initiation, homogeneity of the test diet and stability of the test material in wet and dry diets were analyzed.

Results: A nominal test diet of 10 mg/kg, which was not tested in dogs, was used to test for the homogeneity and stability of HWG 1608 in the test diet. The test diets were found to be homogenous within a range of 97 to 104% for 10 and 5000 mg/kg nominal concentrations, respectively. Test material was stable in dry diets; 106 and 100% were recovered after 14 days of storage at nominal levels of

TABLE 1. Analysis of HWG 1608 in Test Diets at Representative Intervals

Month		Target Concentration (mg/kg) 200	1000	5000
1	Concentration (mg/kg)	214	1070	5350
	Percentage of Target	107	107	107
2	Concentration (mg/kg)	216	1090	5700
	Percentage of Target	108	109	114
4	Concentration (mg/kg)	200	1050	5850
	Percentage of Target	100	105	117

10 and 5000 mg/kg, respectively. Stability was reduced in wet diets; 83 and 95% of the test material was recovered from the same nominal levels after 24 hours. All diets were within 17% of target. Table 1 presents representative analytical data.

3. Food and Water Consumption: Animals received food [Ssniff-HH Sole double-ground diet for dogs (350 g from study weeks 1 to 5, 380 g from study week 6 to study termination)] and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights, food consumption, clinical biochemistry, and organ weights were analyzed using only descriptive statistics.

5. Quality Assurance: A quality assurance statement signed and dated April 30, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected for signs of morbidity and mortality several times daily. Body temperatures were measured and reflex tests (pupil reaction, corneal reflex, patellar tendon reflex, stretch, bending, and righting reflex) were conducted prior to study initiation and during study weeks 3, 7, and 13.

   Results: One high-dose female (animal No. 0656) was found dead on study day 2; the cause of death was considered to be acute circulatory collapse and moderate pulmonary edema. The death was considered possibly due to dosing with the test compound. Diarrhea, vomiting, and pasty stool were observed sporadically in control and dosed animals throughout the study. A few mid- and high-dose males and high-dose females appeared thin when compared with concurrent controls; this finding occurred sporadically from study week 7 to study termination. Two mid-dose animals and five high-dose animals appeared thin or very thin at the time of sacrifice; the study author considered this finding to be compound related. Body temperature, pulse rates, and reflexes were similar in dosed and concurrent control dogs.

2. Body Weight: Dogs were weighed prior to study initiation and weekly thereafter.

   Results: Body weights of mid- and high-dose males were slightly decreased from study week 7 to study termination

(Table 2). Body weights of all dosed females were slightly decreased throughout the study when compared with concurrent controls. Mean body weight gains calculated over 13 weeks were decreased in mid- and high-dose males (25 and 46%, respectively) and females (19 and 48%, respectively) when compared with controls. Body weights and body weight gains of low-dose males and females were similar to concurrent controls. Statistical analyses were not performed on body weight data.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated weekly. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results: Representative food and compound consumption data are summarized in Table 3. The food consumption of high-dose males and mid- and high-dose females was slightly decreased when compared with concurrent controls (6, 9, and 10.9% decrease from controls for high-dose males, mid-dose females, and high-dose females, respectively, at 13 weeks) throughout the study period. The food consumption of low-dose animals was similar to concurrent controls. Compound intake was 73.7, 368.3, and 1749.1 mg/kg for low-, mid-, and high-dose males, respectively, and 73.4, 351.8, and 1724.8 for low-, mid-, and high-dose females, respectively. The control feed contained no test compound.

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to study initiation and during study weeks 3, 7, 10, and 12; mid- and high-dose dogs were also examined during study week 14. Eye funduses were photographed prior to study initiation and during study week 12; eye funduses of high-dose dogs were also photographed during weeks 10 and 14. One mid-dose male (animal No. 0655) was treated for traumatically induced injury to the cornea of one eye during study weeks 9 and 10.

Results: Lens opacity of varying intensity and extent was found in 3/8 high-dose animals (1 male, 2 females) at study week 7; at study week 10 this finding was exhibited in 8/8 high-dose animals. The intensity and extent of the lens opacity, especially in females, increased until study termination. The study author considered this finding to be the result of compound-induced morphological degeneration of the anterior wall of the lens. The incidence of bilateral lens stars in control and dosed males and

TABLE 2. Representative Results of Mean Body Weights (± S.D.) and Mean Body Weight Gains of Dogs Fed HWG 1608 for 13 Weeks[a]

Dose (ppm)	Mean Body Weight (kg) at Week				Mean Body Weight Gain (kg)[b] (Weeks -1 to 13)
	-1	3	7	13	
			Males		
0	7.9 ± 0.72	8.6 ± 0.66	9.5 ± 0.59	10.3 ± 0.61	2.4
200	7.9 ± 0.98	8.4 ± 1.06	9.2 ± 1.17	10.2 ± 1.25	2.4
1000	8.0 ± 0.81	8.6 ± 0.76	9.2 ± 0.89	9.8 ± 1.49	1.8
5000	7.9 ± 0.62	8.4 ± 0.48	8.9 ± 0.46	9.2 ± 0.55	1.3
			Females		
0	7.7 ± 0.36	8.4 ± 0.89	9.2 ± 0.70	10.3 ± 0.83	2.7
200	7.0 ± 0.53	7.8 ± 0.51	8.6 ± 0.52	9.6 ± 0.36	2.7
1000	7.5 ± 1.22	8.3 ± 1.23	8.9 ± 1.22	9.7 ± 1.64	2.2
5000	7.2 ± 0.13	7.5 ± 0.29	8.0 ± 0.45	8.6 ± 0.34	1.4

[a] Based on four dogs/sex/dose.

[b] Calculated by reviewers as group means of individual body weight gains.

TABLE 3. Representative Food and Compound Consumption of Dogs Fed HWG 1608 for 13 Weeks[a]

Dose (ppm)	Mean Food Consumption (kg/animal/week ± S.D.) at Week 1	7	13	Mean Compound Consumption (mg/kg body weight/day)
		Males		
0	2.45 ± 0.00[b]	2.66 ± 0.00	2.66 ± 0.00	0
200	2.45 ± 0.00	2.66 ± 0.00	2.66 ± 0.00	73.7
1000	2.45 ± 0.00	2.66 ± 0.00	2.66 ± 0.00	368.3
5000	2.37 ± 0.11	2.49 ± 0.27	2.50 ± 0.25	1749.1
		Females		
0	2.45 ± 0.00	2.66 ± 0.00	2.66 ± 0.00	0
200	2.45 ± 0.00	2.66 ± 0.00	2.54 ± 0.24	73.4
1000	2.37 ± 0.12	2.47 ± 0.22	2.42 ± 0.28	351.8
5000	2.06 ± 0.25	2.60 ± 0.11	2.37 ± 0.42	1724.5

[a]Based on four animals/sex/dose.

[b]Standard deviations of mean food consumption were calculated by reviewers.

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females was considered normal. One control female (animal No. 0670) was found to be blind prior to study initiation. Histological examination revealed a tumor at the base of the brain as the cause of blindness.

5. Hematology and Clinical Chemistry: Blood was collected from the jugular vein prior to study initiation and at 3, 7, and 13 weeks for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)[+]	X	Leukocyte differential count
X	Hemoglobin (HGB)[+]	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)[+]	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)[+]	X	Mean corpuscular volume (MCV)
X	Platelet count[+]	X	Coagulation: thromboplastin time (PT)
X	Reticulocyte count (RETIC)	X	Blood sedimentation rate (BSG)
	Red cell morphology		

Results: Platelet counts were increased in high-dose males at study weeks 7 and 13 and in high-dose females at study weeks 3, 7, and 13 when compared with concurrent controls (Table 4). Platelet counts were particularly high in three high-dose females (animal Nos. 0650, 0648, and 0662). Even though these counts were within the range of historical laboratory controls, this effect was considered to be due to compound treatment since increases were dose related and occurred in both sexes. Platelet counts of one low-dose male (animal No. 0669) appeared to deviate greatly at each testing interval; counts ranged from 340 x 10^9/L prior to study initiation to 102 x 10^9/L at 13 weeks. Signs of severe anisocytosis were reported to occur in 6/8 high-dose animals at study week 13; this effect was considered to be compound related. Other hematological variation was considered random.

[+]ecommended by Subdivision F (October 1982) Guidelines.

TABLE 4. Mean Platelet Counts (± S.D.) in Dogs Fed HWG 1608 for 13 Weeks [a,b]

Dose (ppm)	Mean Platelets (10^9/L) at Week			
	Pretest	3	7	13
		Males		
0	325 ± 25	320 ± 21	279 ± 25	284 ± 25
200	296 ± 57	197 ± 50	205 ± 57	215 ± 77
1000	363 ± 37	331 ± 52	306 ± 67	315 ± 79
5000	304 ± 27	328 ± 62	330 ± 33	357 ± 35
		Females		
0	323 ± 28	282 ± 69	268 ± 61	275 ± 61
200	388 ± 89	314 ± 41	297 ± 42	277 ± 35
1000	397 ± 100	359 ± 68	299 ± 52	341 ± 44
5000	294 ± 60	399 ± 26	410 ± 97	398 ± 79

Historical Laboratory Control Range of Platelet Counts (10^9/L)[c]

	Age (months)		
	0-9	10-12	13-18
Males	148-358	153-477	140-428
Females	181-501	172-424	181-421

[a]Standard deviations were calculated by reviewers.

[b]Based on four dogs/sex/dose.

[c]Historical data taken from study report No. 94984, page 48.

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b. Clinical Chemistry:

	Electrolytes
X	Calcium+
X	Chloride+
	Magnesium+
	Phosphorus+
X	Potassium+
X	Sodium+

	Enzymes
X	Alkaline phosphatase (ALP)
	Cholinesterase
	Creatinine phosphokinase+
	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (SGPT)+
X	Serum aspartate aminotransferase (SGOT)+
X	Gamma glutamyltransferase (GGT)
X	Glutamate dehydrogenase

	Other
X	Albumin+
X	Albumin/globulin ratio
X	Blood creatinine+
	Blood urea nitrogen+
X	Cholesterol+
X	Globulins
X	Glucose+
X	Total bilirubin+
	Direct bilirubin
X	Total protein+
X	Urea

	Liver analyses
X	Cytochrome P-450
X	N-demethylase
X	Triglycerides

Results: Selected clinical chemistry results are presented in Tables 5 and 6. Statistical analyses were not conducted by the study author on these results. Alkaline phosphatase (ALP) activity was increased in high-dose males and females throughout the study when compared with concurrent controls; this effect in males was due in part to outliers of one high-dose male (animal No. 0647) at weeks 7 and 13. The study author considered these changes to be compound related. SGOT and SGPT levels were similar in dosed and control males and females.

Albumin levels were slightly decreased in high-dose males, and beta and gamma globulins were slightly increased in this group throughout the study when compared with concurrent controls. This may have been due, in part, to relative changes in the albumin and globulin fractions of one high-dose male (animal No. 0647). The study author considered these changes to be compound related even though they were within the range of historical controls for the laboratory.

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 5. Selected Clinical Chemistry Results (± S.D.) in Male Dogs Fed HWG 1608 for 13 Weeks[a,b]

Parameter/Week	Dose (ppm) 0	200	1000	5000
Alkaline Phosphatase (U/L)				
Pretest	228 ± 29	221 ± 9	200 ± 39	213 ± 25
3	219 ± 63	214 ± 26	192 ± 32	341 ± 99
7	200 ± 62	212 ± 42	200 ± 39	483 ± 319
13	169 ± 42	181 ± 39	215 ± 60	554 ± 452
Albumin (%)				
Pretest	55.52 ± 1.36	56.05 ± 2.66	56.22 ± 2.54	56.65 ± 1.85
3	57.45 ± 0.70	57.65 ± 2.27	57.17 ± 0.83	54.62 ± 2.39
7	56.80 ± 2.30	56.92 ± 1.07	57.45 ± 1.65	52.17 ± 2.61
13	58.42 ± 2.55	57.92 ± 1.43	58.17 ± 2.66	53.07 ± 5.59
Beta Globulin (%)				
Pretest	17.17 ± 0.81	18.10 ± 1.01	17.30 ± 1.51	17.40 ± 0.56
3	17.67 ± 0.30	17.25 ± 1.39	16.85 ± 1.06	18.65 ± 1.60
7	16.65 ± 1.12	16.95 ± 0.59	16.52 ± 1.39	19.92 ± 1.53
13	17.42 ± 0.76	17.75 ± 0.80	17.95 ± 1.75	20.60 ± 2.99
Gamma Globulin (%)				
Pretest	9.57 ± 0.84	9.20 ± 2.16	10.00 ± 0.95	9.25 ± 1.31
3	8.40 ± 0.68	8.72 ± 1.25	9.60 ± 0.88	9.15 ± 0.92
7	9.37 ± 1.44	9.40 ± 0.97	9.67 ± 0.59	10.90 ± 1.10
13	8.42 ± 0.36	8.50 ± 0.72	9.22 ± 1.41	10.47 ± 2.09
N-demethylase (nmoL/g x min)				
13	54.05 ± 8.33	71.22 ± 12.82	100.67 ± 16.84	191.87 ± 38.41
Cytochrome P-450 (nmol/g)				
13	16.70 ± 1.64	15.80 ± 6.47	20.82 ± 4.29	28.97 ± 4.65

[a]Standard deviations were calculated by reviewers.

[b]Based on four dogs/sex/dose.

TABLE 6. Selected Clinical Chemistry Results (± S.D.) in Female Dogs Fed MUG 1608 for 13 Weeks[a,b]

Parameter/Week	Dose (ppm) 0	200	1000	5000
Alkaline Phosphatase (U/L)				
pretest	243 ± 14	236 ± 54	211 ± 34	219 ± 34
3	206 ± 15	209 ± 20	207 ± 31	321 ± 78
7	171 ± 34	171 ± 24	201 ± 43	470 ± 135
13	151 ± 40	165 ± 35	182 ± 41	333 ± 157
Albumin (%)				
pretest	57.45 ± 1.40	56.75 ± 1.71	54.72 ± 1.53	58.97 ± 2.36
3	57.90 ± 1.14	58.95 ± 1.51	57.12 ± 0.64	57.17 ± 1.09
7	57.57 ± 1.22	57.10 ± 0.54	58.77 ± 2.16	57.20 ± 1.55
13	58.32 ± 1.16	59.25 ± 0.31	59.65 ± 1.63	57.77 ± 1.23
Beta Globulin (%)				
pretest	17.35 ± 0.48	17.25 ± 1.00	18.37 ± 1.02	16.90 ± 0.90
3	17.42 ± 0.33	16.72 ± 0.74	18.22 ± 0.72	17.65 ± 0.83
7	16.55 ± 0.58	16.67 ± 0.53	17.90 ± 0.59	20.55 ± 0.64
13	17.15 ± 0.64	16.95 ± 0.59	17.65 ± 0.58	18.50 ± 1.34
Gamma Globulin (%)				
pretest	8.72 ± 0.70	8.75 ± 1.06	9.25 ± 0.95	9.00 ± 1.44
3	8.72 ± 0.54	8.10 ± 0.33	8.60 ± 0.45	9.40 ± 0.77
7	8.82 ± 0.45	8.72 ± 0.84	8.35 ± 0.83	9.32 ± 0.78
13	8.02 ± 1.63	8.55 ± 0.50	7.55 ± 0.30	9.30 ± 0.63
N-Demethylase (nmol/g x min)				
13	50.85 ± 10.67	74.32 ± 9.11	92.80 ± 26.72	156.62 ± 34.40
Cytochrome P-450 (nmol/g)				
13	19.10 ± 6.08	15.95 ± 3.05	18.25 ± 2.16	37.67 ± 0.83

[a]Standard deviations were calculated by reviewers.

[b]Based on four dogs/sex/dose.

N-demethylase activities in liver tissue measured at 13 weeks were found to be increased in dosed males and females when compared with concurrent controls; these increases were dose related. The N-demethylase activities of high-dose males (192 nmoL/g/min) and females (157 nmol/g/min) exceeded that of the historical range for the laboratory (males--14.9 to 107.3 nmoL/g/min; females--31.1 to 101.1 nmoL/g/min). Cytochrome P-450 activities of high-dose males and females measured at 13 weeks were slightly increased when compared with concurrent controls and historical controls for the laboratory. These changes were considered to be a result of dosing. There were no effects on liver triglycerides.

6. Urinalysis: Urine was collected from fasted animals prior to study initiation and at 3, 7, and 13 weeks. The CHECKED (X) parameters were examined:

X	Appearance[+]	X	Glucose[+]
X	Volume[+]	X	Ketones[+]
X	Specific gravity[+]	X	Bilirubin[+]
X	pH	X	Blood[+]
X	Sediment (microscopic)[-]		Nitrate
X	Protein[+]	X	Urobilinogen

Results: Results of urinalyses were similar in dosed and control males and females. Occasional blood found in the urine was reported to be the result of estrus at the time of urine collection (Week 13) or minor accidental injuries and was not considered to be signs of kidney damage.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

[+]Recommended by Subdivision F (October 1982) Guidelines.

Digestive System	**Cardiovasc./Hemat.**	**Neurologic**
Tongue	X Aorta+	XX Brain+
X Salivary glands+	XX Heart+	Peripheral nerve (sciatic nerve)+
X Esophagus+	X Bone marrow+	Spinal cord (3 levels)
X Stomach+	X Lymph nodes+	X Pituitary+
X Duodenum+	XX Spleen+	X Eyes (4 sections) (optic nerve)+
X Jejunum+	X Thymus+	
X Ileum+		
X Cecum+		
X Colon+	**Urogenital**	**Glandular**
X Rectum+	XX Kidneys+	XX Adrenals+
XX Liver+	X Urinary bladder+	Lacrimal gland
X Gallbladder+	XX Testes+	X Mammary gland+
XX Pancreas+	X Epididymides	XX Parathyroids+
	XX Prostate	XX Thyroids+
	Seminal vesicle	Harderian glands
Respiratory	XX Ovaries	
Trachea+	X Uterus+	
XX Lung+		**Other**
		X Bone (sternum and femur)+
		X Skeletal muscle+
		X Skin
		X All gross lesions and masses

Results:

a. Organ Weights: Representative results of absolute and relative organ weights are presented in Table 7. Absolute and relative spleen weights of high-dose males and females were increased when compared with concurrent controls. Absolute and relative liver weights of high-dose males were slightly increased; females of this group exhibited decreased absolute liver weights when compared with concurrent controls. The study author considered these liver changes to be toxicologically irrelevant due to these contradictory findings. The increase in mean ovary weights of high-dose females was due to the increased ovary weight of one female (animal No. 0648) that was in estrus at the

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 7. Selected Organ Weights (± S.D.) and Organ-To-Body Weight Ratios in Dogs Fed HWG 1608 for 13 Weeks[a,b]

Dose (ppm)	Liver		Spleen		Prostate		Ovaries	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Males								
0	372.8 ± 24.5	36.42 ± 3.93	25.3 ± 6	2.45 ± 0.31	5.13 ± 2.8	0.50 ± 0.26		
200	360.0 ± 29.6	35.75 ± 1.16	31.3 ± 2.2	3.12 ± 0.29	3.66 ± 1.3	0.35 ± 0.10		
1000	401.5 ± 68.5	41.00 ± 1.16	25.3 ± 8.3	2.55 ± 0.74	3.46 ± 1.7	0.37 ± 0.21		
5000	402.8 ± 44.2	44.92 ± 3.71	36.5 ± 6.9	4.10 ± 1.05	2.62 ± 1.2	0.29 ± 0.13		
Females								
0	377.0 ± 32.1	37.02 ± 2.78	33.5 ± 9.1	3.32 ± 1.09			1.06 ± 0.26	0.10 ± 0.02
200	332.0 ± 34.8	34.65 ± 3.38	34.0 ± 9.6	3.55 ± 0.95			1.03 ± 0.50	0.11 ± 0.05
1000	364.3 ± 112.6	37.12 ± 5.55	29.3 ± 1.2	3.10 ± 0.58			0.71 ± 0.09	0.07 ± 0.00
5000	338.5 ± 43.6	40.00 ± 4.08	40.0 ± 7.3	4.75 ± 0.87			1.46 ± 1.36	0.17 ± 0.16

[a]Standard deviations were calculated by reviewers.

[b]Based on four dogs/sex/group.

time of sacrifice. The mean prostate weight of high-dose males appeared to be decreased when compared with that of concurrent controls; however, this decrease was considered incidental since the mean control prostate weight was considered high due to two unusually high individual prostate weights (6.2 and 8.6 g).

b. Gross Pathology: Macroscopic findings were considered incidental and not due to compound treatment.

c. Microscopic Pathology:

1) Nonneoplastic: Selected histological findings are listed in Table 8. Slight degeneration of the posterior wall of the lens of the eye and cataract-like eosinophilic plaques were found in 4/4 high-dose males and 1/4 high-dose females; 3/4 high-dose females exhibited bilateral cataracts. Slight siderosis was exhibited in the livers of 1/4 high-dose males and 4/4 high-dose females (compared with 1/4 and 0/4 control males and females, respectively) and the spleens of 3/4 high-dose males and 2/4 high-dose females (compared with 0/4 and 1/4 control males and females, respectively.) These findings correlate with the incidence of severe anisocytosis in 6/8 high-dose animals and the dose-related increases in platelet counts in these animals. Two of 4 high-dose females, 1/4 mid-dose females, and 1/4 low-dose females exhibited increased vacuole formation of the adrenals as compared with 0/4 control females; this change was not found in control or dosed males. The study author regarded the above findings as induced by the test compound. An astrocytoma of the brain, found in one of the four control females, caused blindness in this animal. Other histological findings were regarded as incidental and unrelated to dosing.

D. STUDY AUTHOR'S CONCLUSIONS:

The author concluded that dietary administration of HWG 1608 produced effects on mean body weights, body weight gains, and food consumption at 1000 and 5000 ppm and ophthalmologic, hematologic, and clinical chemistry changes, as well as changes in spleen weights and histology of the eyes, spleen, liver, and adrenals at 5000 ppm. The NOEL was 200 ppm HWG 1608.

TABLE 8. Representative Histopathological Findings in Dogs Fed MAG 1608 for 13 Weeks

	Dose Group (ppm)							
	Males				Females			
	0	200	1000	5000	0	200	1000	5000
Liver	(4)[a]	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Hemosiderosis in Kupffer Cells	1	1	0	1	0	0	0	4
Kidney	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Atrophy of single glomeruli	1	0	1	0	0	1	1	0
Spleen	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Hemosiderosis	0	1	0	3	1	1	1	2
Brain	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Malignant astrocytoma	0	0	0	0	1	0	0	0
Adrenals	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Vacuoles in cells of zona fasciculata	0	0	0	1	1	1	2	
Thyroid	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
C-cell solidification	1	0	2	0	0	1	1	0
Pituitary	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Cyst	1	0	0	0	0	0	1	1
Eyes	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Degenerative alteration in posterior wall of lens	0	0	0	4	0	0	0	1
Cataract (lens)	0	0	0	0	0	0	0	3
Salivary Glands	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Atrophy of gland end pieces	0	1	0	1	1	1	2	2
Cellular infiltration	1	1	0	0	0	1	0	1

[a]Numbers in parentheses represent number of animals examined.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete, and the conduct of the study was acceptable. However, statistical analyses were not conducted on any testing parameter, with the exception of descriptive statistics. Even so, standard deviations of all testing parameters were calculated by the reviewers. The study author calculated standard deviations only on data which combined results of males and females; however, combined data were not considered acceptable. A nominal test diet of 10 mg/kg was used to test for homogeneity and stability of HWG 1608 in the test diet, but this concentration was not tested in the current dog study. This analysis should have been conducted on the nominal doses used in the current study. A macroscopic finding of an ascaris in the jejunum of one low-dose male (animal No. 0651) was listed on page 38 of the study report; this finding was listed in Table 35 (Comparison of Macroscopic and Microscopic Findings, page 245 of the study report) as occurring in one mid-dose male (animal No. 0671).

We agree with the study author that the histological finding of increased siderosis in the spleen and liver and signs of severe anisocytosis in high-dose males and females at study termination may have indicated an increased breakdown of red cells and a marginal compound-induced anemia. However, a marked compensatory increase in reticulocytes was not apparent (slight increase in high-dose males), and no overt effect was found in the HGB or HCT of these animals.

Signs of a treatment-induced effect on the livers were indicated by increased ALP activity, decreased albumin, increased globulins, and increased cytochrome P-450 activity in high-dose animals. In addition, N-demethylase activity was increased in a dose related manner.

Based on decreases in mean body weights, mean body weight gains, food consumption and increases in N-demethylase activity at 1000 ppm, the LOEL for HWG 1608 is 1000 ppm, and the NOEL is 200 ppm HWG 1608.

007200

EPA No.: 68D80056
DYNAMAC No. 147-K
TASK No.: 1-47K
April 18, 1989

DATA EVALUATION RECORD

TERBUCONAZOLE

Subchronic Oral Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 4/17/89

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007200

EPA No.: 68D80056
DYNAMAC No. 147-K
TASK No.: 1-47K
April 18, 1989

DATA EVALUATION RECORD

TERCUBONAZOLE

Subchronic Oral Toxicity Study in Rats

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-17-89

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Mike Ioannou, Ph.D.
EPA Reviewer, and
Section Head
Review Section I
Toxicology Branch II (H-7509C)

Signature: M. Ioannou
Date: 5-10-89

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity study in rats.

ACCESSION/MRID NUMBER: 407009-30.

TEST MATERIAL: HWG 1608.

SYNONYM(S): Terbuconazole; ethyltrianol; Folicur; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl)-pentane-3-ol.

STUDY NUMBER(S): 94212.

SPONSOR: Mobay Corporation, Stilwell, KS.

TESTING FACILITY: Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, Federal Republic of Germany.

TITLE OF REPORT: Subchronic Toxicological Study with Rats; Feeding for Thirteen Weeks.

AUTHOR(S): Bomhard, E.

REPORT ISSUED: October 27, 1986.

CONCLUSIONS:

Administration of HWG 1608 in feed at concentrations of 100, 400, and 1600 ppm for 13 weeks resulted in decreased mean body weights and mean body weight gains in male and female Wistar rats of the high-dose group. An increased incidence of vacuole formation in zona fasciculata cells in the adrenals of high-dose animals of both sexes and females fed 400 ppm was demonstrated. Similarly high-dose males and females had increased incidences of hemosiderosis. Both lesions were reported by the study author to be treatment related. Adverse compound effects appeared to be more intense in females than males and were attributed to increased female food consumption.

Based on the decreased body weights and body weight gain and histological changes in males fed 1600 ppm, the LEL is 1600 ppm in males and the NOEL is 400 ppm. In females the LEL was established at 400 ppm and the NOEL at 100 ppm.

Classification: CORE Minimum.

A. MATERIALS:

1. Test Compound: HWG 1608; description: whitish-yellow powder; batch No.: 16007/83; purity: 93.4% (plus 4.8% symmetrical isomer).

2. Test Animals: species: Rat; strain: Wistar[BOR: WISW(SPF Cpb)]; age: 6 weeks at study initiation; weight: males--79 g and females--77 g; source: F. Winkelmann, D-4799 Borchen.

B. STUDY DESIGN:

1. Animal Assignment: Animals were randomized, identified by cage cards, and assigned to the following test groups:

Test group	Dose in diet (ppm)	Number Assigned		Sacrificed (13 weeks)	
		Males	Females	Males	Females
1 Control	0	10	10	10	10
2 Low (LDT)	100	10	10	10	10
3 Mid (MDT)	400	10	10	10	10
4 High (HDT)	1600	10	10	10	10

Throughout the study, animals were maintained in an environment controlled for temperature (22 ± 2°C), relative humidity (≈50%), and light (12 hours/day); air was exchanged 10 times/hour.

2. Diet Preparation: Diets containing 0, 100, 400, and 1600 ppm of the test material were prepared weekly for 5 weeks in Altromin 1324 meal; from week 6 to study termination, Wessalon (highly dispersed silicates) was added to the dietary pre-mixes to improve homogeneity. Test diets were analyzed for test material concentrations at monthly intervals for 3 months. Stability determinations at 0, 7, and 14 days were conducted as part of a pilot study. Homogeneity studies were conducted prior to study initiation.

   Results: Results of the chemical analyses indicated that diets containing 400 and 600 ppm of HWG 1608 in basal feed were generally within 10% of the nominal concentration; samples from low-dose diets contained 84 to 91% of the target concentration:

Month	% of Target for Dose Concentrations (ppm)		
	100	400	1600
1	85[a]	98[a]	91[a]
2	84	89	99
3	91	93	91

[a]Mean result from duplicate determination.

Homogeneity and stability data that were presented were not from the present study (Appendix A CBI pp. 172-173); however, the pilot studies indicate acceptable stability and homogeneity.

3. Food and Water Consumption: Animals received the appropriate test diets and water ad libitum for 13 weeks.

4. Statistics: The two-sided Mann-Whitney U test and Wilcoxon method were utilized in analyzing the numerical data. Significance was evaluate at probability levels of 0.05 and 0.01.

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5. Quality Assurance: A quality assurance statement was signed and dated October 26, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected at least twice daily (once daily on weekends) for signs of morbidity and mortality; detailed physical examinations were performed weekly.

Results: The report stated that the appearance and behavior of rats administered the test diets of HWG 1608 did not differ from the control rats; individual animal data were not provided. One male in the control group died at week 4 and a second control group male died at week 12; deaths were reported to be associated with blood sampling. In the high-dose group, one male died during the first week of dosing and one female died during week 4; the author concluded that both deaths were probably due to hemorrhagic diathesis and were considered to be compound related.

2. Body Weight: Body weights were determined weekly.

Results: Mean body weights for males receiving 100 ppm of the test material were generally comparable to the control group throughout the course of the study. No significant differences from the control group were noted in mid-dose male body weight until week 13; however, from weeks 7 to 13, the mean body weight for this group was consistently lower than the control. In high-dose males, significantly decreased body weights were recorded at weeks 1 through 3 and from week 6 to study termination. See Table 1.

Mean male body weight gain was calculated by our reviewers from individual animal body weight data at weeks 0 and 13. Regression analysis performed by our reviewers revealed a significant trend ($p<0.01$) over all doses and a significant ($p<0.01$) reduction in high-dose male body weight gain.

In general, the low dose had no adverse effect on female body weights. Females in the mid-dose group had slight but significantly lower than control mean body weights from weeks 1 to 6; however, mean weight gain for the mid-dose group (106.1 g) was similar to control (108.4 g). The body weights of females receiving 1600 ppm were significantly lower than the control from week 2 to study termination.

TABLE 1. Selected Mean Body Weights, Overall Mean Body Weight Gain, and Food Intake of Rats Fed HWG 1608 for 13 Weeks

Dose Level (ppm)	Mean Body Weight (g ± S.D.) at Week: 0	6	13	Mean Body Weight Gain (g ± S.D.)[a] Weeks 0 to 13	Group Mean Food Intake (g/kg body weight/day) at Week 1	13
			Males			
0	82 ± 8	240 ± 18	334 ± 20	249.5 ± 16.58[b]	140	59
100	81 ± 4	239 ± 5	317 ± 14*	235.7 ± 14.65	135	57
400	79 ± 5	240 ± 10	316 ± 11**	236.6 ± 10.24**	141	61
1600[c]	75 ± 6	217 ± 15*	295 ± 16**	220.1 ± 11.11**	158	81
			Females			
0	78 ± 5	167 ± 9	187 ± 14	108.4 ± 14.32[b]	134	64
100	78 ± 6	162 ± 9*	181 ± 11	103.4 ± 9.81	158	68
400[d]	74 ± 4	157 ± 9**	180 ± 13*	106.1 ± 11.93	178	73
1600[e]	77 ± 4	153 ± 7**	172 ± 7*	95.8 ± 7.45	147	138

[a]Mean body weight gain was calculated by our reviewers from individual animal weights at weeks 0 and 13.

[b]Significant trend over all doses for males at $p<0.01$ and for females at $P<0.05$; calculated by our reviewers.

[c]Significantly decreased ($p < 0.01$) male body weights at weeks 1 through 3 and week 6 to study termination.

[d]Significantly decreased ($p < 0.05$ or < 0.01) female body weights at weeks 1 through 6.

[e]Significantly decreased ($p < 0.05$ or < 0.01) female body weights at week 2 to study termination.

*Significantly lower than the control value ($p < 0.05$).

**Significantly lower than control value ($p < 0.01$).

In agreement with the findings for males, a significant trend ($p<0.05$) in mean body weight gain over all doses was uncovered for the treated females. Although no significant differences in treatment body weight gain compared to the control were observed, the percent difference from control for the high-dose females (12%) was comparable to the significant percent difference between high-dose males and their respective control. The results for high-dose females are striking in light of the marked increased in high-dose female food consumption as discussed below.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated at the same intervals as the weighings. Mean and cumulative test compound intake was calculated. Water consumption was measured weekly.

   Results: Food consumption of males in the low- and mid-dose groups and females in the low-dose group were comparable to the corresponding control group values. Slight increases in food intake were noted for mid-dose females primarily during weeks 1 through 5, while marked increases in food consumption occurred in male and female rats receiving diets containing 1600 ppm from the onset of the study and most weeks thereafter. In females, food consumption of the 1600-ppm diet was 10.6 g/animal/day higher than the control group from week 6 to the end of the study. In contrast, high-dose males consumed 4.0 g/animal/day more feed than the control group during weeks 7 to 13. Food efficiency was not determined. Test compound intake (13 weeks) was 8.6, 34.8, or 171.7 mg/kg/day for males receiving 100, 400, or 1600 ppm in the diet, respectively, and 10.8, 46.5, or 235.2 mg/kg/day for females at the same doses. See Table 1.

4. Ophthalmology: Ophthalmological examinations were performed on 10 males and 10 females in the control and 1600-ppm dose groups at week 4 and prior to termination.

   Results: There were no abnormalities at the 4-week examination. The author stated that there was no indication of substance-induced damage to the eyes of the 1600-ppm dose group; however, one high dose male exhibited corneal erosion and one high-dose female had a right corneal lens cataract.

5. Hematology and Clinical Chemistry: Blood was collected from the venae caudales or retro-orbital venous plexus at 1 and 3 months for hematology and clinical analyses from five animals/sex/group (1 month) and all survivors at 3 months. The CHECKED (X) parameters were examined:

a. Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
Reticulocyte count (RETIC)
Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation: thromboplastin time (PT)
X Thrombin

Results: There were no toxicologically relevant effects on hematologic parameters. Although significant differences were noted for certain parameters, dose-related responses were not observed and the significantly different values were well within the historical background ranges presented by the study author for Wistar rats (see Appendix B).

b. Clinical Chemistry

Electrolytes
Calcium*
Chloride*
Magnesium*
Phosphorus
Potassium*
Sodium*

Enzymes
X Alkaline phosphatase (ALP)
Cholinesterase
Creatinine phosphokinase*
Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

Other
Albumin*
Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
Globulins
X Glucose*
X Total bilirubin*
Direct bilirubin
X Total protein*
X Triglycerides

In addition to the above checked parameters, serum iron levels were determined at the conclusion of the study.

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*Recommended by Subdivision F (October 1982) Guidelines.

Results: The blood urea nitrogen (BUN) level for high-dose females was significantly increased after 1 month of treatment (Table 2). Nonsignificant increases in BUN levels were seen in males treated with 400 and 1600 ppm; both values fell within the background range (two standard deviations) furnished by the author but were higher than the mean reference value (7.52 mmoL/L) presented in Appendix B. Urea concentrations were not elevated in either sex at the 13-week determinations. The author speculated that the significantly reduced 4-week triglycerides (TG) concentration for high-dose males may have been treatment related. We noted that TG levels for all male dose groups were appreciably lower than mean values for the control group and historical data (1.16 mmoL/L) but were within background ranges. Similarly, individual values for the majority of animals in the three treatment groups were below the concurrent control and reference values. The significantly decreased 4-week TG concentration observed in mid-dose females was not dose related. At study termination, TG levels in all groups were generally comparable to the control levels.

Significantly lower than control creatinine levels were seen in all male treatment groups at week 13; the effect was dose related; however, treatment group values were comparable to the historical value (52 µmoL/L) and were, therefore, not considered to represent an adverse effect. Other clinical chemistry parameters indicating statistical significance were found to be generally comparable to mean reference values.

6. Urinalysis: Urine was collected a few days prior to the 4- and 13-week blood sampling intervals. During the 16-hour urine collection period, water was available ad libitum but food was withheld.

The CHECKED (X) parameters were examined:

	Appearance*	X	Glucose*
X	Volume	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein	X	Urobilinogen

Results: Quantitative and semi-quantitative urine determinations did not suggest a treatment-related effect on males and females fed the three dietary preparations of HWG 1608.

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*Recommended by Subdivision F (October 1982) Guidelines.

TABLE 2. Selected Clinical Chemistry Parameters (± S.D.) in Male and Female Rats Fed MUG 1608 for 13 Weeks

Dose Level (ppm)	Urea (mmol/L)		Triglycerides (mmol/L)		Creatinine (mmol/L)	
	4 wk[a]	13 wk[b]	4 wk[a]	13 wk[b]	4 wk[a]	13 wk[b]
			Males			
0	6.59 ± 0.62	7.55 ± 0.79	1.24 ± 0.36	1.75 ± 0.43	56 ± 14	73 ± 17
100	6.24 ± 0.86	7.37 ± 0.89	0.96 ± 0.26	1.96 ± 0.59	50 ± 2	57 ± 5**
400	7.65 ± 0.92	7.12 ± 0.78	0.84 ± 0.46	2.03 ± 0.46	58 ± 24	52 ± 4**
1600	8.41 ± 1.27	7.15 ± 0.70	0.68 ± 0.25*	1.51 ± 0.24	44 ± 2*	49 ± 3**
			Females			
0	7.15 ± 0.71	7.61 ± 0.64	1.06 ± 0.08	1.30 ± 0.48	48 ± 4	69 ± 29
100	7.00 ± 0.44	7.31 ± 0.65	1.18 ± 0.29	1.32 ± 0.49	46 ± 3	52 ± 6
400	6.56 ± 0.67	7.59 ± 0.90	0.71 ± 0.12**	1.12 ± 0.33	45 ± 6	57 ± 13
1600	9.92 ± 0.85**	7.03 ± 0.75	0.81 ± 0.28	1.10 ± 0.31	48 ± 2	54 ± 3

[a]Results from week 4 determination represent mean values for five animals/group.

[b]Results from week 13 determinations represent mean values for all survivors/group.

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

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7. Liver Enzyme Induction: Liver N-demethylase and cytochrome P-450 levels were determined in all survivors at study termination.

Results: As shown in Table 3, N-demethylase activity was significantly increased in the high-dose males; significant induction of cytochrome P-450 also occurred in the males of the mid- and high-dose groups. The author concluded that a clear increase was only apparent in the males receiving 1600 ppm.

In females, no increased N-demethylase induction was seen; while significant increases in cytochrome P-450 induction were calculated for the three female treatment groups, the increase in the high-dose group was only slightly higher than the control.

8. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta	X	Brain[+]
X	Salivary glands[+]	XX	Heart		Peripheral nerve (sciatic nerve)[+]
X	Esophagus[+]	X	Bone marrow[+]		Spinal cord(3 levels)
X	Stomach[+]	X	Lymph nodes[+]	X	Pituitary
X	Duodenum[+]	XX	Spleen[+]	X	Eyes (optic nerve)[+]
X	Jejunum[+]	X	Thymus[+]		
X	Ileum[+]				Glandular
X	Cecum[+]		Urogenital	XX	Adrenals[+]
X	Colon	XX	Kidneys		Lacrimal gland
X	Rectum	X	Urinary bladder[+]		Mammary gland[+]
XX	Liver[+]	XX	Testes[+]	X	Parathyroids[+]
	Gallbladder[+]	X	Epididymides	X	Thyroids[+]
X	Pancreas[+]	X	Prostate		Harderian glands
		X	Seminal vesicle		
	Respiratory	X	Ovaries		Other
X	Trachea[+]	X	Uterus[+]	X	Bone (femur)[+]
XX	Lung[+]			X	Skeletal muscle and Femur[+]
				X	Skin
				X	All gross lesions and masses

All tissues identified above were examined histologically for all animals in the control and 1600-ppm dose group; the livers and adrenals of all low- and mid-dose group animals were also examined microscopically.

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 3. Induction of Liver Enzymes (± S.D.) in Male and Female Rats Fed HWG 1608 for 13 Weeks

Dose Level (ppm)	N-Demethylase[a] (nmoL/g/minute)	Cytochrome P-450[a] (nmoL/g/minute)
	Males	
0	99.2 ± 16.9	30.3 ± 2.7
100	108.7 ± 15.9	33.5 ± 6.3
400	100.2 ± 24.8	38.1 ± 2.4**
1600	149.7 ± 13.7**	62.8 ± 6.0**
	Females	
0	41.9 ± 7.8	27.8 ± 3.5
100	33.3 ± 8.0*	30.5 ± 2.0*
400	31.6 ± 11.9	32.0 ± 4.1**
1600	44.2 ± 13.2	33.0 ± 4.3**

[a]Results for week 13 determination; mean values for all survivors.

*Significantly different from the control ($p < 0.05$).

**Significantly different from the control ($p < 0.01$).

Results:

a. Terminal Body Weights/Organ Weights: Terminal body weights of high-dose group males and females and mid-dose group males were significantly lower than the control group body weights (Table 4).

Absolute liver weights for males treated with 400 and 1600 ppm were significantly reduced. The relative (to body weight) liver weights for these groups were also lower than the control; however, only the mid-dose group finding was significant. The absolute but not relative kidney, spleen, and lung weights of high-dose group males were significantly lower than the control. The author attributed these organ weight differences to the reduced body weights in the high-dose group.

Absolute liver weights for females exposed to 1600 ppm were higher than control; however, significantly reduced absolute and relative liver weights were recorded for the low- and mid-dose group females.

Significantly decreased absolute but not relative kidney weights were seen in females exposed to 400 and 1600 ppm. No other significant differences were noted in female organ weight data.

b. Gross Pathology: Necropsies of treated animals revealed no gross lesions that were considered compound related. At necropsy, the thickness of the medial section of the femur was measured. Results indicated that femur diameters for high-dose males and females were significantly lower than the controls; the author attributed this finding to the significantly reduced body weights for animals in this treatment group.

c. Microscopic Pathology: Table 5 summarizes the most frequent lesions in rats that died or were sacrificed by design after 13 weeks of treatment. As shown, the incidence of vacuole formation in zona fasciculata cells of the adrenals was increased in males and females of the mid- and high-dose groups; the finding was noted in 40 and 90% of females treated with 400 and 1600 ppm HWG 1608, respectively, as compared to lower incidence rates in treated males (≤15%). The author stated that the increased rates of vacuole formation were associated with treatment-related lipid accumulation. Hydronephrosis was noted in two high-dose males. A nondose-related increase in the number of treated male exhibiting minimal to mild hemosiderosis

TABLE 4. Selected Mean Organ Weights (±S.D.) and Organ-to-Body Weight Ratios in Rats Fed HWG 1608 for 13 Weeks[a]

	Organ	Dietary Level (mg/kg/day) 0	100	400	1600
Males	Terminal Body Weight (g)	334 ± 20	317 ± 14	316 ± 11*	295 ± 16**
	Liver (g)	13.74 ± 1.37	12.75 ± 1.02	11.89 ± 0.73**	11.55 ± 0.52**
	(% b. wt.)	4.12 ± 0.30	4.02 ± 0.23	3.77 ± 0.18**	3.92 ± 0.16
	Kidneys (g)	2.20 ± 0.26	2.12 ± 0.97	1.99 ± 0.25	1.86 ± 0.90**
	% b. wt.	0.66 ± 0.06	0.67 ± 0.04	0.63 ± 0.07	0.63 ± 0.04
Females	Terminal Body Weight (g)	187 ± 14	181 ± 11	180 ± 13	172 ± 7*
	Liver (g)	8.00 ± 0.77	7.10 ± 0.70*	7.00 ± 0.35**	8.19 ± 0.85
	(% b. wt.)	4.29 ± 0.19	3.92 ± 0.33*	3.90 ± 0.24**	4.75 ± 0.37*
	Kidneys (g)	1.23 ± 0.07	1.17 ± 0.12	1.13 ± 0.07**	1.12 ± 0.09*
	(% b. wt.)	0.66 ± 0.05	0.63 ± 0.06	0.63 ± 0.08	0.65 ± 0.04

[a]Ten animals/sex/group, with the exception of eight males in the control group and nine animals/sex/group in the 1600-mg/kg/da-dosed groups.

*Significantly different from the control value ($p < 0.05$).

**Significantly different from the control value ($p < 0.01$).

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TABLE 5. Selected Histological Findings in Rats Fed MMG 1608 for 13 Weeks

Organ/Finding	Dose Level (mg/kg/day)							
	Males				Females			
	0	100	400	1600	0	100	400	1600
Adrenals	(10)[a]	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Vacuole formation in zona fasciculata cells	4	4	5	6	0	0	4	9
Kidney	(10)	(0)	(0)	(10)	(10)	(0)	(0)	(10)
Hydronephrosis	0	--	--	2	0	--	--	0
Spleen	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Depletion of splenic red pulp	1	0	0	1	0	0	0	1
Hemosiderosis	1	6	3	5	5	3	5	9

[a]Numbers in parentheses are the number of tissues examined histologically.

was observed in all treatment groups; the highest frequency occurred in the low-dose group. Ninety percent of females treated with 1600 ppm HWG 1608 had varying degrees of hemosiderosis ranging from minimal to mild (six females) to mild (three females) as compared to five control group females showing minimal hemosiderosis. The author concluded that the finding of mild hemosiderosis in the three high-dose females may be indicative of increased erythrocyte destruction.

D. STUDY AUTHOR'S CONCLUSIONS:

The author concluded that, "Under the described conditions HWG 1608 was tolerated without ill-effect by the male rats at doses up to and including 400 ppm, and by the female rats at the dose of 100 ppm."

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

With the exception of several recommended clinical parameters that were not evaluated (i.e., electrolytes and albumin) and several recommended organs/tissues that were not collected for histological examination (i.e., sciatic nerve, mammary gland, and parathyroids), the protocol was adequate for a subchronic toxicity study in rats. The lack of data for the above-listed parameters did not affect the outcome of the study or compromise the results.

We assess that diets were, in general, accurately prepared. Results of the pilot study indicated acceptable homogeneity and stability under the experimental conditions.

We consider the decreased body weights in conjunction with the significant reduction in body weight gain in males of the 1600 ppm-dose group to be indicative of adverse compound effects. Similarly, the consistent trend of decreased body weights in mid- and high-dose females and the marked reduction in high-dose female body weight gain are indicative of compound effects. Although the increased incidence of vacuole formation in the adrenals, of females exposed to 400 and 1600 ppm and males to 1600 ppm was not accompanied by a significant decrease in eosinophiles, we concur with the author that the effect was compound induced.

The significantly decreased absolute and relative male liver weights for the mid- and high-dose groups did not correlate with increased liver enzyme induction. We conclude, therefore, that organ weight changes in both sexes were generally associated with decreased terminal body weights and their relevance is doubtful in the absence of gross or histological

effects. In the absence of histopathological changes in kidney tissue and lack of correlation with other parameters that are indicative of renal dysfunction, we assess, in agreement with the author, that the transiently increased BUN levels seen in the high-dose group animals at week 4 are not suggestive of a compound effect.

The incidence of minimal to mild hemosiderosis was increased in males and females of the high-dose group and, in a nondose-related manner, in the low- and mid-dose males. The biological significance of the finding is unclear since significant decreases in RBC, HG, or HCT were not observed. The author concluded, however, that hemosiderosis was compound induced since the two reported deaths in the high-dose group (one male and one female) appeared to be related to hemolytic processes.

Based on the above considerations we agree with the author that the NOEL is 400 ppm in males and 100 ppm in females.

The difference in the intensity of compound effects noted among males and females can be attributed to the increased food consumption by mid- and high-dose females as compared to the males.

We did not note any discrepancies between reported and individual animal data.

The protocol required histological evaluation of low- and mid-dose group livers and adrenals. We assume, however, that the lack of microscopic lesions in the livers of animals treated with 1600 ppm and the frequency of hemosiderosis in spleens prompted the change in organs selected for histology.

F. CBI APPENDIX: Appendix A, Homogeneity and Stability Results, CBI pp. 172-173; Appendix B, Reference Values for Wistar Rats, CBI pp. 53-54; Appendix C, Materials and Methods, CBI pp. 10-23.

APPENDIX A
Homogeneity and Stability Results
(CBI pp. 172-173)

TEBUCONAZOLE Tox R 007200

Page_____ is not included in this copy.

Pages 315 through 334 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.

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007200

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 11/3/89
Review Section I, Tox. Branch-HFAS (TS-769C)
Secondary Reviewer: Quang Q. Bui, Ph.D. Quang Q. Bui
Review Section I, Tox. Branch-HFAS/HED (TS-769C)

DATA EVALUATION RECORD

STUDY TYPE: 12-month dog (oral)
EPA Guideline 83-1

TOX. CHEM. NO: 463P

ACCESSION NUMBER:

MRID NO.: 407009-40

TEST MATERIAL: HWG 1608; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazole-1-yl-methyl) pentane-3-ol; CAS no. 80-443-41-0; 96.9% purity; batch Fl. 132 (mixed batch of batches 16001/84, 16002/84, 16003/84, 16004/84, 16006/84); colorless to slightly yellowish crystals (solid); stored in closed containers in cooling cells at 4°C

SYNONYMS: terbuconazole; ethyl trianol

STUDY NUMBER(S): T 6018115; report no. 16211; lab. report no. 956090

TESTING FACILITY: BAYER AG, Institute of Toxicology, Federal Republic of Germany

TITLE OF REPORT: HWG 1608, Study of chronic toxicity to dogs after oral administration (twelve-month feeding study)

AUTHOR(S): Dr. E. von Kreuz

REPORT ISSUED: November 11, 1987

CONCLUSIONS:

Under the conditions of this bioassay, dietary administration of terbuconazole at 0, 40, 200 and 1000/2000 (1-39, 40-52 wks, resp.) produced lenticular and corneal opacities in mid and high dose animals. The liver was the site of several subtle forms of toxicity including, elevations in alkaline phosphatase in HDT males and females, elevations in N-demethylase activity and triglycerides at the HDT (both sexes), gross changes in liver appearance (lobulation/swelling(?)) along with an increase in the presence of iron-containing pigments and lipids (MDT, HDT). Blood (moderate anisocytosis), adrenals (increased intra-cytoplasmic vacuoles of z. fasiculata) kidney and spleen (elevated weights) in both sexes at mid and/or high dose levels may also be associated target organ/tissue systems. The systemic toxicity LEL, based upon ocular lesions and hepatic toxicity in either sex at the mid and high dose levels is set at 200 ppm. The systemic toxicity NOEL is set at the LDT (40 ppm).

CLASSIFICATION: Core Minimum

A. Materials: (a photocopy of the materials and methods is appended)

1. Test compound: see pg. 1 under test material

2. Test animals: Species: dog, Strain: (strain Bor: Beag), Age: 24-28 weeks, Weight: 7.1-10.5 kg, Source: F. Winklemann, D-4799 Borchen

B. Study Design:

1. Animal assignment

Animals were randomly assigned 8 (4/sex) to the following test groups:

Test group	Dose in diet(ppm)	Main study 12 mos male	Main study 12 mos female	Interim sacrifice mos male	Interim sacrifice mos female
1 control	0	4	4	N/A	
2 low(LDT)	40	4	4	"	
3 mid(MDT)	200	4	4	"	
4 high(HDT)	1000(1-39 wk) 2000(40-52 wk)	4	4	"	

2. Diet preparation

Diet was prepared once per week and stored at room temperature. Reserve samples were taken of each mix. Samples of treated food were analyzed before study initiation to establish that test substance was stable for at least 14 days in dry feed and at least 24 hours in wet feed and was homgeneously distributed in the mixture. Additional analyses during the progress of the study were also performed. Results of these analyses are summarized below (from Tables 1-3, pp. 273-275):

Results-

Nominal concentrations

month/year of analyses	nominal concentrations (mg/kg) 40	200	1000	2000
8/84	35	188	1030	----
11/84	32	212	1070	----
2/85	32	178	900	----
5/85	36	200	990	----
8/85	33	188	---	2080
mean(S.D.)	34(5)	193(7)	998(7)	2080(-)
mean % theoretical	85	97	100	104

The active ingredient concentration was found to be within +/-20% of the nominal concentrations, fluctuations occurring primarily

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at the low dose. For homogeneity, three of five samples were taken (presumably from the homogeneity samples presented above) of which three were analyzed and were 97 to 104 % of the mean theoretical values. Dry food stability was determined to be 100 to 106 % of the nominal concentration while wet food stability was 83 to 95 % of nominal.

3. Animals received food ("Ssniff HH Sole Diet for Dogs, double ground", from Ssniff Versuchstierdiaeten GmbH, D-4770 Soest) and water ad libitum. Animals were fed daily 350 gm for weeks 1-3, 380 gm for weeks 4-22, and 400 gm in weeks 23 to 52. The authors stated that the food ration was raised twice in order to match the beagles' growth curves.

4. Statistics - The following procedures were utilized in analyzing the numerical data: due to the low number of animals per group only the arithmetic means and standard deviations were calculated, and maximums and minimums given.

5. Signed and dated copies of no claims of confidentiality, Good Laboratory Practices statement and a Quality Assurance statement were included.

C. Methods and Results:

1. Observations

Animals were inspected several times daily for signs of toxicity and mortality. Drinking behavior, body temperatures, pulse rates and neurological functions (reflex tests: pupil reaction, corneal reflex, patellar tendon reflex, stretch, righting and bending reflex) were determined.

Toxicity/mortality (survival)

No mortality was observed. Clinical signs observed among all dose-groups were vomiting, pasty feces and diarrhea and were not compound-related. Estrus was observed in 3 to 4 dams in each dose-group. No compound-related effects were evident for drinking behavior, reflexes, body temperature, pulse rate or general nutritional state.

2. Body weight

Animals were weighed weekly (seven day intervals) for the length of the study and mean body weights per week were determined.

No adverse effects upon body weight or body weight gain were observed.

3. Food consumption and compound intake

Consumption was determined daily and individually for each animal. Compound intake was calculated from the consumption and body weight gain data.

Food consumption/compound intake

All animals were fed a constant amount, adjusted for growth, and generally all feed was consumed with no evidence of depressed food consumption.

Mean compound intake (g/animal total or mg/animal/week, resp.) was proportionally increased with dietary intake:

40 ppm	5.6 g,	108.4 mg
200 ppm	28.1 g,	541.0 mg
1000/2000 ppm	175.8 g,	3380.2 mg

4. Opthalmalogical examinations

Performed 2 weeks prior to study initiation and at 13, 26, 32, 39, 46 (controls, HDT) and 52 weeks on all animals.

A summary table of findings (total number of animal given by heading) is presented below (taken from Table 17, pgs. 120-127 of report):

Findings (- 2 weeks)	control	LDT	MDT	HDT
LENS	[1]	[3]	[1]	[1]
-incipient lens star (rt/left)		1		
-slight/faint lens star		1		1
(rt/left); rt central reflecting particles		1		
-weak lens star (rt)		1		
-peripheral opacities; rt two dark pigment patches			1	
TAPETUM LUCIDUM			[3]	[1]
-rt isolated retinal folds			1	
-rt and left more reflecting			2	
-black pigment patch	1			1
BLOOD VESSELS				[1]
- very convoluted/full				1
" Findings" (13-52 weeks)----------				
LENS		[1]	[3]	[1]
-central remnants lens arteri		1		
-central opague structure (stellar)			1	
-peripheral opacities			1	
-part movable towards caudal (fibroid)			1	
-fine stellar opacities/slight lens star			1	1
-normal opacity				1
CORNEA	[2]	[3]	[3]	[4]
-punctiform opacities	2	2	3	3
-mucous deposits		1		1
-mucous suppurative con-junctivitis		1		
-cloudy/filmy opacities		1	2*	
-irregular limited opacity			1	1
-extensive opacity				1
TAPETUM LUCIDUM	[1]	[1]	[3]	[1]
-rt and left light, more reflecting	1	1	3	
-large isolated black pigment				1
FUNDUS				[1]
-mucous deposits				1
BLOOD VESSELS, convolutd/full				[1]

* one of two dogs (P 145) observed only at week 26

Ophthalmological exams prior to study initiation revealed the presence of at least one finding of incipient, slight or weak lens stars in all dose groups with 3 animals of the mid and one animal of the high dose groups with various changes in tapetum lucidum including isolated retinal folds, increased reflecting or black pigment patch. No corneal changes were seen in any dose group at -2 weeks.

There was an apparent increase in the presence of lenticular lesions in the treated as opposed to the control group. Animal P 160 and P 146 (MDT) had a right central opaque (stellar) structure (week 26-52) or right and left slight lens stars (from week 32-52), respectively. One animal, P 168, (HDT) was observed with right and left central fine stellar lens opacities from week 26-52.

Corneal opacities were also apparently elevated in the mid and high dose groups. Although corneal punctiform opacities of a minimal nature were observed in two controls, more severe corneal findings of cloudy cornea (P 179/MDT, wk 32, 39) and extensive right corneal opacity (P 149/HDT, wk 39-52) were reported. All compound-treated groups (3/group) but no controls received treatment with an eye ointment (Leukomycin@) for such things as mucous deposit on cornea, inflammation of the eye, mucous-purulent conjunctivitis, punctiform, hazy or cloudy opacities of the cornea. The relationship of compound-related ocular effects to the use of an eye salve is unclear but is probably not a significant factor in the detection of ocular toxicity since the treatments were for only a limited number of days (3-5 days).

5. a. Hematology

Blood was collected two weeks before treatment and at 6, 13, 26, 39, 46 (controls, HDT) and 52 weeks for hematology and clinical analysis from all animals. The checked (X) parameters were examined:

X		X	
x	hematocrit (HCT)*	x	leukocyte differential count *
x	hemoglobin (HGB)*	x	mean corpuscular HGB (MCH)
x	leukocyte count(WBC)*	x	mean corpuscular HGB conc. (MCHC)
x	platelet count*	x	mean corpuscular volume (MCV)
	blood clotting measurements	x	reticulocyte count
x	-thromboplastin time	x	blood sedimentation rate (BSG)
	-clotting time		
	-prothrombin time		

* required for subchronic and chronic studies

There were no apparent changes in most hematological parameters measured during the test period, including hematocrit, hemoglobin concentration, white and red blood cell count, leukocyte differential count, mean corpuscular count, mean corpuscular concentration, mean corpuscular volume, reticulocyte count or blood sedimentation rate. Thrombocyte counts (10^9/L) may have been slightly elevated in HDT females as compared to control by week 46 and 52 (e.g., week 52: 286.5/control vs 391.0/HDT). However, thromboplastin times were not affected in either male or female dose groups as compared to their respective controls.

Red blood cell types

A summary table of RBC cell aberrations are presented below (from Table 21, pgs. 150-157):

CONTROL	polychromatic n.b.	anisocytosis	slight polychromasia	isolated anulocytes	lymphoc.	oxiphile n.b.
- 2 wks	-----	-------	-------	------	----	-----
6-52 wks	4	2(A1)	1	------	----	4
LDT						
-2 wks	------	1(A1)	-------	------	----	-----
6-52 wks	2	8(A1),1(A2)	1	1	----	-----
MDT						
-2 wks	-----	-------	-------	------	----	-----
6-52 wks	3	6(A1,2(A2)	-------	1	1	1
HDT						
-2 wks	1	-------	-------	------	----	-----
6-52 wks	4	3(A1),5(A2)	------	1	1	3

A1 = slight anisocytosis; A2 = moderate anisocytosis; ; n.b. = normoblast; lymphoc. = lymphocytes often in groups of 2-4 cells

There was a possible increase in the mid and high dose group incidence of moderate anisocytosis (RBC size variation) as compared to controls during weeks 6-52 of compound treatment (i.e., 0/control vs 2/MDT, 5/HDT). The general incidence of anisocytosis (slight predomination at LDT and MDT) was also increased in all dose groups receiving terbuconazole as compared to controls.

5.b. Clinical Chemistry (x indicates analyzed for)

Electrolytes:	Other:
x calcium*	x albumin*
x chloride*	x blood creatinine*
magnesium*	x blood urea nitrogen*
phosphorus*	x cholesterol*
x potassium*	x globulins
x sodium*	x glucose*
Enzymes	x total bilirubin*
x alkaline phosphatase	x total serum protein*
cholinesterase#	x triglycerides
creatinine phospho-	x serum protein electrophoresis
kinase*@	x cytochrome P-450 (liver tissue)
lactic acid dehydrogenase	x N-demethylase (liver tissue)
x serum alanine aminotransferase (also SGPT)*	
x serum aspartate aminotransferase (also SGOT)*	
gamma glutamyl transferase (GGTP)	
x glutamate dehydrogenase	

* required for subchronic and chronic studies
# should be required for OP: plasma, erthrocyte ChE conducted 2X prior to study initiation, 3 and 6 mos. and prior to terminal sacrifice
@ not required for subchronic studies

Magnesium, phosphorus and creatinine phosphokinase parameters were not analyzed.

A summary of selected clinical chemistry values and liver enzyme values are presented below:

Alkaline phophatase levels significantly diminished over the test period of 52 weeks in the controls, low and mid dose groups (e.g., control males/50% of -2 week value%) of both sexes but less so in the HDT animals (e.g., HDT males/93% of -2 week values).

Liver N-demethylase activity but not cytochrome P-450 activity was significantly elevated in both males and females of the high dose group as compared to the controls. Triglycerides were also elevated in male and female HDT groups as compared to respective controls.

Clinical chemistries/liver enzymes, TGs(from Table 26, pp. 198-202):

MALES/ FEMALES		AP(U/L)	N-DEMETH (NMOLxGxMIN)	CYT.P450 (NMOL/G)	TRIGLY.(UMOL/G)
CONTROL	W-2	231.5/181.3	--/--	--/--	--/--
	W 6	169.5/131.5	--/--	--/--	--/--
	W13	169.5/106.8	--/--	--/--	--/--
	W26	146.3/ 93.8	--/--	--/--	--/--
	W39	104.8/ 70.8	--/--	--/--	--/--
	W46	139.8/ 82.0	--/--	--/--	--/--
	W52	115.0/ 84.5	60.175/46.875	31.92/27.67	4.47/3.66
% con.		(50%) (47%)			
LDT	W-2	224.8/187.5	--/--	--/--	--/--
	W 6	180.5/170.8	--/--	--/--	--/--
	W13	154.5/146.8	--/--	--/--	--/--
	W26	120.8/118.5	--/--	--/--	--/--
	W39	87.8/ 92.5	--/--	--/--	--/--
	W46	----/----	--/--	--/--	--/--
	W52	95.5/113.0	43.050/35.075	27.45/20.37	4.25/4.33
% con.		(42%) (60%)			
MDT	W-2	224.0/211.8	--/--	--/--	--/--
	W 6	226.0/168.3	--/--	--/--	--/--
	W13	214.8/154.5	--/--	--/--	--/--
	W26	189.5/137.5	--/--	--/--	--/--
	W39	131.0/114.3	--/--	--/--	--/--
	W46	----/----	--/--	--/--	--/--
	W52	153.8/123.8	59.100/46.100	25.37/22.87	4.54/4.35
% con.		(69%) (58%)			
HDT	W-2	188.0/188.3	--/--	--/--	--/--
	W 6	165.8/177.5	--/--	--/--	--/--
	W13	166.3/168.8	--/--	--/--	--/--
	W26	159.0/146.0	--/--	--/--	--/--
	W39	154.3/131.5	--/--	--/--	--/--
	W46	184.8/152.0	--/--	--/--	--/--
	W52	175.5/159.3	115.175/93.375	24.72/26.65	5.37/5.89
% con.		(93%) (85%)	(191%) (199%)		(120%)(161%)

Serum protein electrophoresis of albumin/globulins

No noticeable compound-related effect upon albumin/globulin ratios, serum albumin, alpha-1, alpha-2, beta and gamma globuins was evident.

6. Urinalysis

Urine was collected from 6-16 hour fasted animals at 2 weeks prior, 6, 13, 26, 39, 46 (control, HDT) and 52 weeks. The Checked (X) parameters were examined.

X		X	
x	appearance*	x	glucose*
x	volume*	x	ketones*
x	specific gravity*	x	bilirubin*
x	pH	x	blood*
x	sediment (microscopic)		nitrate
x	protein*		urobilinogen

* required for chronic studies

There was no evidence of any compound-related alteration for the urine parameters examined.

7. Sacrifice and pathology-
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	X
Digestive system	Cardiovascular/hematopoietic
-tongue	x-aorta*
x-salivary glands*	xxheart*
x-esophagus*	x-bone marrow*
x-stomach*	x-lymph nodes*
x-duodenum*	xxspleen*
x-jejunum*	x-thymus*
x-ileum*	Urogenital
x-cecum*	xxkidneys*1
x-colon*	x-urinary bladder*
x-rectum*	xxtestes*1
xxliver*1	-epididymides
x-gall bladder*@	xxprostate
x-pancreas*	-seminal vesicle
Respiratory	xx-ovaries*1
-trachea*	x-uterus*
xxlung*	Neurologic
-nose#	xx-brain*1 (cerebr.,cerebellum,brain stem)
-pharynx#	x-peripheral nerves*@
-larynx#	-spinal cord (3 levels)*@
	x-pituitary*
	x-eyes (optic n.)*@
Glandular	
xxadrenals*	x-tonsils
-lacrimal gland*@	x-altered organs or organ parts
x-mammary gland*@	
xxparathyroids*2	
xxthyroids*2	
Other	
x-bone*@	
x-skeletal muscle*@	
x-skin*@	
-all gross lesions and masses*	

* required for subchronic and chronic studies
# required for chronic inhalation studies
@ in subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
1 organ weights required in subchronic and chronic studies
2 organ weights required for non-rodent studies

Histopathology was not performed for the trachea and lacrimal glands as required in EPA guidelines.

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a. organ weight

A summary table of selected mean absolute organ weights (gm)/organ to body weights (gm/kg) are presented below (from Table 37, pg. 57 of report):

Dose (ppm)	Liver	Kidney	Spleen	Prostate
MALES				
0	367.3/30.60	57.8/4.82	32.8/2.72	7.337/.6127
40	364.8/28.97	63.5/5.10	34.5/2.75	8.585/.6955
200	409.9/34.52	62.0/5.27	33.0/2.82	9.600/.7832
1000/2000	397.5/32.87	62.0/5.15	36.5/3.07	9.857/.7957
FEMALES				
0	386.5/34.52	49.5/4.45	29.8/2.67	--------
40	357.3/28.17	58.3/4.60	39.5/3.12	--------
200	354.8/30.47	53.5/4.65	34.0/3.00	--------
1000/2000	385.5/35.77	57.0/5.27	38.0/3.07	--------

Absolute and relative liver weights appeared elevated in mid and high dose males (e.g., 32.87/HDT vs 30.60/control, relative wts.). Relative kidney weights in either mid and/or high dose males and females were also elevated while both absolute and relative spleen weights in HDT males and in all treated females (LDT, MDT, HDT) appeared elevated.

b. Gross pathology

Selected gross observations in the liver are presented below (from Table 32, pp. 226-228 of report).

Dose group:	Observations
control	no findings
40 ppm	no findings
200 ppm	P 179: consistency of liver slightly brittle P 146: overall distinct lobulation P 160: overall distinct lobulation
1000/2000 ppm	P 149: distinct lobulation P 169: distinct lobulation (especially diaphragm surface). Cut surface likewise distinct lobulation; consistency unchanged P 156: overall with distinct lobulation P 168: overall with distinct lobulation P 170: with incipient lobulation

There was a consistent change in the appearance of livers in 3/8 and 5/8 of the MDT and HDT groups, respectively, as compared to the controls. The primary alteration was an increase in the lobulation of the livers, being defined as either incipient or distinct lobulation. This may be a reflection of liver enzyme induction (i.e., N-demethylase).

c. Microscopic pathology

1) Non-neoplastic

Summary dose-group incidence data (male/female) are presented below (from Table 40, pp. 255-258):

Observations (# animals examd.):	0 ppm 4/4	40 ppm 4/4	200 ppm 4/4	1000/2000 ppm 4/4
LIVER				
-siderin phagocytosis	0/2	0/2	1/2	3/0
-ORO-positive lipids in Kupffer c.	0/0	0/0	1/0	1/0
SPLEEN				
-increased siderin content	1/1	0/3	1/2	2/3
ADRENALS				
-intra-cytoplasmic vacuoles (z. fasiculata)	0/0	0/0	0/2	0/2
PROSTATE GLAND				
-round c. infiltration	0/-	1/-	1/-	2/-
OVARIES				
-yellow body cysts	-/0	-/0	-/1	-/1
STOMACH				
-pylorus mucosa hemorrhage	0/0	0/0	0/0	1/0
-round c. infiltration (pylorus)	0/0	0/0	0/0	1/1

An increase in the minimal presence of iron-containing pigment (siderin) in liver phagocytes in mid and high dose males along with a possible increase in ORO-positive lipids in Kupffer cells is suggestive of a subtle hepato-toxic effect of terbuconazole. The spleen of males at the HDT and females at all dose levels appears to have an increase in siderin as compared to controls. The adrenals are also apparently affected at the mid and high dose levels as evidenced by the presence in two dogs/group of intra-cytoplasmic vacuoles in the zona fasiculata. Round cell infiltration was also reported in male prostates (all doses) as well as in the pylorus of the stomach of HDT females as compared to zero incidences in respective controls. One female each of the mid and high dose groups had an ovarian yellow body cyst.

2) Neoplastic

No neoplasms were observed in any dog.

D. Discussion

Terbuconzole was administered orally in the diet to beagle dogs at concentrations of 0, 40, 200 and 1000(1-39 wks)/2000(40-52 wks) ppm for 52 weeks. No significant deficiencies were noted in the study materials, methods or data presentation and analysis.

No apparent compound-related effects upon mortality, adverse clinical signs, body weight gain or food consumption were noted. Numerous toxicological manifestations of an overt or more subtle nature were observed.

Ophthamological alterations of an apparent compound-related nature were observed in the mid and high dose groups. Lenticular lesions (opacities, lens stars) were noted in two animals of the MDT as well a one dog at the HDT. In addition, corneal opacities (cloudy, extensive) were also apparently elevated in one dog each of the MDT and HDT groups.

The liver is a target organ for both sexes based upon several findings of a moderate nature: alkaline phosphatase levels remained elevated over the course of the study in HDT males and females, liver N-demethylase but not cytochrome P-450 activity (both sexes) activity was significantly elevated in HDT groups as well as liver triglycerides, elevations in absolute and relative liver weights in MDT and HDT males, a consistent change in the appearance of the liver in 3/8 and 5/8 dogs of MDT and HDT groups, respectively as well as a slight increase in the presence of siderin in mid (1/4) and high-dose (3/4) males and ORO-positive lipids in one dog each of the mid and high-dose groups.

Other potential target organs/tissues are the hematopoietic system (increased presence of moderate anisocytosis in mid and high dose animals), the adrenals (increased incidence of intra-cytoplasmic vacuoules of zona fasiculata) the kidneys (relative weights elevated in mid and/or high dose males and females) and the spleen (elevation in both absolute and relative weights of both HDT males and females).

007200

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 12/20/88
Section I, Toxicology-Herbicide, Fungicide, Antimicrobial
Support Branch (T.H.F.A.S.B.) (TS-769C)
Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T. Quang Bui 12/30/88
Section I, T.H.F.A.S.B./HED(TS-769C)

DATA EVALUATION RECORD

STUDY TYPE: Rat chronic; EPA Guideline 83-1 TOX. CHEM. NO: 463P

ACCESSION NUMBER: MRID NO.: 407009-39

TEST MATERIAL: HWG 1608; 1-(4-chlorophenyl)-3-(1,2,4-triazol-1-yl-methyl)-4,4-dimethyl-pentane-3-ol

SYNONYMS: Terbuconazole; Folicur®

STUDY NUMBER(S): BAYER report no. 16375; Lab Proj. ID 96711

TESTING FACILITY: BAYER AG, Toxicology Division, FRG

TITLE OF REPORT: HWG 1608, Study for chronic toxicity and cancerogenicity in Wistar rats (Administration in diet for two years)

AUTHOR(S): Dr. E. Bomhard, Dr. W. Ramm

REPORT ISSUED: January 25, 1988

CONCLUSIONS: Dietary administration of terbuconazole (0, 100, 300, 1000 ppm) for 2 years produced a slight but statistically significant depression in MDT and HDT female body weights. Hematological alterations were noted in MDT and HDT females (depressions in hemoglobin, hematocrit, MCHC, MCV) associated with apparent enhanced clearance of RBCs in the spleen (HDT, increased incidence of hemosiderosis). Dose-related depressions in female adrenal weights were noted at all dose levels in association with dose-related decrease in adrenal cortical hemorrhagic degeneration. Also noted in females were statistically significantly elevations in liver microsomal enzyme at all dose levels as compared to controls. In males there was a statistically significant elevation in the combined incidences of thyroid C-cell adenoma, carcinoma and hyperplasia but not of adenoma or carcinoma alone. Based upon parafollicular tumors from eleven studies, the findings in treated animals were within the historical range and this is not considered an oncogenic response. Systemic LOEL, NOEL = 300, 100 ppm, resp.

CLASSIFICATION: MINIMUM

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A. Materials: (a photocopy of methods is attached)

1. Test compound:HWG 1608, solid/light yellow crystals; mixed Batch/Fl. no. 132, Purity approx. 95%

2. Test animals: Species: rat, Strain: Bor:WISW(SPF Cpb), Age: 5-6 weeks, Weight: males, 97 g(80-112); females, 90 g (71-111), Source: Winkelmann, Borchen.

B. Study Design:

1. Animal assignment

Animals were assigned randomly (using random number lists generated by computer program from Scientific Subroutine Package, IBM, Institute of Biometrics, Bayer, AG) to the following test groups:

Test group	Dose in diet(ppm)	Main study 24 mos		Interim sacrifice 12 mos	
		male	female	male	female
1 control	0	50	50	10	10
2 low(LDT)	100	50	50	10	10
3 mid(MDT)	300	50	50	10	10
4 high(HDT)	1000	50	50	10	10

2. Diet preparation

Diet was prepared weekly and stored (temperature not stated). Reserve samples of dietary mix with test substance were taken for possible reanalyses and kept for a minimum of six weeks under refrigeration and then destroyed. The test substance content was checked at approximately 3 month intervals. Homogeneity and stability (period of seven days) of dietary test mixture were determined from sample mixes analyzed prior to study initiation.

Results-

Summary tables of percent nominal, homogeneity and stability analyses are presented below.

Analysis of samples of dietary test mixture indicated that the average per cent of nominal concentrations were within 15% of target concentrations (88-91% for the three dose levels). Homogeneity and stability analyses were within exceptable values with mean % nominal concentrations of 50 and 5000 ppm being 92 and 104% for homogeneity, respectively; stability at 7 days of storage (presumably at room temperature) was 92 and 96% of nominal values of 50 and 3000 rpm, respectively.

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Nominal concentrations (from p. 90 of report)

Month/year	nominal conc. (mg/kg) 100	300	1000
10/84	93	267	900
1/85	86	267	900
4/85	88	273	980
7/85	94	276	890
10/85	91	270	880
1/86	90	282	850
4/86	87	279	890
7/86	82	267	950
10/86	83	270	950
mean	88	272	910
rel S.D.(%)	5	2	5
mean % (nominal)	88	91	91

Homogeneity was determined for five samples (50-100 gm) of food mix taken from a rectangular plastic bowl from front left(sample 1), front right(sample 2), middle (sample 3), back left (sample 4) and back right (sample 5).

Homogeneity (from p. 91 of report)

sample no.(random)	nominal conc. (mg/kg) 50	5000
1	45	5100
3	48	5150
4	44	5300
mean	46	5183
max. deviation(%) relative to mean	4	2
relative S.D.(%)	5	2
mean(%) nominal	92	104

Stability (from p. 92 of report)

storage period(days)	nominal conc. (mg/kg) 50	3000
0	46	2880
7	--	2670
14*	43	2520
active ingredient conc. in % nominal relative to storage period*	86	84

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3. Animals receive food (fixed formula standard diet: acclimatization period, Altromin@ 1324 pellets, and study period, Altromin@ 1321 meal, manufacturer Altromin GmbH, Lage) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

a) for clinical/hematology examinations, animal weights, food intake data and organ weights the arithmetic group means, standard deviations and 95 and 99 conficence limits (organ weights only) were determined. Collective numbers were compared against the controls with H.B. Mann and D.R. Whitney's signficance test (U test) or by F. Wilcoxon's method using signficance of $p<0.05$ or $p<0.01$, two-tailed.

b) for incidence data (mortality, clinical signs, etc.) was processed with Fisher's exact test, $p<0.05$ or $p<0.01$, two-tailed.

5. Statements of Data Confidentiality, GLP declarations and Quality Assurance were included with dated signatures.

C. Methods and Results:

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality (once on weekends and public holidays). Detailed individual examinations were performed once a week.

Toxicity/mortality (survival)

No compound-related increase in mortality was noted in the main or satellite groups. Male survival at 102 weeks was 82, 86, 84 and 94 % in 0, 100, 300 and 1000 ppm, respectively, suggesting a slight enhancement in male survival rate.

Clinical signs of toxicity were not apparently treatment-related. Lens opacities (p. 400 of report) were a common finding across all dose groups of both sexes, i.e., Males: 9/50, 12/50, 10/50, 12/50; Females: 4/50, 4/50, 5/50, 6/50, in respective dose groups noted under mortality discussion).

2. Body weight

Each animal was weighed prior to study initiation and then weekly up to and including week 12 and thereafter at biweekly intervals from week 15 to study termination. Extra body weights were recorded immediatedly before planned sacrifices for relative organ weight determinations.

Selected mean body weights (gm) are presented below.

Mean body weights were not statistically significantly different in treated males versus controls over the period of compound administration, although initially lower in the HDT prior to study initiation. There was a consistent but small depression in HDT and MDT females mean body weights (7-9%/HDT, 4-5%/MDT) observed by week one of compound administration in the HDT (data not shown) and by week 15 for the MDT. These decreases (statistically significant) are noted throughout the period of compound administration, and are considered compound-related, since they are not accounted for by significant changes in mean food consumption (g/kg b. wt./day)

MEAN BODY WTS

Dose (ppm)	0	15	27	55	81	101
			Week 6			
MALES						
0	99(7)[a]	339(21)	374(24)	404(31)	420(33)	403(31)
100	99(7)	345(26)	383(31)	413(34)	431(37)	412(38)
300	96(7)	333(25)	371(29)	410(38)	422(42)	411(44)
1000	95(7)**	327(25)*	369(29)	399(34)	416(35)	398(36)
FEMALES						
0	91(7)	201(17)	222(19)	243(23)	262(27)	261(30)
100	90(7)	201(15)	220(16)	241(21)	258(26)	262(28)
300	89(6)	195(12)*	212(14)**	232(16)*	248(19)*	254(19)
1000	90(6)	187(14)**	202(16)**	223(20)**	237(24)**	241(26)**

a = mean (standard deviation)
*, ** = statistically significant difference from respective controls at $p<0.05$, 0.01, respectively

3. Food consumption and compound intake

Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Food consumption/food efficiency/compound intake

Selected food intakes (g/kg body weight/day, g/animal/day) are presented below (pp. 114-118):

Dose (ppm)	1	15	27	55	81	101
			Week			
MALES						
0	64	53	40	43	58	47
	9.1	17.9	15.1	17.5	24.3	19.0
100	110	49	42	45	51	50
	15.6	16.9	16.1	18.6	22.2	20.6
300	105	54	41	44	46	48
	14.5	17.8	15.3	18.0	19.3	19.8
1000	94	54	42	47	52	48
	12.2	17.6	15.6	18.7	21.8	19.2

FEMALES (food consumption summary data tables continued)

0	54	74	72	75	67	85
	6.2	14.8	16.0	18.3	17.4	22.1
100	136	70	61	71	70	81
	15.3	14.1	13.4	17.1	18.2	21.3
300	113	75	63	73	76	87
	12.4	14.6	13.3	16.9	18.9	22.1
1000	116	83	69	91	88	87
	12.2	15.5	13.9	20.3	20.7	21.0

Mean food intake (g/kg body wt./day) over the course of the study was as follows: Males, 54.6, 52.8, 53.1, 55.0; Females, 74.8, 73.7, 76.1, 86.3, respectively.

Mean compound intake (mg/kg body weight/day) over the course of the study are as follows: Males, 5.3, 15.9, 55.0 and Females, 7.4, 22.8, 86.3 in 100, 300 and 1000 ppm, respectively. The relatively higher female mean compound intake at 1000 ppm was due to the consistently higher food consumption observed in the HDT females. The reason for this increased food consumption is not apparent although it is of interest to note that HDT females (primarily) had depressed mean body weight gains over the course of the study.

4. Opthalmological examinations

Performed before study initiation, at 52 weeks and terminal sacrifice on ten animals/sex of control and 1000 ppm dose groups.

Findings at terminal sacrifice (ten animals/group) are presented below (p. 439 of report):

Findings	Males (0 ppm)	Females	Males (HDT)	Females
-no pupil reflex (both sides)	3	1	0	0
-fundus badly or not appraisable (one, both sides)	6	3	7	1
-total to almost total lens opac.	3	1	2	1
-slight to moderate opacity	---	1	4	1
-corneal dystrophy/damage	5	3	2	2
-focal opacity (one, both)	1	1	---	1
-rt. inclusion in vitreous body	---	1	---	1

There was no evidence of dose-related eye changes at 52 weeks in either treated sex as compared with controls. Various eye alterations (lack of pupillary reflex, fundus not appraisable, lenticular opacities, corneal dystrophy/damage) were observed in both control and HDT animals at similar incidences. Examination of the summary histopathology table at terminal kill indicate widespread evidence of progressive retinal atrophy in all dose groups of both sexes (i.e., males: 45/49, 44/48, 46/49, 43/50 at respective doses; female: 47/50, 43/48, 42/47, 42/48, in respective dose groups).

5. a. Hematology

Blood was collected after 6, 12, 18 and 24 months for hematology and clinical analysis from 10 animals per dose group. The checked (X) parameters were examined:

X	X
X hematocrit (HCT)*	X leukocyte differential count *
X hemoglobin (HGB)*	X mean corpuscular HGB (MCH)
X leukocyte count(WBC)*	X mean corpuscular HGB conc. (MCHC)
X platelet count*	X mean corpuscular volume (MCV)
blood clotting measurements	X reticulocyte count
X -thromboplastin time	X-RBC count
-clotting time	X (RBC morphology)
-prothrombin time	

* required for subchronic and chronic studies

Selected values are presented below:

There are generally no consistent hematology changes noted in treated males. In treated females, there are small, consistent, but generally statistically significant depressions in hemoglobin, hematocrit values associated with lowered mean corpuscular volumes and concentrations. These effects are most evident by 79 and 104 weeks of compound adminstration with statistically significant decreases in both the MDT and HDT females for Hb, Hct, MCV and MCH (e.g., Hb: 145/MDT, 144/HDT vs 149/con). These small alterations are still evident, although not statistically significant (except for MCH), in the mid and high dose groups at 104 week analyses.

(HEMATOLOGY SUMMARY): (from Table 4, p. 47)

DOSE(PPM) Week 27:	HB (g/L)	HCT (L/L)	MCV (fL)	MCH (pg)	THROMBOPLASTIN TIME seconds
0 M	157	0.442	54	19.2	32.6
100	156	0.447	54	18.7	31.3
300	158	0.445	53	18.7	32.3
1000	160	0.458	53	18.4**	32.3
0 F	158	0.457	58	20.0	28.8
100	156	0.457	59	20.1	29.6
300	156	0.454	57	19.7	29.1
1000	155	0.450	56*	19.4*	28.6
Week 52:					
0 M	151	0.474	55	18.2	35.3
100	147*	0.459*	55	18.1	33.1
300	151	0.470	54	17.9	32.4
1000	149	0.475	55	17.6	33.4
0 F	141	0.438	61	20.2	29.6
100	143	0.427	59	20.3	28.9
300	141	0.423	59	20.0	28.8
1000	146	0.418*	57**	20.5	29.6
Week 79:					
0 M	157	0.496	59	18.5	31.2
100	153	0.482	59	18.5	30.2
300	153	0.482	57	18.2	28.0
1000	153	0.482	57	18.0	32.4
0 F	149	0.466	65	20.5	31.0
100	148	0.460	63	19.9	28.0**
300	145**	0.452**	61**	19.5*	28.1**
1000	144*	0.453*	60**	19.1**	28.9
Week 104:					
0 M	147	0.459	58	18.6	33.4
100	152	0.472	58	18.6	31.1*
300	146	0.460	58	18.3	32.4
1000	151	0.478	57	17.9	28.9
0 F	146	0.454	53	20.3	31.4
100	148	0.453	60	19.4	31.4
300	143	0.441	59	19.1*	31.2
1000	143	0.446	59	19.1**	31.2

*, ** statistically significant difference from controls at $p<0.05$, 0.01, respectively

5.b. Clinical Chemistry (x indicates analyzed for)

Electrolytes:	Other:
x calcium*	x albumin*
x chloride*	x blood creatinine*
magnesium*	x blood urea nitrogen*
x phosphorus*	x cholesterol*
x potassium*	globulins
x sodium*	x glucose*
Enzymes	x total bilirubin*
x alkaline phosphatase	xtotal serum protein*
cholinesterase#	xtriglycerides
x creatinine phospho-	serum protein electrophoresis
kinase*@	x (iron)
x lactic acid dehydrogenase	
x serum alanine aminotransferase (also SGPT)*	
x serum aspartate aminotransferase (also SGOT)*	
gamma glutamyl transferase (GGTP)	
glutamate dehydrogenase	

* required for subchronic and chronic studies
# should be required for OP: plasma, erthrocyte ChE conducted 2X prior to study initiation, 3 and 6 mos. and prior to terminal sacrifice
@ not required for subchronic studies

Selected clinical chemistry values are presented below:

Although statistically significant alterations (both increases and decreases) in ASAT, ALAT, LDH, CK and triglycerides are noted they are inconsistent, sporadic findings which are not dose-related, sex-related and often are in opposite directions.

CLINICAL CHEMISTRIES (Table 5, p. 51 of report)

DOSE(PPM)	ASAT(GOT) U/L	ALAT(GPT) U/L	LDH U/L	CK U/L	TRIG MMOL/L
Week 27:					
0 M	45.1	28.8	86	68	0.59
100	41.2	26.4	82	89	0.63
300	39.1*	27.4	84	92	0.76
1000	43.6	30.5	69	87	0.57
0 F	42.9	26.4	169	91	0.40
100	39.7	22.5	127*	58*	0.37
300	40.3	22.3	96**	39**	0.35
1000	39.5	24.4	65**	35**	0.31*
Week 52:					
0 M	35.2	27.9	88	49	0.95
100	37.4	29.1	101	47	0.78
300	39.6*	30.6	117	59	1.04
1000	64.3**	38.2*	1093**	226	0.70*
0 F	36.9	26.9	126	45	0.59
100	46.9	29.5	250	70	0.64
300	42.1	30.3	157	52	0.45*
1000	41.2	28.2	105	38	0.43**
Week 79:					
0 M	37.4	49.8	184	66	1.88
100	40.0	45.2	174	58	2.00
300	37.5	51.6	162	46	2.04
1000	40.5	52.9	169	52	2.02
0 F	54.5	50.8	447	124	1.33
100	77.0	52.8	1281**	282*	1.71
300	67.6	56.0	804	262*	1.32
1000	68.7*	65.0**	706**	286**	1.16
Week 104:					
0 M	39.6	46.5	176	148	2.49
100	33.8	47.9	168	81	3.17
300	38.8	52.9	117**	68	2.09
1000	42.4	58.6*	706**	70	1.88
0 F	36.9	42.2	108	63	1.45
100	46.0	49.8*	115	52	2.03
300	37.0	41.5	95	63	1.42
1000	38.8	47.3	102	110*	1.07

*, ** statistically significant difference from controls at $p<0.05$, 0.01, respectively

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6. Urinalysis

Urine was collected from fasted animals at 6, 12, 18 and 24 months. The Checked (X) parameters were examined.

X	X
Xappearance*	X glucos*
Xvolume*	X ketones*
Xspecific gravity*	X bilirubin*
XpH	X blood*
Xsediment (microscopic)	nitrate
Xprotein*	X urobilinogen

* required for chronic studies

DOSE(PPM)	PROT	PROT*VOL
Week 27:	g/L	mg
0 M	1.58	5.8
100	1.51	8.6
300	1.59	8.4
1000	1.49	5.3
0 F	0.39	2.4
100	0.33	2.1
300	0.31	1.5**
1000	0.30	1.5**
Week 52:		
0 M	1.72	7.8
100	2.46	12.2*
300	1.71	8.9
1000	1.86	8.5
0 F	0.33	1.6
100	0.34	1.7
300	0.21	1.2
1000	0.17*	1.0*
Week 79:		
0 M	4.32	11.6
100	4.73	18.0
300	2.82	7.9
1000	3.14	8.3
0 F	0.62	3.9
100	0.76	5.3
300	0.37	2.1
1000	0.21**	2.2

(URINALYSIS CONTINUED):

DOSE(PPM)	PROT	PROT*VOL
Week 104:		
0 M	2.28	16.1
100	4.46*	25.8*
300	2.97	17.7
1000	2.64	16.5
0 F	0.93	4.4
100	1.23	8.2
300	1.28	5.8
1000	0.31**	2.0*

*, ** statistically significant difference from controls at $p<0.05$, 0.01, respectively

Selected urinalysis values are presented above.

In HDT females, but not males, there was a generally consistent, often statistically significant, decrease in protein recovered in the urine at all time periods analyzed. This would suggest a possible compound-related effect upon kidney clearance, although no apparent histopathological changes were noted.

7. Sacrifice and pathology-
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organ in addition were weighed.

X	X
Digestive system	Cardiovascular/hematopoietic
x-tongue	x-aorta*
x-salivary glands*	xx-heart*
x-esophagus*	x-bone marrow*(femur,sternum)
x-stomach*	x-lymph nodes*(mandibular, mesenteric)
x-duodenum*	xx-spleen*
x-jejunum*	x-thymus*
x-ileum*	Urogenital
x-cecum*	xx-kidneys*1
x-colon*	x-urinary bladder*
x-rectum*	xx-testes*1
xxliver*1	x-epididymides
x-gall bladder*@	x-prostate
x-pancreas*	x-seminal vesicle
Respiratory	xx-ovaries*1(with oviduct)
x-trachea*	x-uterus*
xxlung*	Neurologic
-nose#	xx-brain*1 (n. ischiadicus)
-pharynx#	x-peripheral nerves*@(n. opticus)
x-larynx#	x-spinal cord (3 levels)*@(cervical,
	x-pituitary* thoracic, lumbar)
	x-eyes (optic n.)*@
Glandular	
xxadrenals*	x-extraorbital glands
-lacrimal gland*@	x-Harder's glands
x-mammary gland*@	x-ureter
-parathyroids*2	x-urethra
x-thyroids*2	x-head (rest)
Other	x-vagina

x-bone*@ (femur, sternum)
x-skeletal muscle*@ (thigh)
x-skin*@
-all gross lesions and masses*

* required for subchronic and chronic studies
# required for chronic inhalation studies
@ in subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
1 organ weights required in subchronic and chronic studies
2 organ weights required for non-rodent studies

a. organ weight

Selected absolute (mg)/relative weights (mg/100 gm b.wt.) are presented below for interim and final sacrifices:

INTERIM Dose(ppm)	B.wt.	lungs	liver	spleen	adrenals	testes/ ovaries
Males:						
0	418	1369/ 328	14437/ 3447	636/ 153	39/ 9	3896/ 932
100	423	1382/ 328	13856 3269	672/ 159	41/ 10	3726/ 884
300	398	1334/ 337	13126/ 3305	622/ 156	36/ 9	3551/ 896
1000	384*	1368/ 356	12881/ 3361	636/ 166	37/ 9	3576/ 932
Females:						
0	229	965/ 422	8638/ 3769	430/ 188	62/ 27	122/ 53
100	227	971/ 428	8140/ 3585	462/ 203*	62/ 27	123/ 54
300	233	970/ 416	8048/ 3454*	416/ 178	59/ 25	114/ 49
1000	232	1116*/ 482*	7812*/ 3365**	504**/ 217**	60/ 26	120/ 52

TERMINAL KILL Dose(ppm)	B.wt.	lungs	liver	spleen	adrenals	testes/ ovaries
Males:						
0	400	1517/ 381	14760/ 3700	814/ 204	51/ 13	3880/ 980
100	403	1596/ 399	14549/ 3613	832/ 207	53/ 13	3807/ 952
300	407	1563/ 387	14448/ 3555	802/ 199	46/ 11	3619/ 887
1000	395	1469/ 375	14256/ 3620	783/ 200	47/ 12	3489/ 883*
Females:						
0	259	1156/ 451	9176/ 3567	548/ 215	78/ 31	142/ 55
100	260	1194/ 464	9248/ 3550	561/ 216	65*/ 25*	142/ 57
300	252	1134/ 454	8843/ 3504	549/ 220	64**/ 26*	138/ 55
1000	242**	1148/ 478	9108/ 3773*	562/ 233	57**/ 24**	137/ 57

*, ** statistically significantly different from controls at $p<0.05$, 0.01, respectively

Interim organ weights (absolute, relative) in males at 52 weeks were not affected by terbuconazole treatment. At terminal kill, the testes weights were depressed (statistically significant for relative weights, $p<0.05$).

A consistent, dose-related depression was noted in female absolute and relative adrenals weights which was statistically significant ($p<0.05$, 0.01) at all dose levels(e.g., absolute: 65/LDT, 64/MDT, 57/HDT vs 78 gm/control). Inconsistent liver and spleen weights were noted between the interim and terminal organ weights with depressed liver weights and elevated spleen weights noted at the HDT at interim kill but not at 2-years (relative liver weights were statistically higher; spleen weights were similar to controls).

b. Gross pathology

Selected findings are presented below:

Dose(ppm):	0		100		300		1000	
Sex:	M	F	M	F	M	F	M	F
# animals	49	50	49	50	50	50	50	50
KIDNEYS								
-cyst,cystic	0	0	0	1	1	1	4	0
LYMPH NODES								
-enlarged	3	0	3	0	2	0	6	0
-reddened	1	0	2	1	1	0	6	0
TESTES								
-shrunken	3	0	2	0	7	0	6	0
-flaccid consistency	2	0	3	0	1	0	0	0
UTERUS								
-thickened	0	8	0	7	0	5	0	8

In HDT males there was an apparent increase in the presence of kidney cyst/cystic kidneys (4/50, HDT vs 0/49, control) and in enlarged or reddened lymph nodes (e.g., reddened: 6/50, HDT vs 1/49, control). The number of testes of MDT and HDT males also appeared to somewhat more shrunken in appearance than control males (7/60, MDT, 6/50, HDT vs 3/49, controls). Thickening of the uterus was found across all dose groups.

c. Microscopic pathology

1) Non-neoplastic

ORGAN/LESION (male/female)	0 PPM T	0 PPM TK	0 PPM ID	100 PPM T	100 PPM TK	100 PPM ID	300 PPM T	300 PPM TK	300 PPM ID	1000 PPM T	1000 PPM TK	1000 PPM ID
ADRENALS	(49	41	8)	(49	41	8)	(50	42	8)	(49	46	3)[a]
	(50	39	11)	(50	38	12)	(50	39	11)	(50	41	9)
-hemorrhagic	3	3	0...4	3		1..4	4		0..1	0		1
degen.(cortex)	23	21	2..15@	12		3..13#	9		4..4**	4		0
LIVER	(49	41	8)	(49	41	8)	(50	42	8)	(50	47	3)[a]
	(49	39	11)	(50	38	12)	(50	39	11)	(50	41	9)
-pale cell	4	3	1...4	3		1..2	2		0..8	8		0
	0	0	0...0	0		0..1	1		0..1	1		0
-Kupffer cell	0	0	0...1	0		1..0	0		0..1	0		1
pigmentation	2	2	0...2	2		0..1	1		0..7	7		0
-single cell	0	0	0...3	1		2..5	5		0..2	2		0
necrosis	1	0	1...3	2		1..3	2		1..3	3		0
LUNG	(49	41	8)	(49	41	8)	(50	42	8)	50	47	3)[a]
	(50	39	11)	(50	38	12)	(50	39	11)	(50	41	9)
-interstitial	17	17	0..14	12		2..16	14		2..17	16		1
pneumonitis	10	8	2..26	21		5..20	17		3..13	12		1
-mineraliza-	21	19	2..29	27		2..33	31		2..22	22		0
tion(bld vessel walls)	20	18	2..23	18		5..24	20		4..21	19		2
LYMPH NODES	(49	41	8)	(49	41	8)	(50	42	8)	(50	47	3)[a]
	(50	39	11)	(50	38	12)	(50	39	11)	(50	41	9)
(MESENTERIC)												
-blood-filled	2	2	0.. 4	3		1.. 4	2		2..5	5		0
sinuses	0	0	0...0	0		0.. 2	2		0..3	2		1
SPLEEN	(49	41	8)	(49	41	8)	(50	42	8)	(50	47	3)[a]
	(50	39	11)	(50	38	12)	(50	39	11)	(50	41	9)
-hemosiderin	0	0	0...0	0		0...1	0		1.. 0	0		0
(increased)	2	0	2...3	2		1...3	0		3..19**	17		2
THYROID												
-C-cell hyperplasia...(see neoplastic lesions)												
URINARY BLADDER	(48	41	7)	(48	41	7)	(50	42	8)	(50	47	3)[a]
	(49	39	10)	(49	37	12)	(50	39	11)	(50	41	9)
-epithelial	2	2	0...1	0		1...3	1		2.. 2	2		0
hyperplasia	1	0	1...1	0		1...1	1		0.. 4	4		0
UTERUS	(50	39	11)	(50	38	12)	(50	39	11)	(50	41	9)
-squamous meta-	4	3	1...8	5		3...5	4		1..10	8		2
plasia												

(NON-NEOPLASTIC LESION CONTINUED)
LIVER ENZYME INDUCTION
FEMALES: 8/37 18/39* 14/40 27/43**

[a] number of male finding/number of female findings: T = total, TK = terminal kill, ID = interim death
*, ** stat. sign. difference from controls at p<0.05, 0.01, resp.
@, # p<.074, .03 (reviewer's statistical analysis, Fisher's exact test)

There was a dose-related, statistically significant decrease in the incidence of adrenal cortical hemorrhagic degeneration in the MDT and HDT females (LDT approached statistical significance) as compared to the controls. An increase in HDT males of liver pale cell (4/49, control vs 8/50, HDT) is suggested as well as increased Kupffer cell pigmentation (2/49, control vs 7/50) in HDT females. Furthermore, an overall treatment but not dose-related increase in male and females for single cell necrosis is suggested at all dose levels.

There was a statistically significant elevation in the finding of increased hemosiderin deposition in HDT females as compared to controls (2/50, control vs 19/50, p<0.01). This is consistent with hematological changes observed in HDT females. The incidence of uterine squamous metaplasia was increased in treated over controls (approaching statistical significance at the high dose level).

Histological changes related to liver microsomal enzyme induction were evident in all female but not male treatment groups as compared to controls--consistent with the known liver enzyme inducing ability of terbuconazole. These increases were statistically significant in the LDT and HDT dose groups (p<0.05, 0.01, respectively).

2) Neoplastic

Selected neoplastic findings are presented below.

There was no evidence of dose-related increases in hepatocellular adenoma or carcinoma and pituitary adenoma/adenocarcinoma. An increased incidence, not dose-related, in atypical carcinoma of the uterus, described as high malignant, was noted in the treated dose groups (0/50, controls vs 3/50, LDT, 2/50, MDT, 1/50, HDT) as compared to the controls.

In males, but not females, C-cell thyroid adenoma and carcinoma were noted in treated but not control groups. These were non-dose-related findings and were not statistically significant. The incidence of thyroid C-cell hyperplasia was somewhat elevated at the MDT and HDT (1/50, control vs 7/50, MDT, 6/50, HDT) being statistically significant (P<0.05 level) at the mid but not high

dose level. Combination hypeplasia/neoplasia increased the statistical significance of the thyroid findings.

ORGAN/LESION (male/female)	0 PPM T	0 PPM TK	0 PPM ID	100 PPM T	100 PPM TK	100 PPM ID	300 PPM T	300 PPM TK	300 PPM ID	1000 PPM T	1000 PPM TK	1000 PPM ID
LIVER	(49	41	8)	(49	41	8)	(50	42	8)	(50	47	3)[a]
	(49	39	10)	(50	38	12)	(50	39	11)	(50	41	9)
-hepatocellular adenoma	0	0	0...0	0	0	0..0	0	0	0..0	0	0	0
	0	0	0...1	1	0	0..3	3	0	0..0	0	0	0
-hepatocellular carcinoma	1	0	1...1	1	0	0..0	0	0	0..0	0	0	0
	1	1	0...0	0	0	0..0	0	0	0..0	0	0	0
THYROID	(50	41	9)	(50	41	9)	(50	42	8)	(50	47	3)[a]
	(49	39	10)	(50	38	12)	(50	39	11)	(50	41	9)
-follicular adenoma	0	0	0...1	1	0	0..0	0	0	0..3	3	0	
	0	0	0...0	0	0	0..1	1	0	0..1	1	0	
-C-cell adenoma	0	0	0...1	1	0	0..3	3	0	0..2	2	0	
	1	1	0...0	0	0	0..1	1	0	0..1	1	0	
-C-cell carcinoma	0	0	0...1	1	0	0..0	0	0	0..1	1	0	
	0	0	0...0	0	0	0..0	0	0	0..0	0	0	
-C-cell hyperplasia	1	1	0...3	3	0	0..7@	5	0	2..6#	6	0	
	1	1	0...2	2	0	0..3	3	0	0..0	0	0	
-combined hyperpl./neopl. (C-cell)	1	1	0...5	5	0	0..10**	8	2	2..9*	9	0	
	2	2	0...2	2	0	0.. 4	4	0	0..1	1	0	
PITUITARY	(50	41	9)	(50	41	9)	(50	42	8)	(50	47	3)[a]
	(50	39	11)	(50	38	12)	(50	39	10)	(50	41	9)
-adenocarcinoma	1	0	1...0	0	0	0..0	0	0	0..0	0	0	
	0	0	0...0	0	0	0..2	0	2	0..1	0	1	
-adenoma	6	5	1...3	3	0	0..6	5	1	0..6	6	0	
	13	12	1..14	10	4	0..14	13	1	1.11	9	2	
UTERUS	(50	39	11)	(50	38	12)	(50	39	11)	(50	41	9)
-atypical carcinoma (highly malignant)	0	0	0...3	0	3	0..2	0	2	0..1	0	1	

[a] number of male finding/number of female findings: T = total, TK = terminal kill, ID = interim death
*, ** stat. sign. difference from controls at p<0.05, 0.01, resp.
@,# p<0.03, 0.06-reviewer's statistical analysis by Fisher's exact test

D. Discussion

Technical terbuconazole was orally administered (diet) for periods up to 24 months at 0, 100, 300 and 1000 ppm. There was no evidence of compound-related increases in mortality, rather the male but not female HDT dose group appeared to have a slight enhancement in survival rate. Minimal but statistically significant depressions in female body weights (MDT, HDT) were noted thoughout the study and were not accounted for by food consumption patterns.

In females, but not males, there was a small but consistent depressions in hemoglobin, hematocrit and altered mean corpuscular concentrations and volumes at 79 and 104 weeks of analyses which correlated with an increased deposition of splenic hemosiderin in HDT females. Dose-related depressions in female absolute and relative adrenal weights (statistically significant at all dose levels) were associated with a dose-related decrease in the incidence of adrenal cortical hemorrhagic degeneration (statistically significant at MDT and HDT). There was also a dose-related increase in liver microsomal enzyme induction at all dose levels tested. This is based upon histological examination not enzymatic analyses.

In HDT males, gross pathology suggested an increase in the presence of kidney cyst/cystic kidneys and an increase in reddened lymph nodes. Histological examination of the lymph nodes indicated a possible elevation of blood-filled sinuses of the mesenteric lymph nodes in HDT males.

In males, but not females, C-cell thyroid adenoma and carcinoma were noted in treated but not control groups. These were non-dose-related findings and were not statistically significant. The incidence of thyroid C-cell hyperplasia was somewhat elevated at the MDT and HDT (1/50, control vs 7/50, MDT, 6/50, HDT) being statistically significant ($P<0.05$ level) at the mid but not high dose level. The pathology report, p. 465, noted that thyroid C-cell neoplasia and hyperplasia can be combined since the differentiation is arbitrary depending mainly on size of the lesions. Combination of hyperplasia and neoplasia increased the statistical significance of the hyperplastic findings which resulted in the following respective incidences(%): 2, 10, 20 and 18%. The authors submitted historical control data (Bomhard et al., 1986: J.E.P.T.O, 7(1/1), 35-52) from the Bayer laboratories for spontaneous tumors in Wistar TNO/W.70 rats from eleven studies initiated between 1973-1976. The range of thyroid parafollicular tumors (which included interstitial or C-cell adenomas) was 0 to 19.3% with an average of 7.4%. Thus the individual and combined C-cell tumors from the present study were essentially within the historical parafollicular control range.

Atypical carcinoma of the uterus (a highly metastasising, malignant tumor of the uterine ligament) was noted in all treated animals in small frequencies (6%, 6%, 2% of respective treated groups) but not controls. This was not a dose-related tumor nor statistically signficantly different from concurrent controls. Historical control data provided by the registrant in Wistar (Han) rats indicates the occurrence of a large number of spontaneous, metastatic uterine adenocarcinomas (39% cf 305 females) from Wistar rats used in a longevity study (Deerberg et al., 1981, Vet. Pathol., 18, 707-713). Therefore, it is urlikely that this is a compound-induced tumor.

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Review Section I, Tox II (TS-769C)
Secondary reviewer: Quang Q. Bui, Ph.D. Quang Q. Bui 12/21/88
Review Section I, Tox II (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Dose-ranging (mice) for oncogenicity; Guideline 82-1

TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 407009-33

TEST MATERIAL: HWG 1608 TECHNICAL; mixed batch, FL No. 132; solid; purity of 96.9%

SYNONYMS: FOLICUR@;(terbuconazole); ethyltrianol(proposed)

STUDY NUMBER(S): report no. T 0018885(8 wk study); report no. T 6018539(5 day study); Lab. proj. ID no. 94211

SPONSOR: Mobay Corporation, Corporate Toxicology

TESTING LABORATORY: BAYER AG, Institut fuer Toxikologie Landwirtschaft, Fachbereich Toxikologie, D 5600 Wuppertal 1, FRG

TITLE OF REPORT: Range-finding toxicological study study with NMRI mice to establish dosage for a chronic study (feeding for eight weeks) and for determinations of enzyme induction in the liver (feeding for five days)

AUTHOR(S): Dr. W. Ramm

REPORT ISSUED: July 7, 1986

CONCLUSIONS: In an eight week dietary dose range-finding study, terbuconazole produced a slight but consistent depression in female but not male body weights at the HDT. The liver is a major target site for toxicity at both 500 and 2000 ppm as evidenced by increased absolute/relative organs weights, elevated total and indirect bilirubin, gross changes in liver appearance (paleness, lobulation) and increased histopathology findings (necrosis, vacuolization, degeneration, lipidosis). Other potential targets are the spleen (increase pigment deposition, increase serum Fe2+, heart (elevated relative weight), kidneys (increased presence of round cell infiltrates) and the adrenals (increased lipid concentrations, sinus dilation). Terbuconazole was an effective inducer of microsomal enzyme after 5 days of treatment at all dose levels evaluated (125, 500, 2000 ppm). Based upon these findings, dose levels of 20, 60 and 180 ppm were selected for the chronic study.

This study is designated as Core supplementary data.

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MATERIALS AND METHODS: (photocopy of methods appended)

1. Eight week study

SPF-bred mice (4-5 weeks old) in groups of 5 males and 5 females per dose level were acclimatized for 7 days and then fed terbuconazole daily for 8 weeks at 0, 500 and 2000 ppm in their diet. Animals were inspected twice daily (once on weekends, public holidays) and alterations noted. Animal weights were recorded at start of study and then weekly with weekly food consumption and water intake being determined. Clinical chemistries were performed at study termination and included bilirubin and iron.

At study termination, gross and histopathology were conducted with the heart, testicles, ovaries, liver, lung, spleen, kidneys and adrenals weighed (see appended methods for organs/tissues examined). All organs/tissues in the control and 2000 ppm dose groups were examined plus the liver, lungs, spleen, kidneys and adrenals of the mid dose.

Arithmetic group means, standard deviations with 95 and 99% upper and lower confidence limits were determined. Collective values were evaluated for statistical significance with Mann, Whitney and Wilcoxon test.

2. Five day enzyme induction test

NMRI mice of the same strain (five of each sex/dose) were treated with 0, 125, 500 and 2000 ppm for five consecutive days via their diet. After five days the animals were sacrificed, the livers removed and frozen for determination of cytochrome P-450, triglycerides, N- and O-demethylase.

GLP CONCERNS: Signed and dated statements regarding data confidentiality and GLPs were included. Individual phases of the study and the final version of the report were not inspected by the QAU unit.

RESULTS/CONCLUSIONS:

Eight week study

MORTALITY/CLINICAL SIGNS

One female in the control (#6) and one male (# 15) in the 500 ppm group died during terminal blood sampling, apparently due to ether overdose or hypovolemia.

No clinical signs data were provided. The authors stated that mice at 500 and 2000 ppm did not differ in appearance and behavior from controls.

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BODY WEIGHTS/FOOD CONSUMPTION/WATER INTAKE

There were no differences among the dose groups for either sex with regard to mean food consumption (g/animal/day) or water intake (ml/animal/day).

Mean body weights for males during the eight week period were similar but female body weights in the HDT as compared to the controls were consistently 1-2 grams lower (e.g., wk 2: control = 28.5 g vs 2000 ppm = 26.8g; wk 6: control = 28.8g vs 2000 ppm = 27.0g).

CLINICAL CHEMISTRIES

There was a statistically significant decrease in total bilirubin and indirect bilirubin concentrations in males at 500 and/or 2000 ppm as well as a statistically significant increase in serum iron (see below; form Table 3, p. 17 of report). No statistically significant changes were observed in treated females although mean iron values were elevated in the HDT as compare to the controls (54.3 vs 47.4, resp.).

	(UMOL/L)			
dose(ppm)	total Bili	direct Bili	indirect Bili	Fe++
0	3.8	1.2	2.6	41.1
500	2.5	1.8	1.0*	44.3
2000	2.5*	1.4	1.1*	51.7**

*,** statistically significantly different from controls ($p<0.05$;<0.01, resp.)

ORGAN WEIGHTS

Selected summary absolute (mg)/relative (mg/100 gm) organ weights are presented below:

DOSE(PPM)	LIVER		ADRENALS	
	Males	Females	Males	Females
0	1995/4802	1555/4971	7/18	14/46
500	2661*/6435*	2125*/6960*	8/19	13/43
2000	2846*/7204**	2090*/7394*	8/21	11*/38

Statistically significant increase in absolute and relative liver weights for both male and female mice were observed at 500 and 2000 ppm. In HDT females, absolute and relative adrenal weights were decreased (statistically significant for absolute weight). Not shown are compound treated male heart weights which were elevated with the relative weights significantly so ($p<0.05$; 488/control vs 601/500 and 593/2000 ppm, respectively).

GROSS PATHOLOGY/HISTOPATHOLOGY

Apparent compound-related gross changes were swollen or increased lobulation of the liver of males (500 ppm, males, females: 4/5, 0/5, resp.; 2000 ppm, males, females: 3/5, 0/5, resp.) and increase in pale livers, primarily in females (500 ppm, males, females: 1/5, 1/5, resp.; 2000 ppm, males, females: 1/5, 4/5, resp.). None of these changes were observed in controls.

A summary of histopathology findings (taken from Table 2, p. 61 of the report) is presented below:

Alterations	0 ppm	500 ppm	2000 ppm
LIVER			
-liver c. degeneration(grade)*	---	2.2	2.7
# affected	0/9	10/10	10/10
-individual necrosis	---	----	0.6
# affected	0/9	0/9	4/10
-focal necrosis	---	----	0.1
# affected	0/9	0/9	1/10
-large vacuoles	---	0.7	0.4
# affected	0/9	4/9	4/10
-fat content(ORO stain)	1.2	3.0	3.5
SPLEEN			
-pigment increased	---	---	1.0
#affected	0/9	9/9	10/10
KIDNEYS			
-round c. infiltrates	0.1	0.3	0.3
# affected	1/9	2/9	3/10
-round c. infiltrates in pelvis	0.3	0.2	0.6
# affected	3/9	2/9	6/10
ADRENALS			
-cortex c. lipid-rich (males)	---	1.5	3.0
# affected	0/5	4/4	5/5
-sinus dilation	0.4	0.4	1.1
# affected	2/9	2/10	5/10
cortical hyperplasia	0.5	0.6	0.3
# affected	3/9	6/9	3/10
brown degeneration of x-zone (females)	2.0	1.0	1.6
# affected	4/5	5/5	5/5

* Grades: 1 = slight; 2 = slight to moderate; 3 = moderate; 4 = moderate to severe; 5 = severe

The liver, spleen, possibly the kidneys and the adrenal glands appear to be sites of compound-induced toxicity at either the 500 and/or 2000 ppm dose levels in males and/or females. As indicated in the summary, there was an increase in the incidence and severity (grade) of liver cell degeneration, liver cell necrosis, focal necrosis and presence of vacuoles and lipid content at either or both doses. At the HDT, there was an increase in the incidence of pigmentation (iron-related pigment) and round cell infiltrates of the kidneys were apparently greater in the HDT as compared to the controls. The adrenals were also significantly affected as evidenced by an increase in cortical cell lipids (both dose groups) and sinus dilation (HDT) of males.

Five day enzyme induction study

Summary data for enzyme induction/triglycerides in liver are presented below (taken from Table 6, p. 22 of report):

Dose(ppm) Males	N-demethyl. (mU/g)	O-demethyl. (mU/g)	P-450 (nmol/g)	Trigly. (umol/g)
0	234.5	47.9	36.7	4.29
125	225.2	48.4	55.8**	12.71**
500	288.9	54.6	107.0**	18.84**
2000	222.1	36.1**	131.9**	23.78**
Females				
0	217.2	48.9	32.1	5.29
125	490.7**	64.4	45.6*	10.84
500	556.6**	77.6**	94.6**	23.45**
2000	364.2**	92.4**	110.5**	31.76**

Terbuconazole induced microsomal enzymes in both sexes at all dose levels as compared to controls. In males, there was a statistically significant increase in O-demethylase (HDT), P-450 and triglycerides (all dose levels). N-demethylase, O-demethylase, P-450 and triglycerides were all significantly elevated in female mice at all dose levels.

007200

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DATA EVALUATION RECORD

STUDY TYPE: Mouse oncogenicity (21 mos) (EPA Guideline 83-2) TOX. CHEM. NO: 463P

ACCESSION NUMBER: MRID NO.: 407009-41

TEST MATERIAL: HWG 1608 technical a.i.; 1-(4-chlorophenyl-4,4-dimethyl-3-(1,2,4-triazole-1-yl-methyl)-pentane-3-ol; CAS 80-443-41-0

SYNONYMS: Terbuconazole

STUDY NUMBER(S): Report no. 16376; Lab. proj. id report no. 96709

TESTING FACILITY: BAYER AG, Toxicology Division, FRG

TITLE OF REPORT: HWG 1608, Study for cancerogenicity in NMRI mice (administration in diet for up to twenty-one months)

AUTHOR(S): Dr. E. Bomhard, Dr. W. Ramm

REPORT ISSUED: January 25, 1988

CONCLUSIONS: Terbuconazole administered in the diet (0, 20, 60, 180 pm) for 21 months produced a slight depression of body weight in male but not female mice at the HDT during the first third of the study. The major target organ is the liver in both sexes with elevations in bilirubin and liver weights in the mid and high groups associated with slight centrilobular and periportal vacuolation and lipid deposition. Mid and high dose females also had increased minimal medullary hemopoiesis and sinusoidal cellularity. In males there was an increase in adrenal cortical cell size and hyperplasia (MDT, HDT); both sexes had an elevation in stomach gastritis (HDT) while females were reported with an increase in pancreatic interstitial edema (mid, high doses). Based on these findings it appears that the HDT (180 ppm) was not high enough to approximate the MTD. A slight apparent elevation in male benign but not malignant liver tumors was reported; the combined incidences of these tumors are within the historical control range submitted from 6 studies. It is concluded that terbuconazole is not oncogenic in NMRI mice under the conditions of this bioassay.

CLASSIFICATION: CORE SUPPLEMENTARY

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A. Materials: (a photocopy of the methods is appended)

1. Test compound: HWG 1608, Description: colorless crystals, Batch # mixed, Fl.no. 132, Purity approx. 95 %, contaminants: not listed

2. Test animals: Species: mouse, Strain: Bor: NMRI (SPF Han), Age: 5-6 weeks, Weight: males, 29 gm (24-34 gm); females, 24 gm (18-31 gm), Source: Winklemann, Borchen. animals

B. Study Design:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test group	Dose in diet(ppm)	Main study 21 mos male	Main study 21 mos female	Interim sacrifice 12 mos male	Interim sacrifice 12 mos female
1 control	0	50	50	10	10
2 low(LDT)	20	50	50	10	10
3 mid(MDT)	60	50	50	10	10
4 high(HDT)	180	50	50	10	10

2. Diet preparation

Diet was prepared weekly and method of storage not stated--presumably at room temperature. Samples of treated food were analyzed for stability, concentration and homogeneity.

Results-

Test substance concentration (from Table 1, p. 77)

	nominal conc. (mg/kg)		
month/year	20	60	180
12/84	20.6	53	173
3/85	20.8	50	175
6/85	22.4	52	160
9/85	22.0	53	171
12/85	21.4	55	194
3/86	22.6	54	185
6/86	23.2	56	191
9/86	19.6	49	153
mean (rel. S.D.,%)	21.6(6)	53(4)	175(7)
mean % nominal	108	88	97

Average per cent of nominal for 20, 60 and 180 mg/kg ranged from 88 to 108% which is within acceptable variabilty for test substance concentrations.

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Homogeneity (from Table 2, p. 78)

sample no. (random nos.)	nominal conc. (mg/g) 20	180
1	23.8	---
2	23.4	169
3	---	164
4	21.2	---
5	---	160
mean	22.8	164
Maximum deviation (%) relative to mean	7	3
rel. S.D. (%)	6	3
mean in % nominal	114	91

Stability (from Table 3, p. 79)

storage period (days)	nominal conc. (mg/kg) 20	180
0	18.2	164
7	19.0	---
14*)	19.6	178
active ingredient conc. in % nominal relative to storage period marked*)	98	99

Five samples of 50 to 100 gm of the food mix were taken from a rectangular bowl from various sites (e.g., front left, front right, etc.) for 20 and 180 mg/kg nominal concentrations. As shown in the table above, the mean per cent of nominal were 114 and 91, respectively. Stability was not affected at either nominal concentration based on per cent of nominal at 14 days of storage (98, 99%, respectively). The storage was presumably at room temperature.

3. Animals received food (acclimatization: Altromin(R) 1324 pellets; study period: Altromin(R) 1321 meal, manufacturer Altromin GmbH, Lage) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

Arithmetic group means and standard deviations were calculated from individual results and for organ weights and some of medical laboratory examinations the upper and lower confidence limits were determined. Collective data were compared with control using the Mann and Whitney U Test or by Wilcoxon's method. Incidence data (mortality, clinical signs, etc.) were analyzed by Fisher's exact test.

5. Statements of data confidentiality(none claimed), adherence to GLPs and Quality Assurance inspections with signatures were included.

C. Methods and Results:

1. Observations

Animals were inspected twice daily (once on weekends and public holidays) for signs of toxicity and mortality.

Summary mortality data are presented below:

Cumulative mortality data (from Table 1, p. 37)

Dose(ppm)		0	20	60	180	0	20	60	180
sex		m	m	m	m	f	f	f	f
n		50	50	50	50	50	50	50	50
weeks:									
1-13		1	0	0	0	0	1	0	0
	%	2	0	0	0	0	2	0	0
1-26		1	0	2	0	1	2	1	0
	%	2	0	4	0	2	4	2	0
1-52		1	0	4	0	5	4	3	4
	%	2	0	8	0	10	8	6	8
1-78		5	10	13	12	18	18	13	23
	%	10	20	26	24	36	36	26	46
1-91		12	22	21	22	33	28	27	32
	%	24	44	42	44	66	56	54	64
1-93		12	22	21	22	33	29	27	32
	%	24	44	42	44	66	58	54	64

Cumulative mortality was lower in male controls for time periods beyond 52 weeks than in all dose groups receiving terbuconazole. This was stated by the authors as being statistically significant ($p<0.05$). No general differences in cumulative mortality were apparent in any of the female dose groups as compared to controls. The finding in male mice is biologically questionable since mortality among the controls is lower than expected and there is no dose-relationship.

A summary of selected clinical findings of possible toxicity are presented below. No unusual clinical findings were noted in the male or female interim dose groups (not shown in table). Findings of rough coat and poor general condition appeared to be elevated among all male dose-groups as compared to the controls but not among compound-treated females. The loss of hair appeared to be a somewhat common occurrence among all dose groups of both sexes.

Clinical findings (from pp. 124-127 of report):

Dose(ppm)	0	20	60	180	0	20	60	180
sex	m	m	m	m	f	f	f	f
n	50	50	50	50	50	50	50	50
Rough coat	12	25	20	23	23	23	21	25
Loss of hair	7	8	9	9	14	16	12	20
Poor general condition	3	16	10	17	22	22	21	21

2. Body weight

Animals were weighed before study initiation, weekly up to and including week 13, and at two-weekly intervals from week 15 until week 89. Extra body weights for calculating relative organ weights were recorded immediatedly before planned sacrifices in weeks 52 and 91/92.

A summary table of mean body weights (gm) is presented below (taken from report, pp. 137-144):

Body weights: mean(S.D.)

MALES Dose: (ppm)	wk0	wk5	wk13	wk53	wk79	wk91/92
0	29(2)	37(3)	41(4)	49(5)	48(4)	46(3)
20	29(2)	35(4)**	40(5)	49(6)	48(5)	47(4)
60	29(2)	36(3)**	40(4)	48(6)	48(5)	46(6)
180	29(2)	35(3)**	39(4)**	48(5)	47(5)	44(4)
FEMALES						
0	24(2)	26(2)	30(3)	37(4)	38(5)	39(5)
20	24(2)	27(2)	30(3)	39(4)*	40(4)	40(4)
60	24(2)	28(2)**	31(3)	38(5)	40(5)	39(4)
180	25(2)	26(2)	31(2)*	38(4)	39(3)	41(4)

There was a minimal (statistically significant in 10 of first 31 weeks on test) depression in mean body weights observed in males (primarily at the HDT) treated with the test compound as compared to the control group. No compound-related effects were apparent in females treated with terbuconazole.

3. Food consumption and compound intake

Weekly consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

Food consumption/compound intake

No apparent alterations (statistically significant) in mean food consumption (g/kg b. wt/day or g/animal/day) were noted in any dose group for either sex as compared to the respective controls.

Food consumption(from pp.101-104)

Dose: (ppm)	wk1	wk5	wk13	wk53	wk79	wk91
0						
MALES	405[a]	379	320	235	258	275
	13.0[b]	14.1	13.2	11.4	12.4	12.8
FEMALES	531	598	503	373	398	492
	13.0	15.6	14.9	13.7	15.3	19.4
20						
MALES	420	397	342	250	287	277
	13.2	13.8	13.7	12.2	13.6	12.9
FEMALES	538	577	503	382	398	372
	13.3	15.3	15.3	14.7	15.9	14.9
60						
MALES	428	380	343	252	292	284
	13.5	13.5	13.7	12.2	14.0	13.3
FEMALES	537	542	472	367	385	371
	13.2	15.0	14.6	14.0	15.3	14.4
180						
MALES	436	373	355	247	261	270
	13.5	13.1	13.8	11.8	12.3	12.3
FEMALES	551	570	498	419	408	393
	13.5	14.9	15.3	15.8	15.8	15.9

a g/kg body weight/day; b g/animal/day

Mean compound intake over the course of the study (mg/kg b.wt./day) were proportionally increased in both sexes as follows:

-males: 5.9, 18.2, 53.1 for 20, 60 and 180 ppm, respectively
-females: 9.0, 26.1, 80.5 for 20, 60 and 180 ppm, respectively

4. Opthalmological examinations

No eye examinations were performed (not required).

5. a. Hematology

Blood was collected at 12 and 21 months for hematology and clinical analysis from 10 animals/dose group of the interim sacrifice and from 10 animals/dose group randomly selected from the main study groups. The checked (X) parameters were examined:

X		X	
x	hematocrit (HCT)*	x	leukocyte differential count *
x	hemoglobin (HGB)*	x	mean corpuscular HGB (MCH)
x	leukocyte count(WBC)*	x	mean corpuscular HGB conc. (MCHC)
x	platelet count* (thrombocyte)	x	mean corpuscular volume (MCV)
	blood clotting measurements	x	reticulocyte count
	-thromboplastin time		
	-clotting time		
	-prothrombin time		

* required for subchronic and chronic studies

Hematology summary table(Table 4, pp. 48,49 of report)

Dose(ppm) (week 51)	RBC 10E12/L	HB G/L	HCT L/L	THRO 10E9/L	RETI %	SEGM %	LYM %
MALES							
0	8.30	137	0.46	1585	19	16.3	83.4
20	8.57	140	0.48	1350*	17	20.7	78.6
60	8.41	136	0.47	1351*	18	21.1	77.7
180	8.73*	140	0.49	1277**	15**	17.7	81.3
FEMALES							
0	8.30	142	0.47	840	22	13.7	84.7
20	8.07	140	0.46	838	23	10.9	88.3
60	8.42	141	0.47	854	18	13.6	84.2
180	7.88*	133**	0.45*	921	20	11.7	87.4
(week 90)							
MALES							
0	8.20	144	0.428	1478	18	20.3	77.7
20	8.55	154	0.432	1432	20	22.9	76.0
60	8.15	145	0.426	1451	18	23.9	73.6
180	7.65**	139	0.410	1445	12	35.1*	61.6*
FEMALES							
0	7.87	143	0.409	975	23	28.9	67.0
20	7.26*	135	0.390	975	21	24.8	70.1
60	7.13	131	0.393	1145	23	31.6	63.4
180	7.56	141	0.392	998	25	18.1	79.7*

Selected hematology values are presented above. There were no consistent blood changes of a compound-related nature. In males, RBCs were statistically significantly elevated at the HDT at the 51 week sampling period but significantly lower at 90 weeks. Females had statistically significantly lower RBCs, Hb and hematocrit at 51 weeks but not at 90 weeks sampling in the HDT.

In treated males, there was a statistically significant depression at all dose levels for thrombocytes as compared to controls at 51 weeks but not at 90 weeks on study. Inconsistent findings were observed for HDT males for reticulocytes, segmented neutrophils and lymphocytes at 51 or 90 weeks; HDT females had elevated lymphocyte counts at 90 weeks.

5.b. Clinical Chemistry (x indicates analyzed for)

Electrolytes:	Other:
calcium*	albumin*
chloride*	x blood creatinine*
magnesium*	x blood urea nitrogen*
phosphorus*	x cholesterol*
potassium*	globulins
sodium*	glucose*
Enzymes	x total bilirubin*
x alkaline phosphatase	xtotal serum protein*
cholinesterase#	triglycerides
creatinine phospho-kinase*@	serum protein electrophoresis
lactic acid dehydrogenase	
x serum alanine aminotransferase (also SGPT)*	
x serum aspartate aminotransferase (also SGOT)*	
gamma glutamyl transferase (GGTP)	
glutamate dehydrogenase	

* required for subchronic and chronic studies
# should be required for OP: plasma, erthrocyte ChE conducted 2X prior to study initiation, 3 and 6 mos. and prior to terminal sacrifice
@ not required for subchronic studies

Selected clinical chemistry values are presented below.

There was a generally consistent compound-related effect upon bilirubin in mid and/or high dose females. At week 53 bilirubin values in HDT females were significantly increased ($p<0.01$) and were also increased at all dose levels ($p<0.01$) as compared to the respective controls at the 92 week analysis. Cholesterol values were significantly depressed ($p<0.01$) in HDT females at 53 weeks and in the mid dose at 92 weeks ($p<0.01$) as compared to controls. Creatine values were significantly elevated at the mid and high dose levels at 92 weeks in females but not at 53 weeks. Treated males had no apparent clinical chemistry changes.

Dose(ppm) (week 53)	ALAT (SGOT)U/L	AP U/L	BILI umol/L	UREA mmol/L	CHOL mmol/L	CREAT umol/L
MALES						
0	35.4	93	3.9	10.56	4.66	41
20	30.3	95	3.8	11.13	4.38	35
60	36.4	84	3.8	9.65	4.36	36
180	45.8	107	3.9	9.75	3.61*	34
FEMALES						
0	59.1	196	2.7	8.98	3.86	39
20	32.48**	260	3.2	9.40	4.32	40
60	44.0	180	3.3	9.52	3.43	41
180	53.3	186	3.7**	9.90	2.44**	42
(week 92)						
MALES						
0	74.3	152	3.3	8.43	4.31	29
20	56.6	141	3.2	7.76	3.93	36
60	49.4	131	3.3	8.14	3.93	32
180	83.4	153	3.4	8.01	3.27	36
FEMALES						
0	72.9	168	2.2	9.45	3.57	26
20	66.0	393*	2.6*	8.35	3.53	26
60	73.8	201	3.4**	8.82	2.97*	32*
180	101.4	227	3.6**	8.77	3.46	36**

6. Urinalysis

Urines were not collected.

7. Sacrifice and pathology-
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination.

X	X
Digestive system	Cardiovascular/hematopoietic
x-tongue	x-aorta*
x-salivary glands*	x-heart*
x-esophagus*	x-bone marrow*(femur,sternum)
x-stomach*	x-lymph nodes*(mandibular, mesenteric)
x-duodenum*	x-spleen*
x-jejunum*	x-thymus*(if present)
x-ileum*	Urogenital
x-cecum*	x-kidneys*1(both)
x-colon*	x-urinary bladder*[ureter,urethra]
x-rectum*	x-testes*1(both)
x-liver*1	x-epididymides
x-gall bladder*@	x-prostate
x-pancreas*	x-seminal vesicle
Respiratory	x-ovaries*1(with oviduct)
x-trachea*	-uterus*
x-lung*	Neurologic
-nose#	x-brain*1
-pharynx#	x-peripheral nerves*@(n.ischiadicus)
[larynx#]	x-spinal cord (3 levels)*@
	x-pituitary*
	x-eyes [optic n.]*@
Glandular	[eyelids]
x-adrenals*(both)	x-extraorbital glands/Harder's gl.
-lacrimal gland*@	[head, rest]
x-mammary gland*@	x-perianal glands
-parathyroids*2	x-sternum
x-thyroids*2	x-vagina/cervix
Other	
x-bone*@ (femur)	
x-skeletal muscle*@ (thigh)	
x-skin*@	
-all gross lesions and masses*	

* required for subchronic and chronic studies
# required for chronic inhalation studies
@ in subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
1 organ weights required in subchronic and chronic studies
2 organ weights required for non-rodent studies
[ ] kept for possible future reference
___ underlined organs were weighed at interim or terminal sacrif.

Ovary weights were not determined as recommended by EPA test guidelines.

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a. Organ Weight

A summary of mean male/female organ weights (mg) is presented below (from Table 6, p. 55 of report):

WEEK 53 Dose (ppm)	BRAIN	HEART	LUNGS	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0	501/	308/	284/	2421/	163/	853/	7/	276/
	502	209	233	1817	202	504	16	---
20	478/	253**/	289/	2148/	172/	753*/	8/	258/
	507	186	218	1665	219	489	14	---
60	487/	297/	266*/	2332/	147/	843/	6/	267/
	510	204	230	1900	201	524	16	---
180	498/	269/	267/	2461/	180/	803/	9/	257/
	497	219	262	1980	212	482	17	---
WEEK 92/93 Dose (ppm)								
0	495/	288/	299/	2294/	167/	865/	9/	239/
	504	206	250	2255	250	533	13	---
20	513/	292/	296/	2281/	167/	839/	13/	221/
	504	214	286	2131	285	548	14	---
60	500/	277/	290/	2325/	178/	829/	9/	237/
	504	211	293	2284	249	539	12	---
180	500/	287/	289/	2423/	142/	858/	9/	225/
	509	221	273	2822	269	574	14	---

RELATIVE LIVER WEIGHTS (mg/100 g)

	Males	Females
WEEK 53		
0 ppm	4987	4932
20 "	4736	4750
60 "	4809	4746
180 "	5123	5260
WEEK 92/93		
0 ppm	4943	5686
20 "	4908	5308
60 "	4970	5804
180 "	5287**	6902

There was a consistent elevation in absolute (mg) and relative liver weights (mg/100 gm b.wt.) for both HDT males and HDT females at week 92 (absolute) and both at 53 and 92 weeks (relative weights; statistically significant only for relative male liver weights at week 92/93) as compared to respective controls. This is consistent with terbuconzole's liver

microsomal enzyme-inducing ability observed in the range-finding study. The net effect of this would be to increase the liver weights. No other consistent changes were observed.

b. Gross pathology

A summary of selected gross pathology findings is presented below (from Table 1, 2 of pathology report, pp. 6-22):

INTERIM SACRIFICE (males) PPM:	0	20	60	180
animal # examd	10	10	10	10
KIDNEYS				
-cyst(s)	0	0	1	2

DEAD(D)/TERMINATION(T) PPM:	0		20		60		180	
Males:	D	T	D	T	D	T	D	T
animal # examd	12	38	20	30	21	29	22	28
LUNGS								
-nodule(s)	2	15	4	13	5	10	3	5
-discolored	4	1	3	1	5	0	9	1
LIVER								
-nodules	0	1	0	2	1	2	2	3
-enlarged	0	0	0	0	2	0	1	0
Females	D	T	D	T	D	T	D	T
animal # examd	33	17	29	21	27	23	31	19
LUNGS								
-nodules	0	2	4	11	6	6	4	3
-discolored	6	0	11	0	10	2	12	0
OVARIES								
-cyst(s)	1	2	4	0	2	0	5	2

In males and females at terminal sacrifice, there was a general, treatment-related but not dose-related increase in discoloration of the lungs (e.g., females: 6/control vs 11/LDT, 12/MDT, 12/HDT). The number of lung nodules grossly observed was also generally greater in treated than control females but not males (i.e., 2/control vs 15/LDT, 12/MDT, 7/HDT). There appeared to be a slight, dose-related elevation in male but not female liver nodules (1/control vs 2/LDT, 3/MDT, 5/HDT).

c. Microscopic pathology (from Table , pp. 334-346, Table 7, 8, pp. 361-385)

1) Non-neoplastic

INTERIM SACRIFICE
Dead(D)/Interim(I)

PPM:	0		20		60		180	
MALES	D	I	D	I	D	I	D	I
animal # examd	1	9	0	10	0	10	0	10
Heart								
-min.myocard. degen.(focal)	0	0	0	0	0	0	0	1
Liver								
-min.focal peri-portal vacuola.	0	0	0	0	0	0	0	4
Liver ORO								
-min.centrilob. lipid depos.(focal)	0	1	0	2	0	3	0	0
-min.centrilob. lipid depos.	0	4	0	3	0	5	0	8
-marked centrilob. lipid depos.	0	0	0	0	0	0	0	1
-min.periportal lipid depos.(focal)	0	0	0	0	0	2	0	3
-min. periportal lipid depos.	0	0	0	0	0	0	0	4
Kidney								
-occas. dil. tub-ules, eosinoph. material	0	0	0	0	0	2	0	1
Adrenals								
-subcapsular prolif. fibrobl.-like cells	0	1	0	6	0	1	0	3
Eyes								
-few periorbital inflammatory c.	0	0	0	3	0	2	0	4
FEMALES	D	I	D	I	D	I	D	I
animal # examd	1	9	0	10	1	9	1	9
Liver								
-min. focal centri-lob. vacuol.	0	0	0	0	0	1	0	2
-min. centrilob. fine vacuol.	0	0	0	0	0	1	0	2
-mod. periportal vacuol.	0	0	0	0	0	0	0	1
-min. focal peri-portal vacuol.	0	0	0	0	0	4	0	5
-min. periportal fine vacuol.	0	0	0	0	0	1	0	0

(INTERIM SACRIFICE, CONTINUED)

	0		20		60		180	
FEMALES	D	I	D	I	D	I	D	I
animal # exam'd	1	9	0	10	1	9	1	9
Liver ORO -various degrees of centrilob./ periportal lipid depos.	0	3	0	2	0	9	0	9
Adrenals -subcapsular prolif. fibrobl.-like cells	0	6	0	10	0	7	1	7

Selected non-neoplastic lesions from interim sacrifice are presented above.

Interim sacrifice indicates the liver as a primary toxicity site with an elevation in both sexes in mid and/or high dose groups of minimal or moderate focal centrilobular vacuolation, minimum focal periportal or minimum periportal fine vacuolation and various degrees of centrilobular/periportal lipid deposition.

Terminal sacrifice histopathology again implicates the liver as a major target site for toxicity as well as possibly the heart, adrenals, pancreas, stomach and uterus in either treated males or females usually at mid or high dose levels.

In males, the most frequent hepatic alterations of a test substance induced nature were minimal focal centrilobular fine vacuolation (0/control vs 2/LDT, 5/MDT, 2/HDT), minimal to marked centrilobular fine vacuolation (0/control vs 1/LDT, 4/MDT, 13/HDT), minimal focal periportal vacuolation (0/control vs 8/HDT), and various forms of lipid deposition (centrilobular, focal, periportal) (3/control vs 4/MDT, 18/HDT). Enlarged adrenal cortical cells or minimal adrenal cortical hyperplasia were also evident in mid and high dose groups as compared to controls. Minimal stomach gastritis was also elevated in the HDT (2/control vs 12/HDT). Myocardial scarring was increased in HDT males as compared to controls (4/control vs 9/HDT).

In female livers, there was an apparent increase in moderate centrilobular vacuolation (0/control vs 2/MDT, 2/HDT), minimal diffuse vacuolation (0/control vs 7/HDT), minimal extramedullary hemopoiesis (2/control vs 5/LDT, 6/MDT, 6/HDT), increased sinusoidal cellularity (1/control vs 5/HDT) and various degrees of lipid deposition (3/control vs 6/MDT, 12/HDT). In addition to hepatoxicity, the pancreas (minimal to moderate interstitial edema), uterus (minimal to moderate cystic hyperplasia of

TERMINAL SACRIFICE: NON-NEOPLASTIC (CONTINUED)

Dead(D), Terminal(T)

PPM:	0		20		60		180	
MALES	D	T	D	T	D	T	D	T
animal # examd	12	38	20	30	21	29	22	28
Heart								
-myocardial scarring	0	4	0	1	0	2	3	6
Liver								
-necrosis	0	2	2	2	1	1	3	1
-single cell necrosis	0	0	0	0	1	0	1	0
-min. centrilob. hepat. degen./inflamm c.	0	0	0	0	1	0	0	1
-min. focal centrilob. fine vacuol.	0	0	0	2	1	4	1	1
-min. centrilob. fine vacuol.	0	0	0	1	0	3	0	8
-mod. centrilob. fine vacuol.	0	0	0	0	0	1	1	3
-marked centrilob. fine vacuol.	0	0	0	0	0	0	0	1
-min. focal centrilob. vacuol.	1	2	0	3	0	0	1	1
-min. centrilob. vacuol.	1	1	1	1	0	0	0	0
-moderate centrilob. vacuol.	0	0	0	0	1	0	0	0
-min. periportal fine vacuol.	0	0	0	0	0	0	0	1
-min. focal periportal vacuol.	0	0	0	0	1	0	3	5
-min. diffuse vacuol.	0	0	0	0	0	0	1	1
-inflamm. c./ degen. hepatoc.	0	1	0	7	0	3	3	2

endometrial glands) and stomach, also noted in males (minimal gastritis), were affected by terbuconazole at the mid and high dose levels compared to control responses.

(TERMINAL SACRIFICE, CONTINUED)
Dead(D)/Terminal(T)

PPM:	0		20		60		180	
MALES	D	T	D	T	D	T	D	T
animal # examd	12	38	20	30	21	29	22	28
Liver(continued)								
-min. focal portal fibrosis	0	0	0	0	0	0	1	0
-prominent mitosis	0	0	0	0	0	0	0	1
Liver ORO								
-min. centrilob. lipid depos.	0	3	0	1	0	4	0	9
-mod. centrilob. lipid depos.	0	0	0	0	0	0	0	3
-trace focal peri-portal lipid depos.	0	0	0	0	0	0	0	3
-min. focal peri-portal lipid depos.	0	0	0	0	0	0	0	4
Adrenals								
-min. cortical hyperplasia	0	0	0	0	2	5	2	3
-enlarged cortical cells	0	0	5	12	4	8	2	8
-min. brown degen.	0	2	2	4	2	0	3	5
Stomach								
-min. gastritis(g)	1	1	1	1	0	1	5	7
-mod. gastritis(g)	0	0	0	0	0	0	0	1
-mod. acanthosis limiting ridge(ng)	0	0	0	0	0	0	0	1
Eyes								
-partial retinal atrophy	0	0	5	2	0	4	0	2

Dead(D)/Terminal(T)

PPM:	0		20		60		180	
FEMALES	D	T	D	T	D	T	D	T
animal # examd	33	17	29	21	27	23	31	19
Heart								
-myocardial scarr.	0	0	2	0	0	2	1	1
-min. epicarditis	0	1	0	0	1	1	1	1
Liver								
-necrosis	3	0	3	3	3	1	2	0
-single cell necrosis	2	4	1	0	0	1	0	4
-min. centrilob. fine vacuol.	1	1	0	1	1	0	1	2
-min. centrilcb. vacuol.	2	0	3	1	1	0	1	1
-mod. centrilob. vacuol.	0	0	0	0	2	0	2	0
-marked centrilob. vacuol.	0	0	0	0	1	0	0	0

TERMINAL SACRIFICE, FEMALES (CONTINUED)

Dead(D)/Terminal(T) PPM:	0		20		60		180	
FEMALES	D	T	D	T	D	T	D	T
animal # examd	33	17	29	21	27	23	31	19
Liver (continued)								
-min. periportal fine vacuol.	1	0	0	0	0	0	0	0
-min. focal peri-portal vacuol.	0	0	0	0	0	1	0	2
-min. periportal vacuol.	0	0	1	0	1	0	0	1
-mod. periportal vacuol.	0	0	0	0	1	0	1	0
-min. diffuse vacuol.	0	0	0	0	0	0	4	3
-min. pleomor-phism	1	4	0	1	0	2	4	5
-min. extramed. hemopoiesis	2	0	4	1	3	3	3	3
-increased sinu-soidal cellularity	1	0	1	0	2	0	1	4
Liver ORO								
-various degrees centrilob., peri-portal, focal, dif-fuse lipid depos.	0	3	0	2	0	6	0	12
Pancreas								
-min. intersti. edema	2	0	3	0	3	3	7	1
-mod. interst. edema	0	0	0	0	0	0	2	0
Kidneys								
-min. glomerulo-nephritis	4	6	8	9	7	8	12	6
-mod. glomerulo-nephritis	2	1	2	1	5	0	6	0
-marked glomerulo-nephritis	5	0	4	0	3	0	2	0
Uterus								
-min. focal cystic hyperpl. endometrial glands	0	0	1	0	0	0	2	3
-mod. focal cystic hyperpl. endometrial glands	1	4	0	3	2	5	5	6
Stomach								
-min. gastritis(g)	1	0	6	5	2	5	2	5
Skin								
-min. subcut. edema	1	0	2	0	3	0	5	0

2) Neoplastic (from Table 5, pp. 347-360)

Dead(D)/Terminal(T)

PPM:	0		20		60		180	
MALES	D	T	D	T	D	T	D	T
animal # examd	12	38	20	30	21	29	22	28
LYMPH NODES								
-deposit of squamous c. carcinoma	0	0	0	0	0	0	0	1
LIVER								
-benign liver c. tumor	0	2	0	2	3	1	2	4
-benign liver c. tumor (two)	0	0	0	0	0	1	0	0
-malignant liver c. tumor	0	1	0	0	0	0	1	0
Combined (%)	6		4		10		14	
ADRENALS								
-pheochromocytoma	0	0	0	0	0	0	0	1
LUNGS								
-pulmonary adenoma	2	8	1	5	4	3	1	6
-pulmonary adenomata (two)	0	2	0	4	0	4	0	0
-pulmonary adenocarci-noma	1	4	2	4	3	4	4	0
-pulmonary adenocarci-noma (two)	0	0	1	1	0	0	0	0

PPM:	0		20		60		180	
FEMALES	D	T	D	T	D	T	D	T
animal # examd	33	17	29	21	27	23	31	19
LIVER								
-benign liver c. tumor	0	1	0	0	0	0	0	0
-malignant liver c. tumor	0	0	0	0	0	0	1	0
ADRENALS								
-pheochromocytoma	0	0	0	0	0	0	1	0
LUNGS								
-pulmonary adenoma	3	1	3	7	4	2	2	4
-pulmonary adenomata (two)	0	0	1	2	1	0	1	0
-pulmonary adenocarci-noma	0	0	2	3	1	2	0	1
-pulmonary adenocarci-noma (two)	0	0	0	0	0	3	1	0

.....

Historical control ranges(from Table 11, p. 65; 1980-84)

Study no.	1	2	3	4	5	6
# animals	50	50	50	45	46	48
Benign+	7	3	9	5	1	6
malignant*(%)	(14)	(6)	(18)	(11)	(2)	(12)

(*combined since different assessment criteria applied by study pathologists)

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Selected neoplastic observations are presented in tabular form above along with submitted historical control data from the registrant.

The incidence (%) of male but not female benign liver tumors was somewhat higher in the mid and high dose groups as compared to the controls or low dose groups (4%/control, 4%/low, 8%/mid, 12%/high). Malignant liver cell tumors were not elevated in treated vs control males (2%/control vs 2%/high). Combined benign + malignant liver cell tumors in control and treated groups were within the range reported by the registrant for combined historical control data from 6 studies (6-18%).

Pulmonary adenomas (one, two per animal) and adenocarcinomas were generally present in all dose groups of both sexes but there is no evidence of a compound- or dose-related effect. These and other reported tumors are considered of a incidental or age-related nature.

D. Discussion

Terbuconazole was administered to NMRI mice (both sexes) for a period of up to twenty-one months in the diet at dose levels of 0, 20, 60 and 180 ppm. There was no dose-related increase in cumulative mortality in either sex or unusual clinical signs reported. A minimal but statistically significant depression for several weeks in male, but not female, mean body weights was observed during the first third of the dosing period. No apparent compound-related changes were observed in either sex for food consumption.

Bilirubin values in mid and/or high dose females were significantly increased at 53 and 92 weeks. Both absolute and relative liver weights of both sexes were elevated at 53 and/or 92/93 weeks reflective of terbuconazole's ability to induce microsomal enzymes (P-450, N- and O-demethylase). Gross pathology suggested a general increase in male liver enlargement and the presence of liver nodules.

Interim and both terminal sacrifice non-neoplastic histopathology indicate the liver as a primary target organ, usually at the mid and high dose levels in both sexes. In males the most frequent liver lesions are vacuolation (centrilobular, periportal) and lipid deposition (centrilobular, focal, periportal); in females, hepatic vacuolation (centrilobular, minimal diffuse), minimal extramedullary hemopoiesis, sinusoidal cellularity and increased lipid depostion were noted. In males, the adrenals were affected at the MDT and HDT with an increased enlargement and hyperplasia of cortical cells and there was an

increase in minimal stomach gastritis in the high dose group. Females also had apparent elevations in the pancreatic interstitial edema and stomach gastritis at the mid or high dose levels.

Although these findings indicate that the HDT resulted in some toxicity in liver and other organs, the nature and severity of such toxicity suggest that the HDT was not high enough and thus the MTD was not achieved in this study.

While in males, but not females, there was an apparent slight elevation in benign but not malignant liver tumors at 60 and 180 ppm (4%/control vs 8%/MDT, 12%/HDT) comparison to combined benign plus malignant liver tumors from 6 studies (1980-1984) indicated that the combined incidences were within the historical control range (6-18%). A breakdown of the historical control data into benign and malignant liver tumors for NMRI male mice was not provided by the registrant, the reason being that different criteria for pathological assessment of the liver lesion were used in the 6 studies. A breakdown of the histopathology would not provide any unexpected biological relevance since combining hepatocellular adenomas and carcinomas are acceptable and the combined data are not significantly different from the historical controls.

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TEBUCONAZOLE Tox R 007200

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Pages 396 through 413 are not included.

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____ 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.

X FIFRA registration data.

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007200

Reviewed by: James N. Rowe, Ph.D., James N. Rowe 12/21/88
Review Section I
Tox. Branch: Herb. Fung. Antimicrobial Support (TS-769C)
Secondary reviewer: Quang Q. Bui, Ph.D.
Review Section I, Tox. Branch: H.F.A.S. (TS-769C) Quang Bui 12/22/88

DATA EVALUATION REPORT

STUDY TYPE: Dose-ranging rabbit teratology (83-3) TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 407009-44

TEST MATERIAL: HWG 1608 TECHNICAL; Batch No. 16002/85; colorless crystals; purity of 98.2%; stored at room temperature in dark

SYNONYMS: FOLICUR@;(terbuconazole)

STUDY NUMBER(S): report no. R4321; Lab. proj. ID no. 97400; RCC proj. no. 074068

SPONSOR: BAYER AG, Institut fuer Toxikologie Landwirtschaft, Fachbereich Toxikologie, D 5600 Wuppertal 1, FRG

TESTING FACILITY: RCC, Research & Consulting Company AG and RCC. Umweltchemie AG, CH 4452 Itingen/Switzerland

TITLE OF REPORT: Dose range-finding embryotoxicity study (including teratogenicity) study with HWG 1608 TECHNICAl in the rabbit.

AUTHOR(S): H. Becker (study director)

REPORT ISSUED: February 4, 1987

CONCLUSIONS:

Oral gavage of Chinchilla rabbits during days 6-18 of presumed gestation at 0, 30, 100 and 300 mg/kg/day produced reduced body weight gains and food consumption during the dosing period in the high and/or mid dose groups. In the high dose group the single pregnant doe had 100% implantations losses while the mid dose animals had an increase in preimplantation losses and post-implantation losses (due to increased fetal resorptions). Based upon these findings, the dosages set in the full developmental toxicity test were 10, 30 and 100 mg/kg/day.

These data are designated as Core supplementary since it is only intended as a range-finding study.

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MATERIALS AND METHODS:

Chinchilla rabbits(Kfm: CHIN, hybrids, SPF quality) were acclimatized for 7 days under test conditions after a veterinary examinination and were 18 weeks of age at delivery. Body weights upon receipt were 3000 gms(+/-500 gms). Twelve mated females, 3 per group were used. Animals were housed individually in stainless steel cages with automatic cleaning system and fed pelleted Kliba 341 rabbit maintenance diet. Water was available ad libitum.

After acclimation, the females were paired overnight with sexually mature males (1:1). After mating was observed (method of determination not stated), the female was removed and housed individually with the day designated as day 0 post coitum. Animals were assigned to the different groups by a random algorithm. Test and control females were gavaged daily (4 ml/kg) from day 6-18 post-coitum in the morning with the following dosage regimen: Group 1/0, Group 2/30, Group 3/100 and Group 4/ 300 mg/kg. Test mixtures were prepared daily.

Mortality (twice daily, minimum), clinical signs(twice daily, minimum), body weights(daily), food consumption(6,11, 15, 19, 24, 28 p.c.), postmortem examinations of dams (day 28 sacrifice), with emphasis upon the uterus, uterine contents, position of the fetuses in the uterus and number of corpora lutea and fetal examination for sexes, weights and gross external abnormalities were determined. Internal examinations of thorax, abdomen, pelvis, organs and cranii of all fetuses were performed. Uteri and contents of all pregnant females were weighed on the scheduled day of necropsy and used to determine the corrected body weight gain. If no implantation sites were evident, the uteri were placed in ammonium sulfide to accentuate possible hemorrhagic sites.

Food consumption data, body weight data and caesarean section data were recorded on-line and evaluated by computer programs. The additional data were recorded on data sheets. Body weight gain from days 0-6 p.c., 6-11 p.c., 11-15 p.c., 6-19 p.c., 19-24 p.c., 24-28 p.c. and 6-28 p.c. were calculated. Corrected body weight gain was calculated as follows: (weight on day 28 p.c.)-(weight on day 6 p.c.) - (uterus wt.). Mean food consumption/ day was calculated as the average (g) per period fed (days). Mean and standard deviations were applied when found appropriate.

RESULTS/CONCLUSIONS:

MORTALITY/CLINICAL SIGNS/NECROPSY

No deaths or adverse signs of toxicity were reported. No gross pathology were noted in any group at necropsy on day 28 p.c.

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MATERNAL BODY WEIGHTS

Mean body weight gain was depressed in the HDT as compared to the control for the treatment period of 6-19 days (+210 gm/control vs -157 gm/HDT). This was followed by a rebound in mean body weight gain for days 19-24 and 24-28 as compared to controls (e.g., days 24-28: +30 gms/control vs +104 gms/HDT). Corrected body weight gains could not be determined in the HDT since only 1/3 does was pregnant and this animal had only implantation site scars at necropsy.

MATERNAL FOOD CONSUMPTION

Mean food consumption (g/animal/day) was reduced in the mid and high dose animals as compared to controls during compound administration on days 6-19 by 14 and 50%, respectively. This was followed by rebounds in these dose groups in food consumption for days 19-24 and 24-28 (e.g., days 24-28: +80 and +82%, respectively).

REPRODUCTIVE/FETAL DATA

As mentioned above, only 1/3 does was pregnant in the high dose group and no live fetuses were observed in this animal. There was an apparent increase in mean per dam preimplantation loss in the MDT as compared to the controls (0.3, control vs 1.7/MDT). Per dam live fetuses (no dead fetuses were seen in any group) were lower in the MDT than the controls (1.0, control vs 2.5, MDT). This was due to an elevation in fetal resorptions (0/dam, control vs 1.0/dam, MDT). Mean fetal weights were not different among the available dose groups nor were fetal sex ratios.

007200

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 12/22/88
Review Section I
Tox. Branch: Herb. Fung. Antimicrobial Support (TS-769C)
Secondary reviewer: Quang Q. Bui, Ph.D.
Review Section I, Tox. Branch: H.F.A.S.(TS-769C) Quang Q Bui 12/23/88

DATA EVALUATION REPORT

STUDY TYPE: Rabbit teratology (83-3) TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 407009-45

TEST MATERIAL: HWG 1608 TECHNICAL; Batch No. 16002/85; colorless crystals; purity of 98.2%; stored at room temperature in dark

SYNONYMS: FOLICUR@;(terbuconazole)

STUDY NUMBER(S): RCC proj. no. 074070; BAYER T 0023302; 96764

SPONSOR: BAYER AG, Institut fuer Toxikologie Landwirtschaft, Fachbereich Toxikologie, D 5600 Wuppertal 1, FRG

TESTING FACILITY: RCC, Research & Consulting Company AG and RCC, Umweltchemie AG, CH 4452 Itingen/Switzerland

TITLE OF REPORT: Embryotoxicity (including teratogenicity) study with HWG 1608 TECHNICAl in the rabbit.

AUTHOR(S): H. Becker (study director)

REPORT ISSUED: February 26, 1987

CONCLUSIONS:

Oral administration of terbuconazole at 0, 10, 30 and 100 mg/kg/day during days 6-18 of gestation in the Chinchilla rabbit produced a minimal depression in mean body weight gain at the HDT associated with a decrease in food consumption. Thus, it is not apparent that any maternal toxicity was exhibited. There was an increase in postimplantation losses (both early and late resorptions), small decreases in the rate of ossification in the right and left digits or toes of the fore- and hindlimb, and frank malformations in 8 fetuses of 5 litters (peromelia, and palatoschisis, malrotation of right hindlimb, agenesis of claws) in the HDT as compared to concurrent controls or historical data. These effects are considered compound-related. Maternal toxicity NOEL is set at 30 mg/kg/day. The developmental toxicity NOEL is set at 30 mg/kg/day, the LOEL at 100 mg/kg/day.

CORE: MINIMUM. It is requested that the investigators explain the meaning of the skeletal finding stated as "various bones".

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A. MATERIALS

(A photocopy of the materials and methods section is appended).

Test compound: Purity: 98.3%
Description: colorless crystals
Lot No: 16002/85
Contaminants (list in CBI appendix): N/A

Vehicle(s): Distilled water with 0.5% Cremophor EL (BASF)

Test animals: Species: rabbit
Strain: Chinchilla rabbit (Kfm: CHIN, hybrids, SPF Quality)
Source: KFM, Kleintierfarm Madoerin AG, CH 4414 Fuellinsdorf/Switzerland
Age: 13 to 18 weeks (at pairing)
Weight: 2426-4231 (post-coitum)

B. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of terbuconazole, when administered by gavage from gestational days 6 through 18, inclusive.

Mating:

After acclimatization for 7 days, females were housed with males (1:1) overnight until mating was observed. After mating, the females were removed and caged individually. The day of mating was designated as day 0 post-coitum.

Group Arrangement:

Test group	Dose level(mg/kg)	Number assigned
Control	0	16 (1-16)
Low dose	10	16 (17-32)
Mid dose	30	16 (33-48)
High dose	100	16 (49-64)

Dosing:

All doses were in a volume of 4 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on body weights adjusted daily during the treatment period.

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Observations

The animals were checked for mortality or abnormal condition twice daily, minimum. Dams were sacrificed on day 28 of gestation. Examinations at sacrifice consisted of: gross macroscopic examination of all internal organs, with emphasis upon the uterus, uterine contents, position of the fetuses in the uterus and number of corpora lutea. The uteri (and contents) of all pregnant females were weighed at necropsy for corrected body weight gain calculations. All uteri of apparently non-pregnant females were placed in aqueous solution on ammonium sulfide to accentuate possible hemorrhagic areas of implantation sites. Liver weights were recorded.

The fetuses were examined in the following manner: the fetuses were sexed, weighed individually, examined for gross abnormalities and prepared for internal examination. Fetal body cavities (thorax, abdomen, pelvis) and the organs were examined; fetuses were sexed. Crania of all fetuses were examined for ossification. Heads were fixed in trichloroacetic acid and formaldehyde and serially sectioned and examined. Trunks were cleared in potassium hydroxide and stained with alizarin red S for skeletal examinations.

Statistical Analysis

The following statistical analysis methods were used:

Univariate one-way analysis of variance was used to assess the significance of intergroup differences if the variables could be assumed to follow a normal distribution. The Dunnett many-one t-test, based on a pooled variance estimate was used for intergroup comparisons (i.e., single treatment groups against the control group).

A one-way univariate analysis of variance based on Wilcoxon ranks together with the Kruskall-Wallis test was applied to the reproduction data parameters.

Fisher's exact test for 2x2 tables was applied if the variables could be dichotomized without loss of information.

Compliance

- A signed Statement of No Confidentiality Claim was provided.

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

- A signed Quality Assurance Statement was provided.

C. RESULTS

1. Maternal toxicity

Mortality

No deaths attributable to treatment in any dose group was reported. One female death in the HDT was reported due to intubation error (p. 21 of report).

Clinical observations

No abnormal signs of compound-related toxicity were reported.

Body weight

The investigators supplied the following data:

Body weights were recorded daily from day 0 to 28 p.c. Body weight gains from days 0-6 p.c., 6-11 p.c., 11-15 p.c.,6-19 p.c., 19-24 p.c. and 6-28 p.c. were calculated. Corrected body weight gains were calculated using the formula: body weight on day 28 p.c. - body weight on day 6 p.c. - uterus weight at necropsy on day 28 p.c. = corrected body weight.

Mean body weight gains are presented below.

Table I: Body weight gains and corrected weight (grams/%)[a]

	Prior to Dosing (d0-6)	Dosing Period (d6-18)	Post-dosing day19-24	day6-28 Gestation period	Corrected[b] BW Gain day6-28(%)
Group:					
Control	205(+7.3)	319(+10.6)	104(+3.1)	464(+15.4)	1.4
LDT	235(+8.5)	262(+8.8)	98(+3.0)	442(+14.8)	0.5
MDT	242(+8.3)	302(+9.6)	95(+2.8)	453(+14.4)	0.6
HDT	205(+6.7)	198(+6.1)	74(+2.1)	341(+10.5)	-0.3

[a] = data extracted from study project number 074070, p.55); % = percent of weight at beginning of stated period
[b] = corrected body weight gain for dosing period = body weight gain for days 6-28 minus gravid uterus weight; % of day 6 weight

Mean body weights gains were depressed somewhat in the HDT as compared to the controls during the dosing period but there was no evidence of a rebound in body weights following treatment. HDT corrected body weight gain (%) was slightly lower than the controls.

Mean organ weights(gms) and organ to body weight ratios (%) are given below in Table Ia. Neither parameter was affected in any treatment group as compared to the controls.

Table Ia: Organ weights(gm)/organ to body weight ratios(%)

Dose:	Control	LDT	MDT	HDT
Body weights(d. 28)	3470	3464	3571	3598
Liver weights	81.88	74.44	76.61	84.62
organ:b.wt.(%)	2.36	2.15	2.14	2.35

Food consumption

The investigators supplied the following data:

Mean daily food consumption was calculated using the following formula, grams of food consumed per period divided by days per period = mean daily food consumption.

Table II: Food Consumption Data (g/animal/day, %*)[a]

	Prior to Dosing Period	Day6-18 Period	Post-Dosing Period day 19-24	day 24-28
Group:				
Control	195	207	191	119
LDT	199(+2.1)	199(-3.9)	157(-17.8)	118(-0.8)
MDT	201(+3.1)	203(-1.9)	186(-2.6)	142(+19.3)
HDT	202(+3.6)	182(-12.1)	197(+3.1)	158(+32.8)

[a] Data extracted from study RCC project number 074070, p. 59
* percent of control values

Food consumption was depressed in the HDT during the dosing period, days 6-18, (-12.1 % of controls) with an apparent rebound during days 24-28 in both the mid and high dose groups.

Gross Pathological Observations

The investigators supplied the following data:

See discussion under observations.

A summary of gross necropsy findings was presented by the investigators (pgs. 95-98).

No compound-related effects were noted. One doe in the LDT had pus in the right uterine horn (#29). In the HDT one female had slight indentations on the kidney surface (both) (#57) and one doe had several dark-red foci (ca. 5 mm diameter) in the lungs (# 61).

Cesarean Section Observations:

Table III: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#animals assigned	16	16	16	16
#animals mated/inseminated	16	16	16	16
Pregnancy rate (%)	94	88	94	88
Maternal wastage				
#died	0	0	0	0
#died/pregnant	0	0	0	1*
#non pregnant	1	2	1	2
#aborted	0	0	0	0
#premature delivery	0	0	0	0
Total corpora lutea	128	125	140	132
Corpora lutea/dam	8.5	8.9	9.3	9.4
Total implantations	121	116	133	124
Implantations/dam	8.1	8.3	8.9	8.9
Total live fetuses	111	113	122	90
Live fetuses/dam	7.4	8.1	8.1	6.4
Total resorptions	10	3	11	34
Early	2	2	5	12
Late	8	1	6	22
Resorptions/dam	0.7	0.2	0.7	2.4
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Mean fetal weight(g)	35.1	33.5	35.0	33.0
Preimplantation loss(%)	5.5	7.2	5.0	6.1
Postimplantation loss(%)	8.3	2.6	8.3	27.4
Sex Ratio (% Male)	53.2	50.4	50.8	51.1

[a] = Data extracted from RCC project 074070, p. 29-31
* intubation error

Pregnancy rates ranged from 88 % to 94 % and were considered acceptable. Mean number of corporea lutea/dam and implantations/dam were not different among the dose groups. There was a reduction in live fetuses/dam observed in the high dose group as compared to the controls (7.4, control vs 6.4, HDT) which was due to an increase in total resorptions/dam (early, late) (0.7, control vs 2.4, HDT). Preimplantation losses, mean fetal weights and sex ratios were not different among the dose groups.

2. Developmental Toxicity

Table IV: External and/or head examinations

Observations[a]	Control	LDT	MDT	HDT
# pups(litters) examined	111(15)	113(14)	122(15)	90(14)
# pups(litters) affected	0	0	0	8(5)[b]
Peromelia: 1) rt. foreleg, no forepaw, 2) rt. foreleg, foreleg shortened with only stump of forepaw, 3) left foreleg, small stump present	0	0	0	5(4)
Palatoschisis	0	0	0	1(1)
Malrotation of rt. hindlimb;hydrocephalus internus (both hemispheres) with an enlarged fontanelle	0	0	0	1(1)
Agenesis of 3 claws (left hindpaw) and of 1 claw (rt. hindpaw)	0	0	0	1(1)

[a] = some observations may be grouped together
[b] = fetal (litter) incidence

An increase in frank malformations including peromelia in 5 fetuses/4 litters, palatoschisis in 1 fetus/1 litter, agenesis of the claws of the hindpaw in 1 fetus/1 litter and malrotation of the right hindlimb in 1 fetus/1 litter with hydrocephalus internus was observed in does treated with 100 mg/kg/day. No malformations were observed in any other dose group. Historical control data (control; inactive treatment) from 1984-1986 are presented below (pgs. 127-136):

	1984	1985	1986
Peromelia	0	0	0
Palatoschisis	0	0	0
Malrotation of hindlimb	0	1/860(.05%)	2/2146(.09%)
Hydrocephalus internus	1/886(.1%)	0	1/2146(.05%)
Agenesis of claws	0	0	0

Based upon the lack of similar malformations in the controls and the significantly lower incidence of malrotation of the hindlimb and hydrocephalus internus and the lack of occurrence of peromelia, palatoschisis and agenesis of the claws in the historical control data, the findings are considered compound-related.

Visceral Examinations

No abnormal findings were noted.

Skeletal Examinations

Skeletal findings are presented below in Table VI. There was a small but consistent effect of terbuconazole upon the rate of ossification as well as a more general finding simply stated as various bones in the HDT as compared to the control group. Specific fetal findings included increased nonossification of digit 5 medial phalanx (left forelimb), incomplete ossification of digit 2 proximal phalanx (right forelimb) and digits 2, 4 medial phalanx (right forelimb), and increased nonossification of left and right hindlimb (toe 4 medial phalanx).

D. DISCUSSION/CONCLUSION

a. Maternal toxicity:

Oral administration of terbuconazole at 0, 10, 30 and 100 mg/kg/day during days 6-18 of gestation in the Chinchilla rabbit produced a minimal depression in mean body weight gain at the HDT associated with a decrease in food consumption. No other significant effects were noted in regards to clinical signs, organ weights or gross necropsy findings.

b. Developmental toxicity:

The mean number of live fetuses/dam were reduced in the HDT as compared to the controls due to an increase in postimplantation losses (both early and late resorptions). Small decreases in the rate of ossification were noted in HDT fetuses in the right and left digits or toes of the fore- and hindlimb. Frank malformations in 8 fetuses of 5 litters (peromelia, palatoschisis, malrotation of right hindlimb, agenesis of claws) were noted in the HDT but not in concurrent controls or at the same frequency in historical data. These effects are considered compound-related.

c.

No significant study deficiencies were noted. However, it is unclear to the reviewer what the investigators mean when they record the skeletal finding of "various bones", and it is requested that this finding be clarified. This is not critical to a determination regarding the NOEL for developmental effects.

E. CLASSIFICATION: CORE MINIMUM DATA. 007200

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 100 mg/kg/day
Developmental Toxicity NOEL = 30 mg/kg/day
Developmental Toxicity LOEL = 100 mg/kg/day

F. RISK ASSESSMENT:

Development of MOS has been determined to be unnecessary at this time.

Skeletal Examinations

Table VI: Skeletal Examinations

Observations[a]	Control	LDT	MDT	HDT
# pups(litters) examined	111(15)	113(14)	127(15)	90(14)
VARIOUS BONES				
Total litters affected	0	1	2	4
Number of fetuses(%):	0	1	2	6
LEFT FORELIMBS (Nonossified)				
Total litters affected	14	14	15	14
Number of fetuses(%):				
Digit 5 medial phalanx	80(72)	84(74)	90(74)	78(87)
RIGHT FORELIMB (Incompletely ossified)				
Total litters affected	8	10	11	9
Number of fetuses(%):				
Digit 1 proximal phalanx	6(5)	7(6)	4(3)	13(14)
Digit 2 medial phalanx	2(2)	3(3)	2(2)	5(6)
Digit 4 medial phalanx	16(14)	19(17)	26(21)	21(23)
LEFT HINDLIMB (Nonossified)				
Total litters affected	9	10	9	10
Number of fetuses(%):				
Toe 4 Medial Phalanx	17(15)	22(19)	23(19)	36(40)
RIGHT HINDLIMB (Nonossified)				
Total litters affected	8	10	8	11
Number of fetuses(%):				
Toe 4 Medial Phalanx	16(14)	21(19)	22(18)	35(39)

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TEBUCONAZOLE Tox R 007200

Page_____ is not included in this copy.

Pages 427 through 433 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.

X FIFRA registration data.

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____ 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.

007200

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 12/21/88
Review Section I
Tox. Branch: Herb. Fung. Antimicrobial Support (TS-769C)
Secondary reviewer: Quang Q. Bui, Ph.D.
Review Section I, Tox. Branch: H.F.A.S. (TS-769C) Quang Bui 12/22/88

DATA EVALUATION REPORT

STUDY TYPE: Dose-ranging rat teratology (83-3) TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 407009-42

TEST MATERIAL: HWG 1608 TECHNICAL; Batch No. 16002/85; colorless crystals; purity of 98.2%; stored at room temperature in dark

SYNONYMS: FOLICUR@;(terbuconazole)

STUDY NUMBER(S): report no. R4322; Lab. proj. ID no. 87327; RCC proj. no. 074046

SPONSOR: BAYER AG, Institut fuer Toxikologie Landwirtschaft, Fachbereich Toxikologie, D 5600 Wuppertal 1, FRG

TESTING FACILITY: RCC, Research & Consulting Company AG and RCC, Umweltchemie AG, CH 4452 Itingen/Switzerland

TITLE OF REPORT: Dose range-finding embryotoxicity study (including teratogenicity) study with HWG 1608 TECHNICAl in the rat.

AUTHOR(S): H. Becker (study director)

REPORT ISSUED: June 1, 1987

CONCLUSIONS:

Oral administration of terbuconazole at doses of 0, 10, 30 and 90 mg/kg durings days 6-15 of gestation to rats was minimally toxic to the dams producing a slight depression in maternal body weight gain during compound administration followed by a slight body weight rebound. Maternal food consumption was depressed in a parallel fashion with a slight increase after compound withdrawal. There was an apparent doubling of resorptions (primarily fetal) in the 90 mg/kg dose group as compared to controls. Based upon these findings the dosage regimen for the full developmental toxicity test was set at 30, 60 and 120 mg/kg. This dosage regimen is scientifically supportable.

This study is designated as Core supplementary data.

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MATERIALS AND METHODS:

Wistar/HAN rats (Kfm: WIST, Outbred, SPF quality) were acclimatized for 7 days under test conditions and were 10 weeks of age (minimum) when mated. Body weights post-coitum ranged from 176-221 grams. Twenty mated females, 5 per group were used. Animals were housed individually in Makrolon cages type-3 with standardized softwood bedding and fed pelleted Kliba 343 rat/mouse maintenance diet. Tap water was available ad libitum.

After acclimation, the females were paired overnight with sexually mature males (1:1). The day on which spermatozoa were observed in the vaginal smear or a vaginal plug was observed was designated as day 0 post-coitum. Animals were assigned to the different groups by a random algorithm. Test and control females were gavaged daily (10 ml/kg) from day 5-15 post-coitum in the morning with the following dosage regimen: Group 1/ 0, Group 2/ 10, Group 3/30 and Group 4/90 mg/kg. Test mixtures were prepared daily.

Mortality, clinical signs, body weights, food consumption, postmortem examinations of dams (day 21 sacrifice), with emphasis upon the uterus, uterine contents, position of the fetuses in the uterus and number of corpora lutea and fetal examination for sexes, weights and gross external abnormalities were determined. Uteri and contents of all pregnant females were weighed on the scheduled day of necropsy and used to determine the corrected body weight gain. If no implantation sites were evident, the uteri were placed in ammonium sulfide to accentuate possible hemorrhagic sites.

Food consumption data, body weight data and caesarean section data were recorded on-line and evaluated by computer programs. The additional data were recorded on data sheets. Body weight gain from days 0-6 p.c., 6-16 p.c. and 16-21 p.c. were calculated. Corrected body weight gain was calculated as follows: (weight on day 21 p.c.) - (weight on day 6 p.c.)-(uterus wt.). Only dams with at least one live fetus were used for the calculations of corrected body weight gain. Mean food consumption/day was calculated as the average (g) per period fed (days). Mean and standard deviations were applied when found appropriate.

RESULTS/CONCLUSIONS:

MORTALITY/CLINICAL SIGNS/NECROPSY

No abnormal clinical signs and no mortality were reported. No abnormal necropsy findings at day 21 post coitum were noted.

MATERNAL BODY WEIGHTS
Mean body weights and corrected body weights for dams were similar among all dose groups. Body weight gain in the high dose group was somewhat less than the control group during days 6-11 of gestation (+8.1%/control vs. +4.9%/HDT) and for days 6-16 of gestation (+19.5/control vs. +15.5/HDT) with a slight suggestion of a rebound in body weight gain in the HDT for days 16-21 post-dosing (+18.9%/control vs +24.1%/HDT).

MATERNAL FOOD CONSUMPTION

Mean food consumption (g/animal/day) was somewhat depressed in the HDT group as compared to the control during compound treatment (21.5/control vs 20.7 (-3.7% of control)/HDT). Mean food consumption rebounded slightly in the HDT after the compound was withdrawn for days 16-21 of gestation (22.2 g/control vs 25.1 g (+13.1% of control/HDT).

REPRODUCTIVE/FETAL DATA

One of five females in the low dose group (10 mg/kg) did not become pregnant during the mating period. There were no apparent differences in mean number of corpora lutea, mean implantations or mean implantation loss per dam. No dead fetuses were reported. The percentages of males and females were similar for each dose group as were mean fetal weights. Embryonic and fetal resorptions (% of implantations) were approximately doubled in the HDT group as compared to the controls (6.2%/control vs 12.1%/HDT). This was primarily due to an increase in mean fetal resorptions (1.5%/control vs 6.1%/HDT).

In summary, oral administration of terbuconazole at doses of 0, 10, 30 and 90 mg/kg durings days 6-15 of gestation to rats was minimally toxic to the dams producing a slight depression in maternal body weight gain during compound administration followed by a slight body weight rebound. Maternal food consumption was depressed in a parallel fashion with a slight increase after compound withdrawal. There was an apparent doubling of resorptions (primarily fetal) in the 90 mg/kg dose group as compared to controls. Based upon these findings the dosage regimen for the full developmental toxicity test was set at 30, 60 and 120 mg/kg.

The dosing selection for the primary study is scientifically supportable.

007200

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 11/14/88
Review Section I
Toxicology Branch: Herb. Fung. Antimicrobial Support (TS-769C)
Secondary Reviewer: Quang Q. Bui, Ph.D. Quang Q Bui 12/5/88
Review Section I, Tox. Branch: H.F.A.S. (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity Guideline Section 83-3 TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 407009-43

TEST MATERIAL: HWG 1608 Technical

SYNONYMS: Folicur@; terbuconazole

STUDY NUMBER: 074057; Lab. Proj. ID No. 96756; BAYER: T 9023301

SPONSOR: BAYER AG, Institut fuer Toxikologie Landwirtschaft, Fachbereich Toxikologie, D-5600 Wuppertal, FRG

TESTING FACILITY: RCC, Research & Consulting Company AG, and RCC, Umweltchemie AG, CH 4452 Itingen/Switzerland

TITLE OF REPORT: Embryotoxicity study (including teratogenicity) with HWG 1608 TECHNICAL in the rat

AUTHOR(S): H. Becker (study director)

REPORT ISSUED: April 28, 1988

CONCLUSIONS:

Oral administration of terbuconazole at 0, 30, 60 and 120 mg/kg/day during days 6-15 of gestation in Wistar rats produced slight maternal toxicity as evidenced by a small depression in mean body weight associated with depressed food consumption. Mean liver weights and liver to body weight ratios were statistically significantly elevated in the mid and high dose groups. The maternal NOEL is set at 30 mg/kg/day.

Developmental toxicity was evidenced at both the mid and high dose groups by delays in ossification of thoracic, cervical and sacral vertebrae, the sternum and fore- and hind limbs along with an increase in supernumerary ribs. Frank malformations were observed in two fetuses of two high dose dams as missing tail, agnatha, microstomia and anophthalmia. The developmental NOEL is set at 30 mg/kg/day.

CLASSIFICATION: CORE MINIMUM

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A. MATERIALS

(A photocopy of the materials and methods section is appended).

Test compound: Purity: 98.3%
Description: colorless crystals
Lot No: 16002/85
Contaminants (list in CBI appendix): N/A

Vehicle(s): Distilled water with 0.5% Cremophor EL (BASF)

Test animals: Species: rat
Strain: Wistar/HAN (Kfm: WIST, Outbred, SPF quality)
Source: KFM, Kleintierfarm Madoerin AG, CH 4414 Fuellinsdorf/Switzerland
Age: 12 weeks, minimum (at pairing)
Weight: 180-235 gms

B. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of terbuconazole, when administered by gavage from gestational days 6 through 15, inclusive.

Mating:

After acclimitization for 7 days, females were housed with males (1:1) overnight until either the daily vaginal smear was sperm-positive or a copulatory plug was observed. The day of mating was designated as day 0 post-coitum.

Group Arrangement:

Test group	Dose level(mg/kg)	Number assigned
Control	0	25 (1-25)
Low dose	30	25 (26-50)
Mid dose	60	25 (51-75)
High dose	120	25 (76-100)

Dosing:

All doses were in a volume of 10 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on body weights adjusted daily during the treatment period.

Observations

The animals were checked for mortality or abnormal condition twice daily, minimum. Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of: gross macroscopic examination of all internal organs, with emphasis upon the uterus, uterine contents, position of the fetuses in the uterus and number of corpora lutea. The uteri (and contents) of all females with live fetuses were weighed at necropsy for corrected body weight gain calculations. All uteri of apparently non-pregnant females were placed in aqueous solution on ammonium sulfide to accentuate possible hemorrhagic areas of implantation sites.

The fetuses were examined in the following manner: the fetuses were sexed, weighed individually, examined for gross abnormalities. One half of the fetuses from each litter were examined for visceral and brain abnormalies using Wilson's slicing technique and the rest were cleared in potassium hydroxide and stained with alizarin red S for skeletal examinations.

Statistical Analysis

The following statistical analysis methods were used:

Univariate one-way analysis of variance was used to assess the significance of intergroup differences if the variables could be assumed to follow a normal distribution. The Dunnett many-one-t-test, based on a pooled variance estimate was used for intergroup comparisons (i.e., single treatment groups against the control group).

A one-way univariate analysis of variance based on Wilcoxon ranks together with the Kruskall-Wallis test was applied to the reproduction data parameters.

Fisher's exact test for 2x2 tables was applied if the variables could be dichotomized without loss of information.

Compliance

- A signed Statement of No Confidentiality Claim was provided.

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

- A signed Quality Assurance Statement was provided.

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C. RESULTS

1. Maternal toxicity

Mortality

No deaths in any dose group was reported.

Clinical observations

No abnormal signs of compound-related toxicity were reported.

Body weight

The investigators supplied the following data:

Body weights were recorded daily from day 0 to 21 p.c. Body weight gains from days 0-6 p.c., 6-11 p.c., 11-16 p.c.,6-16 p.c., 16-21 p.c. and 6-21 p.c. were calculated. Corrected body weight gains were calculated using the formula: body weight on day 21 p.c. - body weight on day 6 p.c. - uterus weight at necropsy on day 21 p.c. = corrected body weight.

Mean body weights were significantly depressed in the HDT as compared to the control group by day 21 (303 gm/HDT vs 320 gm/con.)(see Table Ia). The body weight gains in the HDT dams were somewhat lower during the dosing period or days 6-21 of gestation but there was no indication of a weight rebound in the post-dosing period nor were the corrected body weights significantly different (see Table I).

Table I: Body weight gains and corrected weight (grams/%)[a]

	Prior to Dosing Period	Dosing Period	Post-dosing period	day6-21 Gestation Period	Corrected[b] BW Gain day6-21(%)
Group:					
Control	19(+9.3)	41(+18.3)	55(+20.8)	96(+42.9)	+9.2
LDT	20(+10.0)	40(+18.1)	53(+20.3)	93(+42.1)	+9.8
MDT	20(+9.7)	35(+15.4)	60(+22.9)	95(+41.9)	+9.6
HDT	20(10.0)	29(+13.1)	53(+21.2)	82(+37.1)	+10.0

[a] = data extracted from study project number 074057, p.55)
[b] = corrected body weight gain for dosing period = body weight gain for days6-21 minus gravid uterus weight

Mean liver weights and mean liver to body weight ratios were statistically significantly increased in the MDT and HDT dose groups as compared to the controls (see Table Ia) suggesting a possible compound-related toxicological/pharmacological effect.

Table Ia: Organ weights(gm)/organ to body weight ratios(%)

Dose:	Control	LDT	MDT	HDT
Body weights(d. 21)	320	314	322	303*
Liver weights	11.51	11.55	12.50*	12.49*
organ:b.wt.	3.59	3.68	3.89**	4.12**

*/** Dunnett-Test; significant at 0.05 and 0.01 level, resp.

Food consumption

The investigators supplied the following data:

Mean daily food consumption was calculated using the following formula, grams of food consumed per period divided by days per period = mean daily food consumption.

Table II: Food Consumption Data (g/animal/day, %*)[a]

	Prior to Dosing Period	Day6-15 Period	Post-Dosing Period
Group:			
Control	20.6	21.4	23.0
LDT	20.1(-2.4)	20.9(-2.3)	23.3(+1.3)
MDT	20.5(-0.5)	19.9(-7.0)	24.2(+5.2)
HDT	20.8(+1.0)	18.2(-15.0)	24.3(+5.7)

[a] Data extracted from study RCC project number 074057, p. 41
* percent of control values

Mean daily food consumption was decreased but not statistically significant during days 6-15 of the test period with a small increase (not statistically significant) post-dosing.

Gross Pathological Observations

The investigators supplied the following data:

See discussion under observations.

A summary of gross necropsy findings was presented by the investigators (pgs. 102-105). One non-pregnant female of the MDT dose group had dilation of the right renal pelvis with a 2mm urinary bladder stone associated with 2 mm dark red foci in the mucous membrane of urinary bladder. A consistent finding in 9/24 HDT dams was the uterus filled with black/brown colored fluid. This probably is related to the increased resorption of embryos/fetuses (one to several) which was observed in all of these dams.

Cesarean Section Observations:

Table III: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#animals assigned	25	25	25	25
#animals mated/inseminated	25	25	25	25
Pregnancy rate (%)	96	96	88	96
Maternal wastage				
#died	0	0	0	0
#died/pregnant	0	0	0	0
#non pregnant	1	1	3	1
#aborted	0	0	0	0
#premature delivery	0	0	0	0
Total corpora lutea	357	347	314	370
Corpora lutea/dam	14.9	14.5	14.3	15.4
Total implantations	302	291	277	298
Implantations/dam	12.6	12.1	12.6	12.4
Total live fetuses	288	271	256	232
Live fetuses/dam	12.0	11.3	11.6	9.7
Total resorptions	14	20	21	56
Early	14	20	19	45
Late	0	0	2	21
Resorptions/dam	0.6	0.8	1.0	2.8
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Mean fetal weight(g)	4.7	4.7	4.6	4.2
Preimplantation loss(%)	15.4	16.1	11.8	19.5
Postimplantation loss(%)	4.6	6.9	7.6	22.1
Sex Ratio (% Male)	48.3	47.2	50.8	50.4

[a] = Data extracted from RCC project 074057, p. 28

Pregnancy rates ranged from 88%(MDT) to 96% (remaining dose groups). No abortions or premature deliveries were noted. Mean corpora lutea counts or mean implantations were not significantly different among the treated groups as compared to the controls. Mean live fetuses/dam were decreased in the HDT group as compared to the controls and lower dose groups. This was due to an increase in both early (embryonic) and late (fetal) embryonic resorptions (i.e., total resorptions: 0.6/dam, control vs 2.8/dam, HDT).

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Mean fetal weight was somewhat (not statistically significant) reduced in the HDT as compare with the controls while sex ratios (% males) were not different among the dose groups. The fetal weight depression may be treatment-related since a decrease in mean fetal weight was still observed despite a reduction in litter size in the HDT.

2. Developmental Toxicity

Table IV: External examinations

Observations[a]	Control	LDT	MDT	HDT
# pups(litters) examined	288(24)	271(24)	256(22)	232(24)
# pups(litters) affected	0	0	0	2(2)
Missing tail	0	0	0	0.4(4.2)[b]
Agnatha(lower jaw), microstomia, anophthalmia	0	0	0	0.4(4.2)

a = some observations may be group together
b = fetal (litter) incidence

External examinations revealed the presence of 2 fetuses in 2 dams with either missing tail or a combination of defects (agnatha, microstomia and anopthalmia). Based upon other developmental effects noted in the HDT, without comparable findings in the controls, these may be compound-related malformations.

Visceral findings (presented below in Table V) indicate findings of excess fluid in the thoracic cavity, primarily in the HDT group (4 fetuses of 2 litters). This may also be considered as a compound-related effect.

Table VI below presents the skeletal findings. There was an increase in the number of fetuses (statistically significant) with nonossified or incompletely ossified bones of the thoracic vertebrae, cervical vertebrae, sacral vertebrae, sternum, forelimbs and hind limbs and an increase in supernumerary ribs. These were primarily observed in the HDT. Skeletal effects observed in both the MDT and HDT were nonossified cervical verterbra 2, vertebral arch 6 (right), digit 1 distal phalanx (left), digit 3 proximal phalanx (left), digit 2 proximal phalanx (right), digit 3 proximal phalanx (right), digit 4 proximal phalanx (right) and toe 5 distal phalanx (right).

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Visceral Examinations

Table V: Visceral Examinations

Observations[a]	Control	LDT	MDT	HDT
# pups(litters) examined	144(24)	134(24)	129(22)	116(24)
Excess fluid in thoracic cavity	0	0.7(4.2)[b]	0	3.4(8.3)
Missing tail	0	0	0	0.9(4.2)
Agnatha(lower jaw), microstomia, anophthalmia	0	0	0	0.9(4.2)

[a] = some observations may be grouped together
[b] = fetal (litter) incidence

D. DISCUSSION/CONCLUSION

a. Maternal toxicity:

Oral administration of terbuconazole during days 6-15 of gestation in the female Wistar rat produced a small (5%) but statistically significant depression in body weights at the HDT associated with increased liver weights and liver to body weight ratios (both MDT and HDT). The depressed body weights were not clearly associated with an effect, i.e., fetal alterations, upon the dams using corrected body weight gains. Mean food consumption was slightly depressed during compound administration followed by a small post-dosing rebound.

The HDT dams had 9/24 uteri filled with black/brown colored fluid, probably blood and associated with the increased resorptions noted in this dose group.

b. Developmental toxicity:

Terbuconazole produced an increased in postimplantation loss in the form of both embryonic and fetal resorptions in the 120 mg/kg dose group. Statistically significant increases in nonossified or incompletely ossified bones of the thoracic, cervical and sacral verterbrae, sternum, fore- and hind limbs along with increases in supernumberary ribs were observed in the mid and high dose groups. Two separate fetuses of two HDT litters had major malformations of missing tail and agnatha, microstomia and anophthalmia at the HDT.

c.

No significant study deficiencies were noted.

Skeletal Examinations

Table VI: Skeletal Examinations

Observations[a]	Control	LDT	MDT	HDT
# pups(litters) examined	144(24)	137(24)	127(22)	116(24)
THORACIC VERTEBRAE				
centrum dumbbell shaped (10-13th)	2(2)#	2(2)	2(2)	6(4)
centrum bipartite (10-12th)	0	0	0	4(2)
CERVICAL VERTEBRAE (Nonossified) Total litters affected	18	19	18	23
Number of fetuses(%):				
Cervical vertebra 1	18(3)	21(15)	24(19)	48(41)**
Cervical vertebra 2	29(20)	40(29)	38(30)*	48(41)**
Cervical vertebra 3	9(6)	10(7)	12((9)	16(14)*
Cervical vertebra 4	0	5(4)*	2(2)	13(11)**
Cervical vertebra 5	3(2)	3(2)	3(2)	10(9)*
Cervical vertebra 6	1(1)	2(1)	2(2)	6(5)*
SACRAL VERTEBRAE (Nonossified) Total litters affected	18	18	15	21
Number of fetuses(%):				
Vertebral arch 6, left	1(1)	2(1)	3(2)	14(12)**
Vertebral arch 6, right	0	2(1)	6(5)**	13(11)**
Vertebral arch 7, left	50(35)	53(39)	49(39)	66(57)**
Vertebral arch 7, right	45(31)	53(39)	51(40)	65(56)**
STERNUM (Incompletely ossified) Total litters affected	18	15	15	19
Number of fetuses(%):				
Sternebra 2	4(3)	3(2)	3(2)	15(13)**
Sternebra 6	0	0	1(1)	4(3)*

(continued on next page)

Table VI: Skeletal Examinations[10] (continued from previous page)

Observations[a]	Control	LDT	MDT	HDT
# pups(litters) examined	144(24)	137(24)	127(22)	116(24)
SUPERNUMERARY RIBS (One, left or right) Total litters affected	9	11	13	12
Number of fetuses(%):				
Ribs, left	14(10)	20(15)	18(14)	26(22)**
Ribs, right	15(10)	23(17)	19(15)	24(21)*
FORELIMBS, LEFT OR RIGHT (Nonossified) Total litters affected	23	20	20	19
Number of fetuses(%):				
Digit 1 distal phalanx(l)	27(19)	36(26)	37(29)*	42(36)**
Digit 3 proximal phalanx(l)	0	3(2)	5(4)*	9(8)**
Digit 4 proximal phalanx(l)	2(1)	7(5)	7(6)	11(9)**
Metacarpal 5(l)	0	0	2(2)	4(3)*
Digit 5 distal phalanx(l)	55(38)	56(41)	55(43)	23(20)**
Digit 2 proximal phalanx(r)	27(19)	33(24)	39(31)*	40(34)**
Digit 3 proximal phalanx(r)	0	3(2)	4(3)*	8(7)**
Digit 4 proximal phalanx(r)	1(1)	6(4)	6(5)*	11(9)**
Metacarpal 5(r)	0	0	2(2)	5(4)*
Digit 5 distal phalanx(r)	45(31)	56(41)	47(37)	21(18)*
HIND LIMB, LEFT OR RIGHT (Nonossified) Total litters affected	24	24	22	24
Number of fetuses(%):				
Metatarsal 1(l)	18(13)	24(18)	18(14)	31(27)**
Toe 2 proximal phalanx(l)	107(74)	92(67)	96(76)	103(89)**
Toe 3 proximal phalanx(l)	81(56)	70(51)	78(61)	87(75)**
Toe 4 proximal phalanx(l)	79(55)	65(47)	72(57)	85(73)**
Toe 5 distal phalanx(l)	1(1)	9(7)**	5(4)	7(6)*
Metatarsal 1(r)	18(13)	24(18)	20(16)	32(28)**
Toe 2 proximal phalanx(r)	110(76)	96(70)	105(83)	105(91)**
Toe 3 proximal phalanx(r)	86(60)	75(55)	76(60)	92(79)**
Toe 4 proximal phalanx(r)	81(56)	71(52)	74(58)	91(78)**
Toe 5 distal phalanx(r)	1(1)	9(7)**	6(5)*	7(6)*

fetal #(litter #)
*, ** = Fisher's exact test; statistically significant (p<0.05 or 0.01)

E. CLASSIFICATION: CORE MINIMUM DATA.

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 60 mg/kg/day
Developmental Toxicity NOEL = 30 mg/kg/day
Developmental Toxicity LOEL = 60 mg/kg/day

F. RISK ASSESSMENT:

Development of MOS has been determined to be unnecessary at this time.

TEBUCONAZOLE Tox R 007200

Page_____ is not included in this copy.

Pages 448 through 454 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.

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____ Identity of the source of product ingredients.

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____ A draft product label.

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007200

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 12/22/88
Review Section I
Toxicology Branch: Herb. Fung. Antimicroial Support (TS-769C)
Secondary Reviewer: Quang Q. Bui, Ph.D.
Review Section I, Tox. Branch: H.F.A.S. (TS-769C) Quang Q Bui 12/23/88

DATA EVALUATION REPORT

STUDY TYPE: Special study: maternal toxicity; Guideline Section N/A

TOX. CHEM. NO.: 463P

ACCESSION NUMBER:

MRID NO.: 408215-00

TEST MATERIAL: HWG 1608 Technical

SYNONYMS: Folicur@; terbuconazole

STUDY NUMBER: Bayer report no. 16511; T5025712; Lab. Proj. no. 97411

SPONSOR: Mobay Corporation, Agricultural Chemicals Division

TESTING FACILITY: BAYER AG, Fachbereich Toxikologie, Institute of Toxicology/Agriculture Friedrich-Ebert-Strasse 217-333, D-5600 Wuppertal 1, FRG

TITLE OF REPORT: HWG 1608, Supplementary study of maternal toxicity to mice after oral administration

AUTHOR(S): Dr. M. Renhof and Dr. E. Karbe

REPORT ISSUED: March 9, 1988

CONCLUSIONS: It is tentatively concluded that maternal toxicity/physiological alterations (liver enzyme induction) is demonstrated at all dose levels (10-100 mg/kg) orally administered to female mice during days 6-15 of presumed gestation. This "toxicity" was primarily demonstrated as elevations in serum AST, ALP and AP associated with increased liver weights with hepatic changes including increased mitosis, vacuolation and lipidosis. A compound-related effect upon mean corpuscular volume reflected in a reduction in hematocrit at dose levels of 20-100 mg/kg was also noted. The limited number of animals tested plus the presence in the various test groups of pregnant and non-pregnant mice makes these findings suggestive but not definitive. A maternal toxicity NOEL, based upon the reduction in hematocrit, is set at 10 mg/kg/day. This study necessitates revising the maternal NOEL in the full mouse teratology study (T5021859) to reflect the maternal toxicity NOEL.

CLASSIFICATION: ACCEPTABLE

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A. MATERIALS

(A photocopy of the materials and methods section is appended).

Test compound: Purity: 97.4%
Description: gray-white, powdery crystals
Lot No: 16012/86
Contaminants (list in CBI appendix): N/A

Vehicle(s): Distilled water with 0.5% Cremophor EL (BASF)

Test animals: Species: mice
Strain: NMRI/ORIG Kisslegg
Source: SAVO-Ivanovas GmbH, 7964 Kisslegg, FRG
Age: males, sexually mature; females, sexually mature (nulliparous)
Weight: Males, > or = 35 gms; Females, 24-40 gms

B. STUDY DESIGN

This study was designed to further assess the maternal toxicity potential of terbuconazole, when administered by gavage from gestational days 6 through 15, inclusive.

Mating:

After acclimitization for at least 6 days, 2-3 females were housed with one male for approximately 4 hours during the day. If a vaginal plug was found, this day was designated Gestation day 0.

Group Arrangement: (males were not treated)

Test group	Dose level (mg/kg) (% conc.)		Number assigned
Control	0	(0)	10
Low dose	10	(0.2)	10
Mid 1	20	(0.4)	10
Mid 2	30	(0.6)	10
High dose	100	(2.0)	10

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for stability and homogeneity. Dosing was based on body weights adjusted daily during the treatment period and was performed each day between 8 a.m. and noon.

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Observations

The animals were checked for mortality or abnormal condition daily. Body weights were determined during the treatment period and the entire gestation period. Dams were sacrificed on day 16 postcoitus. One-half of mice internal organs (selected randomly) were grossly examined and the weights of the liver, spleen, kidneys and adrenal were determined. Blood samples were obtained from 5 anesthetized mice/dose group and the following measurements made: aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, GLDH, bilirubin, creatinine, urea, total protein, triglycerides, cholesterol, hematocrit, hemoglobin, erythrocytes, mean corpuscular hemoglobin, leukocytes, MCV, MCHC, and thrombocytes. The livers were fixed in formaldehyde for histopathology.

Statistical Analysis

The following statistical analysis methods were used for clinical chemistry, hematology and liver weights:

0 t-test (method of Welch)

Statistical signficance was set at a probability error of 5%.

Compliance

- A signed Statement of No Confidentiality Claim was provided.

- A signed Statement of Compliance with OECD GLP's was provided.

- A signed Quality Assurance Statement was provided.

C. RESULTS

1. Clinical signs/mortality

Mortality

No deaths in any dose group were reported.

Clinical observations

No abnormal signs of compound-related toxicity were reported except for one dam which experienced wheezing at day 8 p.c. (LDT).

2. Body weight

The investigators supplied the following data: individual body weights for day 0, 6-15 and mean body weight gains for days 6-15. Mean body weights during dosing and gestation are presented below:

Table I: Mean body weights/ b. wt gains (grams)[a]
(pregnant animals only)

Group:	d6	d10	d15	d6-15	# pregnant
Control	35.2	38.4	47.2	12.0	5
LDT	34.7	37.3	45.6	10.9	7
MD1	35.3	37.3	44.2	8.9	6
MD2	35.4	39.2	50.2	14.8	5
HDT	33.1	35.0	41.3	8.2	5

[a] = data extracted from pp.61 and 70-74 of report

There was no evidence of a consistent compound-related effect in the treated dams during the dosing period.

3. Organ weights

Absolute and relative liver weights (gm, mg/gm) are presented below: (includes pregnant + nonpregnant)

Group:	absolute	relative	# pregnant
Control	1988.2(549.3)[a]	49.7	2
LDT	2430.4(506.0)	52.3	4
MD1	2368.8(738.3)	55.1	3
MD2	2547.4(798.6)	55.38	3
HDT	2379.2(342.3)	63.25	3

[a]mean(S.D.); data extracted from p. 75

The absolute liver weights were consistently elevated in treated animals as compared to the controls. This was also reflected in the relative liver weights particularly at the HDT.

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4. Clinical chemistry/hematology

Selected clinical chemistry, liver homogenate triglyceride and hematology values are presented below:

CLINICAL CHEMISTRY/LIVER HOMOGENATE (from pp.63, 65)

Dose(mg/kg)	AST(U/L)	ALT(U/L)	AP(U/L)	CHOL(MMOL/L)	LIVER TG(UMOL/G) #P
Control	262.7	46.8	98.8	1.61	11.61 (3)
(3)	(278.2)[A]	(13.7)	(58.3)	(0.20)	(0.77)
10	534.6	72.2*	61.5	2.21	10.30 (3)
(3)	(448.0)	(18.5)	(50.1)	(0.58)	(1.15)
20	371.1	59.6	106.0	2.56**	12.53 (3)
(3)	(192.2)	(15.0)	(49.7)	(0.68)	(2.27)
30	562.3*	67.3*	110.0	2.06	11.21 (3)
(2)	(168.2)	(18.5)	(7.2)	(0.56)	(0.61)
100	339.5	60.7	113.8	1.40	30.60*(5)
(5)	(148.1)	(13.7)	(35.9)	(0.10)	(12.57)

HEMATOLOGY (from p. 64)

Dose(mg/kg)	HEMATOCRIT(L/L)	MCV(fl)
Control(3)	0.451(0.0125)	54.4(0.89)
10 (3)	0.453(0.0391)	52.6(3.21)
20 (2)	0.432(0.0251)	50.6(3.29)*
30 (2)	0.418(0.020)***	52.2(1.92)*
100 (5)	0.425(0.0135)***	51.6(1.34)#

[A] mean (S.D.); *, **, ***, # : $p<0.05$, $p<0.025$, $p<0.01$, $p<0.005$, respectively; ( ) = number pregnant/group of five

There was a generally consistent, often statistically significant, increase in the liver enzymes, AST and ALT, in all compound-treated groups. Non-statistically significant elevations in 20, 30 and 100 mg/kg dose groups of AP, another liver-related enzyme, were also noted. Cholesterol concentrations in the serum were statistically signficantly elevated at 20 mg/kg as compared to controls. Analysis of liver homogenate triglycerides concentrations indicated a statistically significant increase at the HDT as compared to controls.

Hematocrits were significantly lowered in the 20-100 mg/kg dose groups parallel to a significant depression in mean corpuscular volume as compared to the respective controls.

Variabilities in these clinical and hematological parameters relates to the limited number of samples analyzed as well as to the observation of considerable difference in the physiological state of the female mice (pregnancy state).

5. Gross pathology/histopathology

Upon necropsy the liver was reported as a potential target organ based upon its pale, lobular pattern as evident below:

Finding	control	10"	20"	30"	100 mg/kg
-pale liver	1/5	0/5	0/5	1/5	5/5*
-lobular pattern, liver	0/5	1/5	0/5	1/5	3/5

* one animal had putty colored liver; data from p. 69

The histopathological state of treated animal livers supports the clinical chemisty findings and gross pathology (see table below). Increased mitosis was noted in the low and mid dose groups with the high dose group having a significant increase in vacuolation in all animals (mild to severe in nature) as compared to no such findings in the control livers. The frequency and severity of lipid deposition was also increased in all animals receiving terbuconazole as compared to controls with all HDT animals having moderate to severe lipidosis. These findings are indicative of disruption of the normal metabolic state of the hepatocytes.

Histopathology (data from Table 1, p. 79)

Finding	control	10"	20"	30"	100 mg/kg
-focal necrosis	1/5(3)*	1/5(1)	0/5	0/5	0/5
-cellular infiltrates	1/5(1)	1/5(1)	1/5(2)	1/5(1)	2/5(1)
-increased mitosis	0/5	2/5(1)	1/5(1)	3/5(1)	0/5
-vacuoles	0/5	0/5	0/5	0/5	5/5(2,2,3,3,4)
-lipidosis-ORO stain	2/5(1)	3/5(1)	5/5(1,1,1,1,2)	4/5 (1,1,2,2)	5/5(3,3,3,3,4)

* grade: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

D. CONCLUSIONS

Further evaluation of the maternal toxicity of terbuconazole was performed in NMRI female mice orally gavaged with 0, 10, 20, 30 and 100 mg/kg during days 6-15 of presumed gestation. No consistent alteration in body weight was evident, although the small sample size and the presence of both pregnant and nonpregnant mice makes this finding tentative. The liver was a primary target organ with 1) elevations in AST(SGOT), ALT(SGPT) and AP at all treatment doses associated with a consistent increase in absolute and relative liver weights, 2) an increase in pale, lobular appearance in HDT animals, 3) increased presence of mitosis, vacuolation and lipidosis generally extending through all animals receiving terbuconazole. A significant decrease in hematocrit values from 20-100 mg/kg was noted and was related to a diminished RBC volume. These findings tentatively support the contention of the registrant that maternal toxicity existed in pregnant dams previously tested for developmental toxicity at 10, 30 and 100 mg/kg (Study # T5021859) and in which there was no overt evidence of toxicity based upon body weights, clinical signs and lack of mortality.

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Pages 462 through 466 are not included.

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____ Identity of product inert ingredients.

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007200

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 12/22/88
Review Section I
Toxicology Branch: Herb. Fung. Antimicroial Support (TS-769C)
Secondary Reviewer: Quang Q. Bui, Ph.D.
Review Section I, Tox. Branch: H.F.A.S. (TS-769C) Quang Bui 12/23/88

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity Guideline Section 83-3 TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 408215-00

TEST MATERIAL: HWG 1608 Technical

SYNONYMS: Folicur@; terbuconazole

STUDY NUMBER: Bayer report no. 16527; T5021859; Lab. Proj. no. 97411

SPONSOR: Mobay Corporation, Agricultural Chemicals Division
TESTING FACILITY: BAYER AG, Fachbereich Toxikologie, Institute of Toxicology/Agriculture Friedrich-Ebert-Strasse 217-333, D-5600 Wuppertal 1, FRG

TITLE OF REPORT: HWG 1608, Study of embryotoxic effects on mice after oral administration

AUTHOR(S): Dr. M. Renhof

REPORT ISSUED: March 14, 1988

CONCLUSIONS: Oral gavage of terbuconazole at 0, 10, 30, 100 mg/kg to mice during days 6-15 of gestation did not produce any overt signs of maternal toxicity. However, results from an associated study (T5025712; 97411) tentatively indicated hepatic changes at all dose levels tested (increased release of AST, ALT, AP associated with liver weight increases and altered metabolic/physiology-increased mitosis, vacuolation and lipidosis); as well as a reduction in hematocrit at dose levels of 20-100 mg/kg/day. Developmental toxicity was noted at the mid and high dose levels in the form of retarded growth, increased numbers of runts (fetuses weighing less than 1.3 gm). In addition, the compound produced frank malformations (skull, "neural tube") at the HDT associated with a reduced rate of ossification in the cranium as compared to controls. The maternal toxicity NOEL is set at 10 mg/kg/day and the LOEL is set at 20 mg/kg/day (reduction in hematocrit). The developmental NOEL, based upon increase number of runts, is 10 mg/kg (LDT) and the LOEL is 30 mg/kg.

CLASSIFICATION: CORE MINIMUM

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A. MATERIALS

(A photocopy of the materials and methods section is appended).

Test compound: Purity: 93.6%
Description: colorless crystals
Lot No: 1616002/84
Contaminants (list in CBI appendix): N/A

Vehicle(s): Distilled water with 0.5% Cremophor EL (BASF)

Test animals: Species: mice
Strain: NMRI/ORIG Kisslegg
Source: IVANOVAS
Age: males, sexually mature; females, sexually mature (nulliparous)
Weight: Males, > or = 25 gms; Females, 28-39 gms

B. STUDY DESIGN

This study was designed to assess the developmental toxicity potential of terbuconazole, when administered by gavage from gestational days 6 through 15, inclusive.

Mating:

After acclimatization for at least 6 days, 2-3 females were housed with one male for approximately 4 hours during the day. If a vaginal plug was found, this day was designated Gestation day 0.

Group Arrangement:

Test group	Dose level(mg/kg)	Number assigned
Control	0	25
Low dose	10	25
Mid dose	30	25
High dose	100	25

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for stability and homogeneity. Dosing was based on body weights adjusted daily during the treatment period and was performed each day between 10 a.m. and noon.

Observations

The animals were checked for mortality or abnormal condition daily. Body weights were determined during the treatment period and the entire gestation period. Dams were sacrificed on day 18 of gestation. Gross macroscopic examination of all internal organs was performed at sacrifice. At C-section the following examinations were performed: number of implantations, number of live and dead fetuses/embryos (dams without live fetuses were classified as not pregnant-reason not stated, but not relevant since no dams died during the study), the sex of all live fetuses, individual and mean fetal weights per litter and of stunted fetuses, total and mean placental weight per litter, external malformations, visceral malformations by modified method of Wilson, and evisceration and clearing in potassium hydroxide and staining with alizarin red S for skeletal examinations.

Statistical Analysis

The following statistical analysis methods were used:

- Nonparametric Wilcoxon rank-sum test (U-test of MANN-WHITNEY and WILCOXON), e.g., for body weight gains, number of implantations, number of fetuses and number of resorptions

- Chi-square test (correction of YATES), e.g., for number of stunted fetuses

- Chi-square test (correction of YATES or as Fisher's exact test, depending on frequency anticipated for indices of fertilized and pregnant animals.

Compliance

- A signed Statement of No Confidentiality Claim was provided.

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

- A signed Quality Assurance Statement was provided.

C. RESULTS

1. Maternal toxicity

Mortality

No deaths in any dose group were reported.

Clinical observations

No abnormal signs of compound-related toxicity were reported.

Body weight

The investigators supplied the following data: individual body weights for day 0, 6-18 and mean body weight gains for days 6-15 and day 0-18 of gestation. Mean body weights during dosing and gestation are presented below:

Table I: Mean body weight gains (grams)[a]

	Dosing Period (day 6-15)	Gestation period (day 0-18)
Group:		
Control	12.8	23.0
LDT	13.4	24.9
MDT	13.7	24.6
HDT	13.4	24.9

[a] = data extracted from p.12 of report

There was no evidence of a compound-related effect in the treated dams during the dosing period or during the entire gestation period.

Food consumption

Daily food consumption data was not submitted. Based upon the mean body weight gains it is unlikely that this would have been affected by compound administration.

Gross Pathological Observations

See discussion under observations.

2. Cesarean data are presented below (Table II):

Pregnancy rates ranged from 80% in the HDT to 96% in the control. Implantations/dam and live fetuses/dam were not significantly different among the dose groups. Total resorptions (#/dam) were somewhat elevated in the HDT as compared to the control group due to an increase in late resorption (0.8/control vs 1.3). This was not a statistically significant effect however--probably due to the large standard deviation in the HDT. Mean fetal weight in the HDT also appeared to be lower than the controls (1.36 gm/control vs 1.30/HDT). This is consistent with the observation of an increase in runts (defined on p. 18 of report as weighing less than 1.13 gm;statistically significant, $p<0.05$) in the MDT and HDT as compared to controls. Mean placental weight was slightly but statistically significantly increased in the HDT as compared to controls. Sex ratios (% males) were not different among the dose groups.

Tables III and IV present malformations and skeletal changes observed in the fetuses examined.

There was a statistically significant increase in the total number of malformations observed at the HDT as compared to the control groups with increased malformation rate being evident in the skull (cleft palate, micrognathia, partial dysplasia of parietal bone) and the "neural tube" (enlargement of ventricle, asymmetry of verterbral bodies, dysplasia/abnormal spinal column) (1 fetus/1 litter in control vs 10 fetuses/8 litters). There was an elevation in the number of fetuses/dam with rudimentary ossification centers of the cranium in the HDT vs controls (1/1 in control vs 5/4 in HDT) which is not surprising in light of the malformations of the skull observed.

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Cesarean Section Observations:

Table II: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#animals assigned	25	25	25	25
#animals mated/inseminated	25	25	25	25
Pregnancy rate (%)	24(96)	23(92)	23(92)	20(80)
Maternal wastage				
#died	0	0	0	0
#died/pregnant	0	0	0	0
#non pregnant	1	2	2	5
#aborted	0	0	0	0
#premature delivery	0	0	0	0
Total corpora lutea	----	----	----	----
Corpora lutea/dam				
Total implantations	255	258	247	228
Implantations/dam	10.6	11.2	10.7	11.4
Total live fetuses	236	235	235	202
Live fetuses/dam	9.8	10.2	10.2	10.1
# examd by Dawson method	168	161	166	142
# examd by Wilson method	68	73	68	60
Total resorptions	19	23	12	27
Early	0.08	0.30	0.04	0.00
Late	0.71	0.70	0.48	1.25
Resorptions/dam (S.D.)	0.80(1.0)	1.0(1.6)	0.5(0.8)	1.3(2.2)
Mean fetal weight(g)	1.36(.08)	1.37(.07)	1.37(.13)	1.30(.12)
Mean placental wt.(g)	0.10(.01)	0.10(.01)	0.10(.02)	0.11(.01)*
Postimplantation loss(%)	7.4	8.9	4.9	11.4
Sex Ratio (% Male)	50.8	54.0	48.1	47.5
Mean fetuses/dam (S.D.)				
-minor skel. devia.	0.08(.28)	0.05(.21)	0.17(.49)	0.40(.68)
-malformations (all)	0.04(.20)	0.18(.66)	0.0(0.0)	0.65*(.93)
-runts	0.21(.51)	0.18(.50)	0.91*(1.7)	1.20*(2.12)
-# runts/dose group	5	4	21	24

[a] = Data extracted from pp. 27-32, Tables 1-5
* significantly different from controls ($p<0.05$)

Table III: External/visceral malformations

Observations[a]	Control	LDT	MDT	HDT
total# fetuses(ltrs) exmd	236(24)	234(23)	234(23)	202(20)
# " " (") affected	1(1)	4(2)	0(0)	13(8)
visceral:fetuses/ltr	68(24)	73(23)	68(23)	60(20)
TYPE				
multiple: cleft face, palate, jaw; dysplasia of limbs; deformed spinal column, ribs; shortened tail	1(1)	----	----	----
-skull: cleft palate, micrognathia, partial dysplasia parietal bone	----	4(2)	----	7(6)
-neural tube: brain ventricle enlarged, asymmetry vertebral bodies, dysplasia spinal column, abnormal flexion spinal column	----	----	----	5(4)
-fused ribs, floating ribs	----	1(1)	----	1(1)
-tail: kinked, shortened	----	----	----	1(1)

Skeletal Examinations

Table IV: Skeletal Examinations (Dawson)

Observations[a]	Control	LDT	MDT	HDT
# fetuses(ltrs) examined	168(24)	161(23)	166(23)	142(20)
STERNUM (#fetuses/#litters)				
-ossif. ctrs missing; slightly cleft sternum	1(1)	----	----	----
CRANIUM				
-rudimentary ossif. ctrs.	1(1)	----	2(2)	5(4)
HYOID BONE				
-missing, separate ossif. ctrs.	1(1)	1(1)	----	2(2)
SPINAL COLUMN				
-vertebral bodies	----	----	----	1(1)

D. DISCUSSION/CONCLUSION

a. Maternal toxicity:

Oral administration of terbuconazole during days 6-15 of gestation in the female NMRI mice produced no apparent effect upon body weights during dosing or the entire gestation period. There was no apparent changes in animal health as determined by clinical signs or mortality (see results from associated study, T5025712, on maternal toxicity).

b. Developmental toxicity:

Terbuconazole produced a non statistically significant increase in postimplantation loss in the form of late resorptions in the 100 mg/kg dose group. Statistically significant increases in runts were observed in the mid and high dose groups as compared to controls. There was a statistically significant increase in total malformations in the high dose group primarily in skull (cleft palate, micrognathia, partial dysplasia of parietal bone) and the "neural tube" (enlarged brain ventricle, asymmetry of vertebral bodies, dysplasia/abnormal spinal column) in 10 fetuses/8 litters as opposed to 1 fetus/1litter with multiple malformations. This was associated with an increase in the HDT of rudimentary ossification centers of the cranium.

c.

No significant study deficiencies were noted.

E. CLASSIFICATION: CORE MINIMUM DATA.

Maternal NOEL = 10 mg/kg/day (LDT) (based on special study submitted (T5025712)
Developmental Toxicity NOEL = 10 mg/kg/day (LDT)
Developmental Toxicity LOEL = 30 mg/kg/day

F. RISK ASSESSMENT:

Development of MOS has been determined to be unnecessary at this time.

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007200

Reviewed by: James N. Rowe, Ph.D., James N. Rowe 12/23/88
Review Section I
Tox. Branch-Herb./Fung./Antimicrobial Support (TS-769C)
Secondary reviewer: Quang Q. Bui, Ph.D.
Review Section I, Tox. Branch-H./F./A.S. (TS-769C) Quang Q Bui 12/23/88

DATA EVALUATION REPORT

STUDY TYPE: Two generation rat reproduction, EPA Guideline 83-4

TOX. CHEM. NO.: 463P

ACCESSION NUMBER: MRID NO.: 407009-46

TEST MATERIAL: HWG 1608 TECHNICAL; mixed batch no. Fl. no. 132; whitish-beige powder; purity of 95.2%; refrigerated during the study

SYNONYMS: ethyl-trianol; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazole-1-yl-methyl)pentane-3-ol

STUDY NUMBER(S): report no. 16223; Lab. proj. ID no. 91064; study no. T 5017647

SPONSOR: MOBAY Corporation, Agricultural Chemicals Division

TESTING FACILITY: Toxicology Division, Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333 and PATCO AG, CH-4452 Itingen, Switzerland

TITLE OF REPORT: HWG 1608, Two-generation study in rats

AUTHOR(S): Dr. R. Eiben (study director)

REPORT ISSUED: November 12, 1987

QUALITY ASSURANCE/GLP/CONFIDENTIALITY:

Signed copies of no confidentiality, GLP statement and quality assurance were included.

CONCLUSIONS:

Dietary administration of terbuconazole at dosages of 0, 100, 300 and 1000 ppm to male and female Wistar rats resulted in parental toxicity primarily at the HDT expressed as increased clinical signs of toxicity (loss of hair), depressed body weights, increased severity of spleen hemosiderosis (females only) and decreased liver and kidney weights in both F0 and F1B males and/or females. Decreased pup viability was observed in F0 but not F1B neonates while there was a significant depression in pup body weight of all littering groups (F1a, F1b, F2a, F2b) at the HDT from birth on. A systemic toxicity LOEL (based upon depressed body weights, increased clinical signs of toxicity,

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decreased food consumption, increased spleen hemosiderosis and decreased liver and kidney weights) is set at 1000 ppm and a NOEL is set at 300 ppm. The reproductive LOEL, based upon neonatal birth weight depression, is set at 1000 ppm and the NOEL is set at 300 ppm.

CORE: Minimum

MATERIALS AND METHODS:
(A photocopy of the materials and methods is appended)

1. Dosage

SPF-bred Wistar rats (strain Bor: WISW (SPF Cpb); from Winkelmann, Borchen) were divided into four study groups (25 of each sex/dose group) and given terbuconazole weekly in the diet at 0, 100, 300 and 1000 ppm (control,LDT, MDT, HDT, respectively) throughout the study, including mating period, gestation and pup lactation (total exposure period of approximately 40 weeks). Specific exposure periods for individual study sections are given in appended methods.

2. Experimental Plan (see methods for time sequence, p. 19)

Each FO female was housed singly until about 17 weeks of age and then mated with one male. Rats (1 female/1 male) were kept in one cage during the mating period with the date of insemination determined by vaginal smears or the presence of vaginal plugs. The calculation of the gestation period was based upon the date of sperm finding, as day 0 of gestation. Five days after birth, the litters were reduced, if necessary, to 8 animals. Animals for further treatment were randomly selected. Pups in the F1a generation (and F2a) were reared and then sacrificed at four weeks. After two week waiting periods, the F0 parental animals were mated a second time. The F1b pups were kept and reared for four weeks, like the pups from the first mating, and then separated from the dams. Four week old F1B (F1b) pups were selected for further treatment and matings.

After reaching an average age of 100 days, F1B parental first matings were performed (20 day period) with a 3 week gestation period. F2a pups were kept for a 6 week period and a second mating and gestation period was observed (F2b; 6 weeks duration). Males in the FO and F1B generations were sacrificed after the last mating. F2b pups were reared up to three weeks of age and then sacrificed. Note: FO rats which had not been fertilized twice were additionally mated with fertile males in the same group and the resulting generation was termed F1C (pg. 16), a somewhat misleading term since all rats must be remated after the first pregnancy anyway. These rematings gave no significant additional data.

3. Necropsy of Adults and Pups

The rats which died or sacrificed moribund during the study were dissected and grossly examined. All the FO and F1B parents were sacrificed about one to three weeks after the pups were weaned, dissected and grossly examined. The brain, pituitary, liver, kidney, adrenals, testicles, epididymis, seminal vesicle, prostate, ovaries, uterus, vagina, spleen, mammary glands (females only) and organs with gross alterations were fixed.

F1B parents' livers, spleens, kidneys, adrenals and testicles or ovaries (pairwise) were weighed.

At an age of about 4 weeks, all the F1B which had not been selected, and all the F2B pups (age 3 weeks) were sacrificed and grossly examined with special attention paid to the reproductive organs. The pups which died were not necropsied since they were autolytic and/or could not be appraised due to cannibalism.

4. Bone examination of F1B rats

The right rear femur of all F1B male rats were removed after necropsy and measured for length and diameter at the thinnest point. The bone was fixed in 10% formaldehyde. No reason for examination of only male femurs was given.

5. Statistics

Arithmetic group means, standard deviations and upper and lower confidence limits were determined. The test collective values were compared with the controls by the U test of Mann and Whitney and Wilcoxon. For indices (formed from magnitudes) the confidence limits were calculated by Clopper and Pearson's method.

Test collective indices were compared with controls by Fisher's Exact test (significance of $p<0.5$ or 0.01). Random lists were produced with the aid of a Subprogram Randu from IBM.

RESULTS:

1. Mortality/clinical signs

Five females died or were sacrificed moribund during the test period (2 females/0 ppm (#s 38, 40+), after first mating; 1 female/1000 ppm (#185), after second mating; 1 female/0 ppm (#237+), during lactation, F2A generation; 1 female/1000 ppm (#387), after birth of F2b pups). These were not dose-related effects.

A summary of the major clinical signs is presented below:

4a

CLINICAL SIGN*:	0 ppm		100 ppm		300 ppm		1000 ppm	
F0 adults	m	f	m	f	m	f	m	f
Loss of hair	55	123	0	92	24	81	21	196
Inflammation/changes of the eye	0	2	3	0	0	0	19	0
F1B adults								
Loss of hair	22	39	0	35	39	63	36	72
Inflammation/changes of the eye	0	15	0	9	0	0	0	0
Ill/bad condition	3	1	1	0	0	0	8	0

* total number of observations reported

There was a general increase in the reported incidence of toxicity signs at the high dose for loss of hair in F0 females (HDT/196 days vs Control/123 days). For inflammation of the eyes, treated F0 males appeared elevated over control values (HDT/19 vs Control/2) but control females had a greater incidence then HDT females(control/15 days vs HDT/0 days). This suggests that a treatment related effect for eye inflammation is questionable. The incidence of loss of hair in F1B females was also increased at the HDT and MDT (HDT/72 days, MDT/63 days vs Control/39 days) and possibly an increase in the observation of ill/bad condition for HDT F1B males (HDT/8 days vs Control/3 days).

2. Body weights/food consumption data

Mean body weights for the F0 and F1B adults are presented below:

Week on study	0 ppm	100 ppm	300 ppm	1000 ppm
F0 males:				
0	92(7)[b]	92(7)	93(7)	91(7)
1	131(8)	129(10)	130(9)	118(9)*
17	351(25)	343(22)	348(24)	327(23)**
29	374(23)	370(26)	370(25)	354(26)**
34	380(24)	381(25)	380(25)	364(25)**
F0 females:				
0	88(5)	89(5)	90(6)	89(6)
1	110(6)	111(8)	111(7)	104(7)**
17	206(14)	208(17)	206(16)	196(17)*
d6[a]	218(15)	223(17)	219(18)	208(17)*
d20	284(24)	282(33)	288(36)	263(35)*
29	223(15)	224(19)	222(19)	209(20)*
d6	234(17)	240(22)	233(21)	218(23)*
d20	311(34)	293(37)	286(46)	284(41)*
34	263(15)	255(25)	249(25)	233(25)**
F1B males:				
5	97(11)	92(12)	99(12)	82(16)**
14	317(36)	317(35)	321(27)	286(32)**
27	390(35)	388(36)	381(32)	352(41)**
31	390(35)	391(36)	390(34)	356(42)**
F1B females:				
5	86(7)	83(8)	89(7)	75(14)**
14	192(14)	192(16)	195(13)	180(17)**
d6	211(18)	214(16)	216(15)	199(19)*
d20	278(33)	286(31)	290(26)	265(32)
27	219(15)	222(17)	224(14)	206(17)**
d6	238(20)	237(17)	243(16)	223(19)*
d20	298(35)	302(45)	314(36)	278(32)*
32	247(28)	242(18)	244(17)	225(20)**

[a] d = days after female insemination; [b] mean in grams (standard deviation) (data taken from pp. 53-58 of report)

Mean body weights were consistently depressed in both male and female adult rats exposed to tebuconazole at 1000 ppm prior to mating, after mating, during lactation and following the lactation period in both the F0 and F1B parental generations.

Summary food consumption data (g/animal/day) are presented below:

Week on study	0 ppm	100 ppm	300 ppm	1000 ppm
FO(male/female)				
1	15.09/13.54	15.07/13.73	14.89/14.23	13.73/13.21
5	20.16/14.80	19.28/15.18	21.27/15.15	19.18/14.98
10	21.23/15.43	20.77/16.41	21.57/16.04	20.93/16.42
17	21.15/16.08	19.95/20.76	19.60/22.10	19.17/16.27
Entire period (W1-W17)	21/15	20/16	20/16	19/16
F1B(male/female)				
6	16.98/15.81	16.41/14.54	16.04/13.19	14.83/13.33
10	25.00/18.47	24.46/19.31	25.39/20.31	23.94/18.75
15	24.58/18.90	23.88/17.31	24.70/18.37	22.23/15.99
Entire period (W6-W15)	24/19	24/18	24/19	22/17

(food data up to first mating; FO = pgs. 78, 79; F1B = p. 122)

No statististically significant effects upon food consumption were observed in either parental generation prior to mating. However, there was a small, generally consistent, depression observed in the 1000 ppm males and/or females of both the F0 and F1B parents over the entire measurement period (FO: males/10%; F1B: males/8%, females/11%) as compared to the respective controls.

3. Reproductive indices

A summary table of reproductive indices is presented below. The fertility index ranged from 75% to 96% and fluctuated considerably but did not appear to be a dose-related effect in either the F0 or F1B generations. The insemination index, gestation index or mean gestation period were not different among the dose groups in either generation. There were statistically significant depressions in the viability index of the F0 generations in the F1a males at 100 and 1000 ppm (90.3%, 88.1%, respectively vs 98.5%/control) and in the lactation index male and/or females of F1a or F1b animals at 100 and 1000 ppm (F1b: 76.1%/females vs 90.9% control; F1a, F1b, resp.: 86.3%, 80.3% vs 95.2%, 90.9%/controls). However, in the F1B generation, neither the F2a or F2b litters were affected in a compound-related manner.

FO generations

Dose:	0 ppm		100 ppm		300 ppm		1000 ppm	
Parameters	F1a	F1b	F1a	F1b	F1a	F1b	F1a	F1b
# females	25	23	25	25	25	25	25	25
insemination index	100	100	96.0	92.0	100	96.0	96.0	92.0
gestation index	100	100	100	94.4	100	100	90.5	95.5
gesta. period(d)	22.3	22.0	22.2	21.9	22.3	22.2	22.3	22.1
fertility index	88.0	95.7	75.0	78.3	88.0	75.0	87.5	95.7
viability index	98.5	94.5	90.3**	89.4	95.2	94.2	88.1**	88.5
lactation index	95.2	90.9	88.9	76.1**	91.1	98.2*	86.3*	80.3*
# viable ltrs	22	22	17	17	18	18	19	21
# nonviable ltrs	0	0	1	1	0	0	2	1

F1B generations

Dose:	0 ppm		100 ppm		300 ppm		1000 ppm	
Parameters	F2a	F2b	F2a	F2b	F2a	F2b	F2a	F2b
# females	25	24	25	25	25	25	25	25
insemination index	100	100	100	100	96.0	100	100	100
gestation index	100	91.3	100	100	100	100	100	100
gesta. period(d)	22.3	22.1	22.0	21.7	22.1	21.7	22.0	22.1
fertility index	96.0	95.8	96.0	84.0	95.8	92.0	92.0	84.0
viability index	98.0	91.7	99.6	96.0	97.7	98.9**	96.6	91.1
lactation index	98.4	97.3	99.5	97.0	99.5	97.2	97.2	97.9
# viable ltrs	24	21	24	21	23	23	23	21
# nonviable ltrs	0	2	0	0	0	0	0	0

(Definitions of parameters:

Fertility index = $\frac{\text{number of pregnant females x 100}}{\text{number of mated females}}$

Gestation index = $\frac{\text{number females with live litters x 100}}{\text{number of pregnant females}}$

Viability index = $\frac{\text{number live pups after 5 days x 100}}{\text{number pups born alive}}$

Lactation index = $\frac{\text{number of live pups after 4 weeks x 100}}{\text{number of live pups after 5 days after reduction}}$

Insemination index = $\frac{\text{number of mated females x 100}}{\text{number paired females}}$

4. Neonatal indices (from pp. 60-63, 70-73 of report)

F0: F1a

Dose:	0 ppm	100 ppm	300 ppm	1000 ppm
total #	204	186	225	177
# dead	5	0	16	17
#/litter(d0)	9.0(1.7)	10.3(2.9)*	9.5(2.8)	7.6(3.4)
#/litter(5DBR)	8.9(0.8)	9.3(2.7)	9.0(3.1)	6.7(3.7)
#/litter(5DAR)	7.6(0.7)	7.5(1.7)	7.2(1.9)	5.9(3.0)
#/litter(Wk 1)	7.4(1.3)	7.4(1.7)	6.9(2.2)	5.8(3.1)
#/litter(Wk 4)	7.2(1.8)	6.7(2.4)	6.5(2.2)	5.1(3.3)*
% males	48	51	47	51
Pup wt.(birth)	6.0(0.5)	5.7(0.5)*	5.8(0.6)	5.6(0.4)*
Pup wt.(5DBR)	10.2(1.2)	9.4(1.0)	9.8(1.5)	9.0(1.4)**
Pup wt.(5DAR)	10.4(1.2)	9.6(1.0)	10.0(1.4)	9.0(1.4)**
Pup wt. (Wk 1)	12.6(1.9)	12.4(1.6)	13.2(2.2)	10.7(2.2)**
Pup wt. (Wk 4)	54.1(5.1)	53.7(5.3)	56.6(7.5)	47.8(8.0)**

F0: F1b

total #	210	159	151	160
# dead	9	8	12	12
#/litter(d0)	9.1(2.1)	8.4(3.6)	7.7(3.4)	6.7(3.3)*
#/litter(5DBR)	8.6(2.3)	7.5(3.3)	7.3(3.5)	6.0(3.7)*
#/litter(5DAR)	7.5(1.0)	6.5(2.3)	6.3(2.5)	5.3(3.0)**
#/litter(Wk 1)	7.4(1.0)	6.1(2.6)	6.3(2.5)	4.9(3.0)**
#/litter(Wk 4)	6.8(1.7)	4.9(2.9)*	6.2(2.5)	4.3(3.0)**
% males	50	49	47	51
Pup wt.(birth)	5.7(0.5)	5.6(0.7)	5.7(0.6)	5.6(0.8)
Pup wt.(5DBR)	9.3(1.2)	8.5(1.6)	10.2(1.9)	8.9(1.9)
Pup wt.(5DAR)	9.4(1.2)	8.5(1.6)	10.3(1.9)	8.8(1.9)
Pup wt. (Wk 1)	12.7(1.6)	11.2(2.6)	13.6(2.1)	11.4(2.7)*
Pup wt. (Wk 4)	58.7(10.1)	58.6(8.6)	60.2(8.8)	52.4(8.8)

F1B: F2a

total #	255	271	261	239
# dead	3	3	3	4
#/litter(d0)	10.5(2.0)	11.2(1.0)	11.2(2.1)	10.2(1.5)
#/litter(5DBR)	10.3(1.9)	11.1(1.0)	11.0(2.1)	9.9(1.4)
#/litter(5DAR)	7.9(0.6)	8.0(0.0)	7.9(0.3)	7.9(0.5)
#/litter(Wk 1)	7.8(0.6)	8.0(0.0)	7.9(0.3)	7.8(0.5)
#/litter(Wk 4)	7.7(0.7)	8.0(0.2)	7.9(0.3)	7.7(0.8)
% males	48	45	49	49
Pup wt.(birth)	5.9(0.6)	5.5(0.3)**	5.7(0.5)	5.3(0.3)**
Pup wt.(5DBR)	9.9(1.5)	9.3(0.8)	9.6(1.2)	8.1(1.0)**
Pup wt.(5DAR)	10.1(1.5)	9.4(0.8)*	9.7(1.2)	8.1(0.9)**
Pup wt. (Wk 1)	12.7(1.9)	12.1(1.3)	12.6(1.9)	10.3(1.2)**
Pup wt. (Wk 4)	56.0(6.1)	56.4(4.2)	56.4(5.6)	43.7(5.0)**

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(continued from previous page

F1B: F2b

Dose:	0 ppm	100 ppm	300 ppm	1000 ppm
total #	214	253	273	213
# dead	8	5	11	10
#/litter(d0)	9.0(3.9)	11.8(2.3)**	11.4(2.4)*	9.7(3.3)
#/litter(5DBR)	8.2(4.0)	11.3(2.0)**	11.3(2.4)**	8.8(3.9)
#/litter(5DAR)	6.5(2.7)	7.9(0.7)	7.9(0.6)	6.8(2.5)
#/litter(Wk 1)	6.5(2.8)	7.9(0.7)	7.9(0.6)	6.8(2.5)
#/litter(Wk 4)	6.3(2.9)	7.6(1.2)	7.7(0.7)	6.7(2.5)
% males	45	43	50	51
Pup wt.(birth)	5.7(0.6)	5.7(0.3)	5.7(0.4)	5.3(0.5)*
Pup wt.(5DBR)	9.2(1.5)	9.1(0.9)	9.4(1.1)	8.4(2.7)**
Pup wt.(5DAR)	9.3(1.5)	9.3(0.8)	9.7(1.2)	8.5(2.6)**
Pup wt. (Wk 1)	12.6(2.0)	12.0(1.3)	12.6(1.7)	10.0(1.1)**
Pup wt. (Wk 3)	36.0(4.2)	36.8(4.2)	38.1(3.0)	30.5(3.4)**

5 DBR = day 5, before reduction; 5 = DAR, after reduction; wk = week after birth

Summary tables of neonatal indices are presented above. There was a significant number of dead fetuses observed in the mid and high dose groups of the F1a litter as compared to the controls (5/control vs 16 or 17, respectively) as well as a smaller increase in both dose groups in the F1b littering group (9/control vs 12/MDT, 12/HDT). No consistent increase in number of dead fetuses was observed in the F1B generation. A statistically significant decrease in the mean number fetuses/litter at birth through week four of lactation was observed in both litters of the F0 generation at the HDT (e.g., at week four, statistically significant decrease: 5.1/dam vs 7.2/dam, F1a; 4.3/dam vs 6.8/dam, F1b) as compared to the control values. However, this was not observed in either the F2a or F2b litters of the F1B generation either at birth or at the end of the lactation period. This significant decrease was observed only after the reduction (day 5) in the F1a pup but <u>both</u> before and after culling of F1b pups suggesting fetal toxicity at parturition with continued fetal toxicity through weaning. Neonatal weights from birth through weeks 3 or 4 of lactation were consistently and statistically significantly depressed in both the F0 and F1B generations in all littering groups (F1a, F1b, F2a, F2b) at the HDT and is considered to be a compound-related effect.

5. Gross necropsy/histopathology

a. Gross necropsy

No apparent compound-related gross changes were observed in parental animals.

b. Histopathology

Selected histopathology findings are presented below (taken from pages 241C-243C):

Organ/Finding

	0 ppm		100 ppm		300 ppm		1000 ppm	
F0 adults	m	f	m	f	m	f	m	f
KIDNEYS: # exam.	25	24	25	25	25	25	25	25
-lymphoid c. infiltr.	2	3	9	7	4	7	12	4
-tubular atrophy	18	6	17	10	24	9	21	9
TESTES: # exam.	25		25		25		25	
-tubular atrophy	-		1		2		2	
-Leydig c. hyperplasia	-		-		1		-	
OVARIES: # exam.		25		25		25		25
-cysts(s)		2		4		5		3
SPLEEN: # exam.	24	24	25	25	25	25	24	25
-hemosiderosis	22	23	25	24	25	25	24	25
-hemopoiesis	10	8	14	10	8	14	17	5
F1B adults	m	f	m	f	m	f	m	f
KIDNEYS: # exam.	25	25	25	25	25	25	25	25
-tubular atrophy	20	5	21	10	18	5	20	7
OVARIES: # exam.		24		25		25		25
-cysts(s)		1		2		1		3
SPLEEN: # exam.	25	25	25	25	25	25	25	25
-hemosiderosis	-	1	-	-	-	-	-	9

HEMOSIDEROSIS (SEVERITY)

	0 ppm		100 ppm		300 ppm		1000 ppm	
F0 ADULTS	m	f	m	f	m	f	m	f
-grade 2	9	11	10	7	7	5	3	0
-grade 3	13	11	15	14	17	14	21	6
-grade 4	0	1	0	3	0	4	0	19
F1B ADULTS								
-grade 3	0	1	------		------		0	9

There appears to be a possible elevation in the finding of lymphoid cell infiltration in F0 males in the HDT (12 vs 2/control) but this was not observed in F1B males. An increase in tubular atrophy was apparent in F1B males at the HDT (20 vs 5/control) but not in F0 males. These findings are therefore discounted.

Hemosiderosis of the spleen appears to be a compound-related toxicity. The incidence in F0 males and females is close to 100% but confined to only the control and HDT females of the F1B generation at a much lower incidence rate. However, from examination of the severity (grade) of the lesions it is evident that HDT F0 females (grade 4: 19 vs 1/control) and HDT F1B females (grade 3: 9 vs 1/control) had elevated findings as compared to controls.

6. Organ weights(mg)/organ-to-body-weight ratios (mg/100 gm)

Selected organ weights/organ-to-body-weight ratios for F1B adults are presented below:

F1B males	0 ppm	100 ppm	300 ppm	1000 ppm
Body wt.(gm)	390	391	390	356**
Liver	13694/3512	13898/3546	12613/3230**	12071**/3383
Kidney	2391/613	2360/603*	2408/617	2144**/604
Adrenal	44/11	42/11	40/10*	39*/11
Testes	3804/978	3879/996	3793/974	3729/1057**
F1B females				
Body wt.(gm)	237	238	241	224*
Liver	9402/3960	9485/3988	9923/4110	9196/4110
Kidney	1576/666	1554/652	1612/667	1458*/651
Ovaries	147/62	149/63	155/64	146/65

*, ** statistically significantly different from controls ($p<0.05$, 0.01, respectively)

Mean liver weights (mg)and liver to body weight ratios (%) were decreased in F1B males but not females at the mid and high dose levels, respectively (e.g., males: mid, 12613/3230** vs control, 13694/3512; females: high, 9196/4110 vs control, 9402/3960; $P<0.01$). Kidney weights as well as organ to body weight ratios were also somewhat lower at the HDT as compared to the controls

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in both male and female rats (absolute weights statistically significant, e.g., . males: mid, 2144**/604 vs 2391/613). No consistent findings were evident for male adrenals or testes. Female ovary weights were not altered.

7. Bone examination

A summary data table of right femurs from F1B males is presented below:

Dose(ppm)	length (cm)	diameter (mm)
0	3.65	4.38
100	3.64	4.55
300	3.62	4.46
1000	3.59	4.50

There was no effect of terbuconazole on bone growth of the right femurs from F1B males.

SUMMARY/CONCLUSIONS:

Administration of terbuconazole continuously in the diet of male and female Wistar rats at 0, 100, 300 and 1000 ppm prior to mating, during mating and gestation and lactation produced evidence of parental toxicity, primarily at the high dosage level. There was an increase in the incidence of hair loss in MDT and HDT females (F0, F1B). Mean body weights were depressed in both HDT males and females of both generations and were associated with a small depression in food consumption (primarily in females). HDT females of both generations had an increase in the severity of hemosiderosis of the spleen. Absolute and relative liver and kidney weights were depressed in F1B (primarily HDT) males and/or females.

Inconsistent reproductive effects were observed between the F0 and F1B generations. In the F0 generation, there was a statistically significant decrease in fetal viability based upon a decrease in the mean viability index at 100 and 1000 ppm in F1a males and in the lactation index of male and/or females of F1a and F1b litters at 100 or 1000 ppm. No alteration was observed in the F1B litters. This was reflected in the F0 litters by the decrease in the mean pup counts/litter observed in both F1a and F1b at birth through the end of weaning. No such findings were reported for the F1B litters. These findings may be accounted in part by the considerable variability in the fertility rate observed in the F0 generation. It may also be a result of acclimation of the parental animals to the toxicant (long-term stimulation of liver microsomal enzymes resulting in accelerated detoxification patterns in the F1B litters; terbuconazole is known to stimulate increased liver microsoma metabolism).

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A consistent, statistically significant depression in neonatal weights from birth through lactation (week 3 or 4) was observed in all littering groups of F0 and F1B at the HDT. This is considered an unequivocal compound-related effect upon the pups which may be a post-natal expression of reproductive toxicity (retarded neonatal growth) since it is seen at birth and continues onward through the weaning period. However, it should be noted that there was no indication of growth retardation based upon bone formation in the right femurs of F1B males.

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____ Identity of product inert ingredients.

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____ Description of quality control procedures.

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James N. Rowe 2/2/89
John H.S. Chen 2/2/89

007200

DATA EVALUATION RECORD

I. STUDY TYPE: Salmonella/microsome point mutation; EPA Guideline 84-2

STUDY TITLES: Salmonella/microsome test to evaluate for point mutagenic effects.

Caswell #: 463P
Tox Branch Project: 8-1043
Document:
MRID No. 407009-47; 407009-48

SPONSOR: Mobay Corporation, Agricultural Chemicals Division

TESTING LABORATORY: BAYER AG, Toxicology Division, FRG

STUDY NUMBER: 91068; 91068-1; BAYER AG report no. 16383, 16383 -A

STUDY DATE: January 27, 1988; February 8, 1988

STUDY AUTHOR(S): Dr. B.A. Herbold

TEST MATERIAL: HWG 1608; alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; beige powder; 96.6% pure; stored at 4°C; batch no.1616001 36

VEHICLE: DMSO

POSITIVE CONTROL: 4-nitro-1,2-phenylene diamine (TA1537, TA1538, & TA98), sodium azide (TA1535), nitroflurantoin (TA100) and 2-aminoanthracene (causes reversion of all strains, +S9).

II. CONCLUSIONS:
There was no evidence of a significant increase (2x) in revertants/plate at any dose level (39.5-450 ug/plate) in the presence or absence of S9 mix over the negative control rate. Therefore, under the conditions of this assay, terbuconazole was not mutagenic in the Salmonella/microsome test at the concentrations tested.

Classification: Acceptable

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III. MATERIALS AND METHODS:

The mutagenicity of HWG 1608 at seven concentrations (39.5, 59.3, 88.9, 133.3, 200, 300, & 450 ug/plate) was evaluated by the Salmonella/mammalian-microsome plate incorporation assay in the presence or absence of metabolic activation (Ames et al., Proc. Nat. Acad. Sci. 70: 2281-2285, 1973a; Ames et al., Mutation Res. 31: 347-364, 1975; Maron and Ames, Mutation Res. 113: 173-215, 1983). Five histidine-deficient strains of S. typhimurium (TA98, TA100, TA1535, TA1537, & TA1538) were used in this study. Stock cultures were prepared and checked to determine crystal violet and UV sensitivity, and resistance to ampicllin. Culture media were prepared according to the method described by Ames et al. (1975). The mammalian metabolic activation system consisted of rat liver homogenate (S9 fraction) from Aroclor 1254-treated male Sprague-Dawley rats and cofactor solution described by Ames et al.(1975) The composition of cofactor solution (per 70 ml) contained 162.6 mg $MgCl_2$-$6H_2O$, 246 mg KCl, 179.1 mg Glucose-6-Phosphate disodium salt, 315 mg NADP disodium salt, and 100 mM Phosphate buffer. For the tests recorded 30% or 10% S9 fraction (v/v) were used in the S9 mix. Four plates per dose as well as positive and negative controls were used. DMSO was the negative control; the positive controls were 4-nitro-1,2-phenylene diamine (0.5 ug/plate), sodium azide (10 ug/plate), nitroflurantoin (0.2 ug/plate), and 2-aminoanthracene (3 ug/plate).

IV. RESULTS/DISCUSSION:

Following the exposures to test cultures (TA98, TA100, TA1535, & TA1537) with 7 concentrations of HWG 1608 (37.5, 75, 150, 300, 600, 1200, & 2400 ug/plate) on a selective agar plate for 48 hrs. at 37° C., strong bacteriotoxic effect (decreased number of his^+ revertants) was observed at doses greater than 600 ug/plate. Therefore, the dose levels of the test material from 39.5 ug/plate to 450 ug/plate were chosen for this study (Tables 1, 2, 3, & 4).

The strain specific control compounds (sodium azide, nitroflurantoin, and 4-NPDA) and the positive control (2-AA) to ensure the efficacy of the activation system have given the positive responses as expected. There was no evidence of a significant increase (2X) in revertants/plate at any dose level tested in the presence or absence of S9 mix over the negative control rate (Tables 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, & 16).

Therefore, under the test conditions of this study, terbuconazole (HWG 1608) was not mutagenic to the Ames tester strains of S. typhimurium. The study is acceptable.

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Pages 508 through 523 are not included.

The material not included contains the following type of information:

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Primary Reviewer: James N. Rowe, Ph.D. 4.1.f 5-10-89
Review Section I/HFASB/HED (TS-769C)
Secondary Reviewer: John H.S. Chen, D.V.M. John H S Chen 4/15/89
Review Section I/HFASB/HED (TS-769C)

DATA EVALUATION RECORD

I. STUDY TYPE: CHO-HGPRT forward mutation (in vitro); EPA Guideline 84-2

STUDY TITLE: Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro
Caswell #: 463P
Tox Branch Project: 8-1043
Document:
MRID No. 407009-49

SPONSOR: Mobay Corporation, Agricultural Chemicals Division

TESTING LABORATORY: BAYER AG, Toxicology Division, FRG

STUDY NUMBER: 87318; BAYER AG report no. 16749

STUDY DATE: May 31, 1988

STUDY AUTHOR(S): Dr. H. Lehn

TEST MATERIAL: HWG 1608; alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; white powder; 96.6% pure; stored at 4°C; batch no.1616001/86

VEHICLE: DMSO

POSITIVE CONTROL: ethylmethanesulfonate (-S9); 3-methylcholanthrene (+S9)

TEST ANIMAL: CHO cell line derived from ovary of female Chinese hamster (CHO-K1-BH4 subclone)

II. CONCLUSIONS:

No statistically significant increases in mutant frequency (Mutants/10^6 clonable cells) above the solvent controls were observed in the HWG 1608-treated cultures either with or without S9 mix activation. However, according to the acceptable procedure for performing the CHO/HGPRT assay as recommended by EPA, the highest concentration tested should produce a low level of survival (approximately 10% of that of control) after a treatment time of 5 hours. Since the highest concentrations of the test substance used in this study did not elicit this level of cytotoxicity after 2 hours of exposure at 37° C, the study is unacceptable.

This study is classified as unacceptable.

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[redacted]

III. MATERIALS AND METHODS:

A photocopy of the material and methods is appended. In summary, CHO cell line (CHO-K1-BH4 subclone), derived from female Chinese hamster (Puck and Kao, 1967), were exposed in culture (4 X 10^6 cells/flask) to the test compound at predetermined doses (80, 90, 92.5, 95, 97.5, & 100 ug/ml without S9 mix activation; 12.5, 25, 50, 100, 150, & 200 ug/ml with S9 mix activation) for 2 hours (37° C). After the exposure period, the cultured cells were washed, trypsinized and replated in culture medium at a density of 1.5 X 10^6 cells in 250 ml-flasks and at 200 cells into each of 3 60 mm-petri dishes. These cultures were incubated for 7 days to allow colony development and determination of the cytotoxicity associated with each test compound. At the end of the expression period, the cultured cells were reseeded at 2 X 10^5 cells per 100-mm dish containing 6-thioguanine (10 ug/ml) in the culture medium for 7-8 days of incubation at 37° C (mutant selection). Negative and positive controls were also run concurrently with the test compound. The detailed information about the preparations of culture medium and S9 mix activation were described in the original report (Pages 10 and 11).

Criteria for determination of mutagenesis include:

1) dose-dependent and reproducible increase and reproducible increase in mutant frequency (desirable for at least 3 doses); mutagenic response should be 2X negative controls
2) a reproducible increase (2X) for a single dose near the highest concentration is observed
3) an assay will be negative if none of the doses tested (with at least 30% toxicity) induces a reproducible mutant frequency

Statistical tables of Kastenbaum and Bowman (1970) were used and results were considered significantly different from the negative control at 95% or 99% confidence levels.

Signed and dated data confidentiality, GLPs and QAU statements were included.

IV. RESULTS/DISCUSSION:

Cytotoxcity was demonstrated in the presence and absence of S9 mix for CHO clonal cells. In the nonactivated assay concentrations of 5-125 ug/ml resulted in a relative survivals ranging from 107.2% to 1.7%, respectively. In the activated assay, concentrations of terbuconazole of 3.9-1000 ug/ml resulted in relative survivals of 92.7% (low dose), 37.5% (125 ug/ml) and 0% (250 ug/ml and higher). Based upon these findings, test concentrations in -S9 were 80-100 ug/ml and in +S9, they were 12.5-200 ug/ml.

However, the study is classified as unacceptable due to the following deficiencies:

1. Inconsistent cytotoxicity data are reported in Tables 1, 2, 3, 4, 5, 6, 7, and 8. (i.e., Relative Survival in Table 1 at 100 ug/ml without activation: 14.8%; Relative Survival in Table 2 at 125 ug/ml with activation: 37.5%; Relative Survival in Table 3 at 100 ug/ml without activation: 92.9%; Relative Survival in Table 4 at 100 ug/ml without activation: 128.7%; Relative Survival in Table 5 at 100 ug/ml without activation: 81.7%; Relative survival in Table 6 at 150 ug/ml with activation: 124.2%; Relative Survival in Table 7 at 150 ug/ml with activation: 109.4%; Relative Survival in Table 8 at 150 ug/ml with activation: 7.5%).
2. According to the acceptable procedure for performing the CHO/HGPRT assay as recommended by EPA (EPA Health Effects Test Guidelines 560/6-83-001), the highest concentration of test material used should produce a low level of survival (i.e., normally 10% of control survival). Since the concurrent cytotoxicity data (Tables 3,4,5,6,& 7) do not indicate this level of toxicity was reached, it is questionable whether appropriate highest concentrations (i.e., 100 ug/ml without S9 mix or 150 ug/ml with S9 mix) were chosen for this study.

3. The treatment time of 2 hours used is not considered adequate to support the negative conclusion for this study. Normally, a treatment time of 5 hours is highly recommended for performing the CHO/HGPRT assay (See U.S.E.P.A. Report of Gene-Tox Program for CHO/HGPRT assay; Hsie, A.W., et al., Mutation Res. 86: 193-214, 1981).

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James N. Rowe 11/31/89

John H.S. Chen 1/31/89

007200

DATA EVALUATION RECORD

I. STUDY TYPE: Dominant lethal (male mouse); EPA Guideline 84-2

STUDY TITLE: HWG 1608, Dominant lethal test on the male mouse to evaluate for mutagenic effects

Caswell #: 463P
Tox Branch Project: 8-1043
Document:
MRID No. 407009-50

SPONSOR: Mobay Corporation, Corporate Toxicology

TESTING LABORATORY: BAYER AG, Institute of Toxicology, FRG

STUDY NUMBER: BAYER AG report no. 94404

STUDY DATE: August 20,1986

STUDY AUTHOR(S): B.A. Herbold

TEST MATERIAL: HWG 1608; alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; 93.5% pure; batch no.16007/83

VEHICLE: 1% Cremophor emulsion

POSITIVE CONTROL: None

TEST ANIMAL: NMRI (SPF HAN) male and female mice

II. CONCLUSIONS:

Under the conditions of this assay, a single administration of terbuconzale (2000 mg/kg) to male NMRI mice, followed by 12 periods of mating with new, untreated virginal females, did not result in a significant increases in dominant lethality as indicated by pre-implantation losses and post-implantation losses.

Classification: Unacceptable

(Deficiencies found: lack of positive control; inadequate MTD and number of dosages used)

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III. MATERIALS AND METHODS:

A photocopy of the methods is appended. In summary, NMRI male mice (50/dose group) about 8-12 weeks old, were administered a single oral dose of 2000 mg/kg b.wt. terbuconazole or the same volume of vehicle (1% Cremophor emulsion) alone. After dosing, males were mated with a new untreated virginal females (1 male/1female) for twelve periods of four days each. During these 48 days, theoretically all the germ cell stages present in the male testicles at the time of exposure could be used for insemination and ferilization of eggs. No check was made for a vaginal plug. After approximately 14 days from mating, pre- and post-implantation losses (dead implants, viable implants, corpora lutea) were counted. Dead implants consisted of deciduomata, resorptions and dead fetuses.

The frequency distribution of the various parameters was compared in the control and treated groups with the non-parametric Kolgorov-Smirnov test. The number of dead implants and total implants (square root transformation), and the ratio of dead implants to total implants (angular transformation) were checked with the bifactorial Analysis of Variance. Significance was at the $p \leq 0.05$.

Signed and dated statements of confidentiality, GLPs and quality assurance were included.

The control was also used in testing another unspecified substance (p. 6 of report).

IV. RESULTS/DISCUSSION:

No clinical signs of compound-related toxicity were observed in the male mice after oral administration of terbuconazole. No mortalities were reported (p. 7 of report). Based upon the reported findings of death at 3000 mg/kg in the pilot study, and the high dosage used, an adequate treatment regimen was administered

A summary table of findings is presented below.

Mean fertilization rates were equivalent between the treated and control groups (77.8, 78.5%, respectively). Mean corpora lutea counts, implantations and viable implants were equivalent between the two groups (e.g., c.l., 12.9/control vs 12.8/treated). Mean pre-implantation losses/dam were slightly but not statistically significantly increased (apparently due to the variation in test findings) in the treated vs control animals (0.98, 0.81, respectively) while dead implants/dam were slightly increased over controls (0.87 vs 0.80; statistically significant by Kolmorov-Smirnov test but not by Analysis of variance). The range of mean dead implants during the 12 test periods was 0.61-1.11 in the controls vs 0.69-1.11 in the treated animals.

Summary table (taken from tables [3] 1-3)

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Parameter	Control	Treated
Fertilization (total #/%)	470(78.5)	467(77.8)
Corpora lutea	6423(13.7)[a]	6436(13.8)
Implantations@	6040(12.9)	5979(12.8)
Pre-implantation losses	383(0.81)	457(0.98)
Viable implants	5669(12.1)	5581(12.0)
Dead implants	375(0.80)	407(0.87)*

@ placenta with 2 embryos may be found at one implantation site, therefore the number of implantation sites may be smaller than the sum of live and dead implants

* statistically significant by Kolmogorov-Smirnov test (p= 0.0335) for total test but not individual tests and not by ANOVA

[a] total number (mean/fertilized female)

The evaluation of dominant lethality of HWG 1608 in mice cannot be accomplished due to the following deficiencies:

1. According to the acceptable procedure for performing the dominant lethal assay (EPA Health Effect Test Guidelines 560/6/83-001; Green, S.,Mutation Res. 154: 49-67, 1985), a concurrent positive control (i.e, triethylenemelamine or cyclophosphamide) should be included in each experiment. Lack of the positive control data in this study appeared difficult to ensure that the experimental conditions were properly maintained for conducting the in-vivo assay.

2. A dose-response relationship is a primary criterion used in the evaluation of test data, at least 3 dosages should be used in this study for proper documentation of negative data. In addition, the highest nonlethal dose must show some degree of toxicity.

3. Therefore, the study is unacceptable in the present form.

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Secondary Reviewer: John H.S. Chen, D.V.M.
Review Section I/HFASB/HED (TS-769C)

James N. Rowe 2/9/89
John H Chen 2/9/89

007200

DATA EVALUATION RECORD

I. STUDY TYPE: Micronucleus test; EPA Guideline 84-2

STUDY TITLE: Micronucleus test on the mouse to evaluate for mutagenic effect

Caswell #: 463P
Tox Branch Project: 8-1043
Document:
MRID No. 407009-51

SPONSOR: Mobay Corporation, Agricultural Chemicals Div.

TESTING LABORATORY: BAYER AG, Institute of Toxicology, FRG

STUDY NUMBER: 94529; BAYER AG report no. 13159

STUDY DATE: January 4, 1985

STUDY AUTHOR(S): Dr. B. Herbold

TEST MATERIAL: HWG 1608; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl) pentane-3-ol; 95.3% pure; Technical batch no.16007/83

VEHICLE: 1% Cremophor emulsion

POSITIVE CONTROL: Endoxan (Asta); active ingredient. cyclophosphamide

TEST ANIMAL: Male, female mice, strain Bor: NMRI(SPF HAN)

II. CONCLUSIONS:

There were no significant differences ($P<0.01$) in the micronucleated polychromatic erythrocyte frequencies between the treated animal groups and the corresponding solvent control groups. At all dose levels of HWG 1608 (i.e., 200 mg/kg at 48 hrs; 500 mg/kg at 24 & 72 hrs; 2000 mg/kg at 24 hrs), toxic bone marrow suppressive effects were in evidence as indicated by the shifts in the ratios of normo- to polychromatic erythrocytes. However, such increases in the proportion of NCE in the treatment groups were not sufficient to interfere with detection of any induced micronucleated polychromatic erythrocytes in this study. Therefore, Terbuconazole (HWG 1608; 200, 500, 2000 mg/kg) had negative response in the mouse micronucleus test at all of the intervals (24, 48, & 72 hrs) evaluated.

This study is designated as acceptable.

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III. MATERIALS AND METHODS:

A photocopy of the methods is appended. A summary of the methods is presented as follows.

Ten mice (5/sex) were randomly allocated to negative, positive control and test groups (200, 500, 2000 mg/kg). The test animals were then administered the respective regimens i.p. and sacrificed after 24, 48 and 72 hours by decapitation and femoral marrow was prepared. Smears were prepared by Schmid's method (Mut. Res. 31:9-15, 1975; Deutsche Forshungsgemeinschaft. Kommission fur Mutagenitatsfragen. Mitteilung III, 53-61, 1975).

One thousand polychromatic erythrocytes per animal were counted and the incidence of cells with micronuclei were determined. The ratio of polychromatic to normochromatic erythrocytes were noted since 1) pathological bone marrow depression not induced by the substance can be detected and omitted and 2) indications of general activity of the substance on bone marrow, i.e., erythropoiesis, can be gained. A ratio for a single animal amounting to more than 3000 normochromatic RBCs per 1000 polychromatic RBCs is regarded as pathological and not compound induced. Conclusions as to the general activity of the test substance on bone marrow are drawn only if the ratio for the majority of the animals in one group, compared with the negative control, is clearly reduced.

Statistical evaluation was performed with Wilcoxon's non-parametric rank sum test where appropriate.

Signed and dated copies of statements of data confidentiality, GLP compliance and quality assurance were included.

IV. RESULTS/DISCUSSION:

The maximum tolerated dose (MTD) selection was based on a preliminary pilot test in which three to five animals were orally administered with 5000 mg/kg, 3000 mg/kg, 2000 mg/kg, 1500 mg/kg, 1000 mg/kg, 500 mg/kg, and 250 mg/kg of the test substance. Although the treated mice did not show any external ill-effects after the oral administration of HWG 1608 at doses up to 2000 mg/kg, severe inhibitions of erythrocyte formation (insufficient no. of poly- and normochromatic erythrocytes for evaluation) were detected in the 2000 mg/kg dose group at the sampling times of 48 and 72 hrs.

A summary data table is presented below.

The micronucleus test was sensitive as evidenced by the consistent, statistically significant increase in mean micronuclei/polychromatic erythrocytes in the positive control, Endoxan/cyclophosphamide. There was a significant increase in the ratio of normo- to polychromatic RBCs at all dose levels administered, apparently indicating inhibition of erythropoiesis in the bone marrow. The MTD selected has demonstrated target cell cytotoxicity in this study.

The number of polychromatic RBCs was severely suppressed at 48 and 72 hours at 2000 mg/kg and 48 hours in the 500 mg/kg dose level and did not allow evaluation of the incidence of micronuclei. However, overall, there was no evidence of a significant increase in the incidence of micronucleated polychromatic RBCs in the treated vs the negative controls at any dosage and any time period.

Summary table (from Tables 14-16 of report)

Test grps	# evaluated polychrom. RBCs	# normochromatic RBC/1000 polychr.	micronucleated Cells per 1000 nrmochrom.	polychr.
2000 mg/kg				
neg.con.	10,000	726	1.0	1.1
HWG1608-1	10,000	2050*	1.5	1.9
HWG1608-2	69#	518#	2.7	not relevant
HWG1608-3	154#	500#	3.0	not relevant
pos.con.	10,000	771	2.1	12.1*
500 mg/kg				
neg.con.	10,000	637	1.7	2.2
HWG1608-1	10,000	2084*	1.8	2.5
HWG1608-2	57#	200#	2.5	not relevant
HWG1608-3	10,000	3886*	1.6	1.8
pos.con.	9,000	509	2.5	13.7*
200 mg/kg				
neg.con.	10,000	487	1.7	2.5
HWG1608-2	10,000	1640*	1.0	1.5
pos.con.	10,000	605	1.0	14.4*

* p</= 0.01 in Wilcoxon's non-parametric rank sum test
# insufficient number of poly- and normochromatic RBCs for evaluation at these time points
HWG1608 1,2,3 = 24, 48, 48 hrs., respectively

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Review Section I/HFASB/HED (TS-769C)
Secondary Reviewer: John H.S. Chen, D.V.M.
Review Section I/HFASB/HED (TS-769C)

DATA EVALUATION RECORD

I. STUDY TYPE: Sister chromatid exchange; EPA Guideline 84-2

STUDY TITLE: Sister chromatid exchange assay in Chinese hamster ovary (CHO) cells, Toxicology Report No. 953
Caswell #: 463P
Tox Branch Project: 8-1043
Document:
MRID No. 407009-52

SPONSOR: Mobay Corporation, Corporate Toxicology

TESTING LABORATORY: Microbiological Associates, Inc.

STUDY NUMBER: 94858; T5390.334

STUDY DATE: 9/3/87

STUDY AUTHOR(S): D.L. Putman

TEST MATERIAL: HWG 1608; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl) pentan-3-ol; off-white powder; 96.5% pure; stored at -20°C; Technical batch no.1616001/86

VEHICLE: DMSO

POSITIVE CONTROL: triethylenemelamine; cyclophosphamide

TEST ANIMAL: CHO cells (repository number CCL 61, American Type Culture Collection, Rockville, MD)

II. CONCLUSIONS:

CHO cells (+/-S9) were treated with terbuconazole at concentrations of 4 to 30 ug/ml without activation and 15 to 120 ug/ml with S9. There was no evidence of a increase in sister chromatid exchange from terbuconazole in either the presence or absence of rat liver microsomes.

The study is rated as acceptable.

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III. **MATERIALS AND METHODS:** (a photocopy of the experimental methods is attached)

A summary of the material and methods is presented below.

The CHO cell line was obtained from the American Type Culture Collection (Repository No. CCL 61). The cells were grown in Ham's F12 or McCoy's 5a medium supplemented with 10% fetal bovine serum, 100 units penicillin and 100 ug streptomycin per ml, and 2 mM glutamine.

The in-vitro metabolic activation system contained rat liver enzyme and a cofcator solution necessary for their function (i.e., S9, 15 ml; NADP, 1.4 mg/ml; isocitric acid, 2.7 mg/ml). The preparation of S9 from adult male Sprague-Dawley rats treated previously with Aroclor 1254 was based on the method described by Ames et al. (Mutation Res. 31; 347-364, 1975).

In order to establish the top dose to be used in the SCE assay, a preliminary toxicity test was performed. The test substance effects on cell growth potential and cell cycle delay were studied using 9 doses of HWG 1608 (0.1 thorugh 1000 ug/ml) in the presencc and absence of metabolic activation.

The CHO cells in Log phase of growth (5X105 cells/25 cm^2 flask) were exposed to 4 concentrations of the test substance (4, 8, 15, & 30 ug/ml without metabolic activation; 15, 30, 60, & 120 ug/ml with activation) for 30 hours and 2 hours in the absence and presence of metabolic activation, respectively, at 37° C in a humidified atmosphere of 5% CO_2 in air. After the exposure, the cells were washed with PBS, refed with complete medium containing 0.01 mM BrdUrd and returned to the incubator for an additional 30 hours. Colcemid was added to each flask at a final concentration of 0.01 ug/ml for the last 2 hours of incubation. The metaphase cells were collected by centrifugation, resuspended in 0.075 M KCl for 4-8 min at room temperature, and fixed with Carnoy's fixative. The fixed cells were centrifuged again to collect the cells and resuspended in Carnoy's fixative. Slides were prepared from the collected cells, air-dried, and stained for 10 min with Hoechst 33258 (5 ug/ml) in a pH 6.8 phosphate buffer and were exposed to U.V. light at 60° C for 4 minutes, and then counter stained with Giemsa.

At least 50 differently stained metaphases of the second cell cycle with BrdUrd-substitution were analyzed per dose and control group. The slides were also examined for the presence of delayed cells. One hundread metaphase cells were scanned and classified as M1, M2, and M3 from each dose and solvent controls to give an estimate of cell cycle inhibition.

Cytotoxicity of each treatment was expressed relative to the solvent-treated control (relative cell survival). The mean SCE/cell (+/-S.D.) was calculated for each duplicate treatment as well as the group mean for the treatment group. Dunnett's t-test was used for pairwise comparisons with the solvent control. The test article was considered positive if 1) it induced a doubling in SCE frequency over control at a minimum of two consecutive dose levels or 2) a dose responsive, statistically significant increase is observed over at least two dose levels.

Signed and dated statements of data confidentiality, compliance with GLP standards and quality assurance were included.

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IV. RESULTS/DISCUSSION:

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Cytotoxicity

Dose-ranging assays to determine terbuconazoles's cytoxicity were run at dose levels of 0.1 to 1000 ug/ml (+/-S9). In the assay without S9 signficant cytotoxicity was observed at dose levels of 30 ug/ml and greater with 22% relative cell growth (relative cell growth = mean cells/treatment flask x 100 divided by mean cells/solvent control) at the HDT of 1000 ug/ml. There was also a elimination of cells in second metaphase at these dose levels(e.g., DMSO: 14% in M1, 86% in M2 vs 100% in M1 at HDT).

In S9 activated assays, a significant decrease in cell growth was observed at doses of 100 ug/ml and greater (10% survival at HDT of 1000 ug/ml). The percentage of cells in metaphase 1 increased at 100 ug/ml and decreased in metaphase 2 while at higher doses no cells were observed in any stage of metaphase. Based upon these findings dose levels of 4.0, 8.0, 15 and 30 ug/ml were selected for -S9 assays and 15, 30, 60 and 120 ug/ml were selected for the +S9 assays.

SCE data summary

A table of SCE incidence (from Tables 3, 4 of the report) is presented below:

Treatment (-S9)	SCEs/chromosome	SCEs/cell(+/-SD)	Group mean SCEs/cell(+/-SD)
untreated	0.78	14.80(3.54)	14.76(3.63)
	0.77	14.72(4.13)	
DMSO	0.87	16.52(5.95)	16.00(5.14)
	0.81	15.48(4.60)	
4.0 ug/ml	0.66	12.68(4.72)	13.18(4.60)
	0.71	13.68(4.83)	
8.0 ug/ml	0.80	15.28(2.53)	14.94(3.59)
	0.77	14.60(4.72)	
15 ug/ml	0.83	15.68(5.96)	15.52(4.85)
	0.81	15.36(3.94)	
30 ug/ml	0.88	16.64(4.64)	16.62(4.42)
	0.87	16.60(4.62)	
TEM	5.58	105.44(12.99)	106.44(12.13)**
	5.71	107.44(11.73)	

(continued below)

(SCE summary table continued)

Treatment	SCEs/chromosome	SCEs/cell(+/-SD)	Group mean SCEs/cell(+/-SD)
(+S9)	0.80	15.16(5.54)	14.90(4.61)
untreated	0.76	14.64(3.95)	
DMSO	0.79	14.96(4.07)	15.98(5.38)
	0.89	17.00(6.61)	
15 ug/ml	0.76	14.48(6.15)	15.46(5.76)
	0.87	16.44(5.60)	
30 ug/ml	0.79	14.76(4.72)	15.80(5.01)
	0.88	16.84(5.46)	
60 ug/ml	0.81	15.28(4.14)	16.36(5.00)
	0.91	17.44(5.88)	
120 ug/ml	ND	ND	ND
	ND	ND	
CP	1.95	37.20(6.18)	36.44(6.22)**
	1.86	35.68(6.57)	

**p< 0.01, Student's t test; ND = no data; no metaphase cells available for SCE analysis due to cytotoxicity

The chromosomal SCE assay in CHO cells was responsive to a positive control in the presence or absence of S9 (TEM, cyclophosphamide) with statistically significant increases in mean number of SCEs/cell (106.44, 36.44, respectively).

Terbuconazole in the absence of rat liver microsomal enzyme did not produce an increase in group mean SCEs/cell at any dose tested (4.0 to 30 ug/ml). However, at the HDT, 30 ug/ml, there appeared to be an increase in metaphase 1 cells (e.g., replicate A, B: 68, 74/M1; 32, 26/M2 of HDT vs 10, 14/M1; 80, 82/M2 of DMSO control) as compared to M2 cells which is indicative of general cytoxicity (cell cycle kinetics from Table 3).

With microsomal activation (+S9), terbuconazole did not produce any evidence of an increase in SCEs at any dose level up to 60 ug/ml. At the HDT (120 ug/ml) there were no metaphase cells (no CHO cells) available for SCE analysic due to complete cytotoxicity. As with the -S9 assay, there was evidence of a delay in the progression of metaphase 1 to metaphase 2 cells at the next lowest dose, an indication of an intermediate cytotoxic response to total cell death (cell cycle kinetics from Table 4 of report).

A total of 50 second-division cells (M2), which were scored for the number of SCEs per dose and neagtive control groups at cell harvest time of 30-30 hrs from treatment initiation, are considered adequate for this study.

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Review Section I/HFASB/HED (TS-769C)
Secondary Reviewer: John H.S. Chen, D.V.M. John H.S. Chen 4/17/89
Review Section I/HFASB/HED (TS-769C)

DATA EVALUATION RECORD

I. STUDY TYPE: In vitro cytogenetics; EPA Guideline 84-2

STUDY TITLE: HWG 1608, In vitro cytogenetic study with human lymphocytes for the detection of induced clastogenic effects

Caswell #: 463P
Tox Branch Project: 8-1043
Document:
MRID No. 407009-53

SPONSOR: Mobay Corporation, Agricultural Chemicals Division

TESTING LABORATORY: BAYER AG, Fachbereich Toxicology, FRG

STUDY NUMBER: 95694, BAYER AG report no. 16395

STUDY DATE: February 2, 1988

STUDY AUTHOR(S): Dr. B.A. Herbold

TEST MATERIAL: HWG 1608; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl) pentan-3-ol; beige powder, mild odor; 96.5% pure; stored at 4°C; Technical batch no.1616001/86

VEHICLE: DMSO

POSITIVE CONTROL: mitomycin C(-S9); cyclophosphamide (+S9)

TEST ANIMAL: Human lymphocytes

II. CONCLUSIONS:

There was no evidence of mutagenicity at concentrations of 30-300 ug/ml terbuconazole in the presence of S9 mix in the human lymphocyte in vitro cytogenetics assay. While sufficient dose levels were used in the +S9 assay to produce significant cytotoxicity(inhibition of mitotic index), no cytoxicity was observed at any dose level (10-300 ug/ml) in the -S9 assay. The-S9 assay must be repeated at concentrations sufficient to produce significant cytoxity to adequately test terbuconazole's chromosomal effects.

The study with metabolic activation (+S9 mix) is acceptable but the study without metabolic activation (-S9 mix) is unacceptable and must be repeated.

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III. MATERIALS AND METHODS:

A photocopy of the methods is attached. A summary is presented below.

Human blood was drawn from one male and one female healthy donor. The cells were stimulated to devide by the plant lectin, phytohemagglutinin in the culture medium according to the method described by Moorehead et al. (Exp. Cell. Res. 20, 613-616, 1960).

The S9 fraction used for metabolic activation was derived from the livers of adult male Sprague-Dawley rats induced with Aroclor 1254. The S9 mix was prepared freshly according to the method described by Ames et al. (Mutation Res. 31, 347-364, 1975). Its composition per 100 ml was as follows: $MgCl_2$ X 6 H_2O, 271 mg; KCl, 410 mg; Glucose-6-phosphate disodium salt, 298.5 mg; NADP, 525 mg; Phosphate buffer 100 mM, 50 ml; and S9 fraction, 50 ml.

Each 48-hour human lymphocyte culture was exposed to the test compound for 24 hours at 37° C under the nonactivated test condition. In the case of assay with metabolic activation, S9 mix was added to the appropriate cultures with test compound for an exposure of 2.5 hours at 37° C. After the exposure, cells were washed and then reincubated in complete culture medium for further 21.5 hours. Three hours before termination, cell devision was arrested by the addition of colxemid (0.4 ug/ml) to each culture. The cells were swollen with hypotonic solution (0.56% KCl) at 37° C for 7 minutes, then fixed in ethanol:glycial acetic acid fixative (3:1 v/v), dropped onto clean slides, air-dried, and stained.

The mitotic index was determined by counting 1000 cells per culture and noting the number of mitotic and non-mitotic cells. The spare culture was used to enlarge the data base for this parameter. Approximately 200 metaphases per concentration (+/- S9) were examined for structural chromosome changes (approx. 100 metaphases per sex/test group). Aberration types included gaps, breaks, fragments, deletions, exchanges and multiple aberrations were determined (see appended methods for definitions) as well as chromosome disintegrations (1000x magnification using light microscope with planapochromatic lenses).

A one-sided corrected Chi test was used for statistical evaluation ($p<0.05$). A test was considered positive if there was a dose-dependent and statistically significant increase in the aberration rate.

Signed and dated statements of data confidentiality, GLPs and QAU are included.

IV. RESULTS/DISCUSSION:

1. The positive controls, Mitomycin C (0.15 ug/ml) and Cyclophosphamide (15 ug/ml) adequately demonstrated the sensitivity of the cultured lymphocyte system to detect a clastogenic effect.

2. The highest-dose level of HWG 1608 (300 ug/ml) under the activated assay system demonstrated cytotoxicity to deviding lymphocytes, resulting in reduction of mitotic index.

3. Although the preliminary assessment of cell cycle delay was not conducted in this study, the single harvest time (21 hours post-treatment; 72 hour-total culture time) for cells exposed to the test material appeared adequate for the detection of chromosomal aberrations in cultured human lymphocytes.

4. In the experiments with metabolic activation, HWG 1608 was not clastogenic in the cultured lymphocytes at 300 ug/ml (at the toxic level). This result is considered to be acceptable. Since the highest dose level (300 ug/ml) used in the experiments without metabolic activation did not demonstrate any cytotoxicity to the human lymphocytes, the study under the non-activated system is unacceptable in the present form and must be repeated.

Mitotic index (from Table 1, p.22 of report)

Dose group(ug/ml)	+/- S9#	absolute #	% of control
0.0	-	48	100.0
3.0	-	84	175.0
10.0	-	96	200.0
30.0	-	55	114.6
Mitocmycin C (0.15)	-	26	52.2*
0.0	+	88	100.0
30.0	+	88	100.0
100.0	+	56	63.6*
300.0	+	only cell fragments found	
Cyclophosphamide (15.0)	+	46	52.3*

4000 nuclei evaluated; * p</= 0.01 in Chi2 test

Summary of chromosomal aberration data# (Tables 4, 5)

	aber.incl. gaps	aber. excl. gaps	exchanges	polyploid@
-S9				
0 ug/ml	14(7.0)^	7(3.5)	0(0)	1(0.3)
3 " "	12(6.0)	7(3.5)	0(0)	0(0.0)
10 " "	8(4.0)	4(2.0)	0(0)	1(0.3)
30 " "	11(5.5)	7(3.5)	0(0)	0(0.0)
MMC .15"	109*(54.5)	67*(33.5)	12*(6.0)	1(0.3)
+S9				
0 ug/ml	22(11.0)	12(6.0)	0(0)	0(0)
30 " "	24(12.0)	8(4.0)	0(0)	0(0)
100 " "	14(7.0)	4(2.0)	0(0)	0(0)
300 " "	only cell fragments found			
CYCL 15"	82*(41.0)	53*(26.5)	8*(4.0)	0(0)

200 metaphases evaluated per dose; ^ = n (%); @ per 300-400 metaphases; * p<0.01 in Chi2 test

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Secondary Reviewer: John H.S. Chen, D.V.M. John H.S. Chen 2/7/89
Review SectionI/HFASB/HED (TS-769C)

DATA EVALUATION RECORD

I. STUDY TYPE: DNA damage and repair; EPA Guideline 84-4

STUDY TITLE: HWG 1608, POL test on E.coli to evaluate for harmful effects on DNA

Caswell #: 463P
Tox Branch Project: 8-1043
Document:
MRID No. 407009-55

SPONSOR: Mobay Corporation, Agricultural Chemicals Division

TESTING LABORATORY: BAYER AG, Institute of Toxicology, FRG

STUDY NUMBER: BAYER AG report no. 94556

STUDY DATE: July 1,1983

STUDY AUTHOR(S): Dr. B.A. Herbold

TEST MATERIAL: HWG 1608; 1-(4-chlorophenyl)ethyl)-4,4-dimethyl-3-(1,2,4-triazol-1-yl-methyl) pentane-3-ol; 97.1% pure; batch no.16001/83

VEHICLE: DMSO

POSITIVE CONTROL: methyl methane sulphonate

NEGATIVE CONTROL: chloramphenicol

TEST ANIMAL: two mutants of E. coli (k 12)p 3478; W 3110)

II. CONCLUSIONS:

There was no evidence of any inhibition of bacterial growth in either the -/+ DNA repair E. coli at any dose tested (625-10000 ug/plate) with or without S9 mix. Therefore terbuconazole does not produce an alteration in DNA and is not mutagenic under the conditions of this test.

This study is designated as unacceptable since no evidence is presented that quality assurance was performed on the study (Other deficiencies are described in the detailed review).

III. MATERIALS AND METHODS:

A photocopy of the methods is appended. A summary of the methods is presented below.

DNA-damaging capacity of HWG 1608 was investigated by the PolA assay using two strains of Escherichia coli, P3478 (repair deficient strain) and W3110 (repair proficient strain) according to the method described by Rosenkranz et al. (In: Chemical Mutagens, Vol. 6: 109, Plenum Press, New York and London 1980). Both overnight cultures of these two strains of E. coli were transferred to a pair of plates containing 5 ml of medium. The substance was placed on small around filter papers, whcih were then placed on nutrient agar plates already containing soft agar including bacteria, and where necessay S9 mix also (S9 mix was prepared according to the method described by Ames et al., Mutation Res. 31: 347. 1975). The plates were incubated at 37° C. overnight (24 hrs) and then the diameter of growth inhibition zone (mm) was measured (4 plates per dose used; 625, 1250, 2500, 5000, & 10000 ug/plate). Positive (MMS) and negative (chloramphenicol) controls were run concurrently with the test substance. A Reproducible increase in difference in inhibition zone diameters between the two strains of over +2 mm is considered positive.

Signed and dated statements of data confidentiality and GLPs, but not QAU, were included.

IV. RESULTS/DISCUSSION:

Copies of data Table 1 and 2 are attached (Attachement 1, 2). The assay system was responsive to the positive control, MMS, producing mean differential inhibition zones of +16.9 and +11.5 mm with or without S9 mix activation, respectively, in the DNA repair deficient bacterial strain. The negative control, chloramphenicol, produced no increased inhibition in the deficient strain of E. coli, rather it seemed to produce a small increase in the inhibition zone of the normal repair strain of approximately 5-7 mm (+/-S9 mix).

However, the study is judged inadequate due to the following reporting deficiencies:

1. Because of the poorly diffusable property of the test substance dissolved in DMSO, the test substance did not yield interpretable results (i.e., no growth inhibition zone was demonstrated either in the repair proficient strain (W3110), or in the deficient strain (P3478) at any dose tested), the test substance should be further evaluated in the modified liquid suspension method recommended by Rosenkranz et al. (1980).

2. The bacterial cell densities for both strains of E. coli were not reported in this study. According to the acceptable procedure for performing the Pol A assay, both of the repair proficient bacteria or the repair deficient bacteria should be standardized to equally desired density of viable cells prior to testing. (Rosenkranz 1980).

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Secondary Reviewer: John H.S. Chen, D.V.M. John H S Chen 2/7/89
Review Section I/HFASB/HED (TS-769C)

DATA EVALUATION RECORD

I. STUDY TYPE: Unscheduled DNA synthesis (in vitro); EPA Guideline 84-4

STUDY TITLE: Mutagenicity test on HWG 1608 Techn. in the rat primary hepatocyte unscheduled DNA synthesis assay

Caswell #: 463P
Tox Branch Project: 8-1043, 8-1218
Document:
MRID No. 408164-02

SPONSOR: Mobay Corporation, Agricultural Chemicals Division

TESTING LABORATORY: Hazleton Laboratories America, Inc.

STUDY NUMBER: 94988; HLA Study no. 9569-0-447; Sponsor study no. T5024090

STUDY DATE: August 10, 1988

STUDY AUTHOR(S): Maria A.Cifone, Ph.D.

TEST MATERIAL: HWG 1608; alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; pale yellow granules; 96.5% pure; stored at 4°C; Technical batch no.1616001/86

VEHICLE: DMSO

POSITIVE CONTROL: 2-acetyl aminofluorene

TEST ANIMAL: Hepatocytes obtained from an adult male Fischer 344 rat purchased from Charles River Breeding Laboratories, Inc.

II. CONCLUSIONS:

There was a dose-related increase in cytotoxicity in the test compound with around 50% cytoxicity at the highest concentration level of 25 ug/ml and 100% survival below 2.52 ug/ml. Thus an acceptable range of toxicity to adequately challenge the test system was demonstrated. There was no evidence at any concentration of an increase in unscheduled DNA synthesis relative to the solvent control.

This study is designated as acceptable.

III. MATERIALS AND METHODS:

Hepatocytes were obtained from an adult male Fischer 344 rat (150-300 g) by perfusion of the liver in situ with a collagenase solution as described by Williams (Cancer Res., 37: 1845-1851, 1977; In: Chemical Mutagens Vol.6, pp. 61-79, Plenum Press, NY, 1980). Monolayer cultures were established on plastic coverslips in culture dishes containing Williams' medium E supplemented with 5% fetal bovine serum, 2mM L-glutamine, 2.4 uM dexamethasone, 90 u/ml penicillin, 90 ug/ml streptomycin sulfate, and 140 ug/ml gentamicin. All cultures were maintained as monolayers at about 37° C in a humidified atmosphere containing approximately 5% CO_2. The negative control was 1% DMSO in culture medium; the positive control was 2-acetylaminofluorene at 4.48 X 10^{-7} M (0.1 ug/ml).

A range of 15 concentrations (0.025 -1000 ug/ml) was applied initially to the hepatocytes. A viable cell count (trypan blue exclusion method) was obtained about 24 hours after initiation of the treatments. Based on the results of this preliminary toxicity test, treatments above 25.2 ug/ml were lethal. Therefore, the following 6 concentrations were chosen for the UDS assay: 0.504, 1.01, 2.52, 5.04, 10.1, and 25.2 ug/ml (25.2 ug/ml = 55.8% survival; 2.52 ug/ml = 100.9% survival).

The UDS assay was initiated within 3 hours by replacing the media in culture dishes with 2.5 ml WME containing 1% fetal bovine serum, 1 uCi/ml ^{3}H-thymidine and the test material at the desired concentartions. After treatment for 18 to 19 hours, the UDS assay was terminated by washing the cell monolayers twice with WME. Two of the cultures from each treatment were used to monitor toxicity and three other cultures per treatment were swelled in 1% sodium citrate solution, and fixed with a solution of ethanol:acetic acid (3:1). The coverslips were mounted on glass slides (cells up), dipped in an emulsion of Kodak NTB-2 and dried. The coverslips were then stored at 4° C in light-tight box for 7 to 10 days. The emulsions were developed in D19, fixed and stained with Williams" modified hematoxylin and eosin procedure.

UDS was quantified by counting the number of nuclear grains in the nucleus and substracting the average background count from three nucleus-size areas of cytoplasm adjacent to each nucleus. The net number of nuclear grains was determined in 50 randomly selected cells of each coverslip. The mean net nuclear count was determined from triplicate coverslips (150 total nuclei). The test material is considered active in the UDS assay at applied concentrations that cause (p. 16 of report 94988) the following:

1) an increase in the mean net nuclear grain count to at least six grains/nucleus after substraction of background,

2) an increase in the percent of nuclei having six or more net grains to at least 10% of the analyzed population after substraction of the concurrent negative control value, and/or,

3) the percent of nuclei with twenty or more grains to reach or exceed 2% of the analyzed population.

A dose-related increase in UDS for at least two consecutive applied concentrations is also considered.

Dated and signed statements of data confidentiality, GLP compliance and QAU were included.

IV. RESULTS/DISCUSSION:
A summary table (taken from table 1 of the report) is presented below:

Test material	UDS grains/ nucleus*	% nuclei** (>=6 grains)	% nuclei** (>=20 grains)	% sur-vival#
DMSO(1%)	0.99	2.7	0.0	100.0
2-AAF (.1UG/ML)	11.55	78.0	12.0	84.1
HWG 1608				
25.2 ug/ml	0.79	2.0	0.0	55.8
10.1 ug/ml	1.05	2.7	0.0	79.0
5.04 ug/ml	1.10	2.0	0.0	95.6
2.52 ug/ml	0.77	2.0	0.0	100.9
1.01 ug/ml	0.93	2.0	0.0	104.1
0.504 ug/ml	0.83	2.0	0.0	97.5

* UDS = average net nuclear grain counts on triplicate coverslips (150 total cells)
** average values for triplicate coverslips
# survival = number of viable cells per unit area relative to the solvent control x 100%
Mean cytoplasmic grain count for solvent controls = 6.35

There was a significant increase in total mean UDS grains/nucleus and in the average percent nuclei with >= 6 or 20 grains in the positive control , 2-AAF, indicating the responsiveness of the bioassay to mutagenic alterations.
There was a dose-related increase in cytotoxicity in the test compound with around 50% cytoxicity at the highest concentration level of 25 ug/ml and 100% survival below 2.52 ug/ml. This is an acceptable range of toxicity. There was no evidence at any concentration of an increase in inscheduled DNA synthesis relative to the solvent control.

Since the nuclear labeling in the negative (solvent) control was also found within the normal range of net nuclear grain count per nucleus for performing the rat hepatocyte UDS assay (Williams 1980; less than 1 net nuclear grain/nucleus), this study is acceptable.