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Contractor			
Document Title		INITIAL SUBMISSION: DEVELOPMENTAL TOXICITY STUDY WITH MKH 3586 TECHNICAL IN THE SPRAGUE-DAWLEY RAT WITH TSCA HEALTH & SAFETY STUDY COVER SHEET DATED 07/20/99	
Chemical Category		MKH 3586 TECHNICAL	

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24843

TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

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1.0 SUBMISSION TYPE - Contains CBI <input type="checkbox"/> 8(d) <input checked="" type="checkbox"/> 8(e) <input type="checkbox"/> FYI <input type="checkbox"/> 4 <input type="checkbox"/> OTHER: Specify <u>8EHQ-0799-14516</u> XX- Initial Submission - Follow-up Submission <input type="checkbox"/> Final Report Submission Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any: # _____ <input type="checkbox"/> continuation sheet attached		
2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(c); optional for §4, 8(d) & FYI) <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID P917-006-921 99-2-56	2.3 FOR EPA USE ONLY
3.0 CHEMICAL/TEST SUBSTANCE IDENTITY - Contains CBI <i>Reported Chemical Name (specify nomenclature if other than CAS name):</i> CAS# 129909-90-6 <u>IH-1,2,4-Triazole-1-carboxamide, 4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-2-(1-methylethyl)-5-oxo-</u> Purity <u>98</u> +% <input type="checkbox"/> Single Ingredient <input type="checkbox"/> Commercial/Tech Grade <input type="checkbox"/> Mixture Trade Name: <u>MKH 3586</u> <i>Technical</i> Common Name: _____ CAS Number: _____ NAME: _____ % WEIGHT: _____ Other chemical(s) present in tested mixture: _____ <input type="checkbox"/> continuation sheet attached		
4.0 REPORT/STUDY TITLE - Contains CBI <u>Developmental Toxicity Study in the Sprague-Dawley Rat - Study #: 96-612-KJ, AC# 108653</u> <input type="checkbox"/> continuation sheet attached		
5.1 STUDY/TSCATS INDEXING TERMS (CHECK ONE) HEALTH EFFECTS (HE): <input checked="" type="checkbox"/> ENVIRONMENTAL EFFECTS (EE): _____ ENVIRONMENTAL FATE (EF): _____ 5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes) STUDY TYPE: _____ SUBJECT ORGANISM (HE, EE only): <u>RAT</u> ROUTE OF EXPOSURE (HE only): _____ VEHICLE OF EXPOSURE (HE only): _____ Other: <u>Developmental Tox</u> Other: _____ Other: _____		
6.0 REPORT/STUDY INFORMATION <input type="checkbox"/> Contains CBI <input checked="" type="checkbox"/> Study is GLP Laboratory: <u>Agriculture Division Tox Lab</u> Report/Study Date: <u>7/8/99</u> Source of Data/Study Sponsor (if different than submitter): <u>Bayer</u> Number of pages: <u>582</u> <input type="checkbox"/> continuation sheet attached		
7.0 SUBMITTER INFORMATION <input type="checkbox"/> Contains CBI Submitter: <u>Donald W. Lamb, Ph.D.</u> Title: <u>Vice President, Product Safety & Reg. Affairs</u> Phone: <u>412-777-7431</u> Company: <u>Bayer Corporation</u> Address: <u>100 Bayer Road, Pgh, PA 15205-9741</u> Technical Contact: <u>Same as above</u> Phone: () _____ <input type="checkbox"/> continuation sheet attached		
8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS <input type="checkbox"/> Contains CBI <u>This compound is an experimental herbicide</u> <input type="checkbox"/> continuation sheet attached		

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Submitter Signature: Donald W. Lamb

Date: 7/20/99

9.0 CONTINUATION SHEET

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CONTINUED FROM COVER SHEET SECTION # 2.1

The increase in the incidence of non-viable fetuses and the statistically significant decrease in fetal weight in the high-dose group, and the skeletal findings, primarily incompletely ossified bones, observed in the mid- and high-dose groups were associated with statistically significant reductions in body weight gain and food consumption. Therefore, the abnormal fetal findings were probably due to maternal toxicity, and not due to a direct effect of MKH 3586 on the fetuses. However, despite the fact that the fetal findings were probably due to the maternal toxicity of MKH 3586, these results are being reported.

Abstract

This study assessed the potential for MKH 3586 Technical to promote gestational effects and/or alter the growth and development of the conceptus. Inseminated dams were administered nominal doses of 0, 15, 100, or 300 mg MKH 3586/kg body weight (analytically confirmed doses of 0, 13.4, 103.3, and 314.0 mg MKH 3586/kg, respectively) by gavage on days 6 through 19 of gestation. Maternal toxicity, as demonstrated by clinical signs and changes in body weight gain and food consumption during gestation, was characterized. All dams were sacrificed on gestation day 20, at which time gross external and internal necropsies were performed, the uterus was examined, and the fetuses were removed by cesarean section. All fetuses were evaluated for external anomalies. Approximately half of each litter was examined for visceral effects, and the other half underwent a skeletal (including cartilage) examination.

Test compound-related maternal effects were observed in the 100 and 300 mg/kg dose groups. Gravid dams in the 300 mg/kg dose group demonstrated statistically significant decreases in body weight gain, food consumption, net body weight, and net body weight gain (subtraction of gravid uterus). Additionally observed at the high-dose was a statistically significant increase in the relative and absolute liver weights. Maternal effects observed in the 100 mg/kg dose group were statistically significantly decreased body weight gain, net body weight, food consumption, and a statistically significant increase in absolute liver weight. No test compound-related maternal findings were noted in the dams in the 15 mg/kg dose group. Effects observed at cesarean section were limited to the 300 mg/kg dose group and included an increase in the incidence of non-viable fetuses and a statistically significant decrease in fetal weight. No test compound-related effects were noted during fetal external or visceral examinations. Test compound-related fetal skeletal findings, primarily incompletely ossified bones, were observed in the 100 and 300 mg/kg dose groups. No effects on the incidence of total malformations or variations, or any gender-related differences in fetal effects were observed in any dose group.

MKH 3586, administered as described in this study, produced maternal toxicity at doses of 100 and 300 mg/kg body weight. No test compound-related maternal effects were demonstrated in the 15 mg/kg dose group. Developmental effects were observed primarily in the 300 mg/kg dose group and to a lesser extent in the 100 mg/kg dose group. Based on the observation of developmental effects only at dose levels that produced maternal toxicity, the developmental findings are considered secondary to maternal toxicity. Both the maternal and developmental no-observed-effect-levels (NOEL) were 15 mg/kg.

A 05

Agriculture Division
Report Number
108653

**A Developmental Toxicity Study with
MKH 3586 Technical in the Sprague-Dawley Rat**



Data Requirements

US EPA OPPTS 870.3700
OECD Testing Guideline 414
EU 87/302/EEC
Health Canada Part II, Vol.122, No. 2
Japanese MAFF, 59 NohSan No. 3850

Authors

A. Barry Astroff and Angela D. Young

Study Completion Date

July 8, 1999

Test Facility

**Bayer Corporation
Agriculture Division
Toxicology
17745 South Metcalf
Stillwell, Kansas 66085-9104**

Study Number

96-612-KJ

Contain NO CBI

A 06

Bayer Corporation
95-612-KJ

Agriculture Division
Report Number
108653

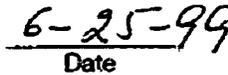
STATEMENT OF DATA CONFIDENTIALITY

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FOIA Section 10(d)(1)(A), (B), or (C).

Company: Bayer Corporation, Agriculture Division

Company Agent: J. H. Thyssen, Vice-President, Toxicology


Signature


Date

A 07

Bayer Corporation
95-612-KJ

Agriculture Division
Report Number
108653

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the FIFRA Good Laboratory Practice Standards of 40 CFR Part 160, the OECD Principles of Good Laboratory Practice, GD(92)32 (Paris, 1992), and the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) "Good Laboratory Practice Standards" (59 NohSan No. 3350).

SUBMITTER

BAYER CORPORATION

J. H. Thyssen:


Vice President, Toxicology
Agriculture Division

Date:

6-24-99

SPONSOR

AGRICULTURE DIVISION

J. H. Thyssen:


Vice President, Toxicology

Date:

6-24-99

STUDY DIRECTOR

A. B. Astroff:


Senior Research Scientist

Date:

7-8-99

A 08

Bayer Corporation
96-612-KJ

Agriculture Division
Report Number
108653

FLAGGING STATEMENT

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

SUBMITTER

BAYER CORPORATION

J. H. Thyssen:


Vice President, Toxicology
Agriculture Division

Date:

6-24-99

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TEST FACILITY

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STUDY DIRECTOR

Barry Astroff, PhD
Toxicology Department
Agriculture Division
Bayer Corporation
17745 South Metcalf
Stilwell, Kansas 66085-9104

DATES

Initiation of mating (first day of co-housing): September 22, 1997
Experimental initiation (first day of dosing): September 29, 1997
Termination (last day of necropsy): October 30, 1997

PERSONNEL AND RESPONSIBILITIES

Toxicology and Sponsor
Representative:

J. H. Thyssen

Toxicology Laboratory:

G. K. Sangha

Study Director and
Report Author:

A. B. Astroff

Report Author:

A. D. Young

Study Conduct:

D. E. Phipps

L. M. Gonzalez

Pathology Services:

B. P. Stuart

Animal Care:

R. E. Mueller

Analytical Chemistry:

K. D. Moore

Quality Assurance:

D. M. Wallace

QUALITY ASSURANCE STATEMENT

Audit reports have been submitted to the study director and laboratory management documenting the status of compliance with applicable departmental standard operating procedures, the study protocol, and Good Laboratory Practice regulations.

The Quality Assurance Unit monitored selected phases of this study, and at least annually functions of all support areas for this study type. The following are the audit dates, phases inspected, auditors, and report dates of quality assurance inspections of this study and, if applicable, of this study type as well as relevant support areas:

AUDITS			REPORT TO STUDY DIRECTOR AND MANAGEMENT
<u>Date</u>	<u>Phase of Study</u>	<u>Auditor</u>	
12/04/96	Protocol Review	C.A. Cox	12/04/96
09/15/98	Animal Shipment Receipt and Exam, Feeding, Cage I.D., Animal Inventory	T.L. Bormann	09/26/97
09/22/97	Animal Quarantine Release for Study	T.L. Bormann	10/02/97
09/22/97	Sacrificing Animals Outside the Body Weight Mean	T.L. Bormann	10/03/97
09/22/97	Animal ID/Tattoo	T.L. Bormann	10/02/97
09/22/97	AM Mortality Check, Cage Cards, Animal ID, Initial Body Weights	T.L. Bormann	10/02/97
09/23/97	AM Vaginal Smears, Transfer of Sperm Positive Females, Tail Marking, Randomizing into Dose Groups, Body Weights, Food Consumption, Observations	T.L. Bormann	10/02/97
09/23/97	Co-Housing	T.L. Bormann	10/02/97
09/24/97	Dosing Vehicle Preparation	T.L. Bormann	11/04/97
09/25/97	Dosage Preparation	T.L. Bormann	11/04/97
09/30/97	Oral Dosing of Animals, Clinical Observations	T.L. Bormann	10/07/97

QUALITY ASSURANCE STATEMENT (Continued)

AUDITS			REPORT TO STUDY DIRECTOR AND MANAGEMENT
<u>Date</u>	<u>Phase of Study</u>	<u>Auditor</u>	
10/17/97	Euthanasia, External Exam & Gross Necropsy of Dams; Corpora Lutea Counts, Organ Weights, Noting/Characterization of Implantations and Resorptions; Counting, Sexing, & Body Weight Collection of Fetuses; Placental Weights, External Exams, Euthanasia, Gross Necropsy, Tagging/ID, Visceral Exams, & Fetal Storage	T.L. Bormann	11/05/97
10/22/97	Fetal Evisceration	T.L. Bormann	11/04/97
10/30/97	Terminal Body Weight	T.L. Bormann	11/14/97
02/03/98	Wilson's Technique	T.L. Bormann	02/19/98
04/28/98	Fetal Processing	C.A. Yount	04/28/98
05/05/98	Skeletal Exam (Bone and Cartilage)	T.L. Bormann	05/05/98
04/17/98	Staining Solution Preparation	T.L. Bormann	04/29/98
04/17/98	Preparation of Alcian Blue and Alizarin Red S Stock Solutions	T.L. Bormann	04/29/98
04/20/98	Macerating Solution Preparation	T.L. Bormann	04/30/98
04/23/98	Holding Solution Preparation	T.L. Bormann	04/30/98
12/1/98- 01/28/99	Final Report Review	T.L. Bormann C.A. Yount	01/28/99
	<u>Phase of Study Type</u>		
05/07/98	Changing Bouin's Solution to Alcohol	T.L. Bormann	05/11/98

QUALITY ASSURANCE STATEMENT (Continued)**AUDITS****REPORT TO
STUDY
DIRECTOR AND
MANAGEMENT**

<u>Date</u>	<u>Analytical Chemistry Support Area Functions</u>	<u>Auditor</u>	
06/13/97	Training Records Review	L.A. Berry	06/13/97
09/25/97, 09/30/97	Analysis for Concentration in Dose Solution	L.A. Berry	09/30/97
12/23/97	Analytical Balance Audit	L.A. Berry	12/23/97
12/30/97	Freezer and Refrigerator Temperature Logbook and Records	L.A. Berry	12/31/97
12/30/97	1997 Chromatographic, Scintillation Counter, and Oxidizer Maintenance Logbook and Records	L.A. Berry	12/31/97
<u>Animal Care Support Area Functions</u>			
01/08/97	Rat Room Filter Check/Change	C.A. Cox	01/09/97
01/08/97	Rat Cage Rack Preparation (Wire Mesh)	C.A. Cox	01/09/97
01/08/97	Cage Rack Preparation (Polycarbonate)	C.A. Cox	01/09/97
01/27/97	Rat Bedding Change (DACB) & Room Maintenance	C.A. Cox	01/27/97
01/28/97	Rat Room Disinfection	C.A. Cox	01/28/97
01/28/97	Polycarbonate Rat Rack/Bedding Change	C.A. Cox	01/30/97
02/19/97, 02/20/97	Cleaning of Rat Wire Mesh Racks, Cages, & Trays	C.A. Cox	02/20/97
02/19/97, 02/20/97	Cleaning of Polycarbonate Racks & Cages	C.A. Cox	02/20/97
03/19/97	Wire Mesh Rat Rack Change	C.A. Cox	03/19/97
05/19/97	Training Records Review	C.A. Yount	05/19/97

QUALITY ASSURANCE STATEMENT (Continued)**AUDITS****REPORT TO
STUDY
DIRECTOR AND
MANAGEMENT**

<u>Date</u>	<u>Animal Care Support Area Functions (cont'd)</u>	<u>Auditor</u>	
07/23/97	Data Review of Water Analysis	C.A. Yount	07/23/97
07/23/97	Data Review of Vermin Control	C.A. Yount	07/23/97
08/05/97	Edstrom Water Filter Changes/Checks & Sanitizing of the Lines	C.A. Yount	08/07/97
12/09/97	Equipment Maintenance (Cage Wash Repair)	C.A. Yount	12/09/97

In compliance with the Good Laboratory Practice regulations, this final report for study number 96-612-KJ has been reviewed by the Quality Assurance Unit. The results presented in this report accurately describe the methods and standard operating procedures and reflect the raw data collected during the conduct of the study.


C.A. Yount, RQAP-GLP, Quality Assurance

6/22/99
Date

ABSTRACT

This study assessed the potential for MKH 3586 Technical to promote gestational effects and/or alter the growth and development of the conceptus. Inseminated dams were administered nominal doses of 0, 15, 100, or 300 mg MKH 3586/kg body weight (analytically confirmed doses of 0, 13.4, 103.3, and 314.0 mg MKH 3586/kg, respectively) by oral gavage on days 6 through 19 of gestation. Maternal toxicity, as demonstrated by clinical signs and changes in body weight gain and food consumption during gestation, was characterized. All dams were sacrificed on gestation day 20, at which time gross external and internal necropsies were performed, the uterus was examined, and the fetuses were removed by cesarean section. All fetuses were evaluated for external anomalies. Approximately half of each litter was examined for visceral effects, and the other half underwent a skeletal (including cartilage) examination.

Test compound-related maternal effects were observed in the 100 and 300 mg/kg dose groups. Gravid dams in the 300 mg/kg dose group demonstrated statistically significant decreases in body weight gain, food consumption, net body weight, and net body weight change (subtraction of gravid uterus). Additionally observed at the high-dose was a statistically significant increase in the relative and absolute liver weights. Maternal effects observed in the 100 mg/kg dose group included statistically significantly decreased body weight and food consumption, and a statistically significant increase in absolute liver weight. No test compound-related maternal findings were noted in the dams of the 15 mg/kg dose group. Effects observed at cesarean section were limited to the 300 mg/kg dose group and included an increase in the incidence of nonviable fetuses and a statistically significant decrease in fetal weight. No test compound-related effects were noted during fetal external or visceral examinations. Test compound-related fetal skeletal findings, primarily incompletely ossified bones, were observed in levels II and III. No effects on the incidence of total malformations or variations, or any gender-related differences in fetal effects were observed in any dose group.

MKH 3586, administered as described in this study, produced maternal toxicity at doses of 100 and 300 mg/kg body weight. No test compound-related maternal effects were demonstrated in the 15 mg/kg dose group. Developmental effects were observed primarily in the 300 mg/kg dose group and to a lesser extent in the 100 mg/kg dose group. Based on the observation of developmental effects only at dose levels that produced maternal toxicity, the developmental findings are considered secondary to maternal toxicity. Both the maternal and developmental no-observed-effect-levels (NOEL) were 15 mg/kg.

MATERIALS**I. The test substance was supplied by the sponsor with the following information:****Test Substance:**

Identification: MKH 3586 Technical

Physical Appearance: White powder

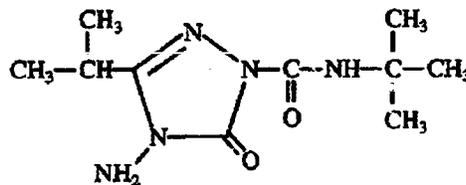
Batch No.: 05262/0005

Purity: 98.5% (date of analysis: April 14, 1997)
98.2% (date of analysis: October 17, 1997)
96.1% (date of analysis: April 27, 1998)

Active Ingredient:

Common Name: MKH 3586

Chemical Name: 4-Amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide

Structural Formula:

CAS No.: 129909-90-6

II. The test substance was stored and archived at freezer conditions.

PURPOSE

The purpose of this study was to assess the potential for MKH 3586 Technical, administered by oral gavage to gravid Sprague-Dawley rats on days 6 through 19 of gestation, to promote gestational effects and/or alter the growth and development of the conceptus.

GUIDELINES

This report was prepared in accordance with US EPA Health Effects Guideline OPPTS 870.3700, Prenatal Developmental Toxicity Study, August, 1998; the OECD Guidelines for Testing of Chemicals, Section 4: Health Effects, Subsection 414, "Teratogenicity", pages 1-6, May, 1981; EU guidelines on Teratogenicity Studies, in the Official Journal of the European Communities, 87/302/EEC, February, 1995; Health Canada, Canada Gazette, Part II, Vol. 122, No. 2, January, 1988; and Japan, Ministry of Agriculture, Forestry, and Fisheries (MAFF), Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration, 59 NohSan No. 4200, January, 1985.

METHODS

Animal Information

Source, Number, Age and Test System Rationale

As described in the protocol (Appendix I, page 96), the study design required 120 young adult female (nulliparous and nonpregnant) and 30 sexually mature male Sprague-Dawley rats (Sasco Inc., Kingston, NY). Females and males were not previously treated and were approximately 12-15 weeks of age at the beginning of the study. Rats were used as the test system as they are one of the US EPA, OECD, EU, and Japanese MAFF suggested species for developmental toxicity studies.

Examination and Acclimation

Upon receipt, animals were examined by trained personnel, and those animals considered acceptable were then placed into individual cages and acclimated to their ambient laboratory conditions for at least six days prior to study initiation. For the holding period, animal care personnel observed the animals at least once daily for moribundity and mortality. Moribund animals were sacrificed if their condition dictated. Necropsies were not required on animals that were either found dead or were sacrificed in a moribund condition at any time prior to initiation of the study. With completion of the acclimation period, a veterinarian reviewed the status of the animals prior to their being release for the study.

Care and Housing

Male rats, and females prior to being declared sperm positive, were individually housed in suspended stainless steel cages. Sperm positive females were housed individually in plastic cages with corn cob bedding. The room, stainless steel cages, and cage racks were thoroughly cleaned and disinfected before arrival of the animals. Deotized Animal Cage Board was used in the bedding trays and changed at least three times weekly. The stainless steel cages and racks were replaced at least once every two weeks with clean, disinfected cages and racks. Clean plastic cages were provided every week and bedding was changed at least twice weekly. The room was disinfected at least once every two weeks. Municipal tap water and Purina Mills Rodent Lab Chow 5001-4 was provided for ad libitum consumption. Water and feed are periodically analyzed for possible contaminants and the results are compared to the allowable limits in "Lab Chows Animal Diet Reference Guide" (Publication SP2437M-87010 dated 1987) from Purina Co., St. Louis, Missouri. The animal room was maintained at 18 to 26 °C with 30 to 70% relative humidity and a 12-hour photoperiod. As noted in protocol deviation 2 (see Appendix I) on several occasions there were variations in the light cycle, these did not appear to have had any effect during the study and are not expected to adversely affect the interpretation of the results.

Identification

Males were identified by cage card and tail marking. Females prior to being declared sperm positive were identified by cage card and tattoo. Once declared sperm positive, females were additionally identified by tail marking.

Animal Selection

Females were put on study if their body weight was within +/- 20% of the mean body weight for all females.

Rationale for Dose Selection

The doses selected for this study were based on a dose range-finding developmental toxicity study with MKH 3586 Technical in the Sprague-Dawley rat [2]. In this study gravid Sprague Dawley rats were administered nominal doses of 0, 5, 15, 50, 150, or 300 mg of MKH 3586/kg body weight by oral gavage on gestation days 6 through 15. Maternal toxicity, demonstrated by statistically significantly decreased body weight gain and food consumption, were observed in the 300 mg/kg dose group. Although not statistically significant, a 22 and 23% decrease in food consumption was observed during gestation days 6-8 in the 50 and 150 mg/kg dose groups, respectively. No embryo or fetal effects were observed in any dose group.

Based on these results, the doses proposed for the definitive developmental toxicity study were 0, 15, 100, and 300 mg MKH 3586/kg body weight. This dose range should produce evidence of maternal toxicity at the high dose and no maternal, embryo, or fetal effects at the low dose.

Dosage Preparation, Handling, and Analysis

Stock dosing solutions were prepared prior to the first day of dosing by suspending MKH 3586 in an aqueous 0.5% carboxymethylcellulose and 0.4% Tween 80 (CMC) solution. Following preparation, the concentration of the test compound at each dose level was determined [2] and the stock suspensions refrigerated. On the days of dosing an aliquot of each stock suspension was taken, from which the appropriate animals were dosed. Any unused portion of the daily aliquot was discarded. Homogeneity and stability of the test substance in the CMC vehicle at the storage conditions indicated have been verified [1].

Experimental Design

Route/Dose/Number of Animals

One hundred and twenty female rats were assigned to one of four treatment groups (30 animals/group): 0 (vehicle control), 15, 100, and 300 mg MKH 3586/kg body weight. Doses were administered by oral gavage, the suggested route of administration for studies of this type, in the CMC vehicle at a dosage volume of 10 ml/kg, on days 6 through 19 of gestation. Dosing volume was adjusted daily, based on dam body weight during the dosing period.

Animal Co-Housing

Rats were co-housed with a maximum of two females per male at one time. Following cohabitation, morning vaginal smears were taken and examined for the presence of sperm. Females found to be sperm-positive were randomized into groups as described below. The day on which sperm was observed in the vaginal smear was designated day 0 of gestation for that female.

Randomization Procedure

Females were assigned, using randomization chips, to one of the four groups as they became inseminated. Specifically, females found sperm positive were assigned a numbered chip. Chips were then blindly selected and the corresponding females placed into consecutive treatment groups.

Observations/Body Weight/Food Consumption

Inseminated females underwent a detailed examination for clinical signs once daily (AM), and a mortality check twice daily (AM and PM). Mortality checks consisted of a cageside observation that characterized mortality, moribundity, and overt toxicity by viewing the animal in the cage. Dams were observed once (detailed) prior to termination on day 20. A mortality check and a detailed clinical observation were performed daily on weekends and holidays. Mortality checks consisted of a cageside observation that characterized mortality, moribundity, and overt toxicity by viewing the animal in the cage. The detailed evaluation of clinical signs included both observing the animal in the cage and removing the animal to perform a physical examination. Cageside observations characterized mortality, moribundity, behavioral changes, and overt toxicity by viewing the animal in the cage. In the event a possible clinical sign was observed during the cageside evaluation, the animal may have been removed from the cage and a detailed assessment conducted. Dam body weights were taken on days 0, 2, 4, 6 through 19, and 20 of gestation. Food consumption was recorded on gestation days 2, 4, 6 through 19, and 20.

Day 20 Termination/Gross Pathology

On day 20 of gestation, the dams were terminated by carbon dioxide asphyxiation and a gross external examination performed. The abdomen and thoracic cavities were opened and a gross internal necropsy performed. The liver, thyroids (the thyroid weight of dam 215 was missed, see protocol deviation in Appendix I) were excised and weighed. The ovaries were excised, corpora lutea counted, and pregnancy determined. The intact uterus was removed and weighed. The uterus was opened and resorptions, if any, were characterized. Fetuses were removed from the uterine wall and each implant was noted. The placentas were trimmed of extraneous tissue, blotted, and weighed. Fetuses were sacrificed by intraperitoneal injection of 0.01-0.05cc Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI). All fetuses were individually identified, sexed, weighed, and externally examined. Approximately half of all fetuses from each litter were fixed in toto in 70% alcohol, eviscerated, processed, and evaluated for general skeletal development [3], including cartilage. The remaining fetuses were subjected to a gross visceral examination [4] and placed in Bouin's solution. Prior to fetal cranial examination, the fetuses were transferred to 70% alcohol. Sectioning of the head was performed according to the method of Wilson [5].

Necropsy of Non-Gravid, Moribund, or Dams Found Dead

Dams sacrificed on gestation day 20 and suspected to be not pregnant underwent a gross examination. The abdomen was opened and the uterus was flushed with saline or water to verify the patency of the cervical/uterine os. If the openings were patent the uterus was excised and examined for implantation sites to confirm pregnancy. If the dam was not pregnant no further examinations were performed. Dams found dead, moribund, or delivering prematurely while on study, were sacrificed if their status dictated and a gross necropsy was performed.

Evaluated Parameters

Reproductive and Dam Assessment included:

- Fertility Index: # pregnant (with implants) / # sperm-positive x 100
- Gestation Index: # with viable fetuses / # pregnant (with implants) x 100
- Mating Index: # sperm-positive / # co-housed x 100
- Body weight gain (corrected for gravid uterine weight)

- Food consumption
- Clinical signs
- Necropsy findings
- Organ weights: gravid uterus, liver, and thyroids
- Number of corpora lutea
- Total number of implantation's

Litter Assessment included:

- Total number of fetuses
- Number of viable fetuses
- Number of non-viable fetuses
- Fetal sex distribution
- Number/type of resorptions
- Number of affected (i.e., nonviable and malformed) implants
- Number of malformed males or females
- Pre-implantation loss: $\# \text{ corpora lutea} - \# \text{ implants} / \# \text{ corpora lutea} \times 100$
- Post-implantation loss: $\# \text{ implantation's} - \# \text{ viable progeny} / \# \text{ implantation's} \times 100$
- Number of affected litters

Fetal Assessment included:

- Placental weight
- Fetal weight
- Type and incidence of:
 - external malformations and variations
 - visceral malformations and variations
 - skeletal malformations and variations

External, visceral, and skeletal findings were considered either malformations or variations. Malformations include defective or abnormal development that may be life-threatening or life-limiting. Malformations occur at low frequencies in every population. However, they do not typically occur in several litters within one group (treatment or control), unless induced. Malformations, (particularly those of genetic origin) may be spontaneous in origin, and can occur in one to several individuals within a given litter. In contrast to malformations, variations are alterations in development that are commonly observed in a given strain of animal. These changes are not considered meaningful unless they occur in a treatment group, in a dose-dependent fashion, and at significantly higher rates than observed in the control [6].

Statistical Analysis

The data was analyzed with the litter as the primary experimental unit, using applications provided by TASC [7] and/or SAS [8]. Parametric data (including dam body weights and food consumption) was analyzed using an Analysis of Variance (ANOVA), and if significant differences were observed, a Dunnett's Test was performed. Fetal and placental weights were specifically analyzed via the Healy's Test if significance was observed in the ANOVA. Nonparametric data (e.g. litter size and number of corpora lutea) were first analyzed by the Kruskal-Wallis test and then subjected to Dunn's Test if significant differences were identified. Nonparametric dichotomous data (e.g. number normal/abnormal)

were initially analyzed by the Chi-Square Test and if significance was observed between groups then by the Fisher's Exact Test with the Bonferroni adjustment. Differences between the control and test compound-treated groups were considered statistically significant when $p \leq 0.05$.

Historical Controls

The historical control data presented in this study is a compilation of studies conducted at our laboratory using the Sprague-Dawley rat. The control groups from these studies were maintained and evaluated in a similar manner to the animals on the present study with one major exception. In the present study, the fetal skeletal examination included an evaluation of both the cartilage and bone, whereas the fetal skeletal examinations of the historical control considered only the bone. In order to accomplish an examination of the cartilage and bone the fetuses are double-stained with Alcian Blue (cartilage) and Alizarin Red S (bone). During the staining process a skeletal structure composed of both cartilagenous and ossified material would take up both stains, however, as the blue stain is darker it would overshadow the red stain. Thus, a structure that would have been stained red (single stain) and potentially considered completely ossified would now be regarded as incompletely ossified following double-staining. This difference in the interpretation of skeletal ossification may be seen if one compares the mean fetal/litter incidences of incompletely ossified bones in the historical control with the incidences of incompletely ossified bones in the control group of the present study. For 22 of the 29 bones considered (76%), the mean incidence of an incompletely ossified bone was greater in the present study than in the historical control database. Moreover, for 9 of the 22 bones noted above, the mean incidences of incomplete ossification in the control group of the present study were also greater than the upper limit of the historical control range for the affected bones. Therefore, based on the preceding discussion, there appears to be an effect of double staining on the interpretation of fetal skeletal anomalies and the resultant increase in the incidence rate of incompletely ossified bones. The consequences of this effect will be related to the findings of the present study, as appropriate, in the fetal skeletal portion of the results section of this report.

Archival Procedures

The final report, protocol, raw data, tissues, and a sample of the test substance will be archived at locations specified by Bayer Corporation, Agriculture Division, Toxicology, 17745 South Meikart, Stiiweil, Kansas 66085-9104.

RESULTS AND DISCUSSION

Animal Care and Environmental Conditions

There were no deviations from the animal care procedures described in the methods section or the study protocol (Appendix I, page 96). A deviation from the environmental conditions, a variation in the light cycle (see protocol deviation 2) occurred during the study. This deviation is not expected to have had any effect on the study.

Dose Analysis

A summary of the results from the analysis of the dosing suspensions used in the present study, as well as homogeneity and stability analysis, are shown in Appendix II (page 113).

Homogeneity and five-week stability of the test substance in the dosing vehicle at the storage conditions previously described were confirmed for concentrations which encompassed the range of dosages used in this study [1]. The actual concentrations of MKH 3586 in the nominal 0, 1.5, 10, and 30 mg/ml dosing solutions were 0, 1.34, 10.33, and 31.40 mg/ml, respectively. Based on a dosage volume of 10 ml/kg, the actual doses were 0, 13.4, 103.3, and 314.0 mg MKH 3586/kg body weight. In the interest of clarity and consistency between the text, tables, and appendices, the dose levels will be presented throughout the report as the control, 0; level I, 15; level II, 100; and level III, 300 mg/kg nominal doses.

Clinical Signs

A summary of the clinical signs observed prior to, during, and following MKH treatment are presented in Table 1 (page 29). Individual clinical observations are given in Appendix III (page 117).

There were no test compound-related clinical signs observed in the dams of level I. Clinical signs ascribed to treatment included hard stool, observed in both levels II and III, and clonic convulsions, observed on two occasions in two dams from level III. One dam in level III (#434) was found dead.

The no-observed-effect-level (NOEL) for maternal clinical signs was 15 mg/kg.

Gestational Body Weight and Food Consumption

Body weight is summarized in Table 2 (page 31) and illustrated in Figure 1 (page 92). The individual body weight data is shown in Appendix IV (page 126). The summary of food consumption during gestation is given in Table 3 (page 33) with the individual food consumption data in Appendix V (page 134).

A statistically significant decrease in maternal body weight was observed in level III during gestation days 7-20. Also observed in this group were statistically significant effects on body weight gain during gestation days 2-19 and 0-20, decreased 37% and 32% compared to control, respectively. Actual body weight (final body weight - gravid uterine weight) as well as actual body weight gain were also statistically significantly decreased in level III. In level II, statistically significantly decreased body weight was observed similar to level III, albeit to a lesser extent. In contrast to levels II and III, level I demonstrated statistically significantly decreased body weight at all time points, including gestation days 0, 2, and 4, which were measured prior to treatment with the test compound. Therefore, the difference between the mean body weight in the control and level I, which began on gestation day 0, was simply maintained

throughout the study. The maintenance of the difference in body weight throughout the study is evidenced graphically in Figure 1 (page 92). Based on this observation, the differences in body weight between the control and level I are not considered test compound-related. This conclusion is also supported by the observation that for body weight gain during gestation days 2-20 there was a statistically significant difference between control and level I, whereas when one includes the first body weight measurement (gestation day 0) the body weight gain (gestation days 0-20) is no longer statistically significant. As would be expected given the reduced body weight throughout the study, the actual body weight was also statistically significantly reduced in level I. However, no statistically significant effect was observed on actual body weight gain, which supports the previous contention that the differences in body weight observed in level I were not test compound-related.

Statistically significant and biologically meaningful decreases in food consumption were observed in level II (gestation days 6-9) and level III (gestation days 6-12 and 14-15). An incidental, albeit statistically significant, decrease in food consumption was noted in level I on one occasion (gestation days 9-10). However, this value was considered within the normal variation of food consumption typically observed in this rodent strain.

The NOELs for effects on maternal body weight gain and food consumption were both 15 mg/kg.

Maternal Necropsy

Maternal necropsy findings are summarized in Table 4 (page 35), with the individual findings shown in Appendix VI (page 142). A summary of the mean final body weights, liver, and thyroid weights are given in Table 5 (page 38). Maternal organ weights expressed as a percent of the final body weight are summarized in Table 6 (page 40). The individual organ weights are shown in Appendix VII (page 151) and the historical control for maternal organ weights are shown in Appendix VIII (page 156). Table 7 (page 42) summarizes the gravid uterus weights, the net body weight (day 20 body weight - gravid uterine weight), and the net body weight change from day 0 ((day 20 body weight - weight of gravid uterus) - day 0 body weight) at each level. Individual net body weight data may be found in Appendix IX (page 158).

No necropsy findings were ascribed to treatment. Findings, when observed, were sporadic in both frequency and type. Mean final body weights were statistically significantly decreased in all dose groups, however, as discussed previously, the effect noted in level I is not considered test compound-related, but rather a consequence of the reduced body weight already present prior to test compound administration. Absolute and relative liver weight was statistically significantly increased in level III. In contrast, only the relative liver weight was increased in level II. An increase in liver weight was observed in level I, however this observation is not considered test compound-related based on the following discussion. The increase in relative weight may have been due, in part, to the reduced body weight previously discussed. Moreover, the relative liver weight in level I was well within the historical control range for this endpoint (mean relative liver weight for level I was 4.100 compared to the historical control range of 3.608 to 4.374). No effects on thyroid weight, absolute or relative, were observed. Effects observed on net (actual) body weight and net body weight change have been discussed in the previous section of this report.

The NOEL for findings observed at maternal necropsy, including organ weights and net body weight change, was 15 mg/kg.

Reproductive Parameters

A summary of the reproduction data is given in Table 8 (page 44) with individual data shown in Appendix X (page 163). Individual breeding records may be found in Appendix XI (page 168).

The fertility index was 83.3, 93.3, 66.7, and 90.0%, for the control, level I, level II, and level III, respectively. The mating index was 100% for all levels. The results of these two indices indicate that there were an adequate number of litters for evaluation. The gestation index was 100% for the control and levels I and II, and 96.2% for level III.

The NOEL for reproductive parameters was 100 mg/kg.

Embryo Implantation/Resorption

Table 8 (page 44) summarizes the implantation results for each dose group. The historical control data for maternal and fetal data at cesarean section is presented in Appendix XII (page 172).

One dam from level I (#899) had a litter with 10 viable fetuses and 1 non-viable fetus, and in level III one dam (#341) had a litter with 12 viable fetuses and 1 nonviable fetus and another dam (#866) had a litter with two nonviable fetuses only. All litters from the control and level II contained only viable fetuses. Although a clear dose-response relationship was not established between the test compound and nonviable fetuses, the incidence of litters with nonviable fetuses in level III (approximately 7%) was greater than the historical control incidence (approximately 3%) for this endpoint. Therefore, the relationship between the test compound and nonviable fetuses in level III cannot be disregarded. There were no significant differences in pre or post-implantation loss or on early or late resorptions observed between animals of the control and levels I, II, or III.

The NOEL for embryotoxicity was 100 mg/kg.

Litter Effects

The litter data at each dose level are summarized in Table 9 (page 47). Mean fetal body weights are depicted graphically in Figure 2 (page 94). Individual fetal weights are presented in Appendix XIII (page 175).

No statistically significant differences in the litter size, the number and proportion of live fetuses/litter, or the median percent of male fetuses were observed between the control and the test substance-treated groups. Statistically significant decreases in the male, female, and combined (male + female + unknown) mean fetal weights were observed in level III. No effects on fetal weight were observed in levels I or II, and no effect on the mean placental weight was noted at any dose level.

The NOEL for litter effects was 100 mg/kg.

Fetal External Malformations/Variations

A summary of fetal external malformations and variations are given in Table 10 (page 49), with the fetal external findings summarized in Table 11 (page 51). Individual external observations per fetus are shown in Appendix XIV (page 210). The historical control data for fetal external findings may be found in Appendix XV (page 249).

No statistically significant effects on either the fetal or litter incidence of external malformations were observed in any dose group. Specific findings observed in level III included one fetus with anasarca (dam #223) and another fetus with domed head (dam #475). Both findings are relatively common in this strain of rat, and the fetal incidences of both findings were within the historical control incidence ranges for these observations. Hence, the findings are considered incidental to treatment. No fetal external variations were observed in any dose group.

The NOEL for fetal external malformations and variations was 300 mg/kg.

Fetal Visceral Malformations/Variations

A summary of fetal visceral malformations and variations are given in Table 12 (page 53), with the fetal visceral findings summarized in Table 13 (page 55). Individual visceral observations per fetus are shown in Appendix XVI (page 252). The historical control data for fetal visceral findings may be found in Appendix XVII (page 272).

There were no fetal visceral malformations noted in any dose group. Fetal visceral variations were observed independent of dose. The most common finding at visceral examination was hydroureter, observed in approximately 3 to 4% of all fetuses, regardless of treatment group.

The NOEL for fetal visceral malformations and variations was 300 mg/kg.

Fetal Skeletal Malformations/Variations

Table 14 (page 59) summarizes the fetal skeletal malformations and variations for each dose level, with the fetal skeletal findings summarized in Table 15 (page 61). Individual skeletal findings per fetus are shown in Appendix XVIII (page 275). The historical control data for fetal skeletal findings may be found in Appendix XIX (page 574).

There were no test compound-related fetal skeletal malformations observed. An incidental finding, the absence of one pair of lumbar arches, was observed in one fetus from level II.

Fetal skeletal variations were observed in all litters and in virtually all fetuses. Statistically significantly increased variations were observed to the greatest extent in level III and to a lesser extent in level II. Several skeletal variations were also increased, relative to control, in level I. In comparison to the effects observed in levels II and III, the variations noted in level I were typically within the historical control range and/or failed to demonstrate a dose-response relationship with the test compound. For example, unossified caudal arches, observed in 62.9% of the fetuses in level I, were also noted in 63.0% of the fetuses from level II, and both incidence rates fell within the historical control range for this finding. As discussed in the methods section, the incidence of incompletely ossified bones (the principal skeletal variation observed in this study) was artificially increased due to the skeletal staining methodology. This increase may be quantified by comparing the mean incidence of a given finding in the concurrent control with the mean incidence rate for the same finding in the historical control. In general, the mean incidence rate (litter, for example) for incompletely ossified bones in the concurrent control group was typically 20 - 30% greater than the mean in the historical control. Based on this observation, the historical control range would also be expected to increase by a similar margin. Hence, the incompletely ossified bones noted in level I that were just outside the historical control range would probably fall within the range if the historical control database consisted of fetuses processed in a manner similar to the present study. In comparison, most of the findings statistically significantly increased in levels II and III were well outside the historical control ranges for the respective findings. Based on the preceding discussion, the

variations observed in level I are not considered test compound-related but within the normal variation for these endpoints in this test system.

The NOEL for fetal skeletal malformations and variations was 15 mg/kg.

Total Fetal Malformations/Variations

An overall summary of fetal external, visceral, and skeletal malformations and variations is given in Table 16 (page 87).

The summary includes the results from the external examination of all fetuses, the visceral examination of approximately half of each litter, and the skeletal examination of the remaining fetuses from each litter. There were no statistically significant effects on the fetal or litter incidences of total malformations, variations, or affected fetuses.

The NOEL for total fetal malformations, variations, and total affected fetuses was 300 mg/kg.

Relationship Between Gender and Malformations

A comparison of the incidences of malformations between male and female fetuses is presented in Table 17 (page 89).

The comparison considered total malformations and individual malformation categories: external, visceral, and skeletal. No statistically significant differences in the incidence of total malformations, or any individual malformation category were noted between the males and females of any dose group.

The NOEL for gender-related effects on malformations was 300 mg/kg.

SUMMARY AND CONCLUSIONS

This study assessed the potential for MKH 3586 Technical to promote gestational effects and/or alter the growth and development of the conceptus. Inseminated dams were administered nominal doses of 0, 1, 7, or 50 mg MKH 3586/kg body weight (analytically confirmed doses of 0, 13.4, 103.3, and 314.0 mg MKH 3586/kg, respectively) by oral gavage on days 6 through 19 of gestation. Maternal toxicity, as demonstrated by clinical signs and changes in body weight gain and food consumption during gestation, was characterized. All dams were sacrificed on gestation day 20, at which time gross external and internal necropsies were performed, the uterus was examined, and the fetuses were removed by cesarean section. All fetuses were evaluated for external anomalies. Approximately half of each litter was examined for visceral effects, and the other half underwent a skeletal (bone and cartilage) examination.

Test compound-related maternal effects were observed in the 100 and 300 mg/kg dose groups. Gravid dams in the 300 mg/kg dose group demonstrated statistically significant decreases in body weight gain, food consumption, net body weight, and net body weight change (subtraction of gravid uterus). Additionally observed at the high-dose was a statistically significant increase in the relative and absolute liver weights. Maternal effects observed in the 100 mg/kg dose group included statistically significantly decreased body weight and food consumption, and a statistically significant increase in absolute liver weight. No test compound-related maternal findings were noted in the dams of the 15 mg/kg dose group. Fetal effects were noted in the 300 mg/kg dose group and included an increase in the incidence of nonviable fetuses and a statistically significant decrease in fetal weight. No test compound-related effects on fetal weight were observed in the 15 or 100 mg/kg dose groups. No test compound-related effects were noted during fetal external or visceral examinations. Test compound-related fetal skeletal findings, primarily incompletely ossified bones, were observed in levels II and III. Several skeletal variations were noted in level I, however, based on their incidence falling within the historical control, an equivocal dose-response relationship, or their relationship to effects due to skeletal processing methodology, they were not considered test compound-related. No effects on the incidence of total malformations or variations, or any gender-related differences in fetal effects were observed in any dose group.

MKH 3586, administered as described in this study, produced maternal toxicity at doses of 100 and 300 mg/kg body weight. No test compound-related maternal effects were demonstrated in the 15 mg/kg dose group. Developmental effects were observed primarily in the 300 mg/kg dose group and to a lesser extent in the 100 mg/kg dose group. Based on the observation of developmental effects only at dose levels that produced maternal toxicity, the developmental findings are considered secondary to maternal toxicity. Both the maternal and developmental no-observed-effect-levels (NOEL) were 15 mg/kg.

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