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BAYER CORP		
Contractor		
Document Title	INITIAL SUBMISSION: A SUPPLEMENTAL DEVELOPMENTAL TOXICITY STUDY WITH PROPYLENE THIOUREA (PTU) IN THE SPRAGUE-DAWLEY RAT, W/TSCA HEALTH & SAFETY STUDY COVER SHEET DATED 07/13/99	
Chemical Category	PROPYLENE THIOUREA	

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TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

Clearly mark the confidential information with bracketing and check the box in the appropriate section (L Contains CBI). Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

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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-0799-14507</u> <input type="checkbox"/> Initial Submission <input checked="" type="checkbox"/> Follow-up Submission <input type="checkbox"/> Final Report Submission Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any: # Follow-up to our 1/08/98 submission "Developmental Tox Study with Propylene Thiourea in the Sprague-Dawley Rat, Report # 108018" <input type="checkbox"/> continuation sheet attached		
2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e); optional for 8(d) & FYI) <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID Cert# P917.006.920 99-2-55	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#: 2122-19-2 Purity <u>99</u> + <u> </u> % <input checked="" type="checkbox"/> Single Ingredient <input type="checkbox"/> Commercial/Tech Grade <input type="checkbox"/> Mixture Trade Name: _____ Common Name: <u>Propylene Thiourea</u>		
4.0 REPORT/STUDY TITLE - Contains CBI A Supplemental Developmental Toxicity Study with Propylene Thiourea in the Sprague-Dawley Rat Report # 108018-1 <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 SUBJECT ROUTE OF EXPOSURE VEHICLE OF EXPOSURE TYPE: <u>CTOX</u> ORGANISM (HE, EE only): <u>RATS</u> EXPOSURE (HE only): <u>ORAL</u> EXPOSURE (HE only): _____ Other: _____ Other: _____ Other: _____		
6.0 REPORT/STUDY INFORMATION <input type="checkbox"/> Contains CBI <input checked="" type="checkbox"/> Study is GLP Laboratory <u>Bayer Agricultural Toxicology Lab</u> Report/Study Date <u>6/17/99</u> Source of Data/Study Sponsor (if different than submitter) _____ Number of pages <u>267</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>V. P., Prod. Safety & Reg. Affs</u> Phone: <u>412-777-7431</u> Company Name: <u>Bayer Corporation</u> Company Address: <u>100 Bayer Road</u> <u>Pittsburgh, PA 15205-9741</u> Submitter Address (if different): _____ Technical Contact: <u>Donald W. Lamb, Ph.D</u> Phone: <u>(412)777-7431</u> <input type="checkbox"/> continuation sheet attached		
8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS <input type="checkbox"/> Contains CBI <div style="font-size: 2em; font-weight: bold; text-align: center;">Contains No CBI</div> <div style="text-align: right; font-size: 1.5em; font-family: cursive;">MR 24446</div> <input type="checkbox"/> continuation sheet attached		

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Submitter Signature: Donald W. Lamb Date: 7/13/99



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9.0 CONTINUATION SHEET

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Submitter Tracking Number/Internal ID

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99-2-55

CONTINUED FROM COVER SHEET SECTION # 2.1

Although this study did not demonstrate any maternal, embryo, or fetal effects, a prior developmental toxicity study with PTU was reported to the EPA under TSCA 8(e) on 1/8/98 based on a biologically-relevant increase in developmental effects (reduced fetal weight and increased external, visceral, and skeletal anomalies) observed in the study. As a previous developmental toxicity study has been sent to the EPA, the present developmental toxicity study with PTU is being made as a follow up.

Abstract

A developmental toxicity study was conducted with propylene thiourea (PTU) in the Sprague-Dawley rat. Gravid rats were administered nominal doses of 0, 0.3, and 1.2 mg (0, 0.32, and 1.22 mg, analytically confirmed) PTU/kg body weight by gavage, in deionized water, on days 6 through 19 of gestation. Maternal evaluations during gestation included clinical observations and measuring body weight and food consumption. All dams were sacrificed on gestation day 20, at which time gross external and internal necropsies were performed. The gravid uterus was weighed and examined, and the fetuses were removed by cesarean section. Maternal organ weights included the liver, kidneys, spleen, and thyroids. All fetuses were evaluated for external anomalies. Approximately half of all fetuses from each litter were examined for visceral effects at necropsy and also evaluated for cranial effects using the Wilson's technique. The remaining fetuses underwent an evaluation for general skeletal (including cartilage) development.

There were no test compound-related maternal effects observed during gestation. No effects were observed on gravid uterine weight, and no effects were observed on any maternal organ weights. There were no test-compound related effects on the gestation index, or any embryonic endpoints, including pre/post-implantation loss and early/late resorptions. There were no statistically significant effects on litter size or the number of fetuses per implantation site. No test compound-related effects were observed for fetal external, visceral, or skeletal malformations or for fetal external or visceral variations. Several fetal skeletal variations, incompletely ossified frontal and parietal bones and the associated enlargement of the anterior fontanel, were statistically significantly increased in the 1.2 mg/kg dose group. In contrast, the incidence of these variations were not statistically significantly increased in the 0.3 mg/kg dose group. No effects on the incidence of total malformations or variations, or any gender-related differences in fetal effects were observed.

Propylene thiourea, administered as described in this study, demonstrated equivocal fetal effects (two fetal skeletal variations) at the 1.2 mg/kg dose level. The 0.3 mg/kg dose level was free from potential test compound-related effects. Therefore, the no-observed-effect-level (NOEL) for developmental effects was 0.3 mg PTU/kg (0.32 mg/kg, analytically confirmed).

A 05

BAYER CORPORATION
Study Number 96-512-TS

Agriculture Division
Report Number
178018-1

SUPPLEMENTAL SUBMISSION



Study Title

A Supplemental Developmental Toxicity Study with
Propylene Thiourea (PTU) in the Sprague-Dawley Rat

Original Study Title

A Developmental Toxicity Study with
Propylene Thiourea (PTU) in the Sprague-Dawley Rat

Data Requirements

US EPA OPPTS 870.3700, 1998
OECD-Guidelines for Testing of Chemicals, 4: 414, 1981
EU 87/302/EEC, 1995
Health Canada, Canada Gazette, Part II, Vol. 122, No. 2, 1988
Japanese MAFF 59 NohSan No. 4200, 1985

Author of Original Report

A. Barry Astroff

Authors of Supplemental Report

Angela D. Young and A. Barry Astroff

Completion Date of Original Study

December 8, 1997

Completion Date of Supplemental Study

June 17, 1999

Test Facility

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

Original Study Number

97-612-KR

A 06

BAYER CORPORATION
Study Number 98-614-TS

Agriculture Division
Report Number
108018-1

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 FIFRA Section 10(d)(1)(A), (B), or (C).

Company: Bayer Corporation, Agriculture Division

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


Signature

6-17-99
Date

A 07

BAYER CORPORATION
Study Number 98-812-TS

Agriculture Division
Report Number
108018-1

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted and reported in compliance with the FIFRA Good Laboratory Practice Standards, 40 CFR Part 160; the TSCA Good Laboratory Practice Standards, 40 CFR Part 792; the OECD Principles of Good Laboratory Practice, GD(92)32 (Paris, 1992), and the Japanese MAFF Good Laboratory Practice Standards, 59 NohSan No. 3850 (August 10, 1984).

SUBMITTER

BAYER CORPORATION

J. H. Thyssen:


Vice President, Toxicology
Agriculture Division

Date: 6-17-99

SPONSOR

AGRICULTURE DIVISION

J. H. Thyssen:


Vice President, Toxicology

Date: 6-17-99

STUDY DIRECTOR

A. B. Astroff:


Senior Research Scientist

Date: 6-17-99

A 08

BAYER CORPORATION
Study Number 98-612-TS

Agriculture Division
Report Number
108018-1

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: 6-17-99

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SPONSOR

Bayer Corporation
Agriculture Division
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Kansas City, Missouri 64120-0013

TEST FACILITY

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

DATES

Study initiation (protocol signed by study director): October 9, 1998
Initiation of mating (first day of co-housing): January 4, 1999
Experimental initiation (first day of dosing): January 11, 1999
Study termination (last day of fetal examination): April 15, 1999

PERSONNEL AND RESPONSIBILITIES

Toxicology and Sponsor
Representative:

J. H. Thyssen

J. H. Thyssen

Toxicology Laboratory:

G. K. Sangha

G. K. Sangha

Study Director and
Report Author:

A. B. Astroff

A. B. Astroff

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A. D. Young

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L. M. Gonzalez

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Pathology Services:

B. P. Stuart

B. P. Stuart

Animal Care:

R. E. Mueller

R. E. Mueller

Analytical Chemistry:

K. D. Moore

K. D. Moore

Quality Assurance:

D. M. Wallace

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, all phases of this study type including the 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/ MANAGEMENT
	<u>Phase of Study</u>	<u>Auditor</u>	
10/06/98	Protocol Review	T.L. Bormann	10/06/98
11/10/98	Active Ingredient Check	T.L. Bormann	11/10/98
12/28/98	Animal Receipt and Shipment Exam	T.L. Bormann	12/29/98
01/04/99	Animal Release from Acclimation for Study Use	T.L. Bormann	01/08/99
01/04/99	Randomization	T.L. Bormann	01/08/99
01/04/99	Animal ID (Tattoo & Tail Mark), Co-housing	T.L. Bormann	01/08/99
01/08/99	Mortality Check	T.L. Bormann	01/08/99
01/11/99	Test Animal Inventory, Dosing, Gestation Body Weights, Food Consumption, and Clinical Observations	T.L. Bormann	01/15/99
01/12/99	AM Vaginal Smears, Transfer of Inseminated Females, Tail Marking	T.L. Bormann	01/15/99
02/01/99	Dam Terminal Body Weights, Euthanasia, Necropsy and Fetal Necropsy	L.A. Berry	02/05/99
02/19/99	Changing Storage Solutions (Bouin's to Alcohol)	T.L. Bormann	02/26/99
02/25/99	Wilson's Technique	T.L. Bormann	02/26/99
04/14/99	Skeletal/Cartilage Examination	T.L. Bormann	04/20/99
05/11/99- 05/12/99	Final Report Review	M.W. Dietrich A.F. Uelner	05/12/99

QUALITY ASSURANCE STATEMENT (continued)

AUDITS		REPORT TO STUDY DIRECTOR/ MANAGEMENT	
<u>Phase of Study Type</u>	<u>Auditor</u>		
09/24/98	Staining Solution Preparation	T.L. Bormann	10/09/98
09/25/98, 09/28/98, 10/01/98, 10/02/98	Changing of Fetal Processing Solutions	T.L. Bormann	10/09/98
09/28/98	Macerating Solution Preparation	T.L. Bormann	10/09/98
10/01/98	Clearing Solution Preparation	T.L. Bormann	10/09/98
10/01/98- 10/02/98	Holding Solution Preparation	T.L. Bormann	10/09/98
12/15/98	Skeletal Exams for Bone and Cartilage	T.L. Bormann	12/15/98
<u>Analytical Chemistry Support Functions</u>			
11/18/98	Annual Balance Records Review	L.A. Berry	11/18/98
11/18/98	Chemist Training Records Review	L.A. Berry	11/18/98
11/19/98	Analytical Standards Records Review	L.A. Berry	11/19/98
11/30/98	Chromatographic Maintenance Records Review	L.A. Berry	12/01/98
01/06/99	Analysis For Concentration in Dose Solution	T.L. Bormann	01/06/99
<u>Animal Care Support Functions</u>			
01/28/98	Rat DACB Bedding Change & Room Maintenance	C.A. Yount	01/28/98
05/13/98	Rat (Wire Mesh) Rack Change	C.A. Yount	05/13/98
10/26/98- 10/27/98	Rack Check Documentation Review	C.A. Yount	10/27/98
10/27/98	Vermin Control Documentation Review	C.A. Yount	10/27/98
10/27/98- 10/28/98	Equipment Maintenance and Repair Documentation Review	C.A. Yount	10/28/98
10/28/98	Data Review of Water Analyses Reports	C.A. Yount	10/28/98

QUALITY ASSURANCE STATEMENT (continued)**AUDITS****REPORT TO
STUDY
DIRECTOR/
MANAGEMENT**

	<u>Animal Care Support Functions (cont.)</u>	<u>Auditor</u>	
01/07/99	Edstrom Water Flushing & Filter Changes	C.A. Yount	01/14/99
01/27/99	Polycarbonate Rat Cage: Bedding/Rack Change, Room Maintenance	C.A. Yount	01/29/99
02/09/99	Rat Rack Preparation (Wire Mesh)	C.A. Yount	02/09/99
02/09/99	Rat Rack Preparation (Polycarbonate)	C.A. Yount	02/09/99
02/10/99	Cleaning of Rat (Wire-Mesh) Racks and Cages:	C.A. Yount	02/12/99
02/12/99	Cleaning of Rat Feeders	C.A. Yount	02/12/99
02/12/99	Cleaning of Rat Polycarbonate Cages	C.A. Yount	02/12/99
04/07/99	Rat Room Disinfection & Air Filter Change	C.A. Yount	04/12/99

In compliance with the Good Laboratory Practice regulations, this final report for study number 98-612-TS 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.

D.M. Wallace 11/11/99
D.M. Wallace, RQAP-GLP, Quality Assurance

5/3/99
Date

ABSTRACT

A developmental toxicity study was conducted with propylene thiourea (PTU) in the Sprague-Dawley rat. Gravid rats were administered nominal doses of 0, 0.3, and 1.2 mg (0, 0.32, and 1.22 mg, analytically confirmed) PTU/kg body weight by oral gavage, in deionized water, on days 6 through 19 of gestation. Maternal evaluations during gestation included clinical observations and measuring body weight and food consumption. All dams were sacrificed on gestation day 20, at which time gross external and internal necropsies were performed. The gravid uterus was weighed and examined, and the fetuses were removed by cesarean section. Maternal organ weights included the liver, kidneys, spleen, and thyroids. All fetuses were evaluated for external anomalies. Approximately half of all fetuses from each litter were examined for visceral effects at necropsy, and also evaluated for cranial effects using the Wilson's technique. The remaining fetuses underwent an evaluation for general skeletal (including cartilage) development.

There were no test compound-related maternal effects observed during gestation. No effects were observed on gravid uterine weight, and no effects were observed on any maternal organ weights. There were no test-compound related effects on the gestation index, or any embryonic endpoints, including pre/post-implantation loss and early/late resorptions. There were no statistically significant effects on litter size or the number of fetuses per implantation site. No test compound-related effects were observed on fetal external, visceral, or skeletal malformations or on fetal external or visceral variations. Several fetal skeletal variations, incompletely ossified frontal and parietal bones and the associated enlargement of the anterior fontanel, were statistically significantly increased in the 1.2 mg/kg dose group. In contrast, the incidence of these variations were not statistically significantly increased in the 0.3 mg/kg dose group. No effects on the incidence of total malformations or variations, or any gender-related differences in fetal effects were observed.

Propylene thiourea, administered as described in this study, demonstrated equivocal fetal effects (two fetal skeletal variations) at the 1.2 mg/kg dose level. The 0.3 mg/kg dose level was free from potentially test compound-related effects. Therefore, the no-observed-effect-level (NOEL) for developmental effects was 0.3 mg PTU/kg (0.32 mg/kg, analytically confirmed).

MATERIALS

I. The test substance was supplied by the sponsor with the following information:

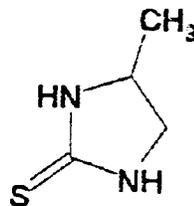
Test Substance:

Identification:	Propylene Thiourea
Physical Appearance:	White powder
Batch No.:	NLL 3790-4
Purity:	99.7% (date of analysis: October 9, 1998); 99.8% (date of analysis: May 28, 1999)
* Stability at Storage Conditions:	At least 7 months (based on % purity)

Active Ingredient:

Common Name:	Propylene Thiourea (PTU)
Chemical Name:	4-Methyl-2-imidazolidinethione

Structural Formula:



CAS No.:	2122-19-2
----------	-----------

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

PURPOSE

The purpose of this study was to supplement the results of a previous developmental toxicity study with propylene thiourea (PTU) [1]. Of particular interest was the potential relationship between the test compound and fetal skeletal development (see dose selection rationale section of this report). To this end, gravid Sprague-Dawley rats were administered 0, 0.3, or 1.2 mg PTU/kg body weight on gestation days 3 through 19. The results from this study will then supplement the findings of the previous developmental toxicity study with PTU.

GUIDELINES

This report was prepared in accordance with the US EPA Health Effects Test 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, adopted May 12, 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

The study design required 90 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, Health Canada, 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 the start of the study. 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 sacrificed in a moribund condition at any time prior to initiation of the study. Upon completion of the acclimation period, a veterinarian reviewed the status of the animals prior to their release for study.

Care and Housing

Male rats, and females prior to being declared sperm positive, were individually housed, except during the co-housing phase where a maximum of two females were housed with one male on a given day, 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 was periodically analyzed for possible contaminants. The results were compared to the allowable limits in "Lab Chows Animal Diet Reference Guide", 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.

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. DATATOX Software [2] was used in this determination.

Rationale for Dose Selection

The present study was conducted to supplement the findings of a previous developmental toxicity study with PTU [1]. In the former study, PTU was orally administered to gravid Sprague-Dawley rats (0, 1, 7, or 50 mg PTU/kg body weight) on gestation days 6 through 19. The author concluded that both the maternal and developmental no-observed-effect-levels were 1 mg/kg. Upon review, a question arose as to the potential relationship between the test compound and several fetal skeletal observations noted in the 1 mg/kg dose group. As discussed in the original report, the incidences of these skeletal variations were within the historical control ranges. Nevertheless, in order to clearly characterize the potential relationship between the fetal skeletal findings and the test compound, the present study was conducted.

Dosage Preparation, Handling, and Analysis

Stock dosing solutions were prepared prior to the first day of dosing by dissolving propylene thiourea in deionized water. Following preparation, the concentration of the test compound at each dose level was determined and the stock solutions refrigerated. On the days of dosing, an aliquot of each stock solution 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 water vehicle, at the storage conditions indicated, have been previously verified for the 1.2 mg/kg dose level [1], using liquid chromatography [3]. However, the homogeneity and stability of a concentration that brackets the lower dose of 0.3 mg/kg was verified as a part of the current study.

Experimental Design

Route/Dose/Number of Animals

Ninety female rats were assigned to one of three treatment groups (30 animals/group): 0 (vehicle control), 0.3, and 1.2 mg PTU/kg. Doses were administered by oral gavage, the suggested route of administration for studies of this type, in deionized water 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. A deviation from this dosing regimen occurred on one occasion, when dam 718 was administered less than the prescribed volume (see protocol deviation 1).

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 randomized into dose groups based on body weight [2]. Once found to be sperm positive, the dams were then assigned a random number generated by SAS [4].

Observations/Body Weight/Food Consumption

Inseminated females underwent a detailed examination for clinical signs once daily (AM when possible) on gestation days 0-20. Mortality checks were performed twice daily (AM and PM), during the workweek and once daily on weekends and holidays. Mortality checks consisted of a cage-side 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. In the event a possible clinical sign was observed during the cage-side 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, kidneys, and spleen 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 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 (including cartilage) [5,6]. The remaining fetuses were subjected to a gross visceral examination [7] 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 [8].

Necropsy of Non-Gravid Dams

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.

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, spleen, kidneys, and thyroids

Reproductive and Dam Assessment (continued):

- Number of corpora lutea
- Total number of implantations

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: # corpora lutea - # implants / # corpora lutea x 100
- Post-implantation loss: # implantations - # viable progeny / # implantations x 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 [9].

Statistical Analysis

The data was analyzed with the litter as the primary experimental unit, using applications provided by TASC [10]. 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) was 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) was initially analyzed by the Chi-Square Test and if significance was observed

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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 $p \leq 0.05$.

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 Metcalf, Stilwell, Kansas 66085-9104.

RESULTS AND DISCUSSION

Animal Care and Environmental Conditions

There were no deviations from the environmental conditions described in the methods section of this report, the study protocol (Appendix I, page 79), or the animal care report (Appendix II, page 92).

Dose Analysis

A summary of the results from the analysis of the dosing solutions are shown in Appendix III (page 95).

Homogeneity and stability of the test compound at concentrations that bracketed the dose levels used in the present study were either previously verified [1] or conducted with this study. The analytical confirmed concentrations of propylene thiourea (PTU) in the nominal 0, 0.03, and 0.12 mg/ml dose solutions were 0, 0.032, and 0.122 mg/ml, respectively. These concentrations correspond to doses of 0, 0.32, and 1.22 mg/kg based on a dosage volume of 10 ml/kg. However, in the interest of clarity and consistency between the text, tables, and appendices, the dose levels will be presented throughout the report as the nominal 0, 0.3, and 1.2 mg/kg doses.

Clinical Signs

A summary of the clinical signs observed during gestation are presented in Table 1 (page 27).

There were no test compound-related clinical signs observed during the study. On day 6 of gestation, dam #108 was sacrificed for humane reasons following severe dehydration after getting caught in the feeder.

The NOEL (no observed effect level) for maternal clinical signs was 1.2 mg/kg.

Gestational Body Weight and Food Consumption

Body weight is summarized in Table 2 (page 31) and illustrated in Figure 1 (page 75). The individual body weight data is shown in Appendix IV (page 97). The summary of food consumption during gestation is given in Table 3 (page 33) with the individual food consumption data in Appendix V (page 104).

There were no statistically significant effects on body weight in either test compound-treated group. A statistically significant decrease in food consumption was observed in the 1.2 mg/kg dose group on one occasion during gestation, days 14-15. This finding is considered incidental to treatment and within the normal range of variability expected with this endpoint in this strain of rat.

The NOEL for effects on maternal body weight and food consumption was 1.2 mg/kg.

Maternal Necropsy

Maternal necropsy findings are summarized in Table 4 (page 35), with the individual findings shown in Appendix VI (page 111). Table 5 (page 37) 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 uterine and net body weight data may be found in Appendix VII (page 118). A summary of the mean final body weights, liver, kidney, spleen, and thyroid weights for those dams sacrificed on gestation day 20 are given in Table 6 (page 39). Maternal organ weights expressed as a percent of the final body weight are summarized in Table 7 (page 41). Individual organ weight data is shown in Appendix VIII (page 122).

There were no statistically significant findings noted at necropsy. There were no statistically significant effects on uterine weight, net weight, or net weight change. No effects on organ weights, absolute or relative to body weight, were noted in either dose group.

The NOEL for findings observed at maternal necropsy, net body weight, or organ weights, was 1.2 mg/kg.

Reproductive Parameters

A summary of the reproduction data is given in Table 8 (page 43) with individual data shown in Appendix IX (page 126). Individual breeding records may be found in Appendix X (page 130).

The fertility index was 63.3 for the control and 0.3 mg/kg dose group and 70.0 for the 1.2 mg/kg dose group, and indicates that there were a sufficient number of gravid dams to evaluate. The mating and gestation indices were 100% for all groups.

The NOEL for reproductive parameters was 1.2 mg/kg.

Embryo Implantation/Resorption

Table 8 (page 43) summarizes the implantation results for each dose group. The historical control data for maternal and fetal data at cesarean section is presented in Appendix XI (page 134).

All gravid dams terminated on gestation day 20 from all groups had viable fetuses. No statistically significant differences were seen in the number of corpora lutea or the number of implantation sites. There were no statistically significant effects on resorptions, early or late; or pre or post-implantation loss.

The NOEL for embryotoxicity was 1.2 mg/kg.

Litter Effects

The litter data at each dose level are summarized in Table 9 (page 46). Mean fetal body weights are depicted graphically in Figure 2 (page 77). Individual fetal and placental weights are presented in Appendix XII (page 136).

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. No statistically significant effects were observed on fetal or placental weights.

The NOEL for litter effects was 1.2 mg/kg.

Fetal External Malformations/Variations

A summary of fetal external malformations and variations are given in Table 10 (page 48), with the fetal external findings summarized in Table 11 (page 50). Individual external observations per fetus are shown in Appendix XIII (page 157). The historical control data for fetal external findings may be found in Appendix XIV (page 180).

No statistically significant effects on the fetal or litter incidences of external malformations or variations were observed in either dose group.

The NOEL for fetal external malformations and variations was 1.2 mg/kg.

Fetal Visceral Malformations/Variations

A summary of fetal visceral malformations and variations are given in Table 12 (page 52), with the fetal visceral findings summarized in Table 13 (page 54). Individual visceral observations per fetus are shown in Appendix XV (page 182). The historical control data for fetal visceral findings may be found in Appendix XVI (page 195).

No statistically significant effects on either the fetal or litter incidences of visceral malformations were observed in either dose group.

The NOEL for fetal visceral malformations and variations was 1.2 mg/kg.

Fetal Skeletal Malformations/Variations

Table 14 (page 57) summarizes the fetal skeletal malformations and variations for each dose level, with the fetal skeletal findings summarized in Table 15 (page 59). Individual skeletal findings per fetus are shown in Appendix XVII (page 197). The historical control data for fetal skeletal findings may be found in Appendix XVIII (page 262).

There were no fetal skeletal malformations observed in any group.

Skeletal variations, of one form or another, were observed in virtually all fetuses examined.

Several findings were statistically significantly increased in level II, namely, incompletely ossified frontal and interparietal bones, and the related enlargement of the anterior fontanel. These findings are considered within the normal variation for these endpoints in this rodent strain based on the following discussion. These skeletal variations have been described to occur spontaneously, with relatively high frequency, in this strain of rat [11, 12]. The incidence rates in the present study, both fetal and litter, were well within the historical control ranges for these observations. Moreover, the mean fetal incidences of these findings: 6.7% for incompletely ossified frontal bones, 42.2% for incompletely ossified interparietal bones, and 6.7% for enlarged anterior fontanel, were not only within the historical control ranges but were also at or below the mean historical control incidences: 15.0% for incompletely ossified frontal bones, 41.5% for incompletely ossified interparietal bones, and 16.6% for enlarged anterior fontanel. Furthermore, the statistical significance of these observations may have also been exacerbated by the extremely low incidence rates for these findings in the control group. If one compares either the fetal or litter incidences of the aforementioned findings in the current control with the incidences in the historical control, for all three observations the incidence in the current control was less than the lowest incidence in the historical control range. Irrespective of the preceding information, a conservative interpretation of these results indicate that the skeletal variations observed in the 1.2 mg/kg dose group may have been test compound-related. In contrast, the incidence of these findings in the 0.3 mg/kg dose group was essentially that observed in the control group. The only other statistically significant fetal skeletal finding was enlargement of the posterior fontanel, noted in level I, which, based on the absence of a dose-response relationship, and the relatively high spontaneous occurrence of this finding [11, 12], is not considered treatment related.

The NOEL for fetal skeletal malformations and variations was 0.3 mg/kg.

Total Fetal Malformations/Variations

An overall summary of fetal external, visceral, and skeletal malformations and variations is given in Table 16 (page 70).

The summary includes the results from the external examination of all fetuses, the visceral (necropsy) examination of approximately half of each litter (all dose levels), the visceral (Wilson's technique) examination of approximately half of the fetuses from the control and dosed groups, and the skeletal examination of approximately half of the fetuses from each litter of the control and dosed groups. No statistically significant increases were observed in either dose level.

The NOEL for total fetal malformations, variations, and total affected fetuses was 1.2 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 72).

The comparison considered total malformations and individual malformation categories: external, visceral, and skeletal. No gender-related differences in the incidence of total malformations or the incidence of individual malformation categories: external, visceral, or skeletal, were observed.

The NOEL for gender-related effects on malformations was 1.2 mg/kg.

SUMMARY AND CONCLUSIONS

This study assessed the potential for propylene thiourea (PTU) to promote gestational effects and/or alter the growth and development of the conceptus. Of particular interest was the potential relationship between the test compound and fetal skeletal development. In a previous developmental toxicity study with PTU in the Sprague-Dawley rat [1], fetal skeletal variations were observed at all dose levels, including 1.0 mg/kg. Based on the relatively low incidence of these findings and that the incidence of all variations at this dose level were within the historical control ranges for these anomalies, the author concluded that they were incidental to treatment. However, as previously discussed, this interpretation of the results was not unequivocally accepted. In order to evaluate the potential relationship between PTU and fetal skeletal development the present study was conducted. Nominal dose levels included 0.3 mg/kg and 1.2 mg/kg (0.32 and 1.22 mg/kg, analytically confirmed). These dose levels were selected to bracket the 1.0 mg/kg dose level in the previous study. Specifically, gravid Sprague-Dawley rats were administered 0, 0.3, or 1.2 mg PTU/kg body weight via oral gavage on gestation days 6 through 19. Maternal evaluations during gestation included clinical observations and changes to body weight gain and food consumption. All dams were sacrificed on gestation day 20, at which time gross external and internal necropsies were performed. Maternal organ weights included the liver, kidney, spleen, and thyroids. The gravid uterus was weighed and examined, and the fetuses were removed by cesarean section. All fetuses were evaluated for external anomalies. Approximately half of all fetuses from each litter were examined for visceral effects at necropsy, and also evaluated for cranial effects using the Wilson's technique. The remaining fetuses underwent an evaluation for general skeletal (including cartilage) development.

There were no test-compound related maternal effects observed during gestation. No effects were noted on the gestation index, or any embryonic endpoints, including pre/post-implantation loss and early/late resorptions, in either dose group. There were no statistically significant effects on litter size or the number of fetuses per implantation site. No test compound-related effects were observed on fetal external, visceral, or skeletal malformations or on fetal external or visceral variations. Several fetal skeletal variations, incompletely ossified frontal and interparietal bones and the related enlargement of the anterior fontanel, were statistically significantly increased in the 1.2 mg/kg dose group. Although the incidence of these variations were well within the historical control ranges for these findings, their relationship to the test compound cannot be dismissed. In contrast, the incidence of these variations was not statistically significantly increased in the 0.3 mg/kg dose group. No effects on the incidence of total malformations or variations, or any gender-related differences in fetal effects were observed.

Propylene thiourea, administered as described in this study, demonstrated equivocal fetal effects (two fetal skeletal variations) at the 1.2 mg/kg dose level. The 0.3 mg/kg dose level was free from potentially test compound-related effects. Therefore, the no-observed-effect-level (NOEL) for developmental effects was 0.3 mg PTU/kg (0.32 mg/kg, analytically confirmed).

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