227 pp

OPPT-2002-0029-0033
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Mammalian 2-Generation – PTU Special Study

Tier II

Content

This report is also available at the web site.
http://epa.gov/scipoly/oscpendo/edmvs

This report and the Appendixes will be available in Docket 2002-0029 following this meeting.

Note: The PTU Special Study has an appendix that is a 3 megabyte Acrobat (PDF) file. This appendix is available upon request due to its size. If you would like this document electronically, please contact Jane Smith at smith.jane@epa.gov or 202 564-8476.

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BOOK B

Appendix 2

Two-Generation Reproduction Toxicity Study of Propylthiouracil when Administered to Sprague-Dawley Rats in the Drinking Water

Protocol and Amendments

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THERIMMUNE
Research Corporation

STUDY PROTOCOL

TWO-GENERATION REPRODUCTION TOXICITY STUDY OF PROPYLTHIOURACIL WHEN ADMINISTERED TO SPRAGUE-DAWLEY RATS IN THE DRINKING WATER

Approved by:

TherImmune:

Gary W. Wolfe 4/27/00
Study Director/Date

4-27-00
Reproductive Toxicologist//Date

NIEHS:

RE Chapin 4-26-00
Project Officer/Date

Reviewed By:

4-27-00
Quality Assurance Auditor/Date

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TWO-GENERATION REPRODUCTION TOXICITY STUDY
OF PROPYLTHIOURACIL WHEN ADMINISTERED
TO SPRAGUE-DAWLEY RATS IN THE DRINKING WATER

1.0 INTRODUCTION

1.1 Proposed Investigations/Rationale for Dose Selection

Propylthiouracil (CAS No. 51-52-5) is a thyroid hormone-synthesis inhibitor and antithyroid agent for the treatment of hyperthyroidism. Propylthiouracil (PTU) has been shown to decrease T3 and T4 while increasing TSH. PTU is being used to validate a One-Generation Study to Model proposed to identify potent and weak thyroid toxicants. The dose levels selected for the study are 0.0001, 0.0004, and 0.0015% (w/v). The high dose of 0.0015% should result in increased TSH, decreased T3 and T4, thyroid pathology, and decreased growth. The remaining dose levels should result in less toxicity a no effect at 0.0001% (w/v).

1.2 Regulatory Compliance

This study will be conducted according to Health Effects Test Guidelines OPPTS 870.8300 Reproduction and Fertility Effects and in compliance with the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (1987). This study will be conducted according to TherImmune Standard Operating Procedures.

1.3 Quality Assurance

The protocol, in-life phases, data, and the final report will be audited by TherImmune Quality Assurance. Critical phases to be audited for each generation will be selected by the Director of Quality Assurance.

1.4 Testing Facility

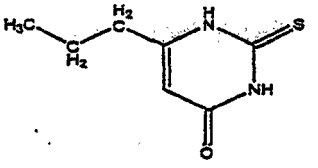
TherImmune Research Corporation
15 Firstfield Road
Gaithersburg, MD 20878

2.0 TEST ARTICLE

2.1 Characterization of Test Articles (From Dose Formulation Report)

Identity:	6-Propyl-2-Thiouracil
R.OW. ID No.:	1340
Source:	Sigma Chemical Co.
CAS No.:	51-52-5
Lot No.:	47H2500
Molecular Wt:	170.20
Formula:	$C_7H_{10}N_2OS$

Structure:



Purity: 99.8%

Storage:

Test Article: Room Temperature (~25°C) and protected from light

Formulation: Store in Nalgene™ carboys protected from light at ~5°C

Stability:

Test Article: Analyze every 24±2 weeks to verify stability

Formulation: Dose formulations (5μg/ml in tap water) are stable for 36 days at 5°C in sealed amber glass container. Under conditions which simulate animal dosing (room temperature), the dose formulation (5 μg/ml in tap water) showed no significant loss over 7 days (recovery of day 7 sample was 91.2%).

Certificate of analyses: Each batch of each test article will be accompanied by a certificate of analysis. The Sponsor will determine for each batch of test article the strength, purity, and composition or other characteristics which appropriately define the test article. A copy of the dose formulation report will be attached (Appendix 2).

Bulk Chemical Samples: Prior to use, two 0.5 g samples of the bulk of each test article will be collected into glass bottles with Teflon® coated lids, sealed and stored in the freezer (-20°C) protected from light for possible future reanalysis.

A bulk test article sample of 0.5 grams will be collected and sent to TherImmune Analytic Service Division for purity and stability testing within 30 days of receipt, and thereafter at 24±2 week intervals. A 5 g aliquot will be sent within 30 days prior to the start of any study.

2.2 Safety and Handling

2.2.1 Emergency First Aid Procedures

Eye: First check the victim for contact lenses and remove if present. Flush victims' eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Assure adequate flushing. Do not put any ointments, oils, or medication in the victims' eyes without specific instructions from a physician. Immediately transport the victim to a hospital even if no symptoms (such as redness or irritation) develop.

Skin: IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently wash affected skin areas thoroughly with soap and water. If symptoms such as inflammation or irritation develop, IMMEDIATELY call a physician or go to a hospital for treatment.

Inhalation: IMMEDIATELY leave the contaminated area and take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop, call a physician and be prepared to transport the victim to a hospital.

Ingestion: If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. If the victim is convulsing or unconscious, ensure that the victims's airway is open and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY TRANSPORT THE VICTIM TO A HOSPITAL.

2.2.2 Protective Equipment

Eye: Safety glasses/goggles

Gloves: Two pairs of dissimilar protective gloves (latex over Nitrile) shall be worn when handling the neat chemical, and dosing solutions.

Clothing: Minimally, a disposable laboratory suit (e.g. Tyvek ®), bouffant, and shoe covers shall be worn, as specified in the most current NTP Statement of Work or the NTP Health and Safety Minimum Requirements which is located in Health Safety Officer's Office.

Respiratory Protection: A NIOSH-approved chemical cartridge respirator with an organic vapor, acid gas and high-efficiency particulate filter cartridge. Use only in well ventilated areas.

2.2.3 Monitoring Procedures

Not required.

2.2.4 Spills and Containment

The Health and Safety Officer shall be informed in the event of any spillage. If the spillage is containable (at the discretion of the Health and Safety Officer) the following steps shall be taken:

1. A HAZORB® Chemical Spill Kit will be used.
2. Place HAZORB® control pillows around the spill area.
3. Place additional pillow over spill and allow absorption to occur.
4. Dispose of all absorbed material as hazardous waste.

2.2.5 Decontamination of Laboratory Equipment

Computer Terminal/
General Equipment — Whenever feasible, a protective covering (e.g., plastic wrap) shall be placed over the keyboard when in use. Before removing general laboratory equipment (i.e., lab carts, portable hoods and balances) from animal dosing rooms and/or chemical preparation areas, clean work surfaces with a 1.0% T.B.Q (quaternary ammonium) solution.

2.2.6 Disposal Procedures

Waste Disposal: Securely package and label, in double bags, all waste material. All potentially contaminated material (i.e., carcasses, bedding, soiled disposable clothing) shall be disposed of by incineration in a chemical incinerator equipped with an after burner and scrubber in a manner consistent with federal (EPA), state, and local regulations.

The precautions necessary when handling any test article or the prepared formulations of the test substance are based on the Material Safety Data Sheet (MSDS) supplied by the Sponsor. The MSDS will be retained in the study file.

2.3 Dose Formulation and Analysis

The quantity of propylthiouracil to prepare a solution in deionized water will be accurately weighed in a volumetric flask. Deionized water will be added to the flask and the contents added to a calibrated carboy. Deionized water will be added to the required volume and the solution will be mixed thoroughly to insure complete dissolution. The formulation for each group will be stored in Nalgene™ carboys protected from light at ~5°C. Each formulation will be dispensed into amber glass water bottles with sipper tubes and stoppers on the day of use.

Each water bottle will be color coded by group. The tray used to hold the water bottles will be labeled with the TherImmune Study No., Task No., R.O.W. ID No., Test article Name, Group, Dose Level, Vehicle, Mix Number, Preparation Date, Expiration Date, Storage conditions, Study and Group Color Code (see SOP No 506.0 Storage Sampling, and Labeling of Control and Test Diets and Mixtures and SOP No.121.0 Color-Coding for Study Identification and Dose Groups).

Each time a new mix or batch is prepared two (2) 50 ml archival samples of each dose level of test article formulation will be collected and stored at TherImmune in amber glass bottles with Teflon® lined lids protected from light in the refrigerator. One sample from each dose level and a 5g sample of the bulk test article will be forwarded on ice packs to TherImmune Analytical Service Division for dose concentration analysis at the following times: initial, mid, and final formulations for F_0 Evaluation and initial mix for F_1 Evaluation and at other periods specified by the Sponsor and communicated to the Study Director. Archival samples which are not selected for analysis will be discarded as hazardous waste as following requirements in Section 2.2.6 after at least ninety days following preparation.

3.0 EXPERIMENTAL DESIGN

3.1 Test System

Species:	Sprague-Dawley Outbred CD® Rats Crl:CD® BR
Rationale:	The Sprague-Dawley rat was selected as the test system due to its established quality as a breeder and the availability of historical toxicologic data for reference.
Supplier:	Charles River Breeding Laboratories, Inc. (Portage, Michigan, or Raleigh, North Carolina)
Sex:	Male and Female
Age (study initiation):	Approximately 5-6 weeks (F_0 Evaluation)
Weight Range (study initiation):	F_0 Evaluation: Males: >100 g Females: > 75 g

3.2 Animal Husbandry

All laboratory animal care will be in accordance with the Guide for the Care and Use of Laboratory Animals, TherImmune Standard Operating Procedures, and applicable EPA, FDA and USDA regulations.

Acclimation period:	At least 5 days
Animal housing during acclimation:	1 to 3/cage
Lighting:	12/12 hour light/dark cycle
Temperature:	71±3°F
Relative Humidity:	30-70%
Observations:	Twice daily observations for general health and availability of adequate food and water.
Cage changes:	At least twice a week, unless the animals are individually housed in 19" x 10½" x 8" (group-housed) cages which may be changed once a week.
Feeder/bottle changes:	At least once per week
Procedure for Individual Animal Identification:	All animals will be uniquely identified by tail tattoo and by cage cards.

Housing Requirements:

Cage Type:	Polycarbonate
Cage Measurement:	19" x 10 1/2" x 8" (group housed) 9" x 8 1/2" x 8" (single housed)
Bedding Material:	"Sani Chip" Hardwood Laboratory Bedding (P.J. Murphy Forest Products Corp., Montville, N.J.). All bedding will be autoclaved prior to use.
Feed:	NIH-07 (Pellets), manufactured by Harlan Teklad (Madison, Wisconsin).
Frequency:	*Ad Libitum.*
Analysis:	The feed is analyzed for nutrients, aflatoxins, nitrosamines, heavy metals, chlorinated hydrocarbons, organophosphates, PCB's, nitrates, nitrites, BHA, BHT, total bacterial plates, coliforms, E. coli and Salmonella by the vendor prior to release.
Water:	Deionized Water
Frequency:	*Ad Libitum.*
Analysis	A water quality sample is analyzed for total dissolved solids, heavy metals, chlorinated hydrocarbons, organophosphates, nitrates, nitrites, microbiological content, and total trihalomethanes at least semi-annually to conform with the Safe Drinking Water Act. None of the contaminants are expected to be at levels sufficient to interfere with the study.
Health Screening Requirements:	Prior to initiation of the study two F_0 males and two F_0 females will be sent to AnMed/Biosafe Laboratories (Rockville, MD) for serological tests: Pneumonia Virus of Mice (PVM) Respiratory Enteric Orphan III (REO3) Toolan's H-1 (parvovirus) (TH1) Encephalomyelitis (GD7) Sialodacryoadenitis Virus (coronavirus)(SDAV/RCV) Sendai (SEN) Mycoplasma Pulmonis (MYCO) Lymphocytic Choriomeningitis (LCM) Kilham's Rat Virus/rat Orphan Parvovirus (KRV/rOPV)

4.0 STUDY DESIGN

4.1 General Study Design

F_0 and F_1 breeding pairs will be maintained and allowed to produce one litter per pair. Selected F_1 animals will be reared to adulthood and be allowed to produce one litter (F_2).

4.1.1 Mortality

Any animals found dead or killed *in extremis* on the study will be subject to necropsy. The following tissues will be retained and placed in 10% neutral-buffered formalin unless otherwise specified:

liver	gross lesions
kidneys	ovaries (transfer to 70% ethanol within 24 hours)
left testis	vagina/uterus/cervix
left epididymis	adrenals
prostate (ventral and dorso-lateral lobes)	stomach
seminal vesicles/coagulating glands	pituitary
brain	thyroid/parathyroids
spleen	

Histopathology of the unscheduled deaths/sacrifices will be performed at the discretion of the Sponsor.

4.1.2 Necropsy

All necropsies are performed according to TherImmune Standard Operating Procedures.

4.1.3 Histopathology

Histopathological examination of fixed tissues for animals found dead or killed *in extremis* will not be conducted unless indicated by a protocol amendment. Tissues will be transferred to Pathology Associates International (PAI) located in Frederick, MD under subcontract to TherImmune. If pathology is conducted, the findings will be incorporated in the final report.

4.2 F_0 Evaluation (F_0 Parents/F_1 Pups)

At initiation of treatment (Study Day 1), animals will be singly housed. On Study Day 1 (SD1) the males and females will be dosed via the drinking water and will continue to be dosed daily until necropsy. On SD 71 (after 10 weeks of exposure), male and female rats from the same group will be paired (1 female to 1 male). Vaginal smears will be performed daily for confirmation of mating. When sperm or plug positive (GD 0) or after 14 days of cohabitation, whichever comes first, the females will be separated from the males. Pups from the F_1 litters will be maintained with the dam until weaning (PND 21). Observations of these pups will be made on PND 1, 4, 7, 14 and 21. Pups will be evaluated for structural effects and sexual development. Selected pups will be used to produce the F_2 litter.

4.2.1 Number of Animals

Group	Dose Level (% w/v)	Number of Males	Number of Females
1	0.0000	20	20
2	0.0001	20	20
3	0.0004	20	20
4	0.0015	20	20

4.2.2 Allocation

Eighty males and eighty females plus an appropriate number of extra animals (~10/sex), will be ordered for the F_0 Mating Trial. Approximately seven days before treatment initiation, animals will be randomly assigned to one of four groups by a computer generated randomization procedure. The randomization procedure will ensure equal weight distribution between the groups. The males and females will be randomized separately.

4.2.3 Treatment

Treatment will be administered in the drinking water starting on SD 1 for the males and SD 15 for the females and continue until necropsy. Control animals will receive the vehicle, deionized drinking water only.

4.2.4 Measurements

Parental

Observations for mortality and signs of toxicity: Twice daily

Body Weight:

- Males: At randomization; Weekly thereafter
- Females: At randomization; Weekly; At littering; F_0 dams during F_1 lactation — PND 1, 4, 7, 14, and 21 for all dams with F_1 litters.

Physical Examination: At randomization; At initiation; Weekly thereafter.

Food Consumption:

- Males: Weekly when housed individually.

Females	Weekly when housed individually. F_0 dams during F_1 lactation — PND 1-4, 4-7, 7-11, 11-14, and 14-18, 18-21 for all dams with F_1 litters.

Water Consumption:

Males	Weekly when housed individually.
Females	Weekly when housed individually. F_0 dams during F_1 lactation — PND 1-4, 4-7, 7-11, 11-14, and 14-18, 18-21 for all dams with F_1 litters.

Vaginal Cytology: Conducted on all F_0 dams for a minimum of 14 consecutive days prior to cohabitation.

Pup Observations

The following pup observations will be made on PND 1, 4, 7, 14, and 21 for the F_1 pups

Number of live pups
Number of dead pups
Number of males
Total body weight of males
Number of females
Total body weight of females
Anogenital distance including individual pup weights (PND 1 only)

Male and female pups will be examined for pinna detachment and eye opening beginning on PND 2.

Male pups will be examined for retained nipples on PND 12 and 13 only, pay special attention to the areola.

The following sexual development observations will be made for the F_1 animals selected on PND 16 for the F_1 Evaluation:

Testes Descent (male) starting on PND 16.
Vaginal Opening (female) starting on PND 25.
Preputial Separation (male) starting on PND 35.

4.2.5 Disposition of Offspring from F_0 Parents

PND 16 -- Two males and two females from each litter will be randomly selected for the F_1 Mating Trial. These animals will be assigned a unique identification number and tail tattooed. On PND 21, these animals will be separated from the dam and housed. Testes Descent (male) observations will start on PND 16. Vaginal Opening (female) observations will start on PND 25. Preputial Separation (male) observations will start on PND 35. Approximately one week before PND 81 ± 12, one male will be assigned to one female to form 20 mating pairs per group. Sibling matings will be avoided.

Three additional males and females from all litters will be randomly selected on PND 16 for the PND 21 necropsy. Remaining F_{1c} animals will be euthanized by carbon dioxide asphyxiation and discarded without necropsy following examination for external reproductive abnormalities on PND 21.

4.2.6 Terminal Procedures

■ F_1 Males selected for Necropsy PND 21

Schedule: PND 21

Group: The necropsy will be performed on up to 3 males selected per litter.

Procedure: The pups selected for the PND 21 necropsy will be weighed, sacrificed by carbon dioxide, exsanguinated and necropsied according to the following procedures.

Organ Weights: brain
spleen
thymus

Tissue Preservation: The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

left testis and epididymis (preserved in 2% paraformaldehyde/3% glutaraldehyde solution for 3-5 days, then transferred to phosphate-buffered saline.)
adrenals
kidneys
liver (median lobe)
pituitary
prostate, ventral
prostate, dorsolateral
seminal vesicles/coagulating glands
thyroid/parathyroids
gross lesions

Histopathology: Possible histopathology will be determined by Sponsor after review of data.

Sperm Analysis: Testicular spermatid head count will be determined on all necropsied male rats (F_1). The right testis will be frozen (~ -80 °C) and used for spermatid head counts.

■ F_{1c} Females Selected for Necropsy PND 21

Females :

Schedule: Scheduled on PND 21

Groups: The necropsy will be performed on up to 3 females selected per litter.

Procedure: Necropsy following terminal body weight and sacrifice by carbon dioxide asphyxiation and exsanguinated.

Organ Weights: brain
spleen
thymus

Tissue Preservation: The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

adrenals
kidneys
liver (median lobe)
ovaries (Bouin's and transfer into 70% ethanol within 24-48 hours)
pituitary
thyroid/parathyroids
vagina/uterus/cervix
gross lesions

Histopathology: Possible histopathology will be determined by Sponsor after review of data.

■ F_0 Males

Males:

Schedule: Scheduled following completion of weaning of the F_1 pups.

Groups: The necropsy will be performed on 20 control and 20 treated F_0 males from each dose group.

Blood Collection: Blood will be collected by jugular puncture and plasma obtained and frozen at - - 80 °C. The frozen plasma will be forwarded to Anilytics, Inc. (Gaithersburg, MD) for determination of TSH, T3, and T4.

Procedure: Necropsy following terminal body weight, sacrifice by carbon dioxide asphyxiation, and exsanguinated.

Organ Weights: brain
adrenals (paired)
cauda epididymis (right)
epididymis (right)
kidneys (paired)
liver
pituitary
prostate, ventral
prostate, dorsolateral
seminal vesicles/coagulating glands
spleen
testis (right)

thyroid /parathyroids

Tissue Preservation: The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

left testis and epididymis (preserved in 2% paraformaldehyde/3% glutaraldehyde solution for 3-5 days then transferred to phosphate-buffered saline.)
adrenals
brain
kidneys
liver (median lobe)
pituitary
prostate, ventral
prostate, dorsolateral
seminal vesicles/coagulating glands
thyroid/parathyroids
gross lesions

Histopathology: The left testis and epididymis from the first 10 surviving males/group will be embedded in glycol methacrylate (GMA), stained with Periodic Acid-Schiffs's and hematoxylin and examined microscopically. The thyroid/parathyroid, and gross lesions from the first 10 surviving males/group will be embedded in paraffin, sectioned, and examined microscopically by the study pathologist.

Sperm Analysis: Computer-assisted sperm motion analysis using the Hamilton Thorne Integrated Visual Optics System, epididymal sperm density, sperm morphology, and testicular spermatid head count will be determined on all necropsied male rats (F_0). The right cauda epididymis will be used for sperm density and morphology; the right vas deferens will be used for sperm motion analyses; and the right testis will be frozen (~ -80 °C) and used for spermatid head counts.

■ **F_0 Females**

Schedule: Scheduled following completion of weaning of the F_1 pups.

Groups: The necropsy will be performed on 20 control and 20 treated females from each dose group.

Blood Collection: Blood will be collected by jugular puncture and plasma obtained and frozen at ~ -80 °C. The frozen plasma will be forwarded to Anilytics, Inc. (Gaithersburg, MD) for determination of TSH, T3, and T4.

Procedure: Necropsy following terminal body weight, sacrifice by carbon dioxide asphyxiation, and exsanguinated.

Organ Weights: adrenals (paired)

brain
kidneys (paired)
liver
ovaries (paired)
pituitary
spleen
thyroid/parathyroids
vagina/uterus/cervix

Tissue Preservation: The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

adrenals
brain
kidneys (paired)
liver
ovaries (Bouin's and transfer into 70% ethanol within 24-48 hours)
pituitary
spleen
thyroid/parathyroids
vagina/uterus/cervix
gross lesions

Histopathology: The thyroid and uterus/vagina/uterus the first 10 surviving animals/group will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist.

4.3 F_1 Evaluation (F_1 Parents/F_2 Pups)

The F_1 pups will be used to form breeding pairs to produce the F_2 pups. On PND 81 ± 12, male and female rats from the same group will be paired (1 female to 1 male). Vaginal smears will be performed for confirmation of mating. When sperm or plug positive (GD 0) or after 14 days of cohabitation, whichever comes first, the females will be separated from the males. Pups from the F_2 litters will be maintained with the dam until weaning (PND 21). Observations of these pups will be made on PND 1, 4, 7, 14 and 21. Pups will be evaluated for structural effects and sexual development.

4.3.1 Number of Animals

Group	Dose Level (% w/v)	Number of Males	Number of Females
1	0.0000	20	20
2	0.0001	20	20
3	0.0004	20	20
4	0.0015	20	20

male will be assigned to one female to form 20 breeding pairs per group. Sibling matings will be avoided.

4.3.3 Treatment

Treatment will be administered in the drinking water starting on PND 21 and continue until necropsy. Control animals will receive the vehicle, deionized drinking water only.

4.3.4 Measurements

Parental

Observations for mortality and signs of toxicity:	Twice daily
Body Weight:	
Males	At randomization Weekly thereafter
Females	At randomization Weekly At littering F_1 dams during F_2 lactation — PND 1, 4, 7, 14, and 21 for all dams with F_2 litters.
Physical Examination:	At randomization At initiation Weekly thereafter
Food Consumption:	
Males	Weekly when housed individually.
Females	Weekly when housed individually. F_1 dams during F_2 lactation — PND 1-4, 4-7, 7-11, 11-14, and 14-18, 18-21 for all dams with F_2 litters.
Water Consumption:	
Males	Weekly when housed individually.
Females	Weekly when housed individually. F_0 dams during F_1 lactation — PND 1-4, 4-7, 7-11, 11-14, and 14-18, 18-21 for all dams with F_1 litters.
Vaginal Cytology:	Conducted on all F_1 dams for a minimum of 14 consecutive days at least 4 days after weaning of the last F_2 litters.

Pup Observations

The following pup observations will be made on PND 1, 4, 7, 14, and 21 for the F_2 pups

	F_0 dams during F_1 lactation — PND 1-4, 4-7, 7-11, 11-14, and 14-18, 18-21 for all dams with F_1 litters.
Vaginal Cytology:	Conducted on all F_1 dams for a minimum of 14 consecutive days at least 4 days after weaning of the last F_2 litters.

Pup Observations

The following pup observations will be made on PND 1, 4, 7, 14, and 21 for the F_2 pups

Number of live pups
Number of dead pups
Number of males
Total body weight of males
Number of females
Total body weight of females
Anogenital distance including individual pup weights (PND 1 only)

Male and female pups will be examined for pinna detachment and eye opening beginning on PND 2.

Male pups will be examined for retained nipples on PND 12 and 13 only, pay special attention to the areola.

4.3.5 Disposition of Offspring from F_1 Parents

- F_2 Pups

  PND 16 -- Three males and three females from each litter will be randomly selected for the PND 21 necropsy.

  PND 21 -- Remaining unselected male and female pups will be sacrificed by carbon dioxide overdose and discarded without necropsy.

4.3.6 Terminal Procedures

- <u>F_2 Males selected for Necropsy PND 21</u>

Schedule:	PND 21
Group:	The necropsy will be performed on up to 3 males selected per litter.
Procedure:	Necropsy following terminal body weight, sacrifice by carbon dioxide asphyxiation, and exsanguinated.
Organ Weights:	brain spleen thymus
Tissue Preservation:	The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

left testis and epididymis (preserved in 2% paraformaldehyde/3% glutaraldehyde solution for 3-5 days, then transferred to phosphate-buffered saline.)
adrenals
kidneys
liver (median lobe)
pituitary
prostate, ventral
prostate, dorsolateral
seminal vesicles/coagulating glands
thyroid/parathyroids
gross lesions

Histopathology: The left testis and epididymis will be embedded in glycol methacrylate (GMA), stained with Periodic Acid-Schiffs's and hematoxylin, and examined microscopically.

Sperm Analysis: Testicular spermatid head count will be determined on all necropsied male rats (F_2). The right testis will be frozen (~ -80 °C) and used for spermatid head counts.

■ **F_2 Females Selected for Necropsy PND 21**

Females :

Schedule: Scheduled on PND 21

Groups: The necropsy will be performed on up to 3 females selected per group.

Procedure: Necropsy following terminal body weight, sacrifice by carbon dioxide asphyxiation, and exsanguinated.

Organ Weights: brain
spleen
thymus

Tissue Preservation: The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

adrenals
kidneys
liver (median lobe)
ovaries (Bouin's and transfer into 70% ethanol within 24-48 hours)
pituitary
thyroid/parathyroids
vagina/uterus/cervix
gross lesions

Histopathology: The ovaries from the first 2 animals/group will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist.

■ F_1 Males

Males:

Schedule: Scheduled following completion of weaning of the F_2 pups.

Groups: The necropsy will be performed on 20 control and 20 treated F_1 males from each dose group.

Blood Collection: Blood will be collected by jugular puncture and plasma obtained and frozen at - - 80 °C. The frozen plasma will be forwarded to Anilytics, Inc. (Gaithersburg, MD) for determination of TSH, T3, and T4.

Procedure: Necropsy following terminal body weight, sacrifice by carbon dioxide asphyxiation, and exsanguinated.

Organ Weights: brain
adrenals (paired)
cauda epididymis (right)
epididymis (right)
kidneys (paired)
liver
pituitary
prostate, ventral
prostate, dorsolateral
seminal vesicles/coagulating glands
spleen
testis (right)
thyroid /parathyroids

Tissue Preservation: The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

left testis and epididymis (preserved in 2% paraformaldehyde/3% glutaraldehyde solution for 3-5 days, then transferred to phosphate-buffered saline.)
adrenals
brain
kidneys
liver (median lobe)
pituitary
prostate, ventral
prostate, dorsolateral
seminal vesicles/coagulating glands
thyroid/parathyroids

gross lesions

Histopathology: The left testis and epididymis from the first 10 surviving males/group will be embedded in glycol methacrylate (GMA), stained with Periodic Acid-Schiffs's and hematoxylin, and examined microscopically. The thyroid/parathyroid, and gross lesions from the first 10 surviving males/group will be embedded in paraffin, sectioned, and examined microscopically by the study pathologist.

Sperm Analysis: Computer-assisted sperm motion analysis using the Hamilton Thorne Integrated Visual Optics System, epididymal sperm density, sperm morphology, and testicular spermatid head count will be determined on all necropsied male rats (F_1). The right cauda epididymis will be used for sperm density and morphology; the right vas deferens will be used for sperm motion analyses; and the right testis will be frozen (~ -80 °C) and used for spermatid head counts.

■ F_1 females

Schedule: Scheduled following completion of vaginal cytology data collection.

Groups: The necropsy will be performed on 20 control and 20 treated females from each dose group.

Blood Collection: Blood will be collected by jugular puncture and plasma obtained and frozen at ~ - 80 °C. The frozen plasma will be forwarded to Anilytics, Inc. (Gaithersburg, MD) for determination of TSH, T3, and T4.

Procedure: Necropsy following terminal body weight, sacrifice by carbon dioxide asphyxiation, and exsanguinated.

Organ Weights:
adrenals (paired)
brain
kidneys (paired)
liver
ovaries (paired)
pituitary
spleen
thyroid/parathyroids
vagina/uterus/cervix

Tissue Preservation: The following organs will be retained in 10% neutral-buffered formalin unless otherwise specified:

adrenals
brain
kidneys (paired)
liver
ovaries (Bouin's and transfer into 70% ethanol within 24-48 hours)

pituitary
spleen
thyroid/parathyroids
vagina/uterus/cervix
gross lesions

Histopathology: The thyroid, ovaries, and uterus/vagina/uterus the first 10 surviving animals/group will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist.

5.0 ASSESSMENT OF THE RESULTS

5.1 Statistical Analyses

Statistical analyses of the following will be performed:

Data from the main study will be analyzed by a statistical support group under contract to NTP/NIEHS, RTP, NC. A statistical analysis report will be submitted to TherImmune by the contractor for inclusion in the final study report. Most hypotheses will be tested using the nonparametric multiple comparisons procedure of Dunn (1964) or Shirley (1977), as modified by Williams (1986). Shirley's test is designed to detect treatment-related differences when the response to treatment consistently increased (or decreased) with increasing dose. Although the test employs a smoothing algorithm to adjust for dose-response inversions, Dunn's test is more appropriate if the departure from monotonicity was severe. Jonckheere's test (1954) is used to ascertain whether there was sufficient evidence of a dose-related response to apply Shirley's test. If the p-value from Jonckheere's test is less than 0.01, Shirley's test will be used; otherwise, Dunn's test will be applied.

For data expressed as a proportion, such as number fertile/number cohabited, the Cochran-Armitage test (Armitage, 1971) will be used to test for a dose-related trend, and pairwise comparisons will be performed using a chi-square test (Conover, 1971).

Since the number of pups in a litter may influence the average pup weight in a litter, a parametric analysis of covariance (Neter and Wasserman, 1974) will be used to test overall equality in average pup weight, after adjustment for average litter size. Pairwise comparisons will be performed using Dunnett's test. The Cochran-Armitage trend test will be used to test for a dose-related decrease in fertility and mating indices.

Litter sizes and number of litters in dose groups will be compared to controls using Dunn's or Shirley's test. To examine potential differences in treatment effects on males and females, number of male pups, number of female pups and total number of pups in litters in treated groups will be compared to controls.

The ratio of the number of pups born alive to the total number of pups carried to full term will be computed for all fertile pairs. The sex ratio, expressed as the proportion of males, will be computed for all fertile pairs with at least one live pup. Shirley's or Dunn's test will be used to compare dosed groups to controls based on Jonckheere's test, as described above.

To adjust for the potential effect of the number of pups per litter on the average pup weight, an analysis of covariance will be performed. The covariate used will be average litter size, including live and dead

pups. Least squares estimates of dose group means adjusted for litter size will be computed and tested for overall equality using an F-test and pairwise equality using Dunnett's test. Unadjusted weights will be analyzed with Shirley's or Dunn's test. To examine potential differential effects on males and females, analyses on males, females, and both sexes will be performed.

Using either Shirley's or Dunn's test, feed and water consumption data will be analyzed gram per animal per day and gram per kilogram of body weight per day. Absolute organ and body weights, and organ weights relative to body weight will be analyzed by Shirley's or Dunn's test. Sperm parameters will be analyzed by Shirley's or Dunn's test, unless there are only two groups of data, in which case, the sperm parameters will be analyzed by Wilcoxon's test (Conover, 1971).

Clinical chemistry data, which typically have skewed distributions, will be analyzed using the nonparametric multiple comparisons methods of Shirley or Dunn.

6.0 REPORTS

The following reports will be submitted:

Summary Reports

Thirty days after completion of each mating trial, all data for that mating trial will be summarized and provided to the Sponsor.

Draft Study Report

Thirty days after completion of all analysis, all data will be summarized and conclusions on the reproductive toxicity of the test article be submitted to the Sponsor.

Final Study Report

The Final Study Report will be submitted to the Sponsor after the submission of the Draft Study Report.

7.0 STORAGE OF RECORDS

Upon submission of the final report, all original study records, including all original data sheets; the original final report; all biological samples; tissue, sperm morphology; Sperm data CDs; computer printouts generated in the statistical analysis of data; and copies of the final report will be forwarded to the contracting agency, the NIEHS, Research Triangle Park, North Carolina. Copies of the final study report will also be filed with TherImmune.

8.0 PERSONNEL

Project Officer:	Robert E. Chapin, Ph.D. (NTP)
Study Director:	Gary W. Wolfe, Ph.D., D.A.B.T
Reproductive Toxicologist:	Yefan Wang, M.S.
Technical Supervisor:	Meredith James, B.S.
Health and Safety Officer/ Facility Manager:	Robert Blackford, A.A., LATG

Veterinarian:	Edward Greenstein, D.V.M, ACLAM
Quality Assurance Officer:	Jim Carignan, B.S.
Project Leader:	Joy Palabrica, B.S.
Dose Preparation Supervisor:	Gary Holley, B.S.

9.0 SUBCONTRACTORS

Necropsy/Pathology:	PAI, Frederick, MD
Serology	AnMed/Biosafe, Rockville, MD
Clinical Chemistry	AniLytics, Inc., Gaithersburg, MD
Statistic Analyses:	Analytical Sciences Incorporation

10.0 REFERENCES

Armitage, P. (1971). *Statistical Methods in Medical Research.* John Wiley & Sons, New York.

Conover, W.J. (1971). *Practical Nonparametric Statistics.* John Wiley & Sons, New York.

Dunn, O.J. (1964). Multiple Comparisons Using Rank Sums. *Technometrics* **6** 241-252.

Dunnett, W. (1955). A Multiple Comparison Procedure for Comparing Several Treatments with a Control. *JASA* **50** 1096-1211.

Health Effects Test Guidelines, OPPTS 870.3800, Reproduction and Fertility Effects, EPA 712-C-98-208

Gray, T.J.B. and Gangolli, D.S. (1986). Aspects of the testicular toxicity of phthalate esters. *Environ. health Perspect.* **65**: 229-235.

Shirley, E. (1977). A Non-parametric Equivalent of Williams's Test for Contrasting Increasing Dose Levels of a Treatment. *Biometrics* **33**, 386-389.

Williams, D.A. (1986). A Note on Shirley's Nonparametric Test for Comparing Several Dose Levels with a Zero-Dose Control. *Biometrics* **42** 183-186.

TWO-GENERATION REPRODUCTION TOXICITY STUDY
OF PROPYLTHIOURACIL WHEN ADMINISTERED
TO SPRAGUE-DAWLEY RATS IN THE DRINKING WATER

Appendix 1: MILESTONE SCHEDULE THERIMMUNE 7244-601
(To be included as an amendment)

Study No.: 7244-601 ROW ID Nos.: 1340 Test Articles: Propylthiouracil

Study Activity	F_0	F_1	F_2
Animals Arrive			
Initial Dose Prep and Samples to ASD			
Bulk Analysis of Test Article			
Dose Formulation			
Weight Randomization			
Quarantine Release			
Viral Screen			
Animal ID			
Dosing Initiation			
Body Weights			
Phys. Examinations			
Food Consumption			
Water Consumption			
Cohabitation			
Separation			
Pup Observations			
Vaginal Cytology			
Termination/Necropsy			
Sperm Analysis			
Summary Report Due			
Data files to QC			
Data files to ASI			
Draft Final Report			

TWO-GENERATION REPRODUCTION TOXICITY STUDY
OF PROPYLTHIOURACIL WHEN ADMINISTERED
TO SPRAGUE-DAWLEY RATS IN THE DRINKING WATER

Appendix 2: PROPYLTHIOURACIL DOSE FORMULATION REPORT

COPY

Battelle ... Putting Technology To Work

BATTELLE-SPEC

Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-55395
Battelle Project No.: G002840-AGC
NTP ChemTask No.: CHEM04440
CAS No.: 51-52-5

SPECIAL CONFIRMATION OF IDENTITY AND LIMITED DOSE FORMULATION DEVELOPMENT STUDY REPORT

6-PROPYL-2-THIOURACIL

4-106-SPEC-100

February 1, 2000

Written By:

Steven Graves
Study Director

Approved By:

Steven Graves
Principal Investigator

Submitted to:

Dr. Cynthia S. Smith
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T.W. Alexander Dr.
Research Triangle Park, NC 27709-2233

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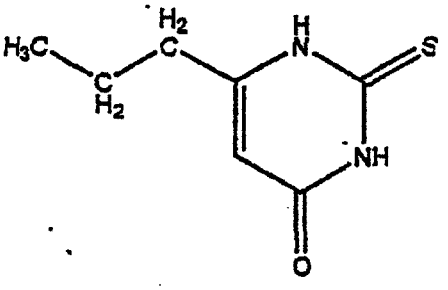
SPECIAL CONFIRMATION OF IDENTITY AND LIMITED DOSE FORMULATION DEVELOPMENT STUDY REPORT

6-PROPYL-2-THIOURACIL

CAS No.: 51-52-5	Lot No.: 47H2500
Battelle Chemical ID Code: 106	Amount Received: 500 g
Battelle Task No.: 4-106-SPEC-100	Receipt Date: 3/18/99
NTP ChemTask No.: CHEM04440	Appearance: White powder
Program Supported: OTH	Submitter: Not applicable
Analysis Dates: 4-1-99 to 5-11-99	Study Lab: Not applicable
Interim Results Date: Not applicable	Vendor: Sigma Chemical Co.
	Vendor Purity: 99.8%(NaOH titration); 99.9% (HLPC)
	Storage Conditions: Room temperature (~25°C)

STRUCTURE	Mol. Wt.	Mol. Formula
	170.20 g/mole	$C_7H_{10}N_2OS$

EXECUTIVE SUMMARY

The identity of the chemical was confirmed as 6-propyl-2-thiouracil by infrared spectroscopy. The information from the supplier indicated it was 99.8+% pure.

A reverse-phase high performance liquid chromatography (HPLC) method with UV detection was developed, evaluated and found to be suitable for the analysis of formulations from 2.5 to 50 ppm (µg/mL).

A stability study at 5 ppm indicated that formulations stored at 5°C in sealed glass bottles were stable for 36 days, but those stored at room temperature were unstable.

A simulated animal room study indicated that a 5 ppm formulation was sufficiently stable to allow for weekly drinking water bottle changes.

QUALITY ASSURANCE STATEMENT

SPECIAL CONFIRMATION OF IDENTITY AND LIMITED DOSE FORMULATION DEVELOPMENT STUDY REPORT

6-PROPYL-2-THIOURACIL

NTP ChemTask No.: CHEM04440

Battelle Project No.: G002840-AGC

Battelle Task No.: 4-106-SPEC-100

Listed below are the phases and/or procedures performed by Battelle that were reviewed by the Quality Assurance Unit during performance of the task described in this report. Adverse findings, if any, were reported to the study director at the time of review.

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Infrared spectroscopy analysis	4/1/99	4/1/99
Audit study file	1/28/00	1/28/00
Audit analytical report	1/28/00	1/28/00

This report reflects the procedures and raw data generated in this study.

In addition to the study-specific audits/inspections cited above, routine inspections of the general facilities and equipment were performed by the QAU and reports were submitted to management as follows:

Facility/Equipment	Date Inspected	Date of Report to Management
Chemistry Technical Center inspection	2/6-2/9/96	2/14/96
	5/8/96	6/11/96
	6/5, 6/12 and 6/24/97	6/25/97
	5/27 and 5/28/98	6/1/98
	7/27/99	7/30/99

2-1-2000

Quality Assurance Unit — Date

Battelle

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1 INTRODUCTION

The purpose of this work was to confirm the identity of this material as 6-propyl-2-thiouracil (PTU); estimate its purity; identify and quantitate, if possible, any significant impurities in the material; develop and evaluate a formulation analysis method; and evaluate the stability of the formulation. This work was done at Battelle, 505 King Avenue, Columbus, OH 43201 in support of OTH studies.

2 CHEMICAL RECEIPT AND STORAGE

6-Propyl-2-thiouracil, Lot No. 47H2500, was received from Sigma on March 18, 1999. Five 1-L amber bottles, nominally containing 100 g each were received. The chemical was homogenized by combining the contents of the bottles in a glass beaker. The chemical was then stirred with a spatula for about five minutes. The 6-propyl-2-thiouracil was repackaged into the original bottles. The net weight was determined to be 510 g.

Samples were taken after repackaging. The number of samples and approximate amounts removed were two 11.5-g "analytical" samples, one 5-g "archive" sample, two 11.5-g "retention" samples. The "archive" and "retention" samples were stored at ≤-20°C. The "analytical" sample was stored at room temperature. The net weight of chemical remaining after sampling was determined to be 462 g. The remaining chemical was stored at room temperature.

A copy of the supplier's Certificate of Analysis for this lot is shown in Figure 1. This states that the purity of the sample is 99.8% by NaOH titration and 99.9% by HPLC.

3 IDENTITY CONFIRMATION BY INFRARED (IR) SPECTROSCOPY

3.1 Method

The infrared spectrum was obtained on a Digilab FTS-60A Fourier Transform infrared spectrometer at 8 cm^{-1} resolution. The sample was prepared as potassium bromide pellet. The spectrum of the test article was compared to a literature reference spectrum found in "Sadtler Research Laboratories, Inc., Pharmaceutical Spectra, Spectrum R573.

3.2 Results

The IR spectrum (Figure 2) was consistent with the literature reference spectrum (Figure 3).

3.3 Conclusions

The sample's identity was confirmed as 6-propyl-2-thiouracil.

Sigma Certificate of Analysis http://199.217.212.43/cgi-bin/gx.c...90c31-471a-11d2-9f2a-0004accii0c16}

SIGMA

Certificate of Analysis

TEST	SPECIFICATION	LOT {047H2500} RESULTS
Product Name	6-N-propyl-2-thiouracil	
Product Number	P3755	
CAS Number	51-52-5	
Formula	$C_7H_{10}N_2OS$	
Formula Weight	170.20	
APPEARANCE	WHITE TO WHITE WITH A YELLOW CAST	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS TO SLIGHTLY HAZY FAINT YELLOW SOLUTION IN 1 M SODIUM	CLEAR COLORLESS SOLUTION AT 200 MG PLUS 4.0 ML OF 1 M SODIUM HYDROXIDE
IR SPECTRUM	CONSISTENT WITH STRUCTURE	CONSISTENT WITH STRUCTURE
PURITY BY SODIUM HYDROXIDE TITRATION	99% MINIMUM	99.8%
PURITY BY HPLC	99% MINIMUM	99.9%
QC ACCEPTANCE DATE		APRIL 1997

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David Feldker, Manager
Analytical Services

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Figure 1 – Certificate of Analysis

Battelle Study No. G002840-AGC 2

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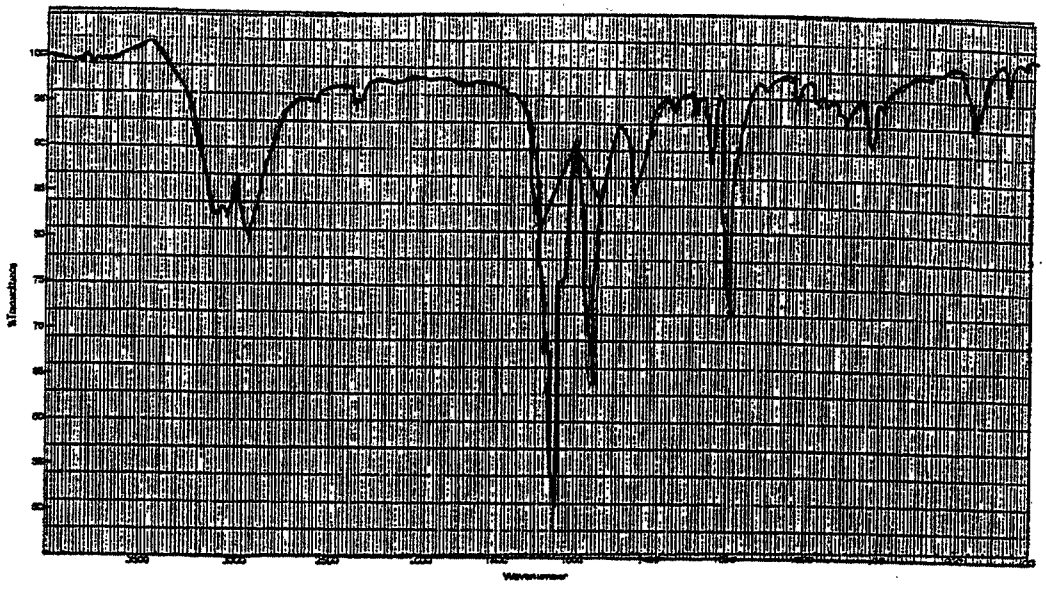

Figure 2 – IR Spectrum of 6-Propyl-2-Thiouracil, Lot No. 47H2500

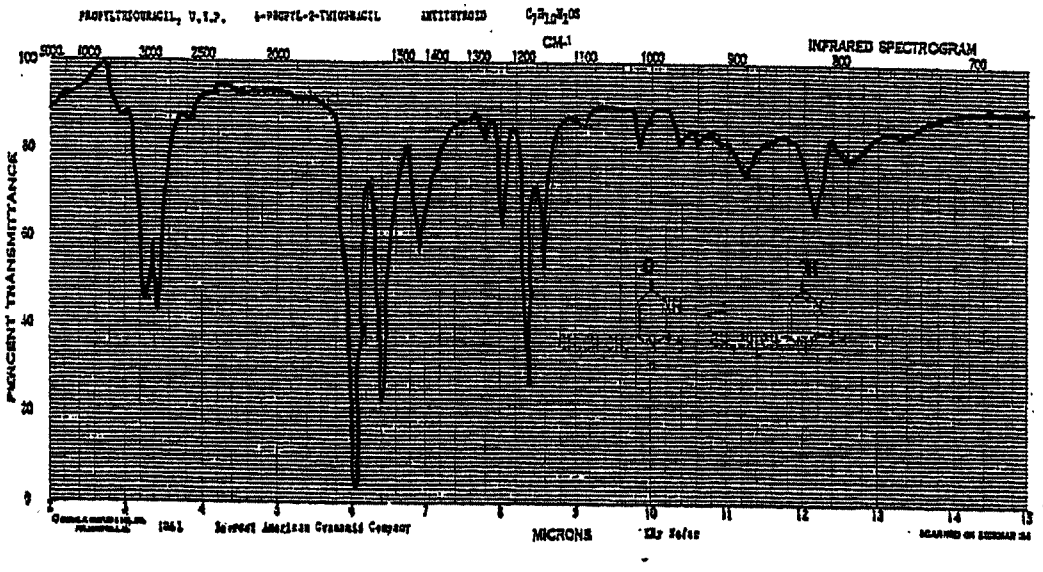

Figure 3 – Literature IR Spectrum of 6-Propyl-2-Thiouracil

4 LIMITED DOSE FORMULATION METHOD VALIDATION

This section describes the evaluation of an analysis method suitable for 6-propyl-2-thiouracil formulations in tap water over the concentration range from 5 to 25 ppm, the results, and the conclusion from this evaluation.

4.1 Method/Experimental Design

A standard curve was prepared containing standards at six concentrations in tap water. Triplicate standards at the highest and lowest concentrations and single standards at the intermediate concentrations were prepared. Triplicate blanks containing internal standard and blanks without internal standard were also prepared.

4.2 Preparation of Internal Standard

An intermediate internal standard (IS) solution was prepared by diluting 50 µL of acetophenone to 50 mL with acetonitrile. This solution was diluted 1-to-100 with tap water to produce the working internal standard.

4.3 Preparation of Standards and Blanks

Two stock standards (A and B) were prepared at target concentrations of 50 and 25 µg/mL by dissolving and diluting approximately 25 and 12.5 mg, respectively, of accurately weighed PTU to 500 mL with tap water. These two solutions also served as the two most concentrated vehicle standards.

Four additional vehicle standards were prepared at target concentrations of 12.5, 5.0, 6.25, and 2.5 µg/mL by diluting 2.5 and 1 mL, respectively, of Stock Standards A and B to 10 mL with tap water.

Working standards were prepared by combining 1 mL of vehicle standard and 1 mL of working IS in individual autoinjector vials. Triplicate working standards were prepared from the highest and lowest concentration vehicle standards. Single working standards were prepared from the other vehicle standards.

Triplicate blanks with IS were prepared by combining 1 mL of tap water and 1 mL of working IS.

Tap water served as the blank.

4.4 Analysis

Single injections were made from each vial using the HPLC system shown in Table 1. Typical chromatograms from a high and low vehicle standard, a blank with internal standard and a blank are shown in Figure 4.

Table 1 – HPLC System

Column	Inertsil ODS (2) 5µ, 150 X 3.0 mm (ID)
Mobile Phase Components	A: Milli-Q Water B: Acetonitrile C: Concentrated Phosphoric Acid
Mobile Phase Composition	90%A:10%B:0.1%C (v:v:v), Isocratic
Flow Rate	1.0 mL/minute
Injection Volume	25 µL
Detector Type and Wavelength	Ultraviolet at 254 nm
Run Time	20 minutes
Retention Times PTU IS	~2.0 minutes ~13.5 minutes

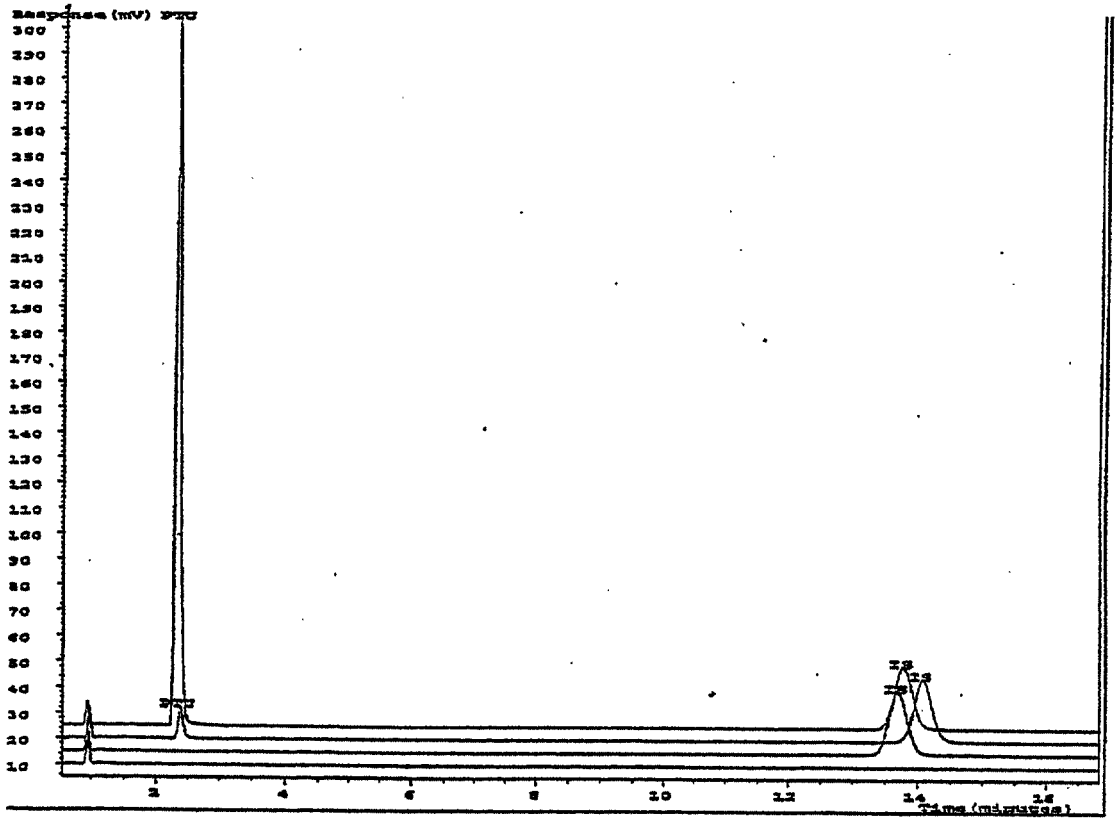

Figure 4 – Representative Chromatograms from High Standard, Low Standard, Blank with IS, and Blank

(Chromatograms are in order from top high standard, low standard, blank with IS to blank)

4.5 Calculations

The integration of the PTU and internal standard (IS) peaks, as done by the chromatography data system, was evaluated and manually adjusted, if necessary to achieve consistent integration. The response ratio of the PTU peak area divided by the IS peak area was calculated. An unweighted linear regression equation was calculated relating the response ratio of the working standards to their actual concentrations. A determined concentration was calculated for each standard using the regression equation and the response ratio for that standard. The relative error for each standard was calculated by subtracting the actual concentration from its determined concentration, dividing the difference by the actual concentration and multiplying the ratio by 100. The average relative error, the standard deviation, and the relative standard deviation were calculated for the low and high standards.

4.6 Results

The results from the analysis of the standard curve are shown in Table 2.

Table 2 – Dose Analysis MPE Results

Nominal Vehicle Std. Conc (µg/mL)	Det'd Vehicle Std. Conc (µg/mL)	Avg Det'd Vehicle Std. Conc (µg/mL)	S (µg/mL)	%RSD	%RE	Avg %RE
	52.34				1.3	
51.66	51.27	51.67	0.58	1.1	-0.8	0.0
	51.40				-0.5	
24.90	24.75	NA	NA	NA	-0.6	NA
12.92	13.06	NA	NA	NA	1.1	NA
6.255	6.205	NA	NA	NA	-0.3	NA
5.166	5.187	NA	NA	NA	0.4	NA
	2.473				-0.7	
2.490	2.505	2.479	0.023	0.9	0.6	-0.4
	2.460				-1.2	

NA = Not Applicable

The limit of detection (LOD), defined as three times the standard deviation of the lowest standard because there was no blank response, was 0.069 µg/mL. The limit of quantitation (LOQ), defined as ten times the standard deviation of the lowest standard because there was no blank response, was 0.232 µg/mL. The experimental limit of quantitation (ELOQ), defined as the lowest standard with acceptable accuracy and precision, was 2.490 µg/mL.

The results of a regression analysis of the standard curve are shown in Table 3. The regression line for the vehicle curve is shown in Figure 5.

Table 3 – Results of Regression Analysis

Slope	Y-Intercept	Correlation Coefficient
0.06228	-0.01769	0.999

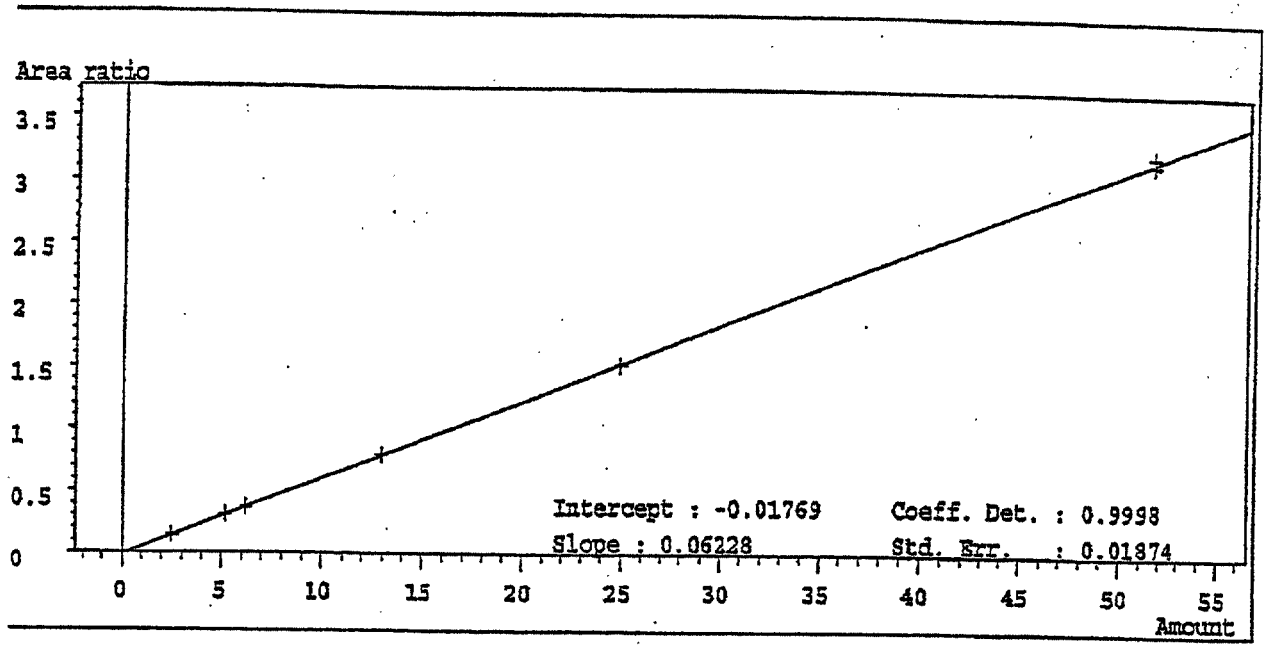

Figure 5 – Standard Curve from MPE

4.7 Conclusions

The method met all acceptance criteria for linearity, precision, accuracy, sensitivity and specificity. It is suitable for the analysis of PTU in tap water at concentrations from 2.5 to 50 µg/mL (ppm).

5 DOSE FORMULATION STUDIES

5.1 Formulation Storage Stability Study

5.1.1 Study Design

A storage stability study was conducted at a target concentration of 5 ppm (µg/mL) in tap water for samples in sealed amber glass bottles for 36 days at room temperature (25°C) and 5°C.

Preparation of Formulation

A stability stock standard was prepared at a target concentration of 250 µg/mL by dissolving and diluting approximately 25 mg of accurately weighed PTU to 100 mL with tap water. The formulation for the stability study was prepared by diluting 20 mL of the stability stock standard to 1-L with tap water. Fifty (50) mL of the formulation was transferred to two 60-mL amber glass bottles. The bottles were sealed. One bottle was stored at room temperature; the other was stored at 5°C.

Preparation of Analytical Standards

A stability stock solution was prepared by dissolving and diluting approximately 25 mg of accurately weighed PTU to 100 mL with tap water on each analysis day. This stock was diluted one-to-fifty with tap water to produce a vehicle standard with a target concentration of 5 µg/mL. Nine working standards were prepared from the vehicle standard on each analysis day by combining 1 mL of the vehicle standard and 1 mL of working IS in nine autoinjector vials. The vials were sealed.

Preparation of Formulation Samples

Triplicate aliquots were taken from the mixing flask on Day 0 and from both glass bottles on the other study days. One (1) mL of the formulation was combined on each analysis day with 1 mL of working IS in autoinjector vials. The vials were sealed.

Analysis

Injections of the standards and formulation samples were made using the chromatography system in Table 1 (Method Validation). At least three standard injections were made before and after the formulation samples. The peak area ratio of PTU/IS was calculated for each standard and these ratios were averaged. The peak area ratio of PTU/IS was calculated for each formulation sample and these ratios were averaged. The formulation samples' individual concentrations were calculated by dividing their peak area ratio by the averaged ratio for the standards and then multiplied by the nominal standard concentration. The average formulation concentrations were calculated.

Results

The results from the storage stability studies are shown in Table 4.

Table 4 – Formulation Storage Stability Study Results (5 µg/mL)

Day	Storage Temp (°C)	Det'd Conc (µg/mL)			Avg Det'd Conc (µg/mL)	% of Day 0 Conc
0	NA	4.903	4.838	4.905	4.882 ± 0.038	100.0
7		4.882	4.765	4.843	4.830 ± 0.060	98.9 ± 1.2
15		4.834	4.896	4.919	4.883 ± 0.044	100.0 ± 0.9
21	5	4.694	4.669	4.663	4.675 ± 0.017	95.8 ± 0.3
29		5.046	4.920	4.968	4.978 ± 0.064	102.0 ± 1.3
36		4.476	4.617	4.570	4.554 ± 0.072	93.3 ± 1.5
7		4.503	4.447	4.416	4.455 ± 0.044	91.2 ± 0.9
15		3.975	3.994	4.028	3.999 ± 0.027	81.9 ± 0.6
21	25	3.639	3.595	3.525	3.586 ± 0.057	73.5 ± 1.2
29		3.484	3.430	3.486	3.467 ± 0.031	71.0 ± 0.6
36		3.201	3.229	3.196	3.208 ± 0.018	65.7 ± 0.4

Discussion and Conclusions

The concentration of the formulation was within 10% of target on Day 0. Based on a pooled relative standard deviation of 0.9%, any individual value would have to be more than 2.1% different from 100% to be statistically significant at the 95% confidence level.

The concentration of the formulation stored at room temperature showed a steady decline over time. The chromatograms for the room temperature samples showed a marked increase in the size of peak eluting before PTU with time. Figure 6 shows chromatograms from room temperature samples on Days 7, 15, 21 and 36.

The concentration of the formulation stored at 5°C had no clear trend with time, although two of the points were lower than the lower control value. The degradation peak increased significantly at room temperature but did not significantly increase at 5°C.

These data indicate that the formulation can be stored for 36 days at 5°C in sealed amber glass containers.

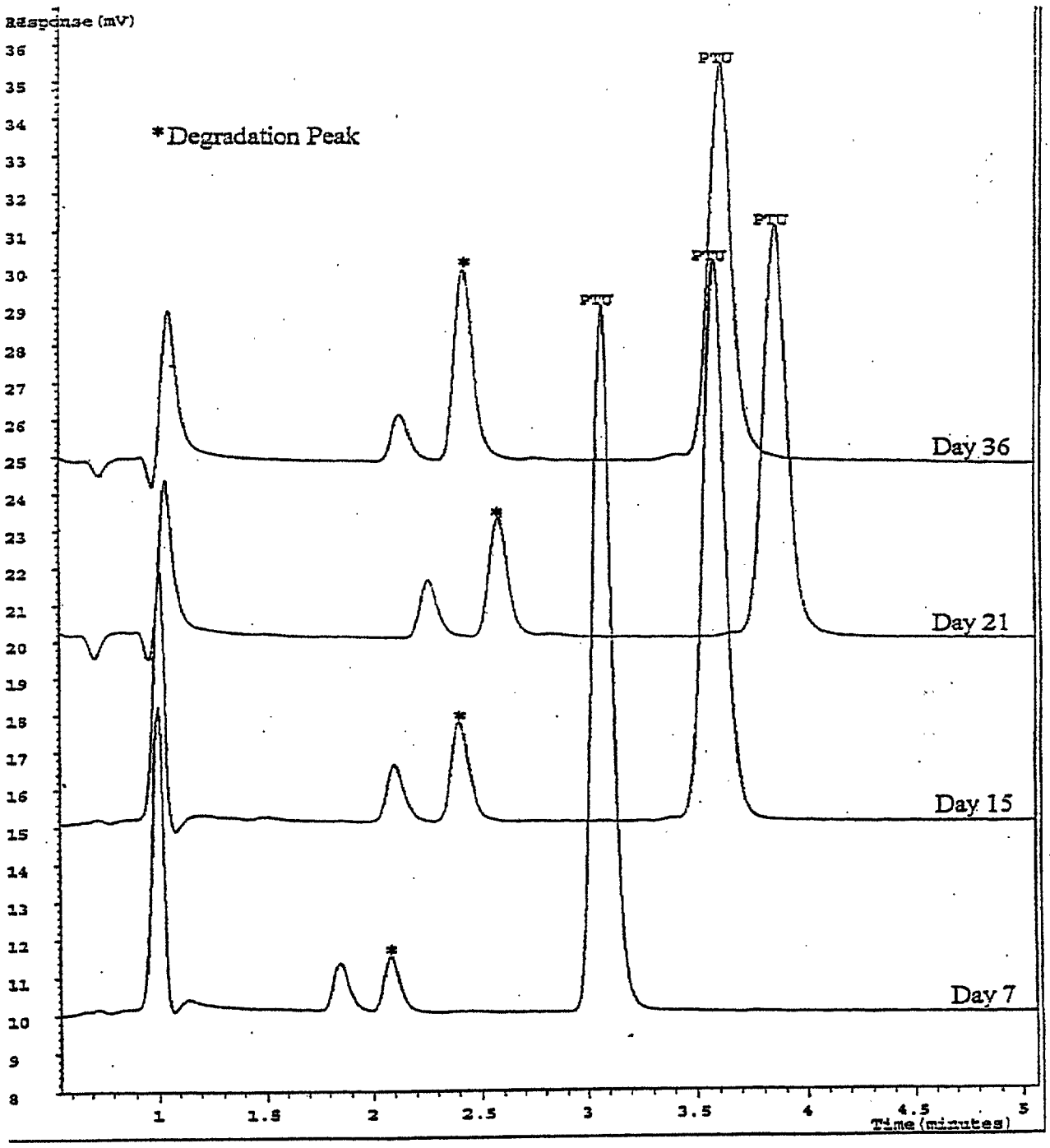

Figure 6 – Representative Chromatograms showing Degradation Peak during Stability Period

5.2 Simulated Animal Room Stability Study

Study Design

A simulated animal room stability study was done in the following manner. A single sample of approximately 200 mL from the same 5 µg/mL formulation used to conduct the storage stability study was placed in a clear glass drinking water bottle with a sipper tube. The bottle was placed in a hood. Triplicate aliquots were removed and analyzed after 4 and 7 days storage identically to the storage stability samples.

Results

The results from the simulated animal room stability study are shown in Table 5.

Table 5 – Simulated Animal Room Stability Study Results

Day	Det'd Conc (µg/mL)			Avg Det'd Conc (µg/mL)	% of Day 0 Conc
0	4.903	4.838	4.905	4.882 ± 0.038	100.0
4	4.545	4.601	4.640	4.596 ± 0.048	94.1 ± 1.0
7	4.714	4.651	4.662	4.676 ± 0.033	95.8 ± 0.7

Conclusions

The results from the simulated animal room showed a small decline in concentration over 7 days. However, the decline was sufficiently small that drinking water bottles can probably be used for 7 days.

6 CONTRIBUTORS

Analytical support for this work was provided by Mr. Tim Hutson and Mr. Dave Koebel. The report was written by Mr. Steve Graves.

TWO-GENERATION REPRODUCTION TOXICITY STUDY
OF PROPYLTHIOURACIL WHEN ADMINISTERED
TO SPRAGUE-DAWLEY RATS IN THE DRINKING WATER

Appendix 3: STANDARD OPERATING PROCEDURES
(At the time of protocol preparation)

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NECROPSY AND EUTHANASIA PROCEDURES

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STANDARD OPERATING PROCEDURES FOR HEALTH AND SAFETY

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	References
Visitors Access to Test Area	117.0, CHP, RPP
Employee Training	119.0, 1001.0, CHP, RPP
Medical Surveillance	1001.0, CHP, RPP
Eye Protection	117.0, 1003.0, CHP
Personal Protective Equipment	1003.0, CHP, RPP
General Housekeeping Practices	1005.0, CHP
Ventilation System Maintenance	715.0, CHP
Storage, Receipt, Transport and	501.0, 502.0, 504.0
Shipping of Study Materials	506.0, 506.1,1005.0, CHP
Spill Clean-Up, Accident and Emergency	1007.0, CHP
Response (including material disasters) and	
fires/explosions	
Dose Preparation	500 Series
Enter and Exit from Limited Access Areas	117.0, CHP, RPP
Respiratory Protection and Fit	RPP

Note: CHP = Chemical Hygiene Plan

RPP = Respiratory Protection Program

STANDARD OPERATING PROCEDURES FOR HEALTH AND SAFETY

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PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:
AMENDMENT NUMBER: 1	
STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water	
DISTRIBUTION:	
STUDY DIRECTOR/Wolfe **OPERATIONS DIRECTOR/Hatcher** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/James, M** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **HEALTH AND SAFETY OFFICER/Blackford** **STUDY NOTEBOOK/Nehrebechyj**	**PAI/Delaney** **CENTRAL FILE/Belardo** **DOSE PREPARATION/Holley** **ACUC Chair/Rocca** **PROJECT LEADER/Palabrica** **TOXICOLOGIST/Wang** **SPONSOR/Chapin**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION:	

1. **Subject: 4.2.3 Treatment**

   Treatment will be administered in the drinking water starting on SD 1 for all male and female animals and continue until necropsy.

   Justification: Clarification.

2. **Suject: 1.1 Proposed Investigations/Rational for Dose Selection**

   PTU is being used to validate a Two-Generation Study to Model proposed to identify potent ans weak thyroid toxicant.

   Justification: Typographic error.

3. **Subject: 4.2.5 Disposition of Offspring From F_0 Parents and 4.3 F_1 Evaluation (F_1 Parents/F_2 Pups)**

   Approximately one week before PND 99±10, one male will be assigned to one female to form 20 pairs per group. On PND 99±10, male and female rats from the same group will be paired (1 female to 1 male).

   Justification: To follow the EPA Guidelines and be consistent with F_0 generation, F_1 animals need to be dosed for at least 10 weeks before the mating period.

Approval:

RE Chapin 5.18.00

Robert E. Chapin, Ph.D. Date
Project Officer

Gary W. Wolfe 5/11/00

Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:
AMENDMENT NUMBER: 2	
STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water	
DISTRIBUTION:	
STUDY DIRECTOR/Wolfe **OPERATIONS DIRECTOR/Hatcher** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/Naawu** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **HEALTH AND SAFETY OFFICER/Blackford** **STUDY NOTEBOOK/Nehrebechyj**	**PAI/Delaney (3)** **CENTRAL FILE/Belardo** **DOSE PREPARATION/Holley** **ACUC Chair/Rocca** **PROJECT LEADER/Okoth** **TOXICOLOGIST/Wang** **SPONSOR/Bishop** **NECROPSY/Hackett**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION:	

1. **Subject: 4.2.6 Terminal Procedures**

   F_0 Females

   Add ovaries on Histopathology list.

   Justification: To be consistent with F_1 generation.

2. **Subject: 4.2.6 and 4.3.6 Termination**

   Histopathology for F_0 and F_1 females

   Thyroid and parathyroids and gross lesions will be evaluated microscopically.

   F_2 females selected for PND 21 necropsy:

   The necropsy will be performed on up to 3 females selected per litter and the ovaries from the first 2 females per litter will be evaluated microscopically.

   F_0 and F_1 Adult Males and F_2 males and Females Selected for PND 21 Necropsy:

   The entire liver will be saved.

   Justification: To be consistent between the generations.

3. **Subject: 4.1.1 Mortality**

   Ovaries will be saved in Bouin's and then transferred into 70% ethanol within 24-48 hours.

   Justification: Clarification.

4. **Subject: 4.2.5 Disposition of Offspring From F_0 Parents**

   On PND 16, 1-2 male and female pups will be randomly selected from each litter (up to 25 pups per sex per group) for the F_1 Mating Trial.

   Justification: To ensure sufficient number of pups selected for both necropsy and F_1 mating trial.

Approval:

Jack Bishop 9-29-2000

Jack Bishop, Ph.D. Date
Project Officer

Gary W. Wolfe 9/12/00

Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:

AMENDMENT NUMBER: 3

STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water

DISTRIBUTION:

STUDY DIRECTOR/Wolfe
OPERATIONS DIRECTOR/Hatcher
FACILITY MANAGER/Blackford
TECHNICAL SUPERVISOR/Naawu
VETERINARIAN/Greenstein
QUALITY ASSURANCE/Carignan
HEALTH AND SAFETY OFFICER/Blackford
STUDY NOTEBOOK/Nehrebechyj
PAI/Delaney (3)
CENTRAL FILE/Belardo
DOSE PREPARATION/Holley
ACUC Chair/Rocca
PROJECT LEADER/Okoth
TOXICOLOGIST/Wang
SPONSOR/Bishop
NECROPSY/Hackett

ORIGINAL FILED IN QA

SPONSOR AUTHORIZATION:

1. **Subject: 4.2.6 and 4.3.6 Termination**

   F_0 and F_1 Adult Males:

   Add spleen on the tissue preservation list.

   Justification: Omitted in the Protocol.

   F_1 and F_2 Males Selected for Necropsy PND 21:

   Dorsolateral, ventral Prostate, and seminal vesical/coagulating gland will be collected as one piece with urinary bladder attached as an indicator. 9-13-00

   Justification: Due to the sizes of the tissues.

Approval:

Jack B Bishop 9/29/2000
Jack Bishop, Ph.D. Date
Project Officer

Gary W. Wolfe 9/13/00
Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:
AMENDMENT NUMBER: 4	
STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water	
DISTRIBUTION:	
STUDY DIRECTOR/Wolfe **OPERATIONS DIRECTOR/Hatcher** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/Naawu** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **HEALTH AND SAFETY OFFICER/Blackford** **STUDY NOTEBOOK/Nehrebechyj**	**PAI/Delaney (3)** **CENTRAL FILE/Belardo** **DOSE PREPARATION/Holley** **ACUC Chair/Rocca** **PROJECT LEADER/Okoth** **TOXICOLOGIST/Wang** **SPONSOR/Bishop** **NECROPSY/Hackett**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION:	

1. **Subject: 4.2.6 and 4.3.6 Termination**

   F_0 and F_1 Males and Females:

   On the day of necropsy, blood will be collected via orbital sinus and serum will be obtained and frozen at ~-80°C.

   Justification: Serum samples are required for determination of hormone levels.

Approval:

Jack Bishop 9.29.2000

Jack Bishop, Ph.D. Date
Project Officer

Gary W. Wolfe 9/21/00

Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:
AMENDMENT NUMBER: 5	
STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water	
DISTRIBUTION:	
STUDY DIRECTOR/Wolfe **OPERATIONS DIRECTOR/Hatcher** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/Naawu** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **HEALTH AND SAFETY OFFICER/Blackford** **STUDY NOTEBOOK/Nehrebechyj**	**PAI/Delaney (3)** **CENTRAL FILE/Belardo** **DOSE PREPARATION/Holley** **ACUC Chair/Rocca** **PROJECT LEADER/Okoth** **TOXICOLOGIST/Wang** **SPONSOR/Bishop** **NECROPSY/Hackett**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION: Based on the 11-1-00 phone conversation	

1. **Subject: 4.1.1 Mortality**

   Histopathology of the unscheduled death for Group 4 Task 4 animals:

   Lower and upper jaws from 10 Group 4 animals (1 animal/sex/litter) will be embedded in paraffin, step sectioned, and examined microscopically by the study pathologist.

   Two skulls (1 male and 1 female from Group 1 at PND 23-24) saved from the study 7244-206 will be used as represented controls.

   Justification: Sponsor request due to a late incision eruption noted in Group 4 animals.

2. **Subject: 4.2.6 and 4.3.6 Termination**

   Histopathology for F_0 and F_1 Females:

   The uterus/vaginal/cervix from the first 10 females in each group will be saved and examined microscopically.

   Justification: Typographic error.

Approval:

Jack B Bishop 11-15-00

Jack Bishop, Ph.D. Date
Project Officer

Gary W. Wolfe 11/16/00

Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:
AMENDMENT NUMBER: 6	
STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water	
DISTRIBUTION:	
STUDY DIRECTOR/Wolfe **OPERATIONS DIRECTOR/Hatcher** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/Naawu** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **HEALTH AND SAFETY OFFICER/Blackford** **STUDY NOTEBOOK/Brown**	**PAI/Delaney (3)** **CENTRAL FILE/Belardo** **DOSE PREPARATION/Holley** **ACUC Chair/Rocca** **PROJECT LEADER/Okoth** **TOXICOLOGIST/Wang** **SPONSOR/Bishop** **NECROPSY/Hackett**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION:	

1. **Subject: 4.3.4 Measurements**

Food and Water Consumption

Females Weekly, after all females are separated.

Body Weights

Males Weekly following the final F_1 litter
At littering

Females Weekly following the final F_1 litter
At littering
F_1 dams during F_2 lactation – PND 1, 4, 7, 14, and 21 for all dams with F_2 litter

Physical Examination

Males and Females Weekly following the final F_1 litter

Justification: Clarification. F_1 females are not randomized so body weights and physical examinations are not required. No weekly consumption data are collected during gestation and lactation since data are collected as part of the lactation phase.

2. **Subject: 4.2.4 Measurements**

Weekly food consumption on all females will be done during weeks 1-14 and 18-19. Weeks 14-18 will be collected as lactation consumption data.

Justification: Clarification.

Approval:

Jack B Bishop 12/26/2000

Jack Bishop, Ph.D. Date
Project Officer

Gary W. Wolfe 12/19/00

Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:
AMENDMENT NUMBER: 7	
STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water	
DISTRIBUTION:	
STUDY DIRECTOR/Wolfe **OPERATIONS DIRECTOR/Hatcher** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/Naawu** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **HEALTH AND SAFETY OFFICER/Blackford** **STUDY NOTEBOOK/Brown**	**PAI/Delaney (3)** **CENTRAL FILE/Belardo** **DOSE PREPARATIO** [redacted] **ACUC Chair/Rocca** **PROJECT LEADER/Okoth** **TOXICOLOGIST/Wang** **SPONSOR/Bishop** **NECROPSY/Hackett**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION:	

1. **Subject: 4.3.6 Termination**

   F_2 Males and Females Selected for Necropsy PND 21

   Histopathology: Possible histopatholgy will be determined by Sponsor after review of data.

   Justification: To be consistent with F_1 generation.

   F_1 Males and Females

   Serum obtained from each animal for TSH, T3, and T4 analysis will be spilt in two aliquots. One aliquot will be sent to AniLytics, Inc for thyroid hormone analysis and the other aliquot will be stored at ~-80°C at TherImmune until results are received from AniLytics.

   Justification: To further evaluate results received for the F_0 animals.

Approval:

Jack B Bishop 1-17-01

Jack Bishop, Ph.D. Date
Project Officer

Gary W. Wolfe 1/15/01

Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

PROTOCOL AMENDMENT

TherImmune No.: 7244-601	NTP/NIEHS Protocol No.:
AMENDMENT NUMBER: 8	
STUDY TITLE: Propylthiouracil: Two-Generation Reproductive Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats In The Drinking Water	
DISTRIBUTION:	
STUDY DIRECTOR/Wolfe **OPERATIONS DIRECTOR/Hatcher** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/Naawu** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **HEALTH AND SAFETY OFFICER/Blackford** **STUDY NOTEBOOK/Brown**	**PAI/Delaney (3)** **CENTRAL FILE/Belardo** **DOSE PREPARATION/Holley** **ACUC Chair/Rocca** **PROJECT LEADER/Nehrebeckyj** **TOXICOLOGIST/Wang** **SPONSOR/Bishop** **NECROPSY/Hackett**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION:	

1. Subject: 4.3.6 Terminal Procedures

F_1 Males

After conducting epididymal sperm density count, approximately 5 mls of sperm suspensions from all necropsied males will be saved, frozen and sent to an NTP subcontractor.

Justification: Sponsor request.

Approval:

Jack Bishop 2/28/01

Jack Bishop, Ph.D. Date
Project Officer

Gary W. Wolfe 2/23/01

Gary W. Wolfe, Ph.D., D.A.B.T. Date
Study Director

Appendix 3

Two-Generation Reproduction Toxicity Study of Propylthiouracil when Administered to Sprague-Dawley Rats in the Drinking Water

Milestone Schedule

CONTAIN NO CBI

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Perperl

THERIMMUNE RESEARCH CORPORATION

Propylthiouracil:

Two Generation Reproduction Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rata In The Drinking Water

Study No.: 7244-601 R.O.W. No.: 1340 Test Article: Propylthioura

Study Activity	F0 Generation	Holding Phase	F1 Generation
Animals Arrive	05/09/00	9/12/00 - 9/28/00 AVG PND 1±10 =8/31/00	12/7/00 (PND 99±10)
Initial Dose Prep and Samples to ASD	Initiation: 4/27/00, Middle mix:7/24/00, Final mix: 10/2/00	NA	11/27/00
Bulk Analysis of Test Article	4/27/00,	NA	10/9/00, 3/26/01
Dose Formulations	Biweekly	Biweekly	Biweekly
Weight Randomization	5/16/00	NA	NA
Quarantine Release	5/17/00	NA	NA
Viral Screen	5/17/00	NA	NA
Animal ID	5/17 - 19/00	9/7/00 - 9/23/00	NA
Dose Initiation	5/23/00	PND 21 (9/12/00 - 9/28/00)	12/7/00 (Week 1=PND 99±10)
Body Weights	Randomization, Initiation, weekly start 5/23/00, Littering, ♀- PND1, 4, 7, 14, 21	Start on 9/28/00, following the final F1 litters, Weekly thereafter	Initiation, 12/7/00 Weekly thereafter
Phys. Exams	Randomization, Initiation, Weekly start 5/23/00	Start on 9/28/00, following the final litters, Weekly thereafter	Initiation, 12/7/00 Weekly thereafter
Food Consumption	♂,♀ - week 1- 10, 5/23/00 - 8/1/00 ♂, ♀ - week 13-20, 8/15/00 - 10/3/00 ♀ - PND 1, 4, 7, 11, 14, 18, 21	NA	♂,♀ week 3-10, 12/21/00 - 2/15/01, ♀ - PND 1, 4, 7, 11, 14, 18, 21
Water Consumption	♂, ♀ - week 1-10, 5/23/00 - 8/1/00 ♂, ♀ - week 13-20, 8/15/00 - 10/3/00 ♀ - PND 1, 4, 7, 11, 14, 18, 21	NA	♂, ♀ - week 3-10, 12/21/00 - 2/15/01 ♀ - PND 1, 4, 7, 11, 14, 18, 21
Cohabitation	8/1/00	NA	12/7/00 (Week 1=PND 99±10)
Confirmation of Mating	8/2/00 - 8/15/00	NA	12/8/00 - 12/21/00
Separation	When sperm positive or on 8/15/00	NA	when sperm positive or on 12/21/00

THERIMMUNE RESEARCH CORPORATION

Propylthiouracil:

Two Generation Reproduction Toxicity Study of Propylthiouracil
When Administered to Sprague-Dawley Rata In The Drinking Water

Study No.: 7244-601 R.O.W. No.: 1340 Test Article: Propylthiouracil

Pup Observations	F1 - 8/23/00 - 9/8/00 (PND1),	NA	F1 - 12/29/00 - 1/14/01 (PND 1)
Pinna Observation	Start on PND 2, 8/24/00	NA	Start on PND 2, 12/30/00
Eye Opening Obs.	Start on PND 2, 8/24/00	NA	Start on PND 2, 12/30/00
Nipple Ret. Obs.	On PND 12 & 13, 9/3/00 - 9/20/00	NA	On PND 12 and 13, 1/9/01 - 1/26/01
Testis Descent Obs	NA	Start on PND 16, 9/7/00	NA
Vaginal Opening Obs	NA	Start on PND 25, 9/16/00	NA
Preputial Sep. Obs	NA	Start on PND 35, 9/26/00	NA
Pup Selection	NA	On PND 16, 9/7/00 - 9/23/00	NA
Pup Separation	NA	on PND 21, 9/12/00 - 9/28/00	On PND 21, 1/18/01 - 2/3/01
Vaginal Cytology	7/18/00 - 7/31/00	NA	2/7/01 - 2/20/01
Blood Collection	At Termination 10/3/00 - 10/5/00	NA	At the termination, 2/21/01 - 2/23/01
Termination/Necropsy	10/3/00 - 10/5/00	on PND 21, 9/12/00 - 9/28/00	2/21/01 - 2/23/01
Sperm Analysis	10/6/00 - 10/27/00	NA	2/26/01 - 3/19/01
Summary Report Due	11/14/00	NA	3/30/01
Data Files to QC	9/00 - 11/00	NA	1/01
Data Files to ASI	9/00 - 11/00	NA	1/01- 3/01
Draft Final Report	NA	NA	4/27/01

DATE ISSUED: 5/10/00 DATE REVISED : 10/25/99

CONTAIN NO CBI

Appendix 4

Two-Generation Reproduction Toxicity Study of Propylthiouracil when Administered to Sprague-Dawley Rats in the Drinking Water

Analytical Chemistry Reports

CONTAIN NO CBI

51-52-5

COPY

Battelle
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BATTELLE-SPEC

Chemistry Support Services for the NTP

NIH Contract No.: N01-ES-55395

Battelle Project No.: G002840-AGC

NTP ChemTask No.: CHEM04440

CAS No.: 51-52-5

SPECIAL CONFIRMATION OF IDENTITY AND LIMITED DOSE FORMULATION DEVELOPMENT STUDY REPORT

6-PROPYL-2-THIOURACIL

4-106-SPEC-100

February 1, 2000

Written By:

Steven Graves
Study Director

Approved By:

Steven Graves
Principal Investigator

Submitted to:

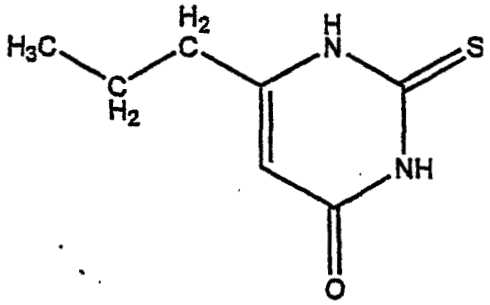
Dr. Cynthia S. Smith
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T.W. Alexander Dr.
Research Triangle Park, NC 27709-2233

7244-601
WOLFE
TIAN
WING-
HOLLEY
PATRICIA

SPECIAL CONFIRMATION OF IDENTITY AND LIMITED DOSE FORMULATION DEVELOPMENT STUDY REPORT

6-PROPYL-2-THIOURACIL

CAS No.: 51-52-5	Lot No.: 47H2500
Battelle Chemical ID Code: 106	Amount Received: 500 g
Battelle Task No.: 4-106-SPEC-100	Receipt Date: 3/18/99
NTP ChemTask No.: CHEM04440	Appearance: White powder
Program Supported: OTH	Submitter: Not applicable
Analysis Dates: 4-1-99 to 5-11-99	Study Lab: Not applicable
Interim Results Date: Not applicable	Vendor: Sigma Chemical Co.
	Vendor Purity: 99.8%(NaOH titration); 99.9% (HLPC)
	Storage Conditions: Room temperature (~25°C)

STRUCTURE	Mol. Wt.	Mol. Formula
	170.20 g/mole	$C_7H_{10}N_2OS$

EXECUTIVE SUMMARY

The identity of the chemical was confirmed as 6-propyl-2-thiouracil by infrared spectroscopy. The information from the supplier indicated it was 99.8+% pure.

A reverse-phase high performance liquid chromatography (HPLC) method with UV detection was developed, evaluated and found to be suitable for the analysis of formulations from 2.5 to 50 ppm (µg/mL).

A stability study at 5 ppm indicated that formulations stored at 5°C in sealed glass bottles were stable for 36 days, but those stored at room temperature were unstable.

A simulated animal room study indicated that a 5 ppm formulation was sufficiently stable to allow for weekly drinking water bottle changes.

QUALITY ASSURANCE STATEMENT

SPECIAL CONFIRMATION OF IDENTITY AND LIMITED DOSE FORMULATION DEVELOPMENT STUDY REPORT

6-PROPYL-2-THIOURACIL

NTP ChemTask No.: CHEM04440

Battelle Project No.: G002840-AGC

Battelle Task No.: 4-106-SPEC-100

Listed below are the phases and/or procedures performed by Battelle that were reviewed by the Quality Assurance Unit during performance of the task described in this report. Adverse findings, if any, were reported to the study director at the time of review.

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Infrared spectroscopy analysis	4/1/99	4/1/99
Audit study file	1/28/00	1/28/00
Audit analytical report	1/28/00	1/28/00

This report reflects the procedures and raw data generated in this study.

In addition to the study-specific audits/inspections cited above, routine inspections of the general facilities and equipment were performed by the QAU and reports were submitted to management as follows:

Facility/Equipment	Date Inspected	Date of Report to Management
Chemistry Technical Center inspection	2/6-2/9/96	2/14/96
	5/8/96	6/11/96
	6/5, 6/12 and 6/24/97	6/25/97
	5/27 and 5/28/98	6/1/98
	7/27/99	7/30/99

2-1-2000

Quality Assurance Unit — Date

Battelle

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1 INTRODUCTION

The purpose of this work was to confirm the identity of this material as 6-propyl-2-thiouracil (PTU); estimate its purity; identify and quantitate, if possible, any significant impurities in the material; develop and evaluate a formulation analysis method; and evaluate the stability of the formulation. This work was done at Battelle, 505 King Avenue, Columbus, OH 43201 in support of OTH studies.

2 CHEMICAL RECEIPT AND STORAGE

6-Propyl-2-thiouracil, Lot No. 47H2500, was received from Sigma on March 18, 1999. Five 1-L amber bottles, nominally containing 100 g each were received. The chemical was homogenized by combining the contents of the bottles in a glass beaker. The chemical was then stirred with a spatula for about five minutes. The 6-propyl-2-thiouracil was repackaged into the original bottles. The net weight was determined to be 510 g.

Samples were taken after repackaging. The number of samples and approximate amounts removed were two 11.5-g "analytical" samples, one 5-g "archive" sample, two 11.5-g "retention" samples. The "archive" and "retention" samples were stored at ≤-20°C. The "analytical" sample was stored at room temperature. The net weight of chemical remaining after sampling was determined to be 462 g. The remaining chemical was stored at room temperature.

A copy of the supplier's Certificate of Analysis for this lot is shown in Figure 1. This states that the purity of the sample is 99.8% by NaOH titration and 99.9% by HPLC.

3 IDENTITY CONFIRMATION BY INFRARED (IR) SPECTROSCOPY

3.1 Method

The infrared spectrum was obtained on a Digilab FTS-60A Fourier Transform infrared spectrometer at 8 cm^{-1} resolution. The sample was prepared as potassium bromide pellet. The spectrum of the test article was compared to a literature reference spectrum found in "Sadtler Research Laboratories, Inc., Pharmaceutical Spectra, Spectrum R573.

3.2 Results

The IR spectrum (Figure 2) was consistent with the literature reference spectrum (Figure 3).

3.3 Conclusions

The sample's identity was confirmed as 6-propyl-2-thiouracil.

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SIGMA

Certificate of Analysis

TEST	SPECIFICATION	LOT {047H2500} RESULTS
Product Name	6-N-propyl-2-thiouracil	
Product Number	P3755	
CAS Number	51-52-5	
Formula	$C_7 H_{10} N_2 O S$	
Formula Weight	170.20	
APPEARANCE	WHITE TO WHITE WITH A YELLOW CAST	WHITE POWDER
SOLUBILITY	CLEAR COLORLESS TO SLIGHTLY HAZY FAINT YELLOW SOLUTION IN 1 M SODIUM	CLEAR COLORLESS SOLUTION AT 200 MG PLUS 4.0 ML OF 1 M SODIUM HYDROXIDE
IR SPECTRUM	CONSISTENT WITH STRUCTURE	CONSISTENT WITH STRUCTURE
PURITY BY SODIUM HYDROXIDE TITRATION	99% MINIMUM	99.8%
PURITY BY HPLC	99% MINIMUM	99.9%
QC ACCEPTANCE DATE		APRIL 1997

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Sigma brand products are sold exclusively through Sigma-Aldrich, Inc.

David Feicker, Manager
Analytical Services

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Figure 1 – Certificate of Analysis

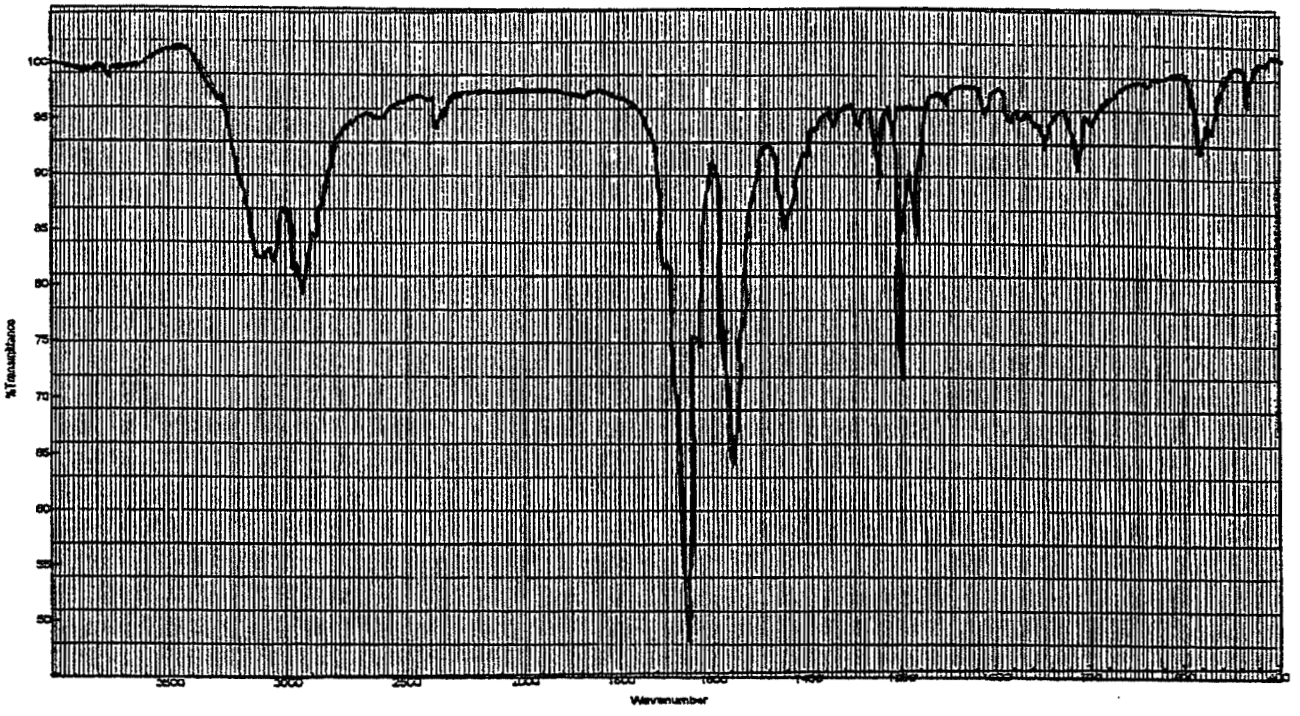

Figure 2 – IR Spectrum of 6-Propyl-2-Thiouracil, Lot No. 47H2500

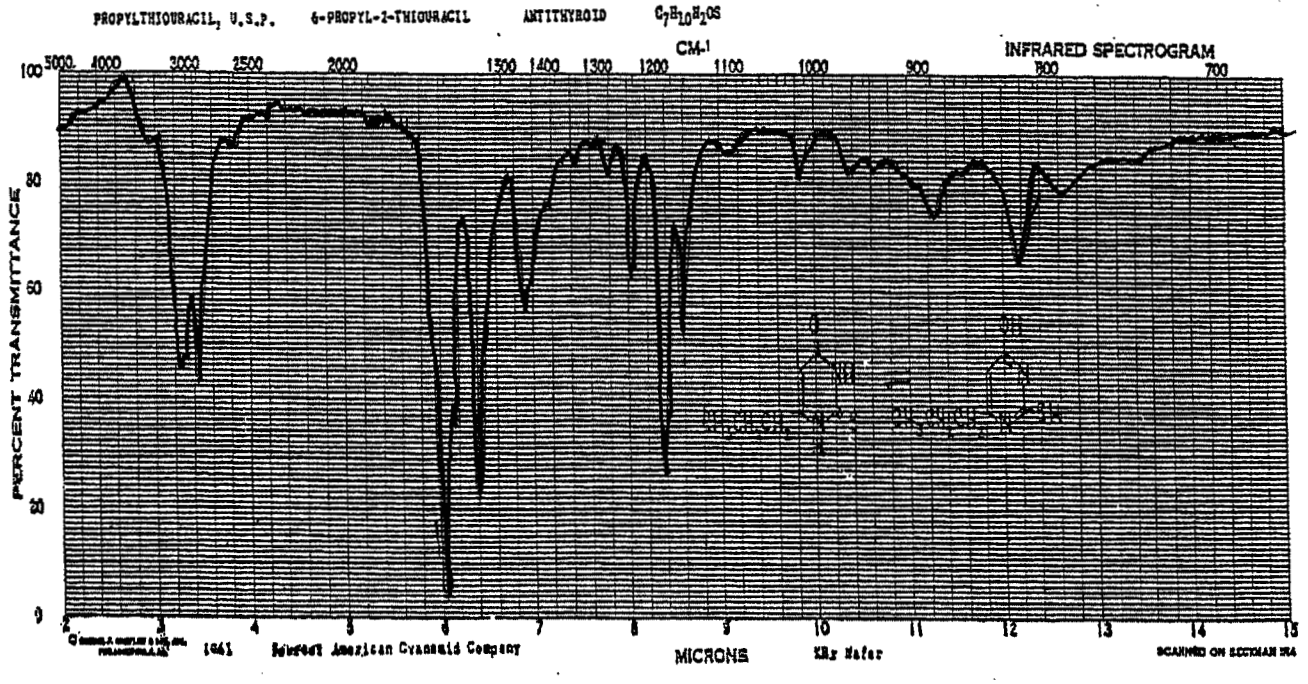

Figure 3 – Literature IR Spectrum of 6-Propyl-2-Thiouracil

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4 LIMITED DOSE FORMULATION METHOD VALIDATION

This section describes the evaluation of an analysis method suitable for 6-propyl-2-thiouracil formulations in tap water over the concentration range from 5 to 25 ppm, the results, and the conclusion from this evaluation.

4.1 Method/Experimental Design

A standard curve was prepared containing standards at six concentrations in tap water. Triplicate standards at the highest and lowest concentrations and single standards at the intermediate concentrations were prepared. Triplicate blanks containing internal standard and blanks without internal standard were also prepared.

4.2 Preparation of Internal Standard

An intermediate internal standard (IS) solution was prepared by diluting 50 µL of acetophenone to 50 mL with acetonitrile. This solution was diluted 1-to-100 with tap water to produce the working internal standard.

4.3 Preparation of Standards and Blanks

Two stock standards (A and B) were prepared at target concentrations of 50 and 25 µg/mL by dissolving and diluting approximately 25 and 12.5 mg, respectively, of accurately weighed PTU to 500 mL with tap water. These two solutions also served as the two most concentrated vehicle standards.

Four additional vehicle standards were prepared at target concentrations of 12.5, 5.0, 6.25, and 2.5 µg/mL by diluting 2.5 and 1 mL, respectively, of Stock Standards A and B to 10 mL with tap water.

Working standards were prepared by combining 1 mL of vehicle standard and 1 mL of working IS in individual autoinjector vials. Triplicate working standards were prepared from the highest and lowest concentration vehicle standards. Single working standards were prepared from the other vehicle standards.

Triplicate blanks with IS were prepared by combining 1 mL of tap water and 1 mL of working IS.

Tap water served as the blank.

4.4 Analysis

Single injections were made from each vial using the HPLC system shown in Table 1. Typical chromatograms from a high and low vehicle standard, a blank with internal standard and a blank are shown in Figure 4.

Table 1 – HPLC System

Column	Inertsil ODS (2) 5µ, 150 X 3.0 mm (ID)
Mobile Phase Components	A: Milli-Q Water B: Acetonitrile C: Concentrated Phosphoric Acid
Mobile Phase Composition	90%A:10%B:0.1%C (v:v:v), Isocratic
Flow Rate	1.0 mL/minute
Injection Volume	25 µL
Detector Type and Wavelength	Ultraviolet at 254 nm
Run Time	20 minutes
Retention Times PTU IS	 ~2.0 minutes ~13.5 minutes

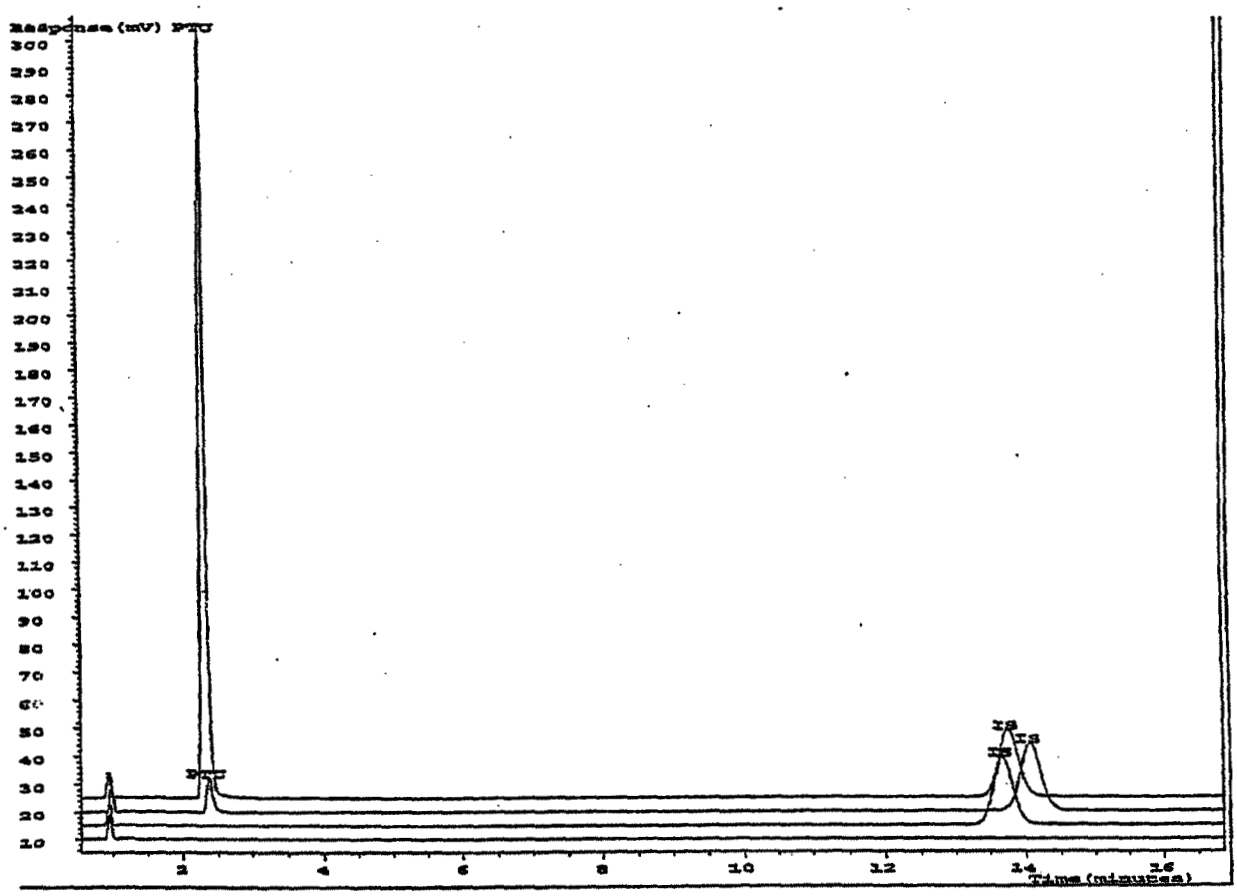

Figure 4 – Representative Chromatograms from High Standard, Low Standard, Blank with IS, and Blank

(Chromatograms are in order from top high standard, low standard, blank with IS to blank)

4.5 Calculations

The integration of the PTU and internal standard (IS) peaks, as done by the chromatography data system, was evaluated and manually adjusted, if necessary to achieve consistent integration. The response ratio of the PTU peak area divided by the IS peak area was calculated. An unweighted linear regression equation was calculated relating the response ratio of the working standards to their actual concentrations. A determined concentration was calculated for each standard using the regression equation and the response ratio for that standard. The relative error for each standard was calculated by subtracting the actual concentration from its determined concentration, dividing the difference by the actual concentration and multiplying the ratio by 100. The average relative error, the standard deviation, and the relative standard deviation were calculated for the low and high standards.

4.6 Results

The results from the analysis of the standard curve are shown in Table 2.

Table 2 – Dose Analysis MPE Results

Nominal Vehicle Std Conc (µg/mL)	Det'd Vehicle Std Conc (µg/mL)	Avg Det'd Vehicle Std Conc (µg/mL)	S (µg/mL)	%RSD	%RE	Avg %RE
	52.34				1.3	
51.66	51.27	51.67	0.58	1.1	-0.8	0.0
	51.40				-0.5	
24.90	24.75	NA	NA	NA	-0.6	NA
12.92	13.06	NA	NA	NA	1.1	NA
6.255	6.205	NA	NA	NA	-0.3	NA
5.166	5.187	NA	NA	NA	0.4	NA
	2.473				-0.7	
2.490	2.505	2.479	0.023	0.9	0.6	-0.4
	2.460				-1.2	

NA = Not Applicable

The limit of detection (LOD), defined as three times the standard deviation of the lowest standard because there was no blank response, was 0.069 µg/mL. The limit of quantitation (LOQ), defined as ten times the standard deviation of the lowest standard because there was no blank response, was 0.232 µg/mL. The experimental limit of quantitation (ELOQ), defined as the lowest standard with acceptable accuracy and precision, was 2.490 µg/mL.

The results of a regression analysis of the standard curve are shown in Table 3. The regression line for the vehicle curve is shown in Figure 5.

Table 3 – Results of Regression Analysis

Slope	y-Intercept	Correlation Coefficient
0.06228	-0.01769	0.999

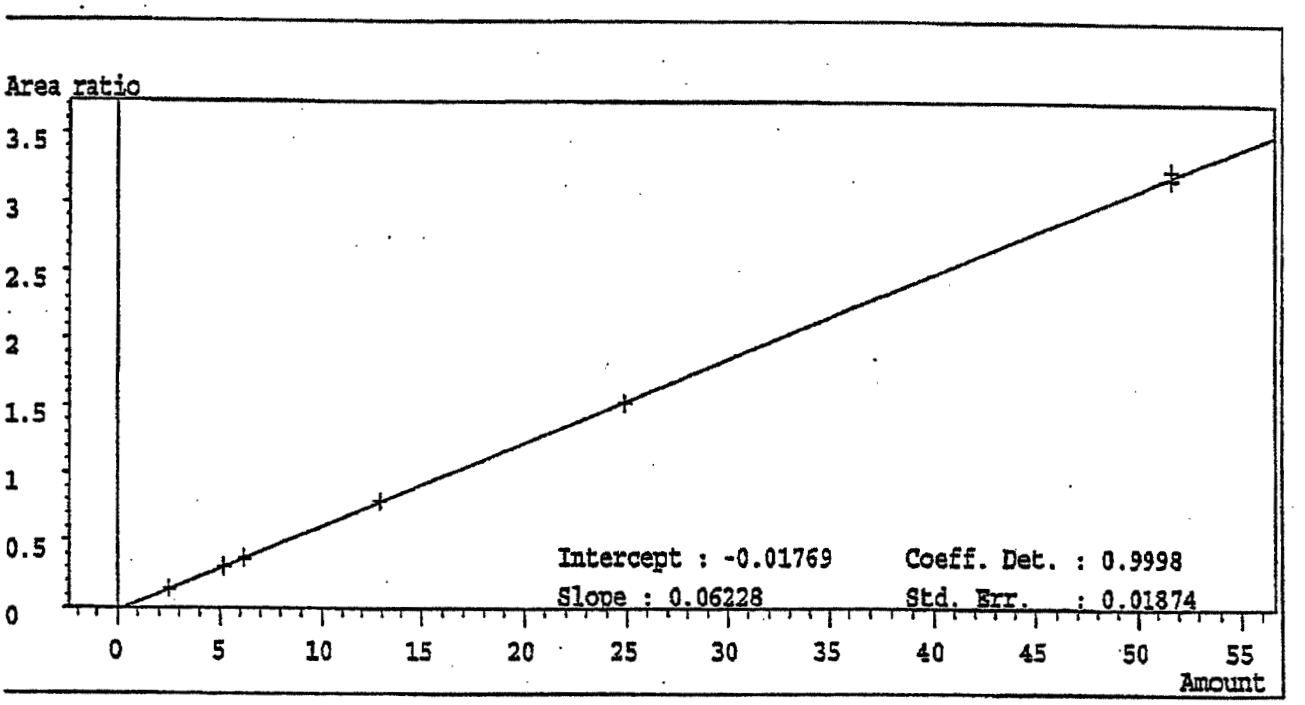

Figure 5 – Standard Curve from MPE

4.7 Conclusions

The method met all acceptance criteria for linearity, precision, accuracy, sensitivity and specificity. It is suitable for the analysis of PTU in tap water at concentrations from 2.5 to 50 µg/mL (ppm).

5 DOSE FORMULATION STUDIES

5.1 Formulation Storage Stability Study

5.1.1 Study Design

A storage stability study was conducted at a target concentration of 5 ppm (µg/mL) in tap water for samples in sealed amber glass bottles for 36 days at room temperature (25°C) and 5°C.

5.1.2 Preparation of Formulation

A stability stock standard was prepared at a target concentration of 250 µg/mL by dissolving and diluting approximately 25 mg of accurately weighed PTU to 100 mL with tap water. The formulation for the stability study was prepared by diluting 20 mL of the stability stock standard to 1-L with tap water. Fifty (50) mL of the formulation was transferred to two 60-mL amber glass bottles. The bottles were sealed. One bottle was stored at room temperature; the other was stored at 5°C.

5.1.3 Preparation of Analytical Standards

A stability stock solution was prepared by dissolving and diluting approximately 25 mg of accurately weighed PTU to 100 mL with tap water on each analysis day. This stock was diluted one-to-fifty with tap water to produce a vehicle standard with a target concentration of 5 µg/mL. Nine working standards were prepared from the vehicle standard on each analysis day by combining 1 mL of the vehicle standard and 1 mL of working IS in nine autoinjector vials. The vials were sealed.

5.1.4 Preparation of Formulation Samples

Triplicate aliquots were taken from the mixing flask on Day 0 and from both glass bottles on the other study days. One (1) mL of the formulation was combined on each analysis day with 1 mL of working IS in autoinjector vials. The vials were sealed.

5.1.5 Analysis

Injections of the standards and formulation samples were made using the chromatography system in Table 1 (Method Validation). At least three standard injections were made before and after the formulation samples. The peak area ratio of PTU/IS was calculated for each standard and these ratios were averaged. The peak area ratio of PTU/IS was calculated for each formulation sample and these ratios were averaged. The formulation samples' individual concentrations were calculated by dividing their peak area ratio by the averaged ratio for the standards and then multiplied by the nominal standard concentration. The average formulation concentrations were calculated.

5.1.6 Results

The results from the storage stability studies are shown in Table 4.

Table 4 – Formulation Storage Stability Study Results (5 µg/mL)

Day	Storage Temp (°C)	Det'd Conc (µg/mL)			Avg Det'd Conc (µg/mL)	% of Day 0 Conc
0	NA	4.903	4.838	4.905	4.882 ± 0.038	100.0
7		4.882	4.765	4.843	4.830 ± 0.060	98.9 ± 1.2
15		4.834	4.896	4.919	4.883 ± 0.044	100.0 ± 0.9
21	5	4.694	4.669	4.663	4.675 ± 0.017	95.8 ± 0.3
29		5.046	4.920	4.968	4.978 ± 0.064	102.0 ± 1.3
36		4.476	4.617	4.570	4.554 ± 0.072	93.3 ± 1.5
7		4.503	4.447	4.416	4.455 ± 0.044	91.2 ± 0.9
15		3.975	3.994	4.028	3.999 ± 0.027	81.9 ± 0.6
21	25	3.639	3.595	3.525	3.586 ± 0.057	73.5 ± 1.2
29		3.484	3.430	3.486	3.467 ± 0.031	71.0 ± 0.6
36		3.201	3.229	3.196	3.208 ± 0.018	65.7 ± 0.4

5.1.7 Discussion and Conclusions

The concentration of the formulation was within 10% of target on Day 0. Based on a pooled relative standard deviation of 0.9%, any individual value would have to be more than 2.1% different from 100% to be statistically significant at the 95% confidence level.

The concentration of the formulation stored at room temperature showed a steady decline over time. The chromatograms for the room temperature samples showed a marked increase in the size of peak eluting before PTU with time. Figure 6 shows chromatograms from room temperature samples on Days 7, 15, 21 and 36.

The concentration of the formulation stored at 5°C had no clear trend with time, although two of the points were lower than the lower control value. The degradation peak increased significantly at room temperature but did not significantly increase at 5°C.

These data indicate that the formulation can be stored for 36 days at 5°C in sealed amber glass containers.

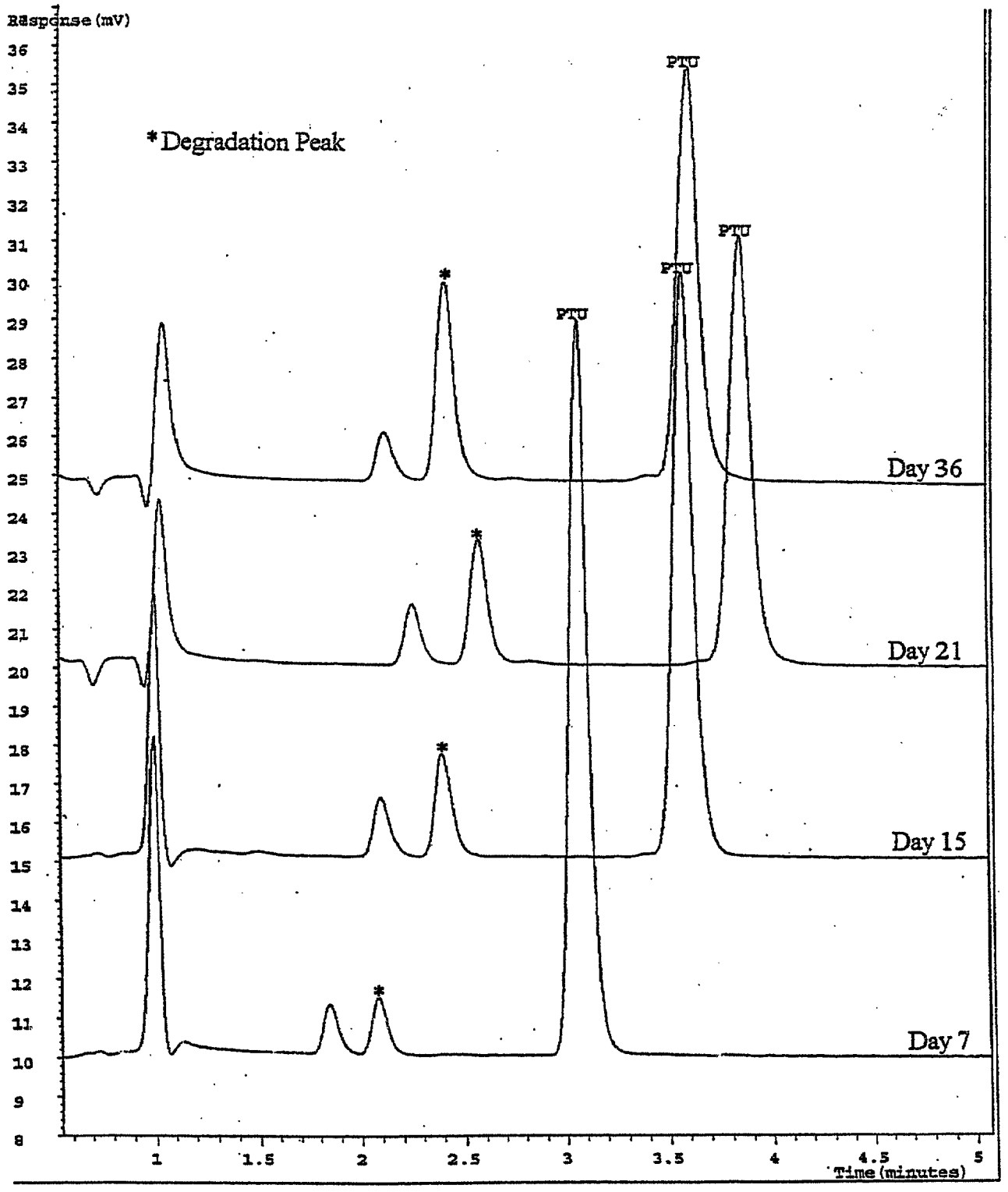

Figure 6 – Representative Chromatograms showing Degradation Peak during Stability Period

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5.2 Simulated Animal Room Stability Study

5.2.1 Study Design

A simulated animal room stability study was done in the following manner. A single sample of approximately 200 mL from the same 5 µg/mL formulation used to conduct the storage stability study was placed in a clear glass drinking water bottle with a sipper tube. The bottle was placed in a hood. Triplicate aliquots were removed and analyzed after 4 and 7 days storage identically to the storage stability samples.

5.2.2 Results

The results from the simulated animal room stability study are shown in Table 5.

Table 5 – Simulated Animal Room Stability Study Results

Day	Det'd Conc (µg/mL)			Avg Det'd Conc (µg/mL)	% of Day 0 Conc
0	4.903	4.838	4.905	4.882 ± 0.038	100.0
4	4.545	4.601	4.640	4.596 ± 0.048	94.1 ± 1.0
7	4.714	4.651	4.662	4.676 ± 0.033	95.8 ± 0.7

5.2.3 Conclusions

The results from the simulated animal room showed a small decline in concentration over 7 days. However, the decline was sufficiently small that drinking water bottles can probably be used for 7 days.

6 CONTRIBUTORS

Analytical support for this work was provided by Mr. Tim Hutson and Mr. Dave Koebel. The report was written by Mr. Steve Graves.

Project No.:	7244-601	Page No.:	
Interval:	Mix 27	SD:	Wolfe
Date:	10/6/00	Analyst:	WC

Results

Sample Name	Peak Area	Amount Detected (ng)	Calc Factor	Calc Amount (ug/mL)	Target Amount (ug/mL)	% Target
Gr.2A	107052	44.44	0.02000	0.8889	1.000	88.9
Gr.2B	107066	44.45	0.02000	0.8890	1.000	88.9
Bracket	304634	127.0	0.02000	2.539	2.490	102
Gr.3A	465468	194.1	0.02000	3.883	4.000	97.1
Gr.3B	466338	194.5	0.02000	3.890	4.000	97.2
Bracket	304604	126.9	0.02000	2.539	2.490	102
Gr.1	460	----	0.02000	----	0.0000	NA
Bracket	304578	126.9	0.02000	2.539	2.490	102

NOTE:

The small peak present in the control that elutes at the same time as the peak of interest, shows a peak area of 460. The peak area of 460, accounts for < 0.5%, of the lowest peak area response for the Group 2A (107052).

The Group 1 result detected, would be displayed as a negative value, it is beyond the linear range of the Standard Curve, and will not be reported. Fields will be filled in with dashed lines (----).

Athey, Patricia M

To: Gary Wolfe (E-mail); Veronica Godfrey (E-mail)
Cynthia Smith Ph. D. (E-mail); Graves, Steve; Athey, Patricia M

Subject: Preliminary Results for CHEM05666 (6-Propyl-2-Thiouracil FA Task), G004110-AUA

The results of analysis of the samples of formulations of 6-propyl-2-thiouracil in deionized water (Study # 7244-601, Mix 27) prepared on 10/5/00 and received from TherImmune on 10/18/00 are summarized below.

Gp. #	Target Conc. %	Det'd Conc. %	Avg. Det'd Conc. %	E/O	%RE	Avg. %RE
1	0	BLOQ BLOQ	BLOQ	NA	NA NA	NA
2	0.0001	0.00008948 0.00009025	0.00008986	0.99	-10.5 -9.8	-10.1
3	0.0004	0.0003996 0.0004011	0.004004	1.00	-0.1 0.3	0.1

BLOQ = Below Limit of Quantitation; no test article in sample.

A copy of this message that can be viewed using the Adobe Acrobat reader is attached.

Pat Athey

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E-mail athey@battelle.org

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51-52-5

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-AWU
NTP ChemTask No.: CHEM05856
CAS No.: 51-52-5

COPY

BULK CHEMICAL REANALYSIS REPORT

6-PROPYL-2-THIOURACIL

6-106-BCR-47

February 13, 2001

Prepared By:

Wendy M. Black
Task Leader

Approved By:

Steven Graves
Principal Investigator

Submitted to:

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Research Triangle Park, NC 27709-2233

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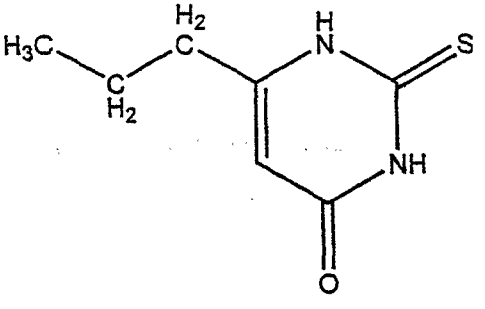
7244-601
WOLFE
HOLLEY
WANG
NEHRBECKLS

BULK CHEMICAL REANALYSIS REPORT

6-PROPYL-2-THIOURACIL

CAS No.: 51-52-5	Lot No.: 47H2500 (TherImmune)
Battelle Chemical ID Code: 106	Samples/Amount Received: 2 15-mL amber glass serum vials/2 g
Battelle Task No.: 6-106-BCR-47	Sample Receipt Date: 1/9/01
NTP Task No.: CHEM05856	Submitter: Not applicable
Program Supported: RDGT	Study Lab: Not applicable
Analysis Date: 1/18/01	Receipt Condition/Appearance: Refrigerated/Solid Powder
Interim Results Date: 1/22/01	Shipping Container: Information not provided
	Storage Conditions (@ Battelle): Room temperature (~25°C)

STRUCTURE	Mol. Wt.	Mol. Formula
	170.20 g/mole	$C_7H_{10}N_2OS$

EXECUTIVE SUMMARY

The purpose of this study was to compare the purity of a sample of the bulk chemical currently in use to the same lot of a frozen reference standard maintained at ≤-20°C. The sample was analyzed by high performance liquid chromatography (HPLC) with UV detection and determined to have a purity of 100.0% relative to the frozen reference, which indicates the purity of the sample is acceptable for continued use.

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1 INTRODUCTION

The purpose of this study was to compare the purity of the 6-propyl-2-thiouracil (PTU) bulk chemical sample currently in use to a frozen reference standard of the same lot maintained at ≤-20°C and to assure that the purity of the chemical is acceptable for continued use. This bulk chemical reanalysis task was performed in support of RDGT studies. This task was conducted at Battelle, 505 King Avenue, Columbus, Ohio 43201.

2 TEST ARTICLE

The bulk chemical reanalysis sample of 6-propyl-2-thiouracil, Lot No. 47H2500, was received at Battelle from TherImmune on January 9, 2001. The frozen reference standard for this analysis was removed from the "archives" sample, which had been stored at ≤-20°C at Battelle following the completion of the chemical handling for this lot.

3 ANALYSIS METHOD

3.1 Preparation of Internal Standard Solution

A solution was prepared by pipetting 100 µL of acetophenone into a 50-mL volumetric flask and dissolving in and diluting to volume with acetonitrile. The flask was sealed and mixed well. One (1) mL of this solution was pipetted into a 100-mL volumetric flask and the flask diluted to volume with Milli-Q water (Milli-Q water has a resistivity of ≥18 megohm-cm). This solution was the working internal standard (IS).

3.2 Preparation of Frozen Reference Standards

Triplicate stock solutions were prepared by transferring 50 ± 5 mg of accurately weighed frozen reference standard into separate 100-mL volumetric flasks. The samples were dissolved in and diluted to volume with Milli-Q water and mixed well. One (1) mL of each of these solutions was pipetted into individual 25-mL volumetric flasks. The contents of the flasks were diluted to volume with Milli-Q water and mixed. One (1) mL of each of these solutions and 1 mL of IS was pipetted into individual autoinjector vials, the vials were sealed and mixed well.

3.3 Preparation of Bulk Chemical Samples

Triplicate stock solutions were prepared by transferring 50 ± 5 mg of accurately weighed bulk chemical sample into separate 100-mL volumetric flasks. The samples were dissolved in and diluted to volume with Milli-Q water and mixed well. One (1) mL of each of these solutions were pipetted into individual 25-mL volumetric flasks. The contents of the flasks were diluted to volume with Milli-Q water and mixed. One (1) mL of each of these solutions and 1 mL of IS was pipetted into individual autoinjector vials, the vials were sealed and mixed well.

3.4 Preparation of Blanks

The blank containing internal standard was prepared by pipetting 1 mL of Milli-Q water and 1 mL of IS into an autoinjector vial, which was then sealed and mixed well. The blank was Milli-Q water.

3.5 Analysis

An aliquot of each frozen reference standard, bulk chemical sample, and blanks was transferred to an autoinjector vial and the vial was sealed. Single injections were made from each vial using the HPLC system shown in Table 1. Typical chromatograms of the frozen reference standard, bulk chemical sample, blank with internal standard and blank are shown in Figure 1.

Table 1 – HPLC System

Analytical Column	Inertsil ODS (2), 5 µ, 150 X 3 mm ID
Guard Column	Inertsil ODS (2), 5 µ, guard cartridge
Mobile Phase	90% Water, 10% Acetonitrile, 0.1% Concentrated Phosphoric Acid, Isocratic
Injection Volume	100 µL
UV Detection Wavelength	254 nm
Run Time	35 minutes
Retention Time	
PTU	~5 minutes
IS	~28 minutes

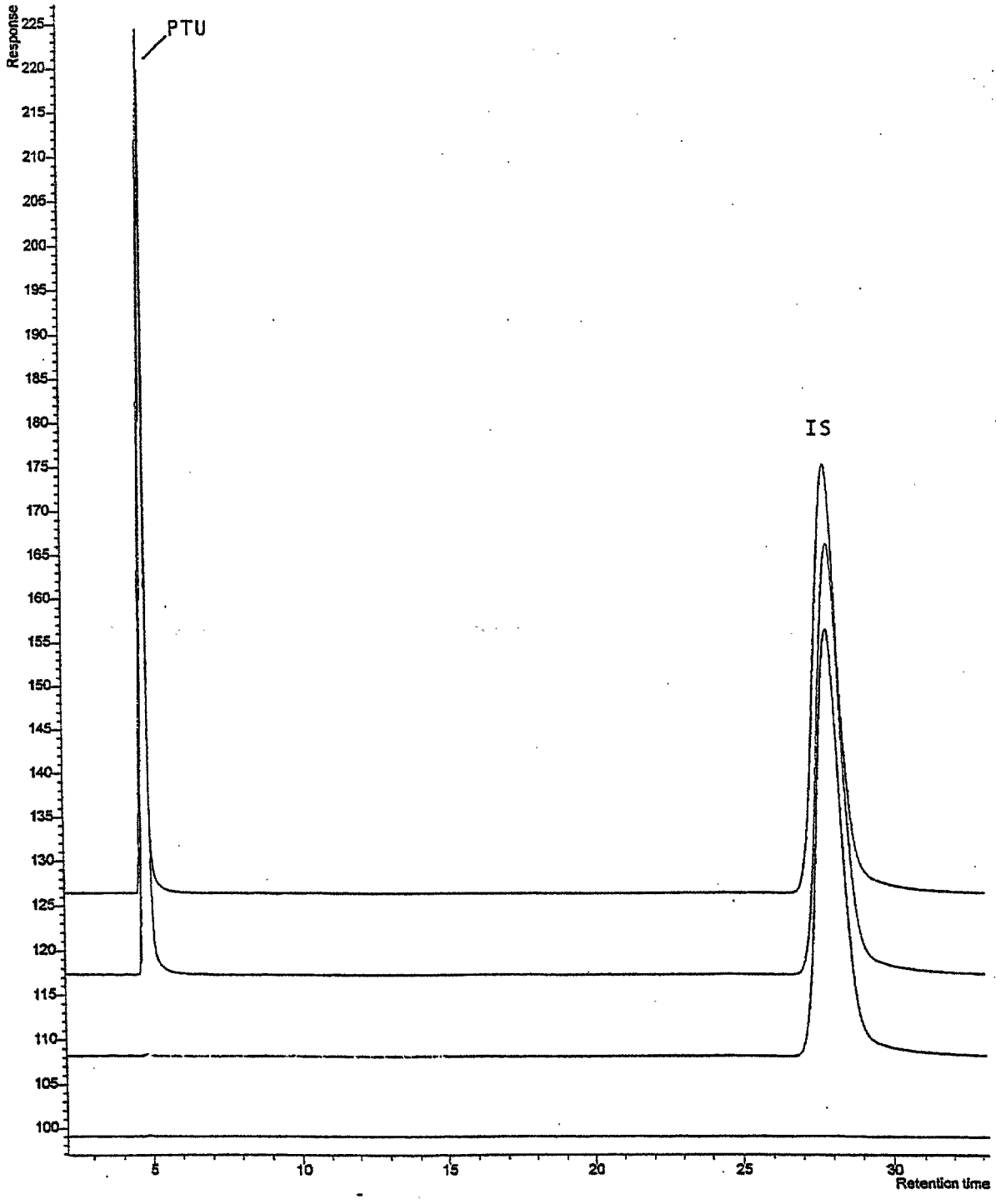

Figure 1 – Representative Overlay of Chromatograms of Frozen Reference Standard, Bulk Chemical Sample, Blank with Internal Standard, and Blank (shown top to bottom)

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4 RESULTS

The integration of the PTU and internal standard peaks by the chromatography data system was examined and manually modified, if necessary. The relative response factor for each reference and bulk chemical was calculated using the peak area ratio (PTU/internal standard) and the individual weight of each sample. The relative response factor for each bulk chemical samples was compared to the average relative response factor for the frozen reference standard. The average relative purity of the bulk chemical sample when compared to the frozen reference standard was 100.0%. The results are shown in Table 2.

Table 2 – Results for Bulk Chemical Reanalysis

Bulk Chemical Sample ID	Relative % Purity	Avg Relative % Purity ± s
1	102.9	
2	102.0	100.0 ± 4.3
3	95.1	

The average percent purity of the bulk chemical sample was compared to the average value of the frozen reference standard using the following formula for the uncertainty value U, where t is the student t variable at the chosen significance level (95% in this case) and the degrees of freedom, s_p is the pooled standard deviation of the method and n is the number of replicates of the means under comparison.

$$U = tsp\sqrt{(na + nb) \div (na \times nb)}$$

5 CONCLUSIONS

The bulk chemical sample is 100.0% pure relative to the frozen reference standard. The calculated value of U (Significant Difference), 10.0% for this analysis, indicated that the average value of the test article was equivalent to the purity of the frozen reference standard. The purity of the bulk chemical is acceptable for continued use.

6 ACKNOWLEDGMENTS

Tudor Fernando performed the analytical work. Wendy M. Black wrote the report. Maria Evascu reviewed the data and report for completeness and accuracy.

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Chemistry Support Services for the NTP

IH Contract No.: N01-ES-05456

Battelle Project No.: G004110-BFO

NTP ChemTask No.: CHEM06526

CAS No.: 51-52-5

COPY

BULK CHEMICAL REANALYSIS REPORT

6-PROPYL-2-THIOURACIL

7-106-BCR-54

December 11, 2001

repared By:

Wendy M. Black

Wendy M. Black
Task Leader

Approved By:

Steven W Graves

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7244-601
WOLFE
PEPPERL, S

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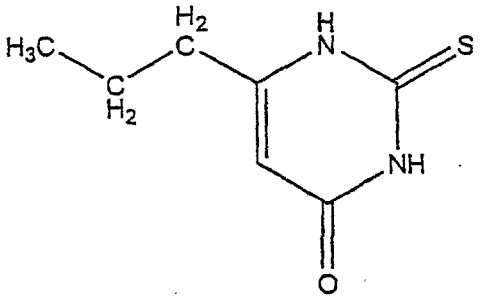
BULK CHEMICAL REANALYSIS REPORT

6-PROPYL-2-THIOURACIL

CAS No.: 51-52-5	Lot No.: 47H2500 (Sigma)
Battelle Chemical ID Code: 106	Samples/Amount Received: One 30-mL amber glass serum vial/5 g
Battelle Task No.: 7-106-BCR-54	Sample Receipt Date: 11/14/01
NTP Task No.: CHEM06526	Submitter: Unknown
Program Supported: RDGT	Study Lab: TherImmune Research Corporation
Analysis Dates: 11/14-11/15/01	Receipt Condition/Appearance: No adverse findings/powder
Interim Results Date: 11/19/01	Shipping Container: Information not provided
	Storage Conditions (@ Battelle): Room temperature (~25°C)

STRUCTURE	Mol. Wt.	Mol. Formula
	170.20 g/mol	$C_7H_{10}N_2OS$

EXECUTIVE SUMMARY

The purpose of this study was to compare the purity of a sample of the bulk chemical currently in use to the same lot of a frozen reference standard maintained at ≤-20°C. The sample was analyzed by high performance liquid chromatography (HPLC) with UV detection and determined to have a purity of 100.7% relative to the frozen reference standard, which indicates the purity of the sample is acceptable for continued use.

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1 INTRODUCTION

The purpose of this study was to compare the purity of the 6-propyl-2-thiouracil (PTU) bulk chemical sample currently in use to a frozen reference standard of the same lot maintained at ≤-20°C and to assure that the purity of the chemical is acceptable for continued use. This bulk chemical reanalysis task was performed in support of RDGT studies. This task was conducted at Battelle, 505 King Avenue, Columbus, Ohio 43201.

2 TEST ARTICLE

The bulk chemical reanalysis sample of 6-propyl-2-thiouracil, Lot No. 47H2500, was received at Battelle from TherImmune Research Corporation on November 14, 2001. The frozen reference standard for this analysis was removed from the "archive" sample, which had been stored at ≤-20°C at Battelle following the completion of the chemical handling for this lot.

3 ANALYSIS METHOD

3.1 Preparation of Internal Standard Solution

A solution was prepared by pipetting 100 µL of acetophenone into a 50-mL volumetric flask and dissolving in and diluting to volume with acetonitrile. The flask was sealed and mixed well. One (1) mL of this solution was pipetted into a 100-mL volumetric flask and diluted to volume with Milli-Q water (Milli-Q water has a resistivity of ≥18 megohm-cm). This solution was the working internal standard (IS).

3.2 Preparation of Frozen Reference Standards

Triplicate stock solutions were prepared by transferring 50 ± 5 mg of accurately weighed frozen reference standard into separate 200-mL volumetric flasks. The samples were dissolved in and diluted to volume with Milli-Q water and mixed well. Two (2) mL of each of these solutions were pipetted into individual 25-mL volumetric flasks. The contents of the flasks were diluted to volume with Milli-Q water and mixed. One (1) mL of each of these solutions and 1 mL of IS were pipetted into individual autoinjector vials, the vials were sealed and mixed well.

3.3 Preparation of Bulk Chemical Samples

Triplicate stock solutions were prepared by transferring 50 ± 5 mg of accurately weighed bulk chemical sample into separate 200-mL volumetric flasks. The samples were dissolved in and diluted to volume with Milli-Q water and mixed well. Two (2) mL of each of these solutions were pipetted into individual 25-mL volumetric flasks. The contents of the flasks were diluted to volume with Milli-Q water and mixed. One (1) mL of each of these solutions and 1 mL of IS were pipetted into individual autoinjector vials, the vials were sealed and mixed well.

3.4 Preparation of Blanks

The blank containing internal standard was prepared by pipetting 1 mL of Milli-Q water and 1 mL of IS into an autoinjector vial, which was then sealed and mixed well. The blank was Milli-Q water.

3.5 Analysis

An aliquot of each frozen reference standard, bulk chemical sample, blank with internal standard, and blank was transferred to an autoinjector vial and the vial was sealed. Single injections were made from each vial using the HPLC system shown in Table 1. Typical chromatograms of the frozen reference standard, bulk chemical sample, blank with internal standard and blank are shown in Figure 1.

Table 1 – HPLC System

Analytical Column	Inertsil ODS (2), 5 µ, 150 X 3 mm ID
Guard Column	Inertsil ODS (2), 5 µ, guard cartridge
Mobile Phase	90% Water, 10% Acetonitrile, 0.1% Concentrated Phosphoric Acid, Isocratic
Injection Volume	100 µL
UV Detection Wavelength	254 nm
Run Time	35 minutes
Retention Time	
PTU	~4 minutes
IS	~25 minutes

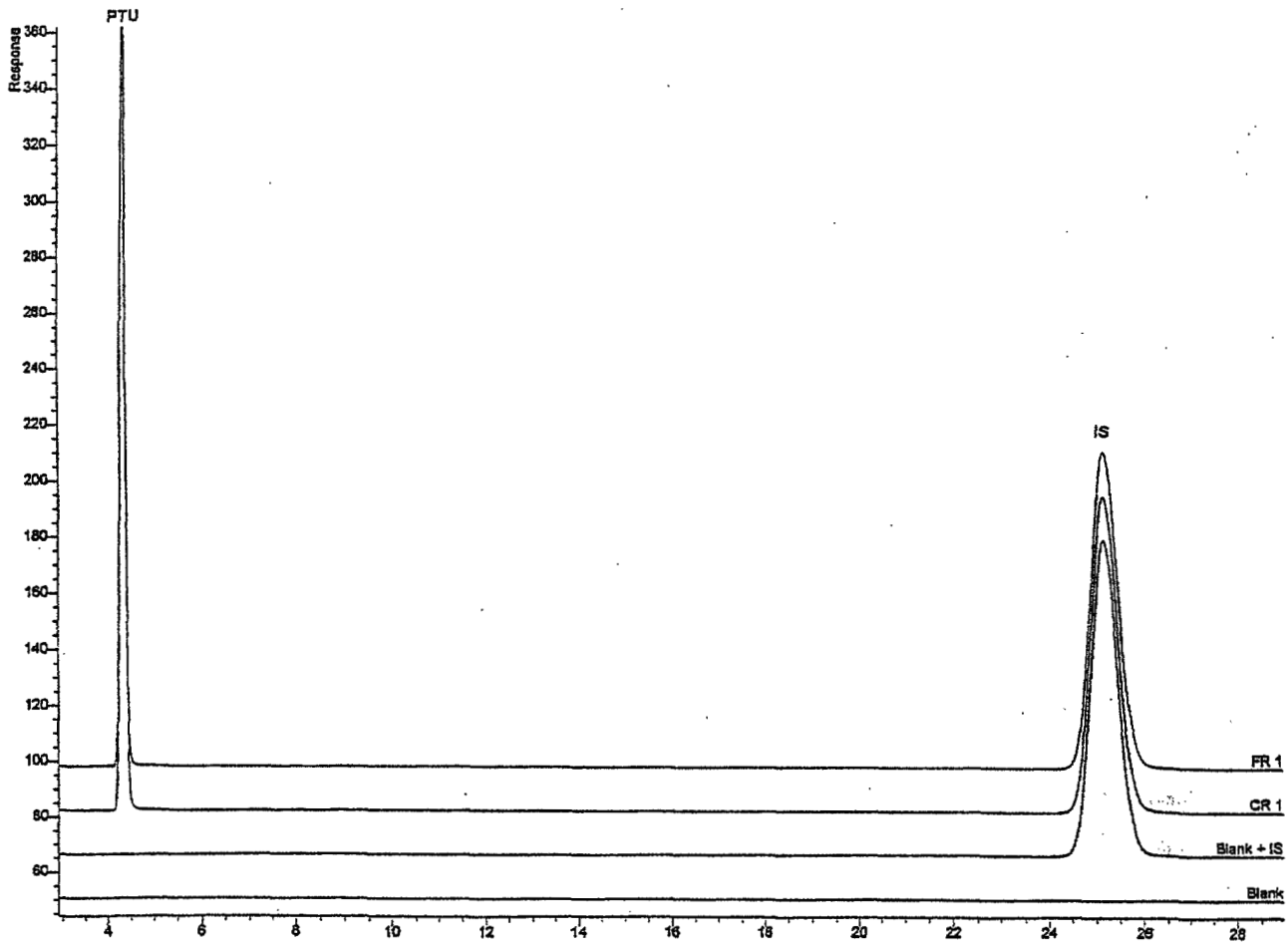

Figure 1 – Representative Overlay of Chromatograms of Frozen Reference Standard, Bulk Chemical Sample, Blank with Internal Standard, and Blank (shown top to bottom)

4 RESULTS

The integration of the PTU and internal standard peaks by the chromatography data system was examined and manually modified, if necessary. The relative response factor for each reference and bulk chemical was calculated using the peak area ratio (PTU/internal standard) and the individual weight of each sample. The relative response factor for each bulk chemical sample was compared to the average relative response factor for the frozen reference standard. The average relative purity of the bulk chemical sample when compared to the frozen reference standard was 100.7%. The results are shown in Table 2.

Table 2 – Results for Bulk Chemical Reanalysis

Bulk Chemical Sample ID	Relative % Purity	Avg Relative % Purity ± s
1	99.4	
2	102.8	100.7 ± 1.8
3	99.8	

The average percent purity of the bulk chemical sample was compared to the average value of the frozen reference standard using the following formula for the uncertainty value U, where t is the student t variable at the chosen significance level (95% in this case) and the degrees of freedom, s_p is the pooled standard deviation of the method and n is the number of replicates of the means under comparison.

$$U = tsp\sqrt{(na + nb) \div (na \times nb)}$$

5 CONCLUSIONS

The bulk chemical sample is 100.7% pure relative to the frozen reference standard. The calculated value of U (Significant Difference), 3.0% for this analysis, indicated that the average value of the test article was equivalent to the purity of the frozen reference standard. The purity of the bulk chemical is acceptable for continued use.

6 ACKNOWLEDGMENTS

Darren Brown performed the analytical work. Wendy M. Black wrote the report. Melissa Cloud reviewed the data and report for completeness and accuracy.

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Chemistry Support Services for the NTP

NIH Contract No.: N01-ES-55395

Battelle Project No.: G002840-AQZ

NTP ChemTask No.: CHEM05414

CAS No.: 51-52-5

DOSE ANALYSIS REPORT

6-PROPYL-2-THIOURACIL

5-106-DA-160

August 24, 2000

Prepared By:

Wendy M. Black

Study Director

Approved By:

Steven Graves

Principal Investigator

Submitted to:

Dr. Cynthia S. Smith

National Institute of Environmental Health Sciences

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111 T.W. Alexander Dr.

Research Triangle Park, NC 27709-2233

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HOLLEY
OKOTH
TIAN

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51-52-5

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BATTELLE-DA

Battelle ... Putting Technology To Work

Chemistry Support Services for the NTP

NIH Contract No.: N01-ES-55395

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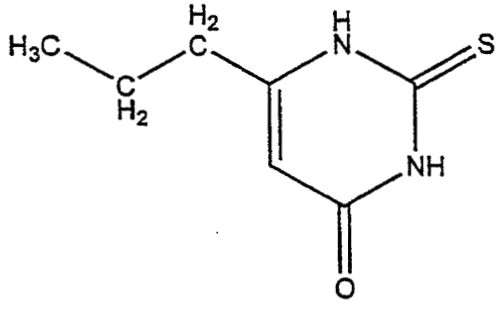
DOSE PREPARATION AND ANALYSIS REPORT

6-PROPYL-2-THIOURACIL

CAS No.: 51-52-5	Lot No.: 47H2500
Battelle Chemical ID Code: 106	Samples Prepared and Analyzed: 7244-601/Group 1/Mix 3/0% Dose/Color Code White; 7244-601/ Group 2/Mix 3/0.0001% Dose/Color Code Green; 7244-601/Group 3/Mix 3/0.0004% Dose/Color Code Blue; 7244-601/Group 4/Mix 3/0.0015% Dose/Color Code Red
Internal Task No.: 5-106-DA-160	Mix Date: 5/22/00
NTP Task No.: CHEM05414	Vehicle: Deionized Water
Program Supported: RDGT	Vehicle Lot No.: NA
Analysis Dates: 6/16 – 6/20/00	Storage Conditions (@ Battelle): Not Taken
Interim Results Date: 6/28/00	

STRUCTURE	Mol. Wt.	Mol. Formula
	170.20 g/mole	$C_7H_{10}N_2OS$

EXECUTIVE SUMMARY

Formulations received for this task had concentrations below the lowest concentration for which the method had been validated. As a result, the method was modified to encompass the range of the formulations by decreasing the low end of the standard curve and validated. This method was used to analyze formulations of 6-propyl-2-thiouracil (PTU), which were received from TherImmune in deionized water at target concentrations of 0, 0.0001, 0.0004 and 0.0015% (w/v) and analyzed in support of an RDGT study. The 0% formulation contained no detectable PTU. The average concentrations of the 0.0001 and 0.0015% formulations were within 10% of target, the NTP acceptance limit. The 0.0004% formulation was outside the normal acceptance limit. It was approximately 16.3% below target.

QUALITY ASSURANCE STATEMENT

DOSE PREPARATION AND ANALYSIS REPORT

6-PROPYL-2-THIOURACIL

NTP ChemTask No.: CHEM05414

Battelle Project No.: G002840-AQZ

Battelle Task No.: 5-106-DA-160

Listed below are the phases and/or procedures performed by Battelle that were reviewed by the Quality Assurance Unit during performance of the task described in this report. Adverse findings, if any, were reported to the study director at the time of review.

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Audit study file	8/21/00	8/21/00
Audit analytical report	8/21/00	8/21/00

This report reflects the procedures and raw data generated in this study.

In addition to the study-specific audits/inspections cited above, routine inspections of the general facilities and equipment were performed by the QAU and reports were submitted to management as follows:

Facility/Equipment	Date Inspected	Date of Report to Management
Dose Formulation Facility inspection	5/22/98	5/26/98
	8/13 and 8/19/99	8/16 and 8/23/99
	6/28/ and 7/26/00	6/30 and 8/1/00
Chemistry Technical Center inspection	5/27 and 5/28/98	6/1/98
	7/27/99	7/30/99
	4/23/00	4/24/00

D. S. Zi 8-23-00

Quality Assurance Unit Date

Battelle

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1 INTRODUCTION

Formulations received for this task had concentrations below the lowest concentration for which the method had been validated. As a result, the method was modified to encompass the range of the formulations by decreasing the low end of the standard curve and validated.

This report contains:

- a description of the validation of the revised method,
- a description of the analysis of formulations of 6-propyl-2-thiouracil (PTU) in deionized water,
- the results of the validation and analysis, and
- our conclusions.

This work was performed at Battelle, 505 King Avenue, Columbus, OH 43201, and supports an RDGT study.

2 TEST ARTICLE AND FORMULATION SAMPLES

Batches of deionized water formulations containing PTU were received at Battelle on May 25, 2000. They were identified with the following group numbers and target concentrations: 7244-601/Group 1/Mix 3/0% Dose/Color Code White; 7244-601/Group 2/Mix 3/0.0001% Dose/Color Code Green; 7244-601/Group 3/Mix 3/0.0004% Dose/Color Code Blue; 7244-601/Group 4/Mix 3/0.0015% Dose/Color Code Red).

All formulations were prepared using PTU, Lot No. 47H2500, according to information provided with the shipment. The stability and identity of this lot was previously determined and reported by Battelle under Battelle Study No. G002840-AIF, ChemTask No. CHEM04550. A sample of the same lot was used as the analytical standard for this work.

3 METHOD REVISION AND VALIDATION

The method was revised to lower the concentration of the lowest standards so that the curve would now contain a standard lower than the lowest concentration of any submitted formulation. The samples, which were received in deionized water were diluted in tap water, as were the standards and blanks. This section describes the revised method and the results and conclusions from its validation.

3.1 Preparation of Standards

3.1.1 Solvent Stocks

Two solvent stock standards were prepared at target concentrations of 45 and 30 µg/mL, respectively, by dissolving approximately 22.5 and 15.0 mg of accurately weighed PTU in 500 mL of tap water.

3.1.2 Vehicle Standards

Three vehicle standards were prepared from the 45 µg/mL standard by pipetting 4, 2 and 1 mL of the 45 µg/mL solvent stock standard into individual 10-mL, 10-mL and 50-mL volumetric flasks respectively, and diluting to volume with tap water. This produced standards with target concentrations of 18, 9 and 0.9 µg/mL, respectively. Three vehicle standards were prepared from the 30 µg/mL standard by pipetting 5, 2 and 1 mL of the 30 µg/mL solvent stock standard into individual 10-mL, 25-mL and 50-mL volumetric flasks respectively, and diluting to volume with tap water. This produced standards with target concentrations of 15, 2.4 and 0.6 µg/mL, respectively.

3.1.3 Working Standards

Working standards were prepared by pipetting 1 mL of each vehicle standard and 1 mL of internal standard (IS) solution (50 µL of acetophenone dissolved in 50 mL of acetonitrile followed by a 1-to-100 dilution with tap water) into individual autoinjector vials. Triplicate working standards were prepared from the highest and lowest concentration vehicle standards. Single working standards were prepared from the other vehicle standards.

The vials were capped and shaken to mix. This produced working standards with target concentrations of 9, 7.5, 4.5, 1.2, 0.45 and 0.3 µg/mL.

3.1.4 Blanks

Triplicate blanks with IS were prepared by combining 1 mL of tap water and 1 mL of working IS in an autoinjector vial. A single blank without internal standard was tap water. The vials were capped and shaken to mix.

3.1.5 Analysis

Single injections were made from each vial using the HPLC system shown in Table 1. Typical chromatograms from a high and low vehicle standard, a blank with internal standard and a blank are shown in Figure 1.

Table 1 – HPLC System

Column	Intersil ODS (2), 150 x 3 mm (ID), 5 µm
Mobile Phase	Water: Acetonitrile: Concentrated Phosphoric Acid (90:10:0.1), Isocratic
Flow Rate	1 mL/minute
Detector Type and Wavelength	Ultraviolet at 254 nm
Injection Volume	100 µL
Run Time	35 minutes
Retention Times	
PTU	~4.4 minutes
IS	~25.5 minutes

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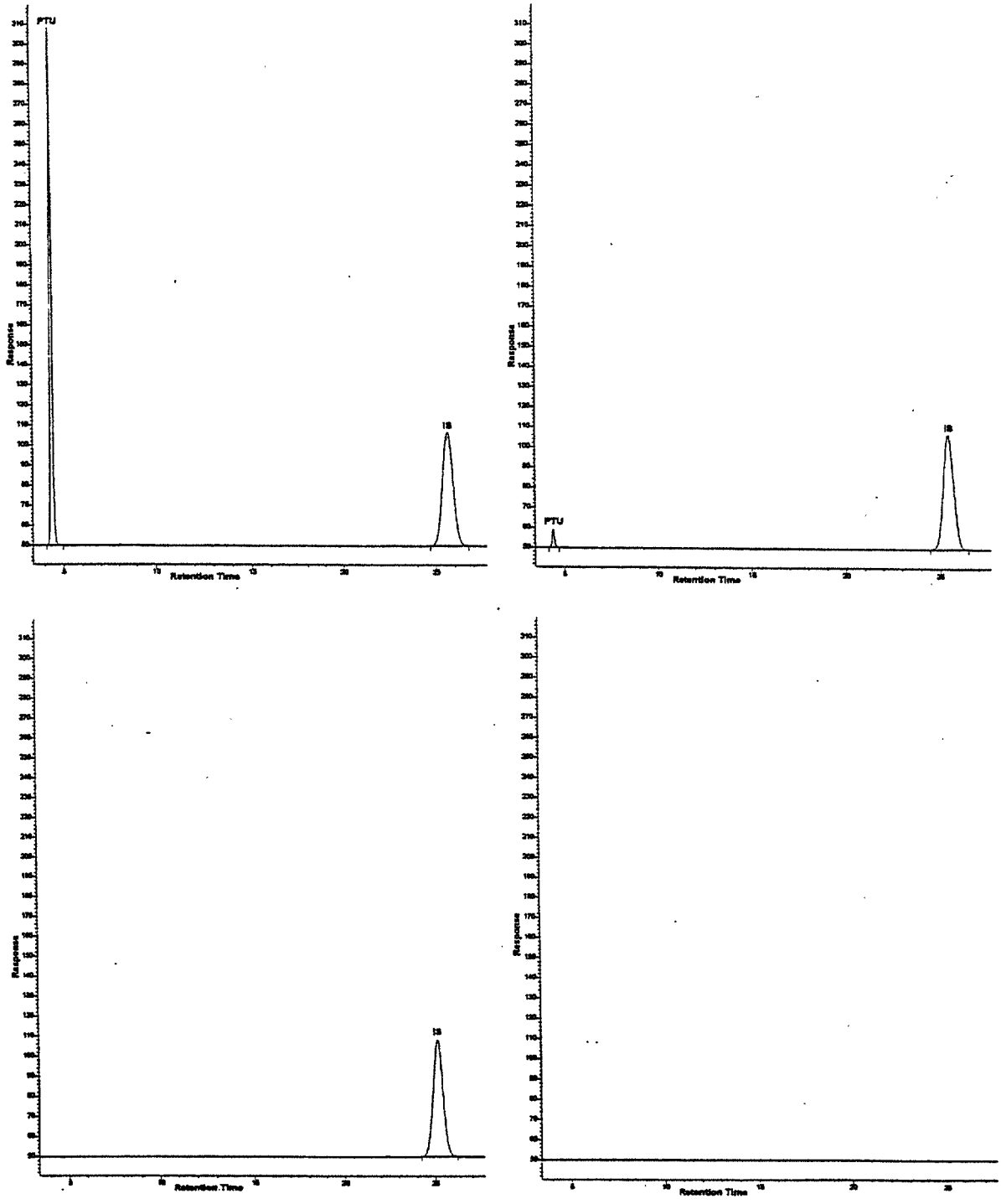

Figure 1 – Representative Chromatograms from High Standard, Low Standard, Blank With Internal Standard and Blank

3.1.6 Calculations

The integration of the PTU and internal standard (IS) peaks done by the chromatography data system, was evaluated and manually adjusted if necessary, to achieve consistent integration. The response ratio of the PTU peak area divided by the IS peak area was calculated. A linear regression equation weighted 1/x was calculated relating the response ratio of the vehicle standards to their nominal concentrations. The use of a 1/x weighting factor was necessary to achieve acceptable accuracy for the low standard. A determined concentration was calculated for each standard using the regression equation and the response ratio for that standard. The relative error for each standard was calculated by subtracting the nominal concentration from its determined concentration, dividing the difference by the nominal concentration and multiplying the ratio by 100. The average relative error, the standard deviation, and the relative standard deviation were calculated for the low and high standards.

3.1.7 Results

The results from the analysis of the standard curve are shown in Table 2. The standard curve and the results of the regression analysis of the standards are shown in Figure 2.

Table 2 – Dose Analysis MPE Results

Nominal Vehicle Std Conc (µg/mL)	Det'd Vehicle Std Conc (µg/mL)	Avg Det'd Vehicle Std Conc (µg/mL)	s (µg/mL)	%RSD	%RE	Avg %RE
	0.5985				-0.5	
0.6016	0.5963	0.5985	0.0022	0.4	-0.9	-0.5
	0.6007				-0.1	
0.9004	0.8995	NA	NA	NA	-0.1	NA
2.406	2.435	NA	NA	NA	1.2	NA
9.004	9.096	NA	NA	NA	1.0	NA
15.04	15.08	NA	NA	NA	0.3	NA
	17.93				-0.4	
18.01	17.93	17.96	0.05	0.3	-0.4	-0.3
	18.02				0.0	

NA = Not Applicable

The limit of detection (LOD), defined as three times the standard deviation of the lowest standard because there was no blank response, was 0.00664 µg/mL. The limit of quantitation (LOQ), defined as ten times the standard deviation of the lowest standard because there was no blank response, was 0.0221 µg/mL. The

experimental limit of quantitation (ELOQ), defined as the lowest standard with acceptable accuracy and precision, was 0.6016 µg/mL.

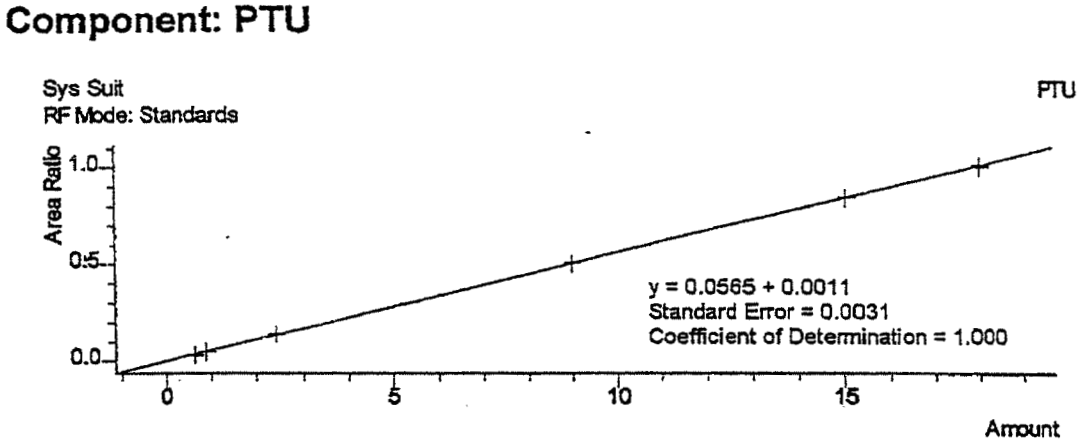

Figure 2 – Standard Curve from Method Validation

3.1.8 Conclusions

The method met all acceptance criteria for linearity, precision and accuracy. It is suitable for the analysis of PTU in tap water at concentrations from 0.6 to 18 µg/mL.

4 DOSE FORMULATION ANALYSIS

The dose formulation analysis was done concurrently with the method validation.

4.1 Preparation of Formulation Samples for Analysis

Duplicate samples from each formulation were prepared for analysis. Each formulation was prepared by pipetting 1 mL of formulation and 1 mL of internal standard into an autoinjector vial. The vials were capped and shaken to mix.

4.2 Analysis

A single injection was made from each vial using the high performance liquid chromatography (HPLC) conditions shown in Table 1.

4.3 Calculations

The integration of the PTU and IS peaks by the chromatography data system was evaluated. Any peaks with inconsistent integration were manually reintegrated to provide consistent integration. The peak areas for the PTU

and IS were then determined for each injection. The regression equation from the validation, the response ratios of the formulations and their dilution factors were used to calculate the concentration in each formulation sample. The error (%E) for each sample was calculated by subtracting the determined value from the target value, dividing by the target value and then multiplying by 100. The average determined concentration and %E were calculated.

4.4 Results

The results from the analysis of the dose formulations are shown in Table 3. A second set of duplicate 0.0004% formulation samples was prepared and analyzed to confirm the original values as low.

Table 3 – Dose Analysis Results

Target Conc (% w/v)	Group No	Determined Concentration (% w/v)				Avg Det'd Conc (% w/v)	Avg %E
0	1	BLOQ		BLOQ		BLOQ	NA
0.0001	2	0.00009566		0.00009639		0.00009603	-4.0
0.0004	3	0.0003325	0.0003342	0.0003359	0.0003359	0.0003346	-16.3
0.0015	4	0.001429		0.001435		0.001432	-4.5

BLOQ = Below limit of quantitation; no test article in sample.

4.5 Conclusions

The 0% formulation contained no detectable PTU. The average concentration of both the 0.0001% formulation and the 0.0015% formulation were within 10% of target, the NTP acceptance limit. The 0.0004% formulation was 16.3% below target, which exceeds the normal acceptance limit.

5 ACKNOWLEDGMENTS

Darren Brown conducted the analytical work. Wendy Black wrote the report.

51-52-5

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hemistry Support Services for the NTP

NIH Contract No.: N01-ES-55395

Battelle Project No.: G002840-ARV

NTP ChemTask No.: CHEM05508

CAS No.: 51-52-5

DOSE ANALYSIS REPORT

6-PROPYL-2-THIOURACIL

5-106-DA-172

August 31, 2000

repared By:

Wendy M. Black

Wendy M. Black
Study Director

Approved By:

Steven Graves
Principal Investigator

Submitted to:

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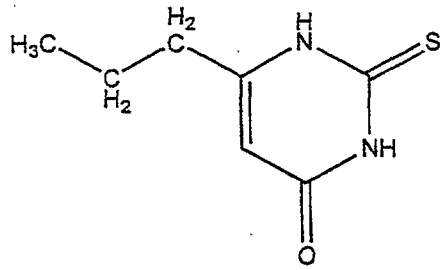
DOSE ANALYSIS REPORT

6-PROPYL-2-THIOURACIL

CAS No.: 51-52-5	Lot No.: 47H2500
Battelle Chemical ID Code: 106	Samples Prepared and Analyzed:
	7244-601/Group 1/Mix 11/0% Dose/Color Code White;
	7244-601/Group 2/Mix 11/0.0001% Dose/Color Code Green;
	7244-601/Group 3/Mix 11/0.0004% Dose/Color Code Blue;
	7244-601/Group 4/Mix 11/0.0015% Dose/Color Code Red;
	7244-601/Group 3/Mix 4/0.0004% Dose/Color Code Blue;
	7244-601/Group 3/Mix 5/0.0004% Dose/Color Code Blue.
Internal Task No.: 5-106-DA-172	Mix Date: 7/17/00 (Mix 11), 5/30/00 (Mix 4) and 6/5/00 (Mix 5)
NTP Task No.: CHEM05508	Vehicle: Deionized Water
Program Supported: RDGT	Vehicle Lot No.: NA
Analysis Dates: 7/28 – 7/29/00	Storage Conditions (@ Battelle): Refrigerated (~5°C)
Interim Results Date: 8/01/00	

STRUCTURE	Mol. Wt.	Mol. Formula
	170.20 g/mole	$C_7H_{10}N_2OS$

EXECUTIVE SUMMARY

Formulations of 6-propyl-2-thiouracil (PTU) were received from TherImmune in deionized water at target concentrations of 0, 0.0001, 0.0004 and 0.0015% (w/v) and analyzed in support of an RDGT study.

For the Mix 11 formulations, the 0% formulation contained no detectable PTU, the average concentration of the 0.0015% formulation was within 10% of target, the NTP acceptance limit, and the 0.0004% and 0.0001% formulations were outside the normal acceptance limit. The 0.0004% formulation was approximately 31% below target and the 0.0001% formulation was approximately 30% above target.

Formulations at target concentrations of 0.0004% from Mix 4 (5/30/00) and Mix 5 (6/5/00) were also submitted and analyzed. The average concentration of both these formulations was found to be within 10% of target, the NTP acceptance limit.

QUALITY ASSURANCE STATEMENT

DOSE ANALYSIS REPORT

6-PROPYL-2-THIOURACIL

NTP ChemTask No.: CHEM05508

Battelle Project No.: G002840-ARV

Battelle Task No.: 5-106-DA-172

Listed below are the phases and/or procedures performed by Battelle that were reviewed by the Quality Assurance Unit during performance of the task described in this report. Adverse findings, if any, were reported to the study director at the time of review.

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Formulation analysis	7/26/00	7/26/00
Audit study file	8/28/00	8/28/00
Audit analytical report	8/28/00	8/28/00

This report reflects the procedures and raw data generated in this study.

In addition to the study-specific audits/inspections cited above, routine inspections of the general facilities and equipment were performed by the QAU and reports were submitted to management as follows:

Facility/Equipment	Date Inspected	Date of Report to Management
Dose Formulation Facility inspection	5/22/98	5/26/98
	8/13 and 8/19/99	8/16 and 8/23/99
	6/28 and 7/26/00	6/30 and 8/1/00
Chemistry Technical Center inspection	5/27 and 5/28/98	6/1/98
	7/27/99	7/30/99
	4/23/00	4/24/00

Kathleen E. Reed 8-30-00

Quality Assurance Unit Date

Battelle

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1 INTRODUCTION

This report contains:

- a description of the analysis of formulations of 6-propyl-2-thiouracil (PTU) in deionized water,
- the results of the analysis, and
- our conclusions.

This work was performed at Battelle, 505 King Avenue, Columbus, OH 43201, and supports an RDGT study.

2 TEST ARTICLE AND FORMULATION SAMPLES

Batches of deionized water formulations containing PTU were received at Battelle on July 24, 2000. They were identified with the following group numbers and target concentrations: 7244-601/Group 1/Mix 11/0% Dose/Color Code White; 7244-601/Group 2/Mix 11/0.0001% Dose/Color Code Green; 7244-601/Group 3/Mix 11/0.0004% Dose/Color Code Blue; 7244-601/Group 4/Mix 11/0.0015% Dose/Color Code Red; 7244-601/Group 3/Mix 4/0.0004% Dose/Color Code Blue; 7244-601/Group 3/Mix 5/0.0004% Dose/Color Code Blue.

All formulations were prepared using PTU, Lot No. 47H2500, according to information provided with the shipment. The stability and identity of this lot was previously determined and reported by Battelle under Battelle Study No. G002840-AIF, ChemTask No. CHEM04550. A sample of the same lot was used as the analytical standard for this work.

3 DOSE ANALYSIS

3.1 Preparation of Standards

3.1.1 Solvent Stocks

Two solvent stock standards were prepared at target concentrations of 45 and 30 µg/mL, respectively, by dissolving approximately 22.5 and 15.0 mg of accurately weighed PTU in 500 mL of tap water.

3.1.2 Vehicle Standards

Three vehicle standards were prepared from the 45 µg/mL standard by pipetting 4, 2 and 1 mL of the 45 µg/mL solvent stock standard into individual 10-mL, 10-mL and 50-mL volumetric flasks respectively, and diluting to volume with tap water. This produced standards with target concentrations of 18, 9 and 0.9 µg/mL, respectively. Three vehicle standards were prepared from the 30 µg/mL standard by pipetting 5, 2 and 1 mL of the 30 µg/mL solvent stock standard into individual 10-mL, 25-mL and 50-mL volumetric flasks respectively, and diluting to volume with tap water. This produced standards with target concentrations of 15, 2.4 and 0.6 µg/mL, respectively.

3.1.3 Working Standards

Working standards were prepared by pipetting 1 mL of each vehicle standard and 1 mL of internal standard (IS) solution (50 µL of acetophenone dissolved in 50 mL of acetonitrile followed by a 1-to-100 dilution with tap water) into individual autoinjector vials. Single working standards were prepared from each vehicle standard.

The vials were capped and shaken to mix. This produced working standards with target concentrations of 9, 7.5, 4.5, 1.2, 0.45 and 0.3 µg/mL.

3.1.4 Blanks

A single blank with internal standard (IS) was prepared by combining 1 mL of tap water and 1 mL of working internal standard (IS) in an autoinjector vial. The blank without internal standard was tap water. The vials were capped and shaken to mix.

3.2 Preparation of Formulation Samples for Analysis

Duplicate samples from each formulation were prepared for analysis. Each formulation was prepared by pipetting 1 mL of formulation and 1 mL of internal standard (IS) into an autoinjector vial. The vials were capped and shaken to mix.

3 Analysis

Single injections were made from each vial using the HPLC system shown in Table 1. Typical chromatograms from a high and low vehicle standard, a blank with internal standard and a blank are shown in Figure 1.

Table 1 – HPLC System

Column	Inertsil ODS (2), 150 x 3 mm (ID), 5 µm
Mobile Phase	Water: Acetonitrile: Concentrated Phosphoric Acid (90:10:0.1), Isocratic
Flow Rate	1 mL/minute
Detector Type and Wavelength	Ultraviolet at 254 nm
Injection Volume	100 µL
Run Time	35 minutes
Retention Times	
PTU	~5 minutes
IS	~29 minutes

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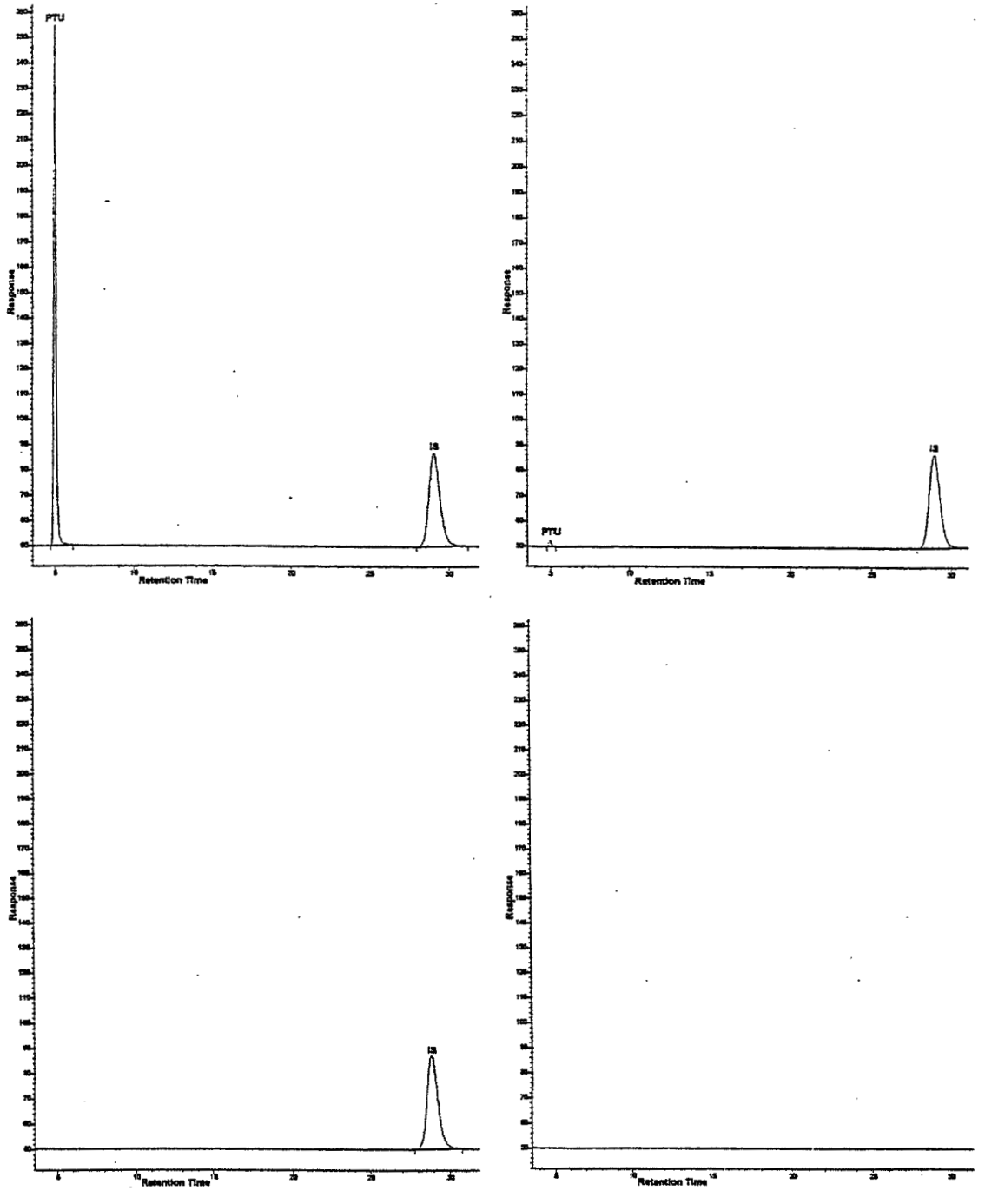

Figure 1 – Representative Chromatograms from High Standard, Low Standard, Blank With Internal Standard and Blank

(shown left to right, top to bottom)

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3.4 Calculations

The integration of the PTU and internal standard (IS) peaks done by the chromatography data system was evaluated and manually adjusted if necessary, to achieve consistent integration. The response ratio of the PTU peak area divided by the IS peak area was calculated. A linear regression equation weighted 1/x was calculated relating the response ratio of the vehicle standards to their nominal concentrations. A determined concentration was calculated for each standard using the regression equation and the response ratio for that standard. The relative error for each standard was calculated by subtracting the nominal concentration from its determined concentration, dividing the difference by the nominal concentration and multiplying the ratio by 100.

3.5 Results

The results from the analysis of the formulations are shown in Table 2. The standard curve and the results of the regression analysis of the standards are shown in Figure 2.

Table 2 – Dose Analysis Results

Group No	Mix No	Target Conc (%)	Det'd Conc (%)		Avg Det'd Conc (%)	Avg % E
1	11	0	BLOQ	BLOQ	BLOQ	BLOQ
2	11	0.0001	0.0001301	0.0001289	0.0001295	29.5
3	4	0.0004	0.0004050	0.0004089	0.0004070	1.7
3	5	0.0004	0.0004107	0.0004135	0.0004121	3.0
3	11	0.0004	0.0002733	0.0002784	0.0002758	-31.0
4	11	0.0015	0.001528	0.001527	0.001527	1.8

BLOQ = Below Limit of Quantitation.

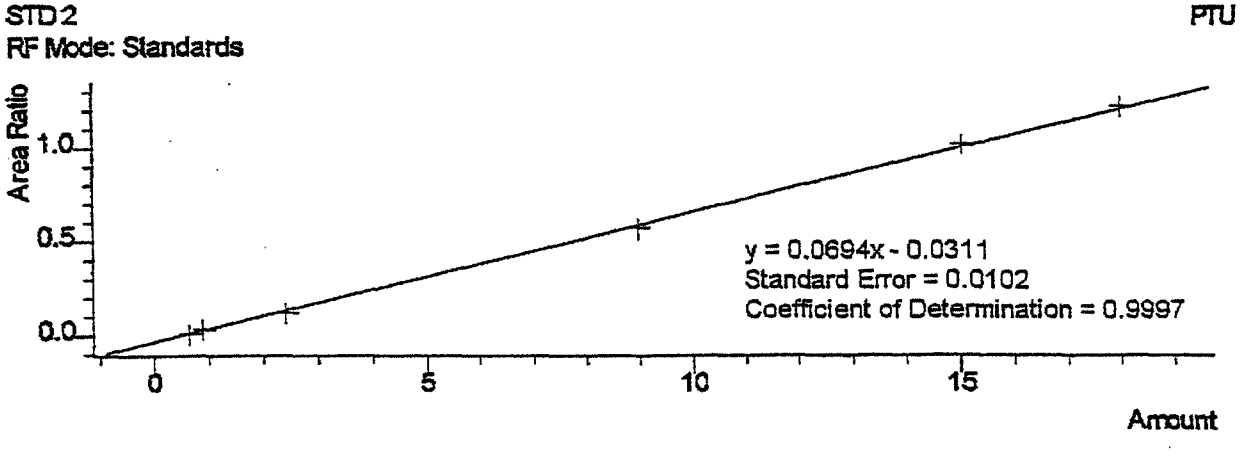

Figure 2 – Standard Curve

3.6 Conclusions

For the Mix 11 formulations, the 0% formulation contained no detectable PTU, the average concentration of the 0.0015% formulation was within 10% of target, the NTP acceptance limit, and the 0.0004% and 0.0001% formulations were outside the normal acceptance limit. The 0.0004% formulation was approximately 31% below target and the 0.0001% formulation was approximately 30% above target.

Formulations at target concentrations of 0.0004% from Mix 4 (5/30/00) and Mix 5 (6/5/00) were also submitted and analyzed. The average concentration of both these formulations was found to be within 10% of target, the NTP acceptance limit.

4 ACKNOWLEDGMENTS

Sandy Runyon conducted the analytical work. Wendy Black wrote the report.

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Appendix 5

Two-Generation Reproduction Toxicity Study of Propylthiouracil when Administered to Sprague-Dawley Rats in the Drinking Water

Dose Formulation Procedure

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THERIMMUNE RESEARCH CORPORATION
DOSE FORMULATION PROCEDURE FORM

R.O.W ID No.: 1340A
Study No.: 7244-601
Task No.: Dose Range-Finding Phase
Test Article: 6-Propyl-2-Thiouracil
Vehicle: Deionized Water
Route of Administration: Drinking Water

Procedure:

Refer to S.O.P. 511 "Preparation of Test Formulations--Solutions/Suspensions, Test Diets, and Dosed Water" for the mixing procedures to be followed.

The quantity of 6-Propyl-2-Thiouracil required to prepare 21 L solutions in deionized water at 0.0001,0.0004 and 0.0015 is listed below:

Group #	Dose Level (%)	Concentration (%)	6-Propyl-2-Thiouracil (g)	Deionized Water (L)	Mix Time (min)
1	0	0	0	21	5
2	0.0001	0.0001	0.021	21	5
3	0.0004	0.0004	0.084	21	5
4	0.0015	0.0015	0.315	21	5

Using glass volumetric flasks, carboys will be calibrated at 21 liters. A permanent mark will be placed on each carboy at the calibration point with an indelible marker. For group 1 (control), the carboy will be filled to the required volume with vehicle (deionized water). Carboys used for dose formulations will be initially filled to approximately 75% of the required volume with vehicle.

Using a stainless steel spatula, the required quantity of 6-Propyl-2-Thiouracil is accurately weighed onto a weigh boat. Pour the weighed test article into a volumetric flask (approximately half filled with vehicle). Rinse the weigh boat at least three times with vehicle and pour the rinse into the flask containing the vehicle and test article. Mix well, until complete dissolution. Pour the solution into a carboy using a funnel. Rinse the flask at least three times with vehicle, each time pouring the rinse into the carboy. Add vehicle to the carboy in order to QS to the required volume. The solution is then mixed with a variable speed stirrer for at least the required time, to insure complete dissolution. Repeat this procedure for each group.

Stability and storage of formulations are based on Battelle's Dose Formulation Developmental Study Report. Formulations will be stored at refrigerated (~5°C) and protected from light. Under these conditions, formulations are stable for 35 days following preparation. Formulations will be dispensed into amber glass bottles, with neoprene stoppers and stainless steel sippers tubes. Dispensed formulations are stable for 7 days, beginning the day of dispensing.

Sampling Procedure:

Take two (2) 50 ml samples of the test article formulation from each dose level of each batch (per mix). Store in glass bottles with teflon coated lids protected from light and refrigerated (1-6°C).

For samples submitted to TherImmune ASD for analysis: Send one sample from each dose level of each batch and a 1 g sample of the bulk test article according to the Sample Submittal Form. The samples should be sent refrigerated with ice packs and shipped according to S.O.P. 506.1 "Shipping of Test Article Samples".

Prepared by: CV 5/3/00 (Initials/date) Approved by: JWW 5/3/00 (Initials/date)

Formulation Technician: Verna Lara 5/3/00 (Initials/date)

CONTAIN NO CBI

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DOSPROCE
5/3/00

Appendix 6

Two-Generation Reproduction Toxicity Study of Propylthiouracil when Administered to Sprague-Dawley Rats in the Drinking Water

Pathology Report (PAI)

CONTAIN NO CBI

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PATHOLOGY ASSOCIATES
A CHARLES RIVER COMPANY

PATHOLOGY REPORT
FOR

TWO-GENERATION REPRODUCTION TOXICITY STUDY OF PROPYLTHIOURACIL WHEN ADMINISTERED TO SPRAGUE-DAWLEY RATS IN THE DRINKING WATER

THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601

PREPARED FOR
THERIMMUNE RESEARCH CORPORATION

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I. Pathology Narrative

DRAFT PATHOLOGY REPORT

Two-Generation Reproduction Toxicity Study Of Propylthiouracil When Administered To Sprague-Dawley Rats In The Drinking Water

TherImmune Study No. 7244-601

INTRODUCTION

The purpose of this study was to validate a Two-Generation Study to Model proposed to identify potent and weak thyroid toxicants using propylthiouracil. The data presented in this report represent the results of pathology support by Pathology Associates, a Charles River Company (PAI) for TherImmune Research Corporation, 15 Firstfield Road, Gaithersburg, MD 20878. The portions of this study performed by PAI were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (1987).

EXPERIMENTAL DESIGN AND METHODS

Eighty male and eighty female Sprague-Dawley (Outbred CD® Rats Crl:CD® BR) rats were randomly assigned to one of four treatment groups. The males and females were randomized separately. The test article, propylthiouracil, was administered *ad libitum* in the drinking water starting on Study Day 1 and continued until necropsy. Control animals received the vehicle, deionized drinking water only. Treatment groups are shown in Text Table I.

Text Table I: Treatment Groups and Dose Levels

Group	Dose Level (% w/v)	Number of Males	Number of Females
1	0.0000	20	20
2	0.0001	20	20
3	0.0004	20	20
4	0.0015	20	20

Task 2 F_0 Adults and Task 4 F_1 Adults

Necropsies were performed on F_0 males and F_0 females surviving to terminal kill following the completion of weaning of the F_1 pups. The animals were sacrificed by carbon dioxide asphyxiation and exsanguinated.

Animals of the F_0 generation found dead or killed in *extremis* were necropsied but not evaluated histopathologically.

Necropsies were performed on F_1 adult males surviving to terminal kill from Groups 1, 2 and 3 following the completion of weaning of the F_2 pups. Necropsies were performed on F_1 females from Groups 1, 2, and 3 surviving to terminal kill following the completion of vaginal cytology data collection. The animals were sacrificed by carbon dioxide asphyxiation and exsanguinated.

The following tissues from the F_0 and F_1 adult animals were preserved in 10% neutral-buffered formalin: adrenals, brain, kidneys, liver, pituitary, ventral prostate, dorsolateral prostate, seminal vesicles/coagulating glands, spleen, thyroid/parathyroids, vagina/uterus/cervix, and gross lesions. The left testis and epididymis were preserved in 2% paraformaldehyde/3% glutaraldehyde solution for 3-5 days then transferred to phosphate-buffered saline. The ovaries were first placed in Bouin's then transferred into 70% ethanol within 24-48 hours.

All fixed tissues were sent to PAI's Frederick, Maryland facility. The left testis and epididymis from the first 10 surviving males/group were embedded in glycol methacrylate (GMA), stained with Periodic Acid-Schiff's and hematoxylin and examined microscopically. The thyroid/parathyroid and gross lesions from the first 10 surviving males/group were embedded in paraffin, sectioned at approximately 5 microns, stained with hematoxylin and eosin, and examined microscopically. The thyroid/parathyroid, ovaries, vagina/uterus/cervix, and gross lesions from the first 10 surviving females/group were embedded in paraffin, sectioned at approximately 5 microns, stained with hematoxylin and eosin, and examined microscopically.

F_1 PND 21 Generation

The male and female pups selected for the PND 21 necropsy were sacrificed by carbon dioxide asphyxiation, exsanguinated, and necropsied.

The following tissues from the F_1 PND 21 animals were preserved in 10% neutral-buffered formalin: adrenals, kidneys, liver, pituitary, ventral prostate, dorsolateral prostate, seminal vesicles/coagulating glands, spleen, thyroid/parathyroids, vagina/uterus/cervix, and gross lesions. The left testis and epididymis were preserved in 2% paraformaldehyde/3% glutaraldehyde solution for 3-5 days then transferred to phosphate-buffered saline. The ovaries were first placed in Bouin's then transferred into 70% ethanol within 24-48 hours. Histopathology was not required for these tissues.

Unscheduled deaths occurred in all Group 4 F_1 weanlings. The lower and upper jaws from Group 4 unscheduled death animals were examined. The jaws were decalcified, embedded in paraffin, step sectioned, and examined microscopically. Two skulls (1 male and 1 female Group 1 PND 23-24) saved from TherImmune Study 7244-214 "3,3', 4,4'-Tetrachloroazobenzene (TCAB): Reproductive

Assessment by Continuous Breeding When Administered to Sprague-Dawley Rats by Oral Gavage" were used as representative controls.

F_2 PND 21 Generation

Selected males and females were sacrificed by carbon dioxide asphyxiation, exsanguinated and necropsied on PND 21. The following tissues from the F2 animals were preserved in 10% neutral-buffered formalin: adrenals, kidneys, liver, pituitary, ventral prostate, dorsolateral prostate, seminal vesicles/coagulating glands, spleen, thyroid/parathyroids, vagina/uterus/cervix, and gross lesions. The left testis and epididymis were preserved in 2% paraformaldehyde/3% glutaraldehyde solution for 3-5 days then transferred to phosphate-buffered saline. Ovaries were placed in Bouin's then transferred into 70% ethanol within 24-48 hours. Histopathology was not required for these tissues.

RESULTS and DISCUSSION

Microscopic findings are summarized in the Project Summary Tables (Section II). Diagnoses for individual animals are tabulated by group, sex, and generation in the Tabulated Animal Data (Section III). Results from the histopathological examination of the lower and upper jaws from the Group 4 Task 4 animals, unscheduled deaths (F_1 weanlings) are presented in Section IV. Microscopic diagnoses are correlated with the gross lesions, when possible, in the Correlation of Gross and Microscopic Findings (Section V). The Comment Report is found in Section VI. The codes used as entries in these tables are explained in the Reports Code Table (Appendix 1). Abbreviations used in the tables are explained in the Abbreviations List (Appendix 2). A comparison of significant findings for the F_0 generation animals and the F_1 generation animals is presented in Text Table II.

Microscopic Observations

F_0 Males and Females

There were several treatment-related microscopic changes in the males. Thyroid follicular cell hyperplasia was seen in 10 of 10 Group 4 males and 7 of 10 Group 3 males. Degeneration of the germinal epithelium of the testes was seen in 2 of 10 Group 4 males, 3 of 10 Group 3 males and 3 of 10 Group 2 males. Multinucleated giant cells were only seen in 1 of the Group 4 males exhibiting degeneration of the germinal epithelium.

Thyroid follicular cell hyperplasia was seen in 10 of 10 Group 4 females and 1 of 10 Group 3 females. In addition, a follicular cell adenoma was seen in 1 of the Group 4 females with follicular cell hyperplasia.

Hardisty and Boorman (1990) state that progression from follicular hyperplasia (focal or diffuse) to adenoma and carcinoma is common among laboratory rodents. As with many other endocrine glands, clear distinction between these categories is sometimes difficult because morphologic criteria are not always predictive of biological behavior. In the thyroid this is further complicated by the fact that removal of TSH stimulation will cause reversal of some but not all lesions.

F_1 Males and Females

There were similar but less severe microscopic changes in the F_1 males. Thyroid follicular cell hyperplasia was seen in 1 of 10 Group 3 males. Degeneration of the germinal epithelium of the testes was seen in 1 of 10 Group 3 males and 1 of 10 Group 2 males.

There were no treatment-related microscopic changes in any of the groups of F_1 females evaluated.

Text Table II: Incidence of Significant Findings in F_0 and F_1 Adults*

	F_0 Males				F_1 Males		
Tissue Microscopic Findings	Group 1 0.0000 (%w/v)	Group 2 0.0001 (%w/v)	Group 3 0.0004 (%w/v)	Group 4 0.0015 (%w/v)	Group 1 0.0000 (%w/v)	Group 2 0.0001 (%w/v)	Group 3 0.0004 (%w/v)
Thyroid: Follicular Cell Hyperplasia	0	0	7	10	0	0	1
Left Testis: Degeneration, Germinal Epithelium	0	3	3	2	0	1	1
Left Testis: Multinucleated Giant Cells	0	0	0	1	0	0	0
	F_0 Females				F_1 Females		
Thyroid: Follicular Cell Hyperplasia	0	0	1	10	0	0	0
Thyroid: Follicular Cell Adenoma	0	0	0	1	0	0	0

* 10 animals examined per group

F_1 Weanlings, Unscheduled Deaths

During necropsy of unscheduled deaths in F_1 Group 4 males and females, a delay in eruption of teeth was noted. Upper and lower jaws were evaluated microscopically. The findings are presented in Section IV. The changes seen seemed to represent damage (depletion and vacuolation) to the odontoblasts and ameloblasts, resulting in a delay in cellular maturation and subsequent tooth eruption. Tissues as examined, although not normal, seemed to be progressing toward normal, albeit delayed, tooth eruption.

CONCLUSIONS

Changes seen in these groups of animals, including thyroid follicular cell hyperplasia and degeneration of the germinal epithelium of the testes, were as expected. Propylthiouracil has been shown in rats to affect each step in thyroid hormone synthesis beyond iodide transport (Jubb *et al.*, 1993). Ultimately, this results in inadequate thyroxine synthesis and decreased levels of thyroxine and triiodothyronine. The decreased levels of thyroxine and triiodothyronine are detected by the hypothalamus and pituitary, and a resultant increase in secretion of thyrotropin results in hypertrophy and hyperplasia of follicular cells. Extrathyroidal changes noted in hypothyroidism include reproductive abnormalities.

As noted in Jubb *et al.* (1993),. abnormalities in reproduction are common. Lack of libido and reduction in sperm count may occur in males, whereas abnormal or absent estrous cycles with reduced conception rates may result in females. The spermatogenic epithelium in the testis often is markedly atrophic in longstanding cases of hypothyroidism.

Found in all groups, ultimobranchial cysts are considered a common and incidental finding in the laboratory rat.

Under the conditions of this study, treatment with propylthiouracil administered in the drinking water dose levels of 0.0001, 0.0004, and 0.0015 % w/v produced testicular degeneration in the germinal epithelium of F_0 and F_1 males. Treatment also produced follicular cell hyperplasia in the thyroid from 0.0004 % w/v in F_0 males and females. Follicular cell hyperplasia in the thyroid was noted in one 0.0004 % w/v F_1 male, F_1 females were not affected. A follicular cell adenoma was observed in one 0.0015 % w/v F_0 male.

Study Pathologist

Jerry L. Quance, DVM, Diplomate, ACVP **Date**

REFERENCES

Hardisty, J. F. and Boorman G. A. (1990). Thyroid Gland. In *Pathology of the Fischer Rat* (G. A. Boorman, S. L. Eustis, M. R. Elwell, C. A. Montgomery Jr., and W. F. MacKenzie, eds.), p 525. Academic Press, San Diego.

Jubb, K. V. F., Kennedy, P. C., and Palmer, N. (1993) , *Pathology of Domestic Animals,* 4th Ed, Vol. 3, pp. 314-320. Academic Press, San Diego.

Appendix 1:
Reports Code Table

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601

Reports Code Table	
N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
U	Tissues unavailable/unsuitable for evaluation
S	Tissues not applicable to animal
*	Tissues not examined/not required by protocol
1	minimal
2	mild
3	moderate
4	marked
( )	focal
[ ]	diffuse
< >	multifocal
P	Present
B	Neoplasm, Benign
M	Neoplasm, Malignant without Metastasis
C	Neoplasm, Malignant with Metastasis
X	Metastatic Site (+)
I	Bilateral
L	Unilateral
_	Diagnosis Not Applicable to Animal/Tissue

Appendix 2:
Abbreviations List

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601

Abbreviations List

STUDY ID	Study Identification
# EX	Number Examined
601F0	7244-601 F0 Generation
601F1	7244-601 F1 Generation
W/C.G.	With Coagulating Glands

II. Project Summary Tables

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

PROJECT SUMMARY

STUDY ID : 7244-601 F0 STUDY NUMBER: 601F0
FATE: Terminal Kill
DAYS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
LEFT TESTIS	# EX	10	10	10	10
Degeneration, germinal epithelium		0	3	3	2
Sperm stasis		0	1	0	0
Multinucleated giant cells		0	0	0	1
LEFT EPIDIDYMIS	# EX	10	10	10	10
Infiltrate, mixed cell		2	0	0	0
Infiltrate, lymphocytic		0	1	1	0
Spermatocele		0	1	0	0
Spermatic granuloma		0	1	0	0
Infiltrate, histiocytic		0	0	1	0
THYROID	# EX	10	10	10	10
Ultimobranchial cyst		2	2	2	0
Mononuclear cell infiltrate		0	1	0	0
Follicular cell hyperplasia		0	0	7	10
PARATHYROID	# EX	10	9	9	8
KIDNEYS	# EX	1	0	0	0
Cyst		1	0	0	0
Chronic progressive nephropathy		1	0	0	0
Mononuclear cell infiltrate		1	0	0	0
Mineralization		1	0	0	0

(1) - 0.0000 (% w/v)
(2) - 0.0001 (% w/v)
(3) - 0.0004 (% w/v)
(4) - 0.0015 (% w/v)

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

PROJECT SUMMARY

STUDY ID : 7244-601 F0 STUDY NUMBER: 601F0
FATE: Terminal Kill
DAYS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
THYROID	# EX	10	10	10	10
Ultimobranchial cyst		5	7	5	2
Follicular cell hyperplasia		0	0	1	10
Mixed cell infiltrate		1	0	0	0
Follicular cell adenoma		0	0	0	1
PARATHYROID	# EX	9	6	6	4
OVARIES	# EX	10	10	10	10
VAGINA	# EX	10	10	10	10
Enlarged vaginal lumen		1	0	0	0
UTERUS	# EX	10	10	10	10
Hydrometra		1	2	0	0
Infiltrate, polymorphonuclear cells		0	0	1	0
CERVIX	# EX	10	10	10	10
Epithelial hyperplasia		1	0	0	0
MAMMARY GLAND(S)	# EX	0	0	1	1
Duct Ectasia		0	0	0	1
KIDNEYS	# EX	1	0	0	0
Cyst		1	0	0	0
Chronic progressive nephropathy		1	0	0	0
Mononuclear cell infiltrate		1	0	0	0
Mineralization		1	0	0	0
URINARY BLADDER	# EX	1	0	0	0
Transitional epithelial hyperplasia		1	0	0	0
Squamous metaplasia		1	0	0	0
Mononuclear cell infiltrate		1	0	0	0
Mineralization		1	0	0	0

(1) - 0.0000 (% w/v)
(2) - 0.0001 (% w/v)
(3) - 0.0004 (% w/v)
(4) - 0.0015 (% w/v)

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

PROJECT SUMMARY

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill
DAYS ON TEST: ALL — SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)
NUMBER OF ANIMALS:		10	10	10	10
		#	#	#	#
PITUITARY	# EX	0	1	0	0

(1) - 0.0000 (% w/v)
(2) - 0.0001 (% w/v)
(3) - 0.0004 (% w/v)
(4) - 0.0015 (% w/v)

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

PROJECT SUMMARY

STUDY ID : 7244-601 F1 STUDY NUMBER: 601F1
FATE: Terminal Kill
DAYS ON TEST: ALL SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)
NUMBER OF ANIMALS:		10	10	10
		#	#	#
LEFT TESTIS	# EX	10	10	10
Degeneration, germinal epithelium		0	1	1
Aspermiogenesis		0	1	0
LEFT EPIDIDYMIS	# EX	10	10	10
THYROID	# EX	10	10	10
Ultimobranchial cyst		4	4	5
Follicular cell hyperplasia		0	0	1
PARATHYROID	# EX	9	8	8
SEMINAL VESICLES W/C.G.	# EX	0	1	0
URINARY BLADDER	# EX	0	1	0

(1) - 0.0000 (% w/v)
(2) - 0.0001 (% w/v)
(3) - 0.0004 (% w/v)

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

PROJECT SUMMARY

STUDY ID : 7244-601 F1 STUDY NUMBER: 601F1
FATE: Terminal Kill
DAYS ON TEST: ALL SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)
NUMBER OF ANIMALS:		10	10	10
		#	#	#
THYROID	# EX	10	10	10
Ultimobranchial cyst		5	6	6
PARATHYROID	# EX	7	9	9
OVARIES	# EX	10	10	10
UTERUS	# EX	10	10	10
VAGINA	# EX	10	10	10
CERVIX	# EX	10	10	10
KIDNEYS	# EX	1	0	0
Adipose tissue, capsular		1	0	0
Mineralization		1	0	0

(1) - 0.0000 (% w/v)
(2) - 0.0001 (% w/v)
(3) - 0.0004 (% w/v)

III. Tabulated Animal Data

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 1: 0.0000 (% w/v)
SEX: MALE

ANIMAL ID:	8981	8982	8983	8984	8985	8986	8987
LEFT TESTIS	N	N	N	N	N	N	N
LEFT EPIDIDYMIS	N	N	N	N	N	N	N
THYROID	N	N	N	N	-	N	N
Ultimobranchial cyst	-	-	-	-	P	-	-
PARATHYROID	N	N	N	N	N	N	N
Non-Protocol Tissues:							
KIDNEYS	-	-	-	-	-	-	-
Cyst	-	-	-	P	-	-	-
Chronic progressive nephropathy	-	-	-	3	-	-	-
Mononuclear cell infiltrate	-	-	-	2	-	-	-
Mineralization	-	-	-	1	-	-	-

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill — GROUP: 1: 0.0000 (% w/v)
DAYS ON TEST: ALL — SEX: MALE

ANIMAL ID:	8988	8989	8990
LEFT TESTIS	N	N	N
LEFT EPIDIDYMIS	-	-	N
Infiltrate, mixed cell	(1)	(1)	-
THYROID	N	N	-
Ultimobranchial cyst	-	-	P
PARATHYROID	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill — GROUP: 2: 0.0001 (% w/v)
DAYS ON TEST: ALL — SEX: MALE

ANIMAL ID:	9021	9022	9023	9024	9025	9026	9027
LEFT TESTIS	N	N	N	N	-	N	N
Degeneration, germinal epithelium	-	-	-	-	<4>	-	-
Sperm stasis	-	-	-	-	<2>	-	-
LEFT EPIDIDYMIS	-	N	N	N	-	N	N
Infiltrate, lymphocytic	(1)	-	-	-	-	-	-
Spermatocele	-	-	-	-	<2>	-	-
Spermatic granuloma	-	-	-	-	<3>	-	-
THYROID	N	-	N	N	N	-	N
Ultimobranchial cyst	-	P	-	-	-	P	-
Mononuclear cell infiltrate	-	-	-	-	-	(2)	-
PARATHYROID	N	N	N	N	U	N	N

See Reports Code Table for Symbol Definitions

LABCAT HP4.33 — 23-APR-2001

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TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill — GROUP: 2: 0.0001 (% w/v)
DAYS ON TEST: ALL — SEX: MALE

ANIMAL ID:	9028	9029	9030
EFT TESTIS	-	N	-
Degeneration, germinal epithelium	(1)	-	<3>
EFT EPIDIDYMIS	N	N	N
HYROID	N	N	N
ARATHYROID	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 3: 0.0004 (% w/v)
SEX: MALE

ANIMAL ID:	9061	9062	9063	9064	9065	9066	9067
LEFT TESTIS	N	N	N	-	-	N	N
Degeneration, germinal epithelium	-	-	-	<4>	<4>	-	-
LEFT EPIDIDYMIS	N	-	N	N	N	N	-
Infiltrate, lymphocytic	-	-	-	-	-	-	(1)
Infiltrate, histiocytic	-	(1)	-	-	-	-	-
THYROID	-	N	-	-	-	-	-
Ultimobranchial cyst	P	-	-	-	-	P	-
Follicular cell hyperplasia	<1>	-	1	1	1	-	1
PARATHYROID	N	N	N	U	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill — GROUP: 3: 0.0004 (% w/v)
DAYS ON TEST: ALL — SEX: MALE

ANIMAL ID:	9068	9069	9070
LEFT TESTIS	-	N	N
Degeneration, germinal epithelium	(4)	-	-
LEFT EPIDIDYMIS	N	N	N
THYROID	-	N	-
Follicular cell hyperplasia	2	-	1
PARATHYROID	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 4: 0.0015 (% w/v)
SEX: MALE

ANIMAL ID:	9101	9102	9103	9104	9105	9106	9107
LEFT TESTIS	-	N	N	N	N	N	N
Degeneration, germinal epithelium	<4>	-	-	-	-	-	-
Multinucleated giant cells	1	-	-	-	-	-	-
LEFT EPIDIDYMIS	N	N	N	N	N	N	N
THYROID	-	-	-	-	-	-	-
Follicular cell hyperplasia	3	2	3	3	2	3	3
PARATHYROID	N	N	N	N	N	U	U

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 4: 0.0015 (% w/v)
SEX: MALE

ANIMAL ID:	9108	9109	9110
LEFT TESTIS	-	N	N
Degeneration, germinal epithelium	(4)	-	-
LEFT EPIDIDYMIS	N	N	N
THYROID	-	-	-
Follicular cell hyperplasia	3	2	2
PARATHYROID	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 1: 0.0000 (% w/v)
SEX: FEMALE

ANIMAL ID:	9001	9002	9003	9004	9005	9006	9007
THYROID	-	N	N	N	N	-	-
Ultimobranchial cyst	P	-	-	-	-	P	P
Mixed cell infiltrate	(2)	-	-	-	-	-	-
PARATHYROID	N	N	N	N	N	U	N
OVARIES	N	N	N	N	N	N	N
VAGINA	N	-	N	N	N	N	N
Enlarged vaginal lumen	-	P	-	-	-	-	-
UTERUS	N	-	N	N	N	N	N
Hydrometra	-	P	-	-	-	-	-
CERVIX	N	-	N	N	N	N	N
Epithelial hyperplasia	-	[1]	-	-	-	-	-
Non-Protocol Tissues:							
KIDNEYS	-	-	-	-	-	-	-
Cyst	-	-	-	-	-	P	-
Chronic progressive nephropathy	-	-	-	-	-	3	-
Mononuclear cell infiltrate	-	-	-	-	-	2	-
Mineralization	-	-	-	-	-	2	-
Non-Protocol Tissues:							
URINARY BLADDER	-	-	-	-	-	-	-
Transitional epithelial hyperplasia	-	-	-	-	-	[4]	-
Squamous metaplasia	-	-	-	-	-	(3)	-
Mononuclear cell infiltrate	-	-	-	-	-	2	-
Mineralization	-	-	-	-	-	2	-

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 1: 0.0000 (% w/v)
SEX: FEMALE

ANIMAL ID:	9008	9009	9010
THYROID	N	-	-
Ultimobranchial cyst	-	P	P
PARATHYROID	N	N	N
OVARIES	N	N	N
VAGINA	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

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TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 2: 0.0001 (% w/v)
SEX: FEMALE

ANIMAL ID:	9041	9042	9043	9044	9045	9046	9047
THYROID	N	-	-	-	-	-	-
Ultimobranchial cyst	-	P	P	P	P	P	P
PARATHYROID	N	U	N	U	N	U	N
OVARIES	N	N	N	N	N	N	N
VAGINA	N	N	N	N	N	N	N
UTERUS	N	N	N	-	N	-	N
Hydrometra	-	-	-	P	-	P	-
CERVIX	N	N	N	N	N	N	N
Non-Protocol Tissues:							
PITUITARY	-	-	-	-	N	-	-

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 2: 0.0001 (% w/v)
SEX: FEMALE

ANIMAL ID:	9048	9049	9050
'HYROID	N	-	N
Ultimobranchial cyst	-	P	-
'ARATHYROID	N	N	U
/ARIES	N	N	N
'AGINA	N	N	N
ITERUS	N	N	N
:ERVIX	N	N	N

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill — GROUP: 3: 0.0004 (% w/v)
DAYS ON TEST: ALL — SEX: FEMALE

ANIMAL ID:	9081	9082	9083	9084	9085	9086	9087
THYROID	N	N	-	-	N	N	-
Ultimobranchial cyst	-	-	P	P	-	-	P
PARATHYROID	N	N	N	N	U	U	N
OVARIES	N	N	N	N	N	N	N
VAGINA	N	N	N	N	N	N	N
UTERUS	N	-	N	N	N	N	N
Infiltrate, polymorphonuclear cells	-	(1)	-	-	-	-	-
CERVIX	N	N	N	N	N	N	N

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TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill — GROUP: 3: 0.0004 (% w/v)
DAYS ON TEST: ALL — SEX: FEMALE

ANIMAL ID:	9088	9089	9090
HYROID	-	-	-
Ultimobranchial cyst	-	P	P
Follicular cell hyperplasia	<1>	-	-
ARATHYROID	U	N	U
VARIES	N	N	N
AGINA	N	N	N
TERUS	N	N	N
ERVIX	N	N	N
Non-Protocol Tissues:			
AMMARY GLAND(S)	N	-	-

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F0
GROUP: 4: 0.0015 (% w/v)
SEX: FEMALE

ANIMAL ID:	9121	9122	9123	9124	9125	9126	9128
THYROID	-	-	-	-	-	-	-
Ultimobranchial cyst	-	-	P	-	-	-	-
Follicular cell hyperplasia	[3]	[3]	[3]	[3]	[2]	[3]	[3]
Follicular cell adenoma	-	-	-	-	-	-	P
PARATHYROID	N	U	U	U	N	U	N
OVARIES	N	N	N	N	N	N	N
VAGINA	N	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N	N
Non-Protocol Tissues:							
MAMMARY GLAND(S)	-	-	-	-	-	-	-
Duct Ectasia	-	-	-	[4]	-	-	-

See Reports Code Table for Symbol Definitions

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TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F0 — STUDY NUMBER: 601F0
FATE: Terminal Kill — GROUP: 4: 0.0015 (% w/v)
DAYS ON TEST: ALL — SEX: FEMALE

ANIMAL ID:	9129	9130	9131
HYROID	-	-	-
Ultimobranchial cyst	-	P	-
Follicular cell hyperplasia	[3]	[3]	[3]
ARATHYROID	U	U	N
/ARIES	N	N	N
AGINA	N	N	N
TERUS	N	N	N
ERVIX	N	N	N

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 1: 0.0000 (% w/v)
SEX: MALE

ANIMAL ID:	1400	1401	1402	1403	1404	1405	1406
LEFT TESTIS	N	N	N	N	N	N	N
LEFT EPIDIDYMIS	N	N	N	N	N	N	N
THYROID	N	N	-	-	N	N	N
Ultimobranchial cyst	-	-	1	1	-	-	-
PARATHYROID	N	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 1: 0.0000 (% w/v)
SEX: MALE

ANIMAL ID:	1407	1408	1409
LEFT TESTIS	N	N	N
LEFT EPIDIDYMIS	N	N	N
THYROID	-	-	N
Ultimobranchial cyst	1	1	-
PARATHYROID	U	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 2: 0.0001 (% w/v)
SEX: MALE

ANIMAL ID:	1480	1481	1482	1483	1484	1485	1486
LEFT TESTIS	N	N	N	N	N	N	N
LEFT EPIDIDYMIS	N	N	N	N	N	N	N
THYROID	N	N	N	-	-	-	N
Ultimobranchial cyst	-	-	-	1	1	1	-
PARATHYROID	N	U	N	N	N	N	N

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TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1 — STUDY NUMBER: 601F1
FATE: Terminal Kill — GROUP: 2: 0.0001 (% w/v)
DAYS ON TEST: ALL — SEX: MALE

ANIMAL ID:	1487	1488	1490
LEFT TESTIS	N	N	-
Degeneration, germinal epithelium	-	-	[4]
Aspermiogenesis	-	-	[4]
LEFT EPIDIDYMIS	N	N	N
THYROID	N	-	N
Ultimobranchial cyst	-	2	-
PARATHYROID	U	N	N
Non-Protocol Tissues: RIGHT TESTIS	-	-	U
Non-Protocol Tissues: SEMINAL VESICLES W/C.G.	-	-	N
Non-Protocol Tissues: URINARY BLADDER	-	-	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1 — STUDY NUMBER: 601F1
FATE: Terminal Kill — GROUP: 3: 0.0004 (% w/v)
DAYS ON TEST: ALL — SEX: MALE

ANIMAL ID:	1560	1561	1562	1565	1566	1567	1568
LEFT TESTIS	N	N	N	N	N	-	N
Degeneration, germinal epithelium	-	-	-	-	-	<1>	-
LEFT EPIDIDYMIS	N	N	N	N	N	N	N
THYROID	-	-	N	-	-	N	N
Ultimobranchial cyst	1	1	-	1	1	-	-
Follicular cell hyperplasia	(1)	-	-	-	-	-	-
PARATHYROID	N	U	N	N	N	U	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1 — STUDY NUMBER: 601F1
FATE: Terminal Kill — GROUP: 3: 0.0004 (% w/v)
DAYS ON TEST: ALL — SEX: MALE

ANIMAL ID:	1569	1570	1572
LEFT TESTIS	N	N	N
LEFT EPIDIDYMIS	N	N	N
THYROID	-	N	N
Ultimobranchial cyst	1	-	-
PARATHYROID	N	N	N

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 1: 0.0000 (% w/v)
SEX: FEMALE

ANIMAL ID:	1440	1441	1442	1443	1444	1445	1446
THYROID	-	-	-	N	N	N	-
Ultimobranchial cyst	1	2	1	-	-	-	1
PARATHYROID	U	N	N	N	N	U	N
OVARIES	N	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N	N
VAGINA	N	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N	N
Non-Protocol Tissues:							
KIDNEYS	-	-	-	-	-	-	-
Adipose tissue, capsular	<2>	-	-	-	-	-	-
Mineralization	<2>	-	-	-	-	-	-

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 1: 0.0000 (% w/v)
SEX: FEMALE

ANIMAL ID:	1447	1448	1449
THYROID	-	N	N
Ultimobranchial cyst	1	-	-
PARATHYROID	N	N	U
OVARIES	N	N	N
UTERUS	N	N	N
VAGINA	N	N	N
CERVIX	N	N	N

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 2: 0.0001 (% w/v)
SEX: FEMALE

ANIMAL ID:	1520	1521	1522	1523	1524	1525	1526
THYROID	-	N	-	-	N	N	-
Ultimobranchial cyst	1	-	1	1	-	-	1
PARATHYROID	N	N	N	N	N	N	N
OVARIES	N	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N	N
VAGINA	N	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 2: 0.0001 (% w/v)
SEX: FEMALE

ANIMAL ID:	1528	1530	1532
THYROID	-	-	N
Ultimobranchial cyst	1	2	-
PARATHYROID	U	N	N
OVARIES	N	N	N
UTERUS	N	N	N
VAGINA	N	N	N
CERVIX	N	N	N

See Reports Code Table for Symbol Definitions

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 3: 0.0004 (% w/v)
SEX: FEMALE

ANIMAL ID:	1600	1601	1602	1603	1604	1605	1606
THYROID	-	N	N	-	-	-	-
Ultimobranchial cyst	1	-	-	1	1	1	1
PARATHYROID	N	N	N	N	U	N	N
OVARIES	N	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N	N
VAGINA	N	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

TABULATED ANIMAL DATA

STUDY ID : 7244-601 F1
FATE: Terminal Kill
DAYS ON TEST: ALL

STUDY NUMBER: 601F1
GROUP: 3: 0.0004 (% w/v)
SEX: FEMALE

ANIMAL ID:	1608	1610	1612
THYROID	N	N	-
Ultimobranchial cyst	-	-	1
PARATHYROID	N	N	N
OVARIES	N	N	N
UTERUS	N	N	N
VAGINA	N	N	N
CERVIX	N	N	N

See Reports Code Table for Symbol Definitions

IV. Histopathology of Lower and Upper Jaws
Group 4, Task 4 Animals: Unscheduled Deaths

PATHOLOGY ASSOCIATES
HISTOPATHOLOGY OF LOWER AND UPPER JAWS
GROUP 4, TASK 4 ANIMALS: UNSCHEDULED DEATHS
FOR THERIMMUNE RESEARCH CORPORATION
STUDY NUMBER: 7244-601

	Group 1 Male: Control Vehicle, Deionized Water				Group 1 Female: Contol Vehicle, Deionized Water				Group 4 Male; Dose Level (%w/v) 0.0015 Propylthiouracil												Group 4 Female: Dose Level (%w/v) 0.0015 Propylthiouracil										
Tissue/Diagnosis	3200	INC			3280	INC			16404	16429	16490	16504	16542	16569	16577	16595	16644	16655	INC			16880	16866	16877	16888	16899	16933	16966	16999	17000	17006	INC		
Lower Jaw	N	1	/	1	N	1	/	1			U			U	U	U			4	/	10			U							U	2	/	10
Depletion, Odontoblasts									1	2		2	1				1	1	6	/	6	1	1		1	1		1	2	2		7	/	8
Vacuolation, Ameloblasts													2					2	2	/	6				1	1	2	1		2		5	/	8
Vacuolation, Odontoblasts																	1	1	2	/	6		1			1	1	1	1	1		6	/	8
Upper Jaw	N	1	/	1	N	1	/	1																							U	1	/	10
Depletion, Odontoblasts									2	2	2	2	3	1	1	1	1	1	10	/	10	1	1	3	1	1	1	1	1	1		9	/	9
Vacuolation, Odontoblasts									2	2		1	2				1	2	6	/	10	1			1	1	1	1	2	2		7	/	9
Vacuolation, Ameloblasts										2	2		3				2	2	5	/	10				2		2	2	1	2		5	/	9
Individual cell necrosis, Odontoblast											1								1	/	10											0	/	9
Depletion, Ameloblasts													3						1	/	10											0	/	9
Note on Animal 1687: teeth still exhibited early stages of development (cap stage) in one section.																																		

N = normal, U = tissues unavailable, 1 = minimal, 2 = mild, 3 = moderate.
Controls were from Study 7244-214

2092-125
4/26/01

V. Correlation of Gross and Microscopic Findings

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: MALE — GROUP: 1: 0.0000 (% w/v)

Animal ID: 8984 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:
KIDNEYS - CYST(S) (TGL): LEFT 14X9MM FLUID FILLED

Related Histopathology:
KIDNEYS - Cyst

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: MALE — GROUP: 2: 0.0001 (% w/v)

Animal ID: 9025 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
LEFT TESTIS - FOCUS 2X2MM ONE WHITE	LEFT TESTIS - No Corollary change detected
LEFT EPIDIDYMIS - ENLARGED CAUDA 8X10X25MM FOUND AT TRIM	LEFT EPIDIDYMIS - Spermatocele; Spermatic granuloma

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: MALE — GROUP: 3: 0.0004 (% w/v)

Animal ID: 9062 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	THYROID - No Corollary change detected
PARATHYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	PARATHYROID - No Corollary change detected

Animal ID: 9063 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MILD (TGL): 5X3MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MILD (TGL): 5X3MM	PARATHYROID - No Corollary change detected

Animal ID: 9067 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	PARATHYROID - No Corollary change detected

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: MALE — GROUP: 4: 0.0015 (% w/v)

Animal ID: 9101 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; PRESENT NO GRADE ASSIGNED (TGL): 6X4MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; PRESENT NO GRADE ASSIGNED (TGL): 6X4MM	PARATHYROID - No Corollary change detected

Animal ID: 9102 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	PARATHYROID - No Corollary change detected

A :D: 9103 — Pathologist: JLQ
Ani... Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	PARATHYROID - No Corollary change detected

Animal ID: 9104 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	PARATHYROID - No Corollary change detected

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: MALE — GROUP: 4: 0.0015 (% w/v)

Animal ID: 9105 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL)	PARATHYROID - No Corollary change detected

Animal ID: 9106 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	PARATHYROID - No Corollary change detected

Animal ID: 9107 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	PARATHYROID - No Corollary change detected

Animal ID: 9108 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL)	PARATHYROID - No Corollary change detected

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: MALE — GROUP: 4: 0.0015 (% w/v)

Animal ID: 9109 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL): 7X4MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL): 7X4MM	PARATHYROID - No Corollary change detected

Animal ID: 9110 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL); 7X3MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL): 7X3MM	PARATHYROID - No Corollary change detected

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: FEMALE — GROUP: 1: 0.0000 (% w/v)

Animal ID: 9002 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
UTERUS - ENLARGEMENT; RIGHT; PRESENT NO GRADE ASSIGNED (TGL): RIGHT HORN OF UTERUS; FILLED WITH BROWNISH FLUID;	UTERUS - Hydrometra
VAGINA - ENLARGED; 10X10MM FLUID-FILLED	VAGINA - Enlarged vaginal lumen

Animal ID: 9006 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - ENLARGEMENT; RIGHT; MODERATE (TGL): FLUID FILLED BROWN	KIDNEYS - Cyst
URINARY BLADDER - ENLARGEMENT; MODERATE (TGL): UROLITHIASIS BLADDER	URINARY BLADDER - Transitional epithelial hyperplasia

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 STUDY NUMBER: 601F0
SEX: FEMALE GROUP: 2: 0.0001 (% w/v)

Animal ID: 9045 Pathologist: JLQ
Animal Fate: Terminal Kill

eference to Necropsy Record:
PITUITARY - ENLARGEMENT; MILD (TGL): 5X4MM

Related Histopathology:
PITUITARY - No Corollary change detected

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: FEMALE — GROUP: 3: 0.0004 (% w/v)

Animal ID: 9083 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	THYROID - No Corollary change detected
PARATHYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	PARATHYROID - No Corollary change detected

Animal ID: 9088 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
MAMMARY GLAND(S) - ENLARGEMENT; RIGHT; PRESENT NO GRADE ASSIGNED (TGL): RIGHT CAUDAL 25X20MM	MAMMARY GLAND(S) - No Corollary change detected

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: FEMALE — GROUP: 4: 0.0015 (% w/v)

Animal ID: 9121 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; PRESENT NO GRADE ASSIGNED (TGL): 6X4MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; PRESENT NO GRADE ASSIGNED (TGL): 6X4MM	PARATHYROID - No Corollary change detected

Animal ID: 9122 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL)	PARATHYROID - (Tissue unavailable)

An. ID: 9123 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	PARATHYROID - (Tissue unavailable)

Animal ID: 9124 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - 6X5X2MM THYROID ENLARGEMENT; BILATERAL; MILD (TGL)	THYROID - Follicular cell hyperplasia
MAMMARY GLAND(S) - DISCOLORATION; YELLOW; RIGHT; FOCAL; MILD (TGL)	MAMMARY GLAND(S) - Duct Ectasia
MAMMARY GLAND(S) - ENLARGEMENT; RIGHT; FOCAL; MILD (TGL)	MAMMARY GLAND(S) - Duct Ectasia

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: FEMALE — GROUP: 4: 0.0015 (% w/v)

Animal ID: 9125 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL): 8X4X4MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL): 8X4X4MM	PARATHYROID - No Corollary change detected

Animal ID: 9126 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL): 9X4MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL): 9X4MM	PARATHYROID - (Tissue unavailable)

Animal ID: 9128 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MILD (TGL)	PARATHYROID - No Corollary change detected

Animal ID: 9129 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MARKED (TGL): 8X7X4MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MARKED (TGL): 8X7X4MM	PARATHYROID - (Tissue unavailable)

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: FEMALE — GROUP: 4: 0.0015 (% w/v)

Animal ID: 9130 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - 7X5X3MM 6X5X3MM. UNKNOWN SIDE. ENLARGEMENT; BILATERAL; MODERATE (TGL)	THYROID - Follicular cell hyperplasia
PARATHYROID - 7X5X3MM 6X5X3MM. UNKNOWN SIDE. ENLARGEMENT; BILATERAL; MODERATE (TGL)	PARATHYROID - (Tissue unavailable)

Animal ID: 9131 — Pathologist: JLQ
Animal Fate: Terminal Kill

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL): 7X5X2MM	THYROID - Follicular cell hyperplasia
PARATHYROID - ENLARGEMENT; BILATERAL; MODERATE (TGL): 7X[illegible]M	PARATHYROID - No Corollary change detected

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F1
STUDY NUMBER: 601F1
SEX: MALE
GROUP: 1: 0.0000 (% w/v)

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F1 STUDY NUMBER: 601F1
SEX: MALE GROUP: 2: 0.0001 (% w/v)

Animal ID: 1490 Pathologist: JLQ
Animal Fate: Terminal Kill

eference to Necropsy Record:	Related Histopathology:
RIGHT TESTIS - SMALL	RIGHT TESTIS - (Tissue unavailable)
URINARY BLADDER - DISTENDED RED FLUID	URINARY BLADDER - No Corollary change detected
SEMINAL VESICLES W/C.G. - LEFT COAGULATING GLAND RED DISCOLORATION	SEMINAL VESICLES W/C.G. - No Corollary change detected
LEFT TESTIS - SMALL, 12 MM L X 8 MM W, FOUND AT TRIM	LEFT TESTIS - Degeneration, germinal epithelium

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PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F1 STUDY NUMBER: 601F1
SEX: MALE GROUP: 3: 0.0004 (% w/v)

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F1 — STUDY NUMBER: 601F1
SEX: FEMALE — GROUP: 1: 0.0000 (% w/v)

Animal ID: 1440 — Pathologist: JLQ
Animal Fate: Terminal Kill

eference to Necropsy Record:
KIDNEYS - RIGHT KIDNEY: 2 TAN TISSUE MASSES PROTRUDING FROM KIDNEY: 2X3 MM 2X1 MM

Related Histopathology:
KIDNEYS - Adipose tissue, capsular

Animal ID: 1449 — Pathologist: JLQ
Animal Fate: Terminal Kill

eference to Necropsy Record:
UTERUS - UTERUS MODERATELY ENLARGED

Related Histopathology:
UTERUS - No Corollary change detected

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F1 STUDY NUMBER: 601F1
SEX: FEMALE GROUP: 2: 0.0001 (% w/v)

No Gross Observations for any animal in this group

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-601 F1 STUDY NUMBER: 601F1
SEX: FEMALE GROUP: 3: 0.0004 (% W/V)

o Gross Observations for any animal in this group

VI. Comment Report

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0
SEX: MALE

STUDY NUMBER: 601F0
GROUP: 1: 0.0000 (% w/v)

Comments for any animal in this group

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0 STUDY NUMBER: 601F0
SEX: MALE GROUP: 2: 0.0001 (% w/v)

Animal ID: 9025 Pathologist: JLQ
Animal Fate: Terminal Kill

TISSUE COMMENTS:
THYROID - One of pair present.
PARATHYROID - One of pair present

Animal ID: 9028 Pathologist: JLQ
Animal Fate: Terminal Kill

TISSUE COMMENTS:
THYROID - One of pair present.
PARATHYROID - One of pair present.

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0 STUDY NUMBER: 601F0
SEX: MALE GROUP: 3: 0.0004 (% w/v)

Animal ID: 9064 Pathologist: JLQ
Animal Fate: Terminal Kill

ISSUE COMMENTS:
EFT TESTIS - When it occurs, the degenerative change is severe. Overall, only a few tubules are affected.

Animal ID: 9065 Pathologist: JLQ
Animal Fate: Terminal Kill

ISSUE COMMENTS:
EFT TESTIS - When it occurs, the degenerative change is severe. Overall, only a few tubules are affected.

Animal ID: 9068 Pathologist: JLQ
Animal Fate: Terminal Kill

IS IMENTS:
EFT IS - When it occurs, the degenerative change is severe. In this case, a single tubule in the section is involved.

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0 — STUDY NUMBER: 601F0
SEX: MALE — GROUP: 4: 0.0015 (% w/v)

Animal ID: 9101 — Pathologist: JLQ
Animal Fate: Terminal Kill

TISSUE COMMENTS:
LEFT TESTIS - When it occurs, the degenerative change is severe. In this case, about 5% of the tubules are affected.

Animal ID: 9108 — Pathologist: JLQ
Animal Fate: Terminal Kill

TISSUE COMMENTS:
LEFT TESTIS - When it occurs, the degenerative change is severe. In this case, one tubule in the section examined exhibits this change.

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0 STUDY NUMBER: 601F0
SEX: FEMALE GROUP: 1: 0.0000 (% w/v)

Animal ID: 9002 Pathologist: JLQ
Animal Fate: Terminal Kill

ISSUE COMMENTS:
AGINA - The vaginal wall is histologically normal, although the vaginal lumen is greatly enlarged. The enlargement corresponds to the gross necropsy observation of a fluid filled vagina.

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0 STUDY NUMBER: 601F0
SEX: FEMALE GROUP: 2: 0.0001 (% W/V)

lo Comments for any animal in this group

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0 STUDY NUMBER: 601F0
SEX: FEMALE GROUP: 3: 0.0004 (% w/v)

Animal ID: 9081 Pathologist: JLQ
Animal Fate: Terminal Kill

ISSUE COMMENTS:
HYROID - One of pair present.
ARATHYROID - One of pair present.

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 7244-601 F0

COMMENT REPORT

STUDY ID: 7244-601 F0 STUDY NUMBER: 601F0
SEX: FEMALE GROUP: 4: 0.0015 (% w/v)

No Comments for any animal in this group

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

COMMENT REPORT

STUDY ID: 7244-601 F1
SEX: MALE

STUDY NUMBER: 601F1
GROUP: 1: 0.0000 (% w/v)

Animal ID: 1401
Animal Fate: Terminal Kill

Pathologist: JLQ

TISSUE COMMENTS:
THYROID - One of pair present.

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

COMMENT REPORT

STUDY ID: 7244-601 F1 — STUDY NUMBER: 601F1
SEX: MALE — GROUP: 2: 0.0001 (% W/V)

Animal ID: 1482 — Pathologist: JLQ
Animal Fate: Terminal Kill

ISSUE COMMENTS:
EFT EPIDIDYMIS - Capta of epididymis not available.

Animal ID: 1490 — Pathologist: JLQ
Animal Fate: Terminal Kill

ISSUE COMMENTS:
Non-Protocol Tissues:
IGHT TESTIS - Right testicle, in accordance with protocol, was frozen and saved for spermatid head counts.

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

COMMENT REPORT

STUDY ID: 7244-601 F1 STUDY NUMBER: 601F1
SEX: MALE GROUP: 3: 0.0004 (% w/v)

lo Comments for any animal in this group

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

COMMENT REPORT

STUDY ID: 7244-601 F1 STUDY NUMBER: 601F1
SEX: FEMALE GROUP: 1: 0.0000 (% w/v)

No Comments for any animal in this group

PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

COMMENT REPORT

STUDY ID: 7244-601 F1 — STUDY NUMBER: 601F1
SEX: FEMALE — GROUP: 2: 0.0001 (% w/v)

Animal ID: 1532 — Pathologist: JLQ
Animal Fate: Terminal Kill

'ISSUE COMMENTS:
'ARATHYROID - One of pair present.

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PATHOLOGY ASSOCIATES
TWO-GENERATION REPRODUCTION TOXICITY STUDY OF
PROPYLTHIOURACIL WHEN ADMINISTERED TO
SPRAGUE-DAWLEY RATS IN THE DRINKING WATER
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-601 F1

COMMENT REPORT

STUDY ID: 7244-601 F1 STUDY NUMBER: 601F1
SEX: FEMALE GROUP: 3: 0.0004 (% w/v)

lo Comments for any animal in this group

VII. Quality Assurance

Pathology Report

Two-Generation Reproduction Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats in the Drinking Water

TherImmune Research Corporation Study Number: 7244-601

QUALITY ASSURANCE STATEMENT

This histopathology project has been inspected and audited by the PAI Quality Assurance Unit (QAU) as required by the Good Laboratory Practice (GLP) regulations promulgated by the U.S. Food and Drug Administration (FDA). The pathology report is an accurate reflection of the recorded data. The following table is a record of the inspections/audits performed and reported by the QAU.

Date of Inspection	Phase Inspected	Date Findings Reported to PAI Management/Study Pathologist
10/23/00	Tissue Trimming	10/23/00
04/24-26/01	Individual Animal Data	04/26/01
04/24-26/01	Draft Pathology Report	04/26/01

Doraine Dundee
Quality Assurance Specialist

April 26, 2001
Date

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DEVIATION REPORT

DATE: April 20, 2001

STUDY NUMBER/TITLE:

7244-601/Two-Generation Reproduction Toxicity Study of Propylthiouracil When Administered to Sprague-Dawley Rats in the Drinking Water

DEVIATION: __X__ PROTOCOL
_____ STANDARD OPERATING PROCEDURE/NO. _____
TITLE: _____

The organ weights, gross observations, and tissue save statuses were not recorded on the day of necropsy (2/22/01) for F1 Animal Nos. 1469 and 1580. All protocol-required tissues were collected and placed in the appropriate fixative. Post-fix weights were recorded.

REASON:
It could not be determined whether this was a technical error or a computer error. This impact of this deviation will need to be determined by the Study Director.

STUDY PATHOLOGIST:

4-20-01
Jerry L. Quance, D.V.M., D.A.C.V.P/DATE

STUDY DIRECTOR:

Gary W. Wolfe 5/1/01
Gary W. Wolfe, Ph.D./D.A.B.T./DATE

cc: Original -Study Notebook (TRC), Study File (PAI), Quance, Anderson, Delaney

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Appendix 7

Two-Generation Reproduction Toxicity Study of Propylthiouracil when Administered to Sprague-Dawley Rats in the Drinking Water

Thyroid Hormone Analysis Methods

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Suite 200
aithersburg, MD 20877

Edition: #3
Effective Date: 1/6/00

STANDARD OPERATING PROCEDURE

TOTAL (T4)

Summary and Principle:

Thyroxine, the principle thyroid hormone is produced in the thyroid gland and normally circulates bound to transport proteins. The exact mechanism of action of thyroid hormone is not known, but is known that in its absence, function of most organs including central nervous system is affected. T4 is also known to cause metabolic rate decline. Thyroxine is not a species specific hormone but the level varies with species. T4 determination in all species is performed using commercially available human reagent system.

T4 is determined using DPC Coat-A-Count Total T4 RIA procedure. In this procedure I(125)-labeled T4 competes with the T4 in the sample for antibody sites immobilized to the wall of the tube. The reaction is allowed to proceed for a fixed time in the presence of blocking agents for thyroid hormone-binding proteins. After the tubes are decanted and counted, T4 concentration is determined from the standard curve.

Specimen Required:

Serum or plasma stored under refrigeration for seven days, or frozen below -15 degrees C for longer storage.

Materials Required:

Gamma counter
Vortex mixer
Micropipets capable of delivering 25 - 1000ul
37 degrees C waterbath
Repipettor

Director
1/4/00
Date

QA Auditor
1-22-00
Date

Reagents:

DPC Coat-a-Count Total T4 RIA Kit

1. T4 Antibody Coated Tubes:
   Ready to use. Store refrigerated and protected from moisture in sealed zip lock bags. Stable at 2-8 degrees C for at least one year from the date of manufacture.

2. I(125) T4:
   Ready to use. Store refrigerated. Stable at 2-8 degrees C for at least 30 days after opening, or until the expiration date marked on the vial.

3. T4 Calibrators:
   Ready to use six vials. Store refrigerated. Stable at 2-8 degrees C for at least 30 days after opening. The calibrators contain 0, 1, 4, 10, 16, 24 ug of T4 per dl respectively. The life of the calibrators may be extended by freezing aliquots to prevent repeated freezing and thawing.

Procedure:

Bring reagents and specimens to room temperature. Mix well.

1. Label coated tubes in duplicate for each calibrator, control, and sample.

2. Pipet 25ul of each calibrator, control, and sample into their respective tubes. Pipet directly to the bottom.

3. Add 1 ml of I(125) T4 to each tube. Vortex.

4. Incubate for 60 minutes in a 37 degrees C waterbath.

5. Aspirate or decant each tube thoroughly.

6. Count each tube for one minute in a gamma counter.

Calculation of Results:

The assay is programmed into the Gamma Counter for automated results.

Alternatively, a calibration curve may be generated manually by using a logit-log graph and T4 concentrations in the test samples read off this curve.

$$\text{Percent Bound} = \frac{\text{Counts}}{\text{MB Counts}} \times 100$$

Expected Values:

Total T4 values are reported ug/dl.

References:

1. DPC T4 kit insert, 5/99.

2. Nakashima, T., etal: Serum T4, T3, and TSH levels in iodine-deficient and iodine sufficient rats before and after exposure to cold. Proc. Soc. Exp. Biol. Med., 167:45, 1981.

3. Burger, A. G., etal: Interrelationship between energy metabolism and thyroid hormone metabolism during starvation in the rat. Acta Endocrinol., 93:322, 1980.

4. Chiu, S. C., etal: Effects of prolonged administration of thyrotropin on serum concentration, release, and synthesis of thyroid hormones in mice. Acta Endocrinol., 103:68, 1983.

5. Kaack, B., etal: Comparative normal levels of serum T3 and T4 in nonhuman primates. Lab. An. Sci., 29:191, 1979.

6. Refettof, S., etal: Parameters of thyroid function in serum of 16 selected vertebrate species. A study of PBI, serum T4, free T4 and the pattern of T4 and T3 binding to serum proteins. Endocrinology, 86:793, 1970.

7. The Clinical Chemistry of Laboratory Animals, Loeb, W.F. and F.W. Quimby, Ed. Taylor & Francis 2nd Ed., 1999.

ALVI LYTICS INCORPORATED

200 Girard Street
Suite 200
aithersburg, MD 20877

Edition: #3
Effective Date: 1/6/00

STANDARD OPERATING PROCEDURE

Total T3

Summary and Principle:

Triiodothyronine (T3) is the second major thyroid hormone. Even though T3 represents approximately five percent of the thyroid hormones, it exerts the major physiologic actions at the cellular level. Measurement of T3 is a valuable tool for evaluating hyperthyroidism thyrotoxicosis.

T3 is not a species specific hormone. Determination of T3 in all species in performed using commercially available human reagent system.

T3 is determined using DPC Coat-A-Count RIA procedure. In this procedure, the reaction is allowed to proceed for a fixed amount of time in the presence of blocking agents which serve to liberate bound T3 from carrier proteins. Thus Total T3 (circulating T3 and that liberated from the proteins) in the sample competes with the radiolabeled T3 for antibody sites immobilized to the wall of the tube. After the tubes are decanted and counted, T3 concentration is determined from the standard curve.

Specimen Required:

Serum or plasma refrigerated for up to 7 days, or frozen below -15 degrees C for longer storage.

Materials Required:

Gamma counter
Vortex mixer
Micropipets capable of delivering 100 - 1000ul
37 degrees C waterbath
Repipettor

Director

1/6/00
Date

QA Auditor

1-22-00
Date

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Reagents:

DPC Coat-a Count Total T3 RIA Kit.

1. T3 Antibody Coated Tubes:

   Ready to use. Store refrigerated and protected from moisture in sealed zip-lock bags. Stable at 2-8 degrees C for at least one year from the date of manufacture.

2. I(125) T3:

   Ready to use. Store refrigerated. Stable at 2-8 degrees C. for at least 30 days after opening, or until the expiration date marked on the vial.

3. T3 Calibrators:

   Ready to use six vials. The calibrators contain 0, 20, 50, 100, 200, and 600 ng/dl of T3 respectively. Store refrigerated. Stable at 2-8 degrees C for at least 30 days after opening, or until the expiration dates marked on the vials.

Procedure:

Bring all reagents and specimens to room temperature. Mix well.

1. Label coated tubes in duplicate for each calibrator, control and sample.

2. Pipet 100ul of each calibrator, control, and sample into their respective tubes. Pipet directly to the bottom.

3. Add 1ml of I(125) T3 to each tube. Vortex.

4. Incubate for 120 minutes in a 37 degree C waterbath.

5. Aspirate or decant each tube thoroughly.

6. Count each tube for 1 minute in a gamma counter.

Calculation of Results:

The assay is programmed into the Gamma Counter for automated results.

Alternatively, a calibration curve may be generated manually by using a logit-log graph and T3 concentrations in the test samples read off this curve.

$$\text{Percent Bound} = \frac{\text{Counts}}{\text{MB Counts}} \times 100$$

Expected Values:

Total T3 values are reported in ng/dl.

References:

1. DPC Kit Insert, 5/98.

2. Rothwell, N.J., etal: Sympathetic and thyroid influences on metabolic rate in fed, fasted, and refed rats. Am. J. Physiol. 243:R339, 1982.

3. Huaug, H.H., etal: Capacity of old versus young male rats to release TSH, T4, and T3 in response to different stimuli. Exper. Aging Res., 6:3, 1980.

4. Chiu, S.C., etal: Effects of prolonged administration of thyrotrophin on serum concentration, release and synthesis of thyroid hormones in mice. Acta. Endocrinol., 103:68, 1983.

5. Kaack, B., etal: Comparative normal levels of serum T3 and T4 in nonhuman primates. Lab. An. Sci., 29:191, 1979.

6. Mual, D.H., etal: Response of T3 and T4 to TSH in adult female baboons. Lab. An. Sci., 32:267, 1982.

7. Kaneko, J.J., Thyroid Function in Clinical Biochemistry of Domestic Animals. Acad. Press 1997, p. 571.

LYTICS
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200 Girard Street
Suite 200
Gaithersburg, MD 20877

Edition: #2
Effective Date: 10/06/96
Responsibility: ALL

STANDARD OPERATING PROCEDURE

Rat/Mouse Thyroid Stimulating Hormone (TSH)

Summary and Principle:

Thyroid Stimulating Hormone (TSH) is a pituitary hormone which through its action on the thyroid gland plays a major role in maintaining normal circulating levels of iodothyronines T3 and T4. Measurement of TSH levels is used as an aid to determine hypo or hyperthyroidism.

TSH is a species specific hormone therefore rat/mouse TSH is determined using species specific hormones.

The assay is performed using an inhouse developed double antibody radioimmunoassay (RIA) procedure. Freshly prepared, 125I-TSH is allowed to react overnight with the specific antibody in the presence and absence of unlabelled hormone in the standard or sample. At the end of reaction time excess amount of second antibody (anti-primary species) containing polyethylene glycol (PEG) is added. The bound and unbound 125I hormone is separated by centrifugation. The supernate is discarded and the counts are measured in the precipitates. A standard curve is generated and the amount of hormone in the samples is quantitated from this curve.

Specimen Requirement:

Frozen Serum. Rat 0.5ml, Mouse 0.25ml.

Supplies and Equipment:

12X75mm glass tubes
Micropipettes capable of accurately delivering 10-1000ul
Pipettes capable of delivering 1-10ml
Repipettor
Vortex
Refrigerated centrifuge
Gamma Counter

Larry R Day
Director

10/6/96
Date

Frank Newman
QA Auditor

11-5-96
Date

Reagents:

PBS -- Phosphate Buffered Saline tablets Cat# P-4417 Sigma Chemical Co.

BSA -- Bovine Serum Albumin, Sigma Chem. Co. or equivalent

NRS -- Normal Rabbit Serum, Antibodies Inc. or equivalent.

Rat TSH (iodination grade), Pituitary Hormones and Antisera Reference Center, UCLA, CA.

Anti-Rat TSH antibody, Pituitary Hormones and Antisera Reference Center, UCLA, CA.

Rat/Mouse standard preparations, Pituitary Hormones and Antisera Reference Center, UCLA, CA.

Precipitating Reagent-Goat Anti-rabbit (GRGG), Diagnostic Products Corp.

125I-Rat TSH, Covance Laboratories, Vienna, VA.

Reagent Preparation:

Buffers:

A. PBS/BSA (1%):
Dissolve 1 tablet of PBS in 200ml of deionized water (DW). Add 2.0gm of BSA and stir until completely dissolved.

B. PBS/BSA/NRS:
Add 0.5ml NRS to 100ml of PBS/BSA buffer, mix well (0.5% NRS).

C. 125I-RAT TSH:
Obtain freshly iodinated hormone. Make appropriate dilution of125I RAT TSH in PBS/BSA/NRS to obtain approximately 25,000 counts per 200ul. (For mouse assay, about 25,000 counts per 50ul).

D. Anti-RAT TSH Antibody preparation:
Using PBS/BSA/NRS buffer make antibody dilutions in the range of 1000-8000. Incubate overnight with a 125I-TSH as prepared above. Precipitate with GRGG and centrifuge. Discard the supernates and determine the 125I counts in the precipitate. Select a dilution of the antibody yielding a binding in the range of 25-35% of the total counts.

E. Standard Curve Preparation:

Rat TSH: Dilute the reference standard in PBS/BSA/NRS appropriately to obtain a standard curve in the range of 50 to 0.39ng/ml.

Mouse TSH: Dilute reference standard in PBS/BSA/NRS appropriately to obtain a standard curve in the range of 14 to 0.4ug/ml.

Procedure:

1. Label tubes in duplicate for total count (TC), nonspecific binding (NSB), maximum binding (B0), standard curve and samples.

2. Assay:

   RAT

   a. Pipet 100ul of each standard into the appropriately labeled tubes followed by 100ul of PBS/BSA/ buffer. Add 200ul of buffer to NSB and B0 tubes.

   b. Pipet 200ul of samples into the correspondingly labeled tubes.

   c. Add 200ul of antibody to all tubes except NSB and TC.

   d. Add 200ul of buffer to NSB tubes. Add 200ul of 125I-Rat TSH to all tubes. Set aside TC tubes. Add 400ul of PBS/BSA buffer to all tubes to bring the final volume to 1.0ml.

   MOUSE

   a. Pipet 50ul of each standard and samples. Add 50ul of buffer to NSB and B0 tubes.

   b. Add 50ul of antibody to all tubes except NSB and TC. Add 50ul buffer to NSB tubes.

   c. Add 50ul of 125I-Rat TSH to all tubes. Set aside TC tubes.

   d. Add 100ul of buffer to all tubes to bring the final volume to 250ul.

3. Vortex all tubes gently, cover with parafilm, and incubate 15-24 hours at room temperature.

4. Rat Assay:
   Add 2.0ml of GRGG to all tubes.

   Mouse Assay:
   Add 0.5ml of GRGG to all tubes.

5. Vortex all tubes gently and incubate 1 hour at room temperature. Centrifuge at 2500-3000 rpm in a refrigerated centrifuge for a minimum of 30 minutes. Carefully aspirate supernatants. Count each tube for 1 minute in a gamma preprogrammed counter to generate a log-logit curve.

Alternatively, graph the standard curve using log-logit parameters with B0 tube as 100%. Read results off the standard curve.

Results:

Rat: The values obtained are divided by two (2) to obtain ng/ml

Mouse: The values are read directly off the curve to obtain ug/ml.

Note: The assay is programmed into the a gamma counter for automated results.

Note: If requested by client, specimens may be assayed in single or on dilution.

Quality Control:

1. NSB cpm should be less than 3% of the TC.
2. Maximum binding should be 25-35% the TC.
3. In each assay, include some of the project samples for linearity/spike recovery. Alternatively, previously assayed specimens may be included. Recovered results should be within 20% of the targeted value.
4. If any of the above QC criteria are not acceptable, inform the director for determination of further action. Record the action taken.

References:

1. Parlow, Albert F., Technical Report, Rat TSH package insert, Pituitary Hormones and Antisera Center.
2. Ottenweller, J. E., Hedge, G. A. (1982), Diurnal Variations of Plasma Thyrotropin, Thyroxine and Triiodothyronine in Female Rats Are Phase Shifted After Inversion of the Photo-Period. Endocrinology 111: 509-514.
3. Loeb, W. F., Quimby, F. W., (eds) The Clinical Chemistry of Laboratory Animals, Pergamon Press, 1989.

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