Final Report - 101

TherImmune Research Corporation

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

THERIMMUNE
Research Corporation

Sponsor:

Environmental Protection Agency
RTP: MD-71 NHEERL
Research Triangle Park, NC 27711

FINAL REPORT

Study Title:

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

Test Articles:

Ethynyl estradiol, Tamoxifen, Propylthiouracil, Ketoconazole, Pimozide, Methoxychlor

Authors:

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Study Completion Date:
June 29, 2000

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TherImmune Study Number:
1143-101

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COMPLIANCE STATEMENT
Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

This study was conducted in compliance with the EPA FIFRA Good Laboratory Practice Standards as set forth in Title 40 of the U.S. Code of Federal Regulations Part 160, issued October 16, 1989, and any applicable amendments. All deviations from the protocol, and SOPs are listed in the raw data. There were no deviations from the aforementioned regulations or protocol which affected the quality or integrity of the study or the interpretation of the results in the report.

Study Director:

M S. Rocca 6.29.00

Meredith S. Rocca, Ph.D./Date

QUALITY ASSURANCE STATEMENT
Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the standard operating procedures of the Quality Assurance Unit and according to the requirements of the EPA FIFRA Good Laboratory Practice Regulations as set forth in Title 40 CFR Part 160. Findings from the inspections and final report review were reported to management and to the study director on the following dates:

Inspections/Review	Findings Reported	Inspector/Reviewer
Protocol 11/21,26,28,29/99	11/30/99	C. Matos-Rosa
Phase Inspection 01/14/00	01/14/00	C. Matos-Rosa
Final Report and Data Audit 04/29 & 05/01-05/00	05/05/00	H. Shaffi
Post-Audit 06/22,23,26/00	06/26/00	H. Shaffi

Quality Assurance Unit:

H. Shaffi H /Date 6/21/00

STUDY IDENTIFICATION

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

TherImmune Study Number:	1143-101
Test Articles:	Ethynyl estradiol, Tamoxifen, Propylthiouracil, Ketoconazole, Pimozide, and Methoxychlor
Sponsor:	Environmental Protection Agency
Sponsor's Representative:	Kenneth H. Elstein, Project Officer Environmental Protection Agency RTP: MD-71 NHEERL Research Triangle Park, NC 27711 Phone: 919-541-3581 Fax: 919-541-1499
Study Director:	Meredith S. Rocca, Ph.D. TherImmune Research Corporation 15 Firstfield Road Gaithersburg, MD 20878 Phone: 301-330-3735
Study Timetable	
Study Initiation:	December 14, 1999
Experimental Start Date:	January 7, 2000
Experimental Termination:	January 28, 2000

STUDY PERSONNEL
Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

Study Director:	Meredith S. Rocca, Ph.D.
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SUMMARY

The purpose of this study was to quantify the effects of endocrine disrupting chemicals on pubertal development and thyroid function in the intact juvenile female rat. An additional goal was to validate this study design and examine the variation between Sprague Dawley (SD) and Long Evans (LE) rats. Female were treated from postnatal day (PND) 22 through 42 or 43, by oral gavage, with 2.5 mL/kg/day corn oil, 0.005 mg/kg/day ethynyl estradiol, 10 mg/kg/day tamoxifen, 240 mg/kg/day propylthiouracil (PTU), 100 mg/kg/day ketoconazole, 30 mg/kg/day pimozide, or 100 mg/kg/day methoxychlor. During treatment females were observed for signs of toxicity, weighed, and examined for vaginal opening daily. Vaginal smears were taken beginning on the day of vaginal opening and examined for stage of estrous. Serum was collected for T4 and TSH analysis at termination. A complete necropsy was performed and the following organs were weighed: uterus, ovaries, liver, pituitary, kidneys, and adrenals. The thyroid, ovaries and uterus were preserved and examined histologically. The data were analyzed by multivariate analysis of covariance (MANCOVA), using body weight at weaning as a covariate. Results are summarized in Figure 1.

Figure 1: Changes in Selected Endpoints Compared to Controls

Test Article	Body Weight	Age at VO	Age at E	Histopathology	TSH	T4
Ethynyl Estradiol	↓LE	↓	↓	✓		
Tamoxifen	↓	↓	↑	✓		↑
Propylthiouracil	↓		↑SD	✓	↑	↓
Ketoconazole	↓LE		↑	✓		
Pimozide	↓			✓		
Methoxychlor	↓LE	↓	↓	✓		

KEY: ↓ = significantly decreased compared to control mean
↑ = significantly increased compared to control mean
✓ = affected histopathology
SD = Sprague Dawley only
LE = Long Evans only
E = first estrus
VO = vaginal opening

This study design accurately identified endocrine disrupting compounds which were estrogenic, anti-estrogenic, inhibitors of steroid and thyroid hormone synthesis, or a dopamine antagonist and quantified their effect on the juvenile female rat. Although there were some differences between strains, when all endpoints were considered the results were consistent. As evidenced by changes observed in body weights, vaginal opening, age of first estrus, vaginal cytology, organ weights, gross and histopathology and TSH and T4 levels, dosing female juvenile rats of the Sprague Dawley or Long-Evans strain from postnatal day 22 through 42 or 43 is a good model for identifying endocrine disrupting compounds.

INTRODUCTION

This study was designed to quantify the effects of endocrine disrupting chemicals on pubertal development and thyroid function in the intact juvenile female rat. The larger goal was to provide preliminary validation of the protocol for future EPA studies, and to assess intra-laboratory and inter-strain variation. Dosing began on January 7, 2000. The last terminal necropsies were performed on January 28, 2000.

TEST AND CONTROL ARTICLES

The control article, corn oil, and the test articles, ethynyl estradiol, tamoxifen, propylthiouracil, ketoconazole, pimozide, and methoxychlor, were received and stored as described below.

Test Article	Lot No.	Date Received	Received From	Purity %	Storage Conditions
Corn Oil	107H1649	1/5/00	Sigma-Aldrich, Inc. St. Louis, Missouri	100	Room Temperature
Ethynyl estradiol	108H0684	1/5/00	Sigma-Aldrich, Inc. St. Louis, Missouri	98	Room Temperature
Tamoxifen	079H1388	1/5/00	Sigma-Aldrich, Inc. St. Louis, Missouri	99	Refrigerate 2-8°C
	079H1388	2/2/00	Sigma-Aldrich, Inc. St. Louis, Missouri	99	Refrigerate 2-8°C, protected from light
Propylthiouracil	099H2509	1/5/00	Sigma-Aldrich, Inc. St. Louis, Missouri	99	Room Temperature
Ketoconazole	079H4087	1/5/00	Sigma-Aldrich, Inc. St. Louis, Missouri	98	Refrigerate 2-8°C
	079H4087	1/6/00	Sigma-Aldrich, Inc. St. Louis, Missouri	99	Refrigerate 2-8°C
	0078353	1/7/00	ICN Biomedicals Aurora, Ohio	99	Room Temperature, protected from light
Pimozide	019H0578	1/5/00	Sigma-Aldrich, Inc. St. Louis, Missouri	100	Refrigerate 2-8°C
Methoxychlor	49H1328	1/5/00	Sigma-Aldrich, Inc. St. Louis, Missouri	95.2	Room Temperature

Reserve samples were taken as follows: 100 mg each of tamoxifen, propylthiouracil, and ketoconazole; and 1 g each of ethynyl estradiol, pimozide and methoxychlor. Ketoconazole was acquired from two different sources due to a shortage of supply from Sigma-Aldrich, Inc., and a reserve sample was taken from each lot. These samples will be stored according to the manufacturer's recommendations to minimize degradation for at least six months after the final report is issued. Information on the methods of synthesis and stability, as well as data on the composition or other characteristics which define the test articles, are on file with the manufacturer.

A 1 mL reserve sample of the initial stock solution made of ethynyl estradiol and 1 mL reserve sample of the first and last dosing solutions administered to the animals was taken and will be stored frozen for at least six months after the final report is issued.

TEST ANIMALS AND HUSBANDRY

Rats were chosen since they have historically been used in safety evaluation studies of this type and are recommended by appropriate regulatory agencies. Twenty-two timed-pregnant female Hsd: Sprague Dawley® SD® Rats and twenty-five timed-pregnant female Long-Evans Hooded Rats, were received on December 7, 1999 (Gestation day 12) from Harlan Sprague Dawley, Inc., Indianapolis, Indiana. They were assigned temporary animal numbers, acclimated to laboratory conditions for 8 days, and released for study use by a staff veterinarian.

Upon receipt, animals were housed individually in polycarbonate cages measuring 19 x 10½ x 8 inches (length x width x height) suspended on stainless-steel racks with an Edstrom automatic watering system providing filtered tap water. Racks were equipped with filter paper liners. Polycarbonate caging contained Sani Chip® heat treated hardwood laboratory bedding. Tap water and TEKLAD™ Certified Rodent Diet 7012C were provided *ad libitum*, except prior to blood collection and terminal necropsy, when the animals were food fasted overnight (with

water available). The water is routinely analyzed for contaminants and specific microbes. The feed is analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specific nutrients. The results of the feed and water analyses are on file at TherImmune Research Corporation. No contaminants were known to be present in the diet or water at levels which might interfere with achieving the objectives of the study.

During the study period, the temperature and relative humidity in the animal rooms were monitored continuously using the Rees™ Scientific Monitoring System and recorded twice daily using a Bacharach® sling psychrometer. The environmental controls in the animal room were set to maintain temperatures between 20 and 24°C and relative humidity between 40 and 50%. Ten or greater air changes/hour and a 14-hour light/10-hour dark cycle were maintained. Exceptions were noted in the raw data and had no adverse effect on the integrity of the study.

METHODS

Observations and Records - Prior to Selection of Study Animals

All of the pregnant females and pups, were observed for mortality, moribundity and clinical observations twice daily, at least six hours apart each day. Observations included skin and fur, eyes and mucous membrane, respiratory system, circulatory system, autonomic and central nervous system, somatomotor pattern, and behavior pattern. Pregnant females were observed at least twice daily for signs of parturition. The pregnant females were allowed to deliver and rear their pups until weaning on postnatal day (PND) 21.

Pups were weighed on PND 1 and weekly thereafter to identify runted and/or unthrifty litters. On PND 4, litters were culled to 10 pups, with approximately the same number of male and female pups. The remaining pups were weaned on PND 21 and randomized into dosage groups (females on this study and males to TherImmune Study No. 1143-100).

Group Assignment and Dose Levels

On PND 21, female pups were initially accepted into the randomization pool based upon physical examinations. They were assigned to study using computer-generated random numbers. Sprague-Dawley and Long Evans rats were randomized separately. The weight variation of selected females did not exceed 2.5 or 3.4 grams above or below the mean body weight of 48.6 or 44.9 grams for Sprague-Dawley (SD) and Long Evans (LE) rats, respectively. The mean weight for each group was not statistically different. During the randomization process, each study animal was assigned a unique number, assigned to groups as shown below, and housed three per cage. All animals not used on study were removed from the study room, with the exception of the male pups chosen for study 1143-100. This protocol was conducted as a "blind study" - the technicians performing the study activities had no knowledge of which test article was administered to which study group.

Group	Treatment	Dosage (per kg/day)	Number of Females/Strain
1	Corn Oil	2.5 mL	6
2	Ethynyl estradiol	0.005 mg	6
3	Tamoxifen	10 mg	6
4	Propylthiouracil (PTU)	240 mg	6
5	Ketoconazole	100 mg	6
6	Pimozide	30 mg	6
7	Methoxychlor	100 mg	6

Test and Control Article Formulation and Administration

Dosing formulations were prepared weekly. The test materials were considered to be 100% pure for formulating purposes, with the exception of methoxychlor, which was adjusted to 100%.

A stock solution of ethynyl estradiol was prepared by dissolving it in ethanol prior to dilution in corn oil. The appropriate amounts of each test article were diluted with the required amount of corn oil and then transferred to amber glass jars. The formulations were stirred continuously for 24 to 48 hours prior to the first dosing. All formulations were stirred continuously during dosing.

Animals were given the appropriate dosing formulation via oral gavage daily on PND 22 through 42 or 43, between 0700 and 0900 h, at a dose volume of 2.5 mL/kg, adjusted daily. Test material was administered using an 18-gauge needle and a 1 mL glass tuberculin syringe. The dosing technician performed the procedure without knowledge of the test article. The test material was administered orally because this is the expected route of human exposure.

Observations and Records - Study Animals

All study animals, were observed for mortality and moribundity twice daily, at least six hours apart each day. Observations included skin and fur, eyes and mucous membrane, respiratory system, circulatory system, autonomic and central nervous system, somatomotor pattern, and behavior pattern. Potential signs of toxicity including tremors, convulsions, salivation, diarrhea, lethargy, coma, limb impairment and resolution, change in fecal and urinary output, or other atypical behavior or appearance were recorded. Detailed clinical observations were recorded weekly.

The females were weighed daily. Beginning on PND 22, the females were examined daily for vaginal opening, with the appearance of a small "pinhole", a vaginal thread, and complete vaginal opening being recorded on the days observed. Beginning on the day of complete vaginal opening, daily vaginal smears were taken, stained, and examined for stage of estrous.

Termination

On PND 42 or 43, between 1300 and 1700, all animals were sacrificed by decapitation and exsanguination. The decapitation was performed in a room separate from the animal room and within 15 seconds of removing the animal from its cage.

Serum Collection and Analysis

Following decapitation, trunk blood was collected from each animal and serum obtained. Approximately 500 μL serum/animal was aliquoted into 1.7 mL siliconized microcentrifuge tubes, stored at approximately -80°C, and later shipped on dry ice by express carrier to Dr. Ralph Cooper, US EPA, Durham, North Carolina. Approximately 550 μL serum/animal was aliquoted into 1.7 mL siliconized microcentrifuge tubes, stored at approximately -80°C, and delivered on dry ice to AniLytics, Inc., Gaithersburg, Maryland, for T4 and TSH analysis.

Necropsy

Necropsies were conducted on each animal by trained personnel and included examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents.

Organ Weights

The following organs were weighed wet from all animals:

ovaries	uterus with cervix
liver	kidney
pituitary	adrenals

The uterus was then placed on a paper towel, slit to allow the fluid contents to leak out, gently blotted dry and then reweighed.

Tissue Preservation

The thyroid, ovaries, and uterus were placed in Bouin's fixative for approximately 24 hours, then rinsed and stored in 70% ethanol.

Histopathology

The preserved thyroid, ovaries, and uterus from all animals were embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically by the pathologist at Pathology Associates International.

Statistical Analyses

Methods used for statistical analysis are presented in Appendix 10. Briefly, data was tested for homogeneity of variance and analyzed by MANCOVA using weight at weaning as a covariate.

For the vaginal opening and vaginal cytology analyses, there were some animals which never reached the stated endpoint (complete vaginal opening or first estrus). In order to perform statistical analysis of these endpoints, these animals were assumed to have reached the endpoint on the day after they were necropsied and at their terminal body weight. Therefore the means for these endpoints in groups including these animals are artificially low. This is noted on the appropriate tables as censored data.

Two endpoints, age at first estrus and dry uterus weight, could not be transformed to achieve variance homogeneity, therefore these endpoints were removed from the MANCOVA and Wilcoxon Rank Sum test was used.

The groups for which the null hypothesis could not be rejected by MANCOVA for organ to body weight ratios were ethynyl estradiol (SD only), PTU, pimozide, and methoxychlor. Therefore, no post-hoc tests were performed for these groups.

"Increased" or "decreased" are used throughout the text of this report to describe the statistical significance at $p \leq 0.05$, unless otherwise indicated.

Record Retention

All records, study protocols, reports, protocol and report revisions, written letters, and specimens generated by TherImmune and/or PAI are retained at TherImmune Research Corporation Archive. Documentation of any transfer of study records and reports will be maintained by TherImmune for a period of one year.

RESULTS

Mortality

Individual day of death for each animal is presented in Appendix 5. All animals survived to scheduled euthanasia.

Clinical Observations

Weekly clinical observations are summarized in Table 1 and presented individually in Appendix 1. No adverse clinical signs were observed - all females appeared normal at their weekly physicals.

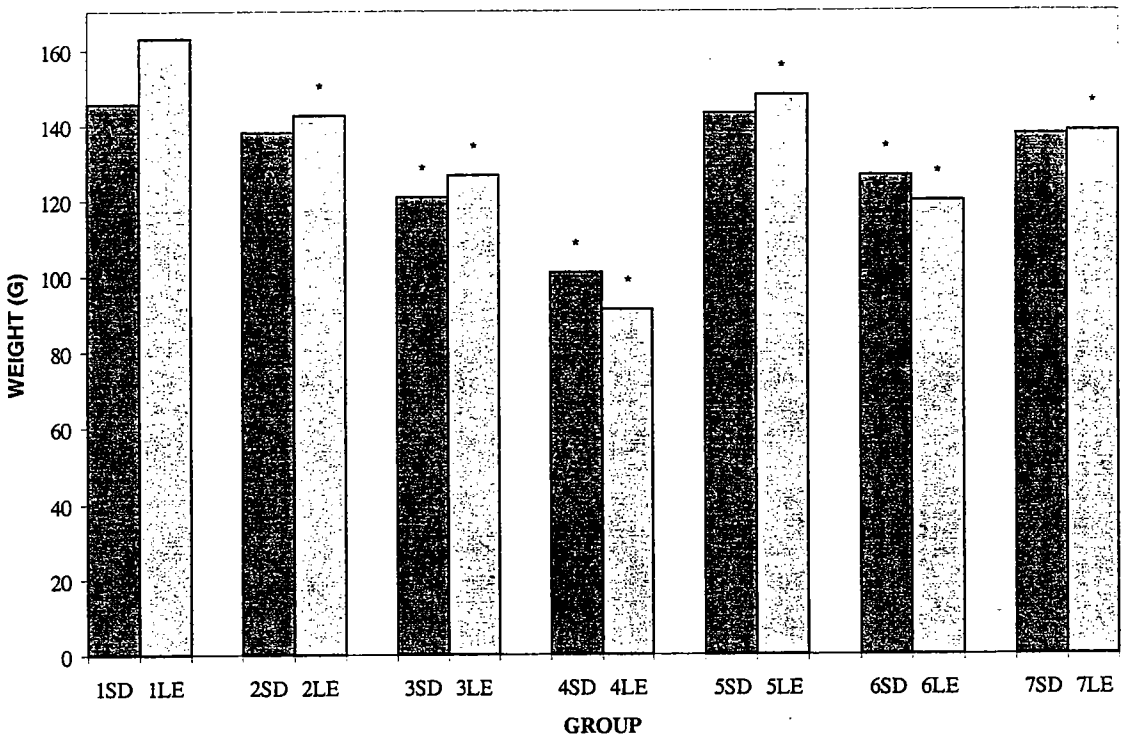
Daily Body Weights

Mean daily body weights are shown in Figure 2, summarized in Table 2 and presented individually in Appendix 2. Mean body weight changes from PND 21 to necropsy are summarized in Table 3.

The mean body weight of females increased daily, with a few exceptions, in all groups except pimozide-treated females. Between PND 22 and 24, pimozide-treated females mean body weight decreased 12.6% for SD and 11.2% for LE rats. Pimozide treatment resulted in PND 42 mean body weights which were significantly lower than controls, being only 86.8 and 73.6% of controls for SD and LE females, respectively. Treatment with tamoxifen and PTU also resulted in decreased PND 42 mean body weights in both strains of rat. The PND 42 mean body weight of tamoxifen-treated females was only 82.8 and 77.7% of their control groups, for SD and LE females respectively. PTU treatment resulted in the greatest decrease in PND 42 mean body weights, with SD and LE females weighing 69.1 and 56.0% of their respective control groups.

Long Evans rats were also significantly affected by ethynyl estradiol (87.4% of mean control body weight), methoxychlor (85.0%), and ketoconazole (90.6%) treatments.

Figure 2: PND 42 Mean Body Weight



KEY: PND = Postnatal Day

GROUP: 1 = 2.5 mL/kg/day Corn Oil
2 = 0.005 mg/kg/day Ethynyl Estradiol
3 = 10 mg/kg/day Tamoxifen
4 = 240 mg/kg/day Propylthiouracil

* = significantly different than control
5 = 100 mg/kg/day Ketoconazole
6 = 30 mg/kg/day Pimozide
7 = 100 mg/kg/day Methoxychlor

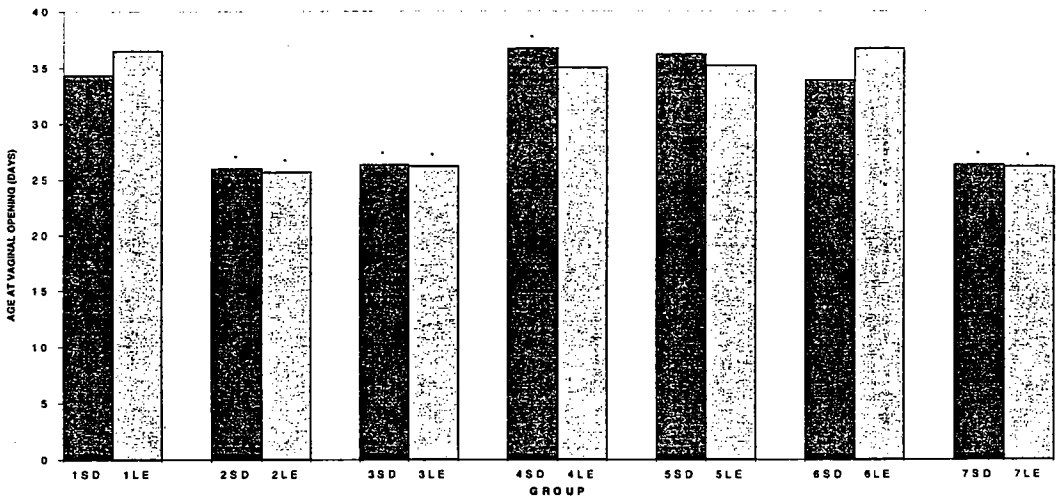
Age and Weight at Vaginal Opening

Vaginal opening data are shown in Figure 3, summarized in Table 4 and presented individually in Appendix 3.

The mean age of vaginal opening was 2 days later in LE females than in SD females. Control females opened on PND 34.3 and 36.5 for the strains, respectively. The mean age of

vaginal opening was significantly decreased to approximately PND 26 in both strains of females treated with ethynyl estradiol, tamoxifen, and methoxychlor. In contrast, PTU treatment increased the mean age of vaginal opening to PND 36.7 in SD females. Ketoconazole or pimozide treatment had no significant effect on the mean age of vaginal opening. The mean for pimozide-treated LE females is actually artificially low as one female never achieved vaginal opening and therefore the day after necropsy was used as the day of vaginal opening for statistical purposes for this female.

Figure 3: Mean Age at Vaginal Opening



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3 = 10 mg/kg/day Tamoxifen

4 = 240 mg/kg/day Propylthiouracil

* = significantly different than control

5 = 100 mg/kg/day Ketoconazole

6 = 30 mg/kg/day Pimozide

7 = 100 mg/kg/day Methoxychlor

PND = Postnatal Day

Vaginal Cytology

Vaginal cytology, including age at first estrus, are shown in Figures 4 and 5, summarized in Table 5 and presented individually in Appendix 4.

The mean age at first estrus was two days later in LE females (PND 37.2) than in SD females (35.2). Females treated with pimozide were not significantly different. Treatment with ethynyl estradiol, however, decreased the mean age at first estrus to PND 26 in both strains. Methoxychlor treatment also significantly reduced the mean age at first estrus to PND 29.0 and 31.2 in SD and LE females respectively.

The mean age at first estrus was significantly increased by tamoxifen (PND 43.5), PTU (SD) and ketoconazole (PND 41.7). The means for tamoxifen, PTU, ketoconazole and pimozide-treated groups are actually artificially low as some females never exhibited estrus and therefore the day after necropsy was used as the day of first estrus for statistical purposes for these females. No tamoxifen-treated females ever exhibited estrus (0%) and only 33% of ketoconazole-treated rats exhibited estrus. Five out of six SD females (83%) in each of the pimozide and PTU-treated groups exhibited estrus. PTU treatment also inhibited estrus in LE females - only 66% exhibited estrus.

Figure 4: Mean Age at First Estrus

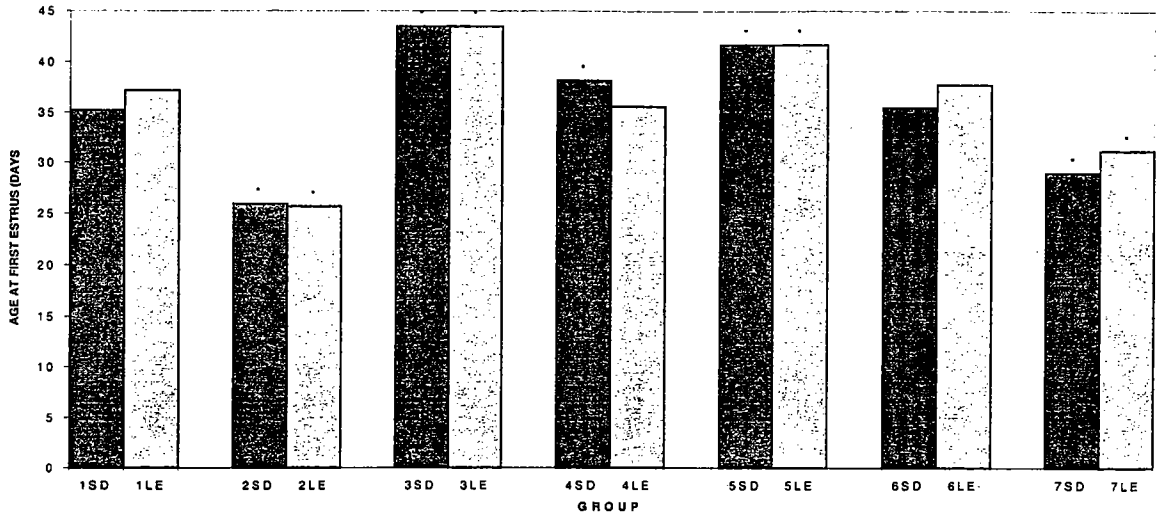
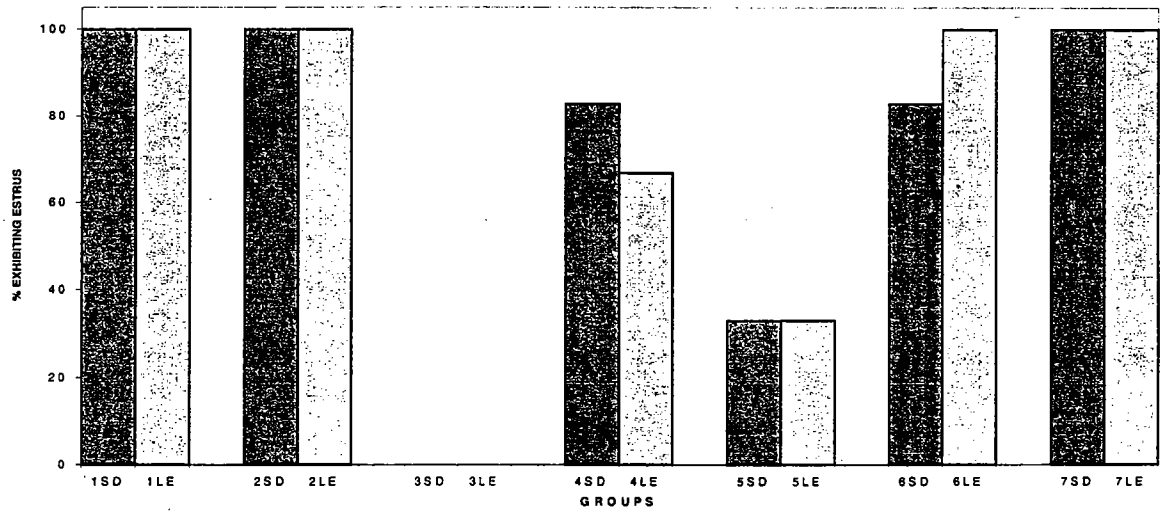


Figure 5: Percent of Females Exhibiting Estrus



KEY: PND = Postnatal Day

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4 = 240 mg/kg/day Propylthiouracil

* = significantly different than control
5 = 100 mg/kg/day Ketoconazole
6 = 30 mg/kg/day Pimozide
7 = 100 mg/kg/day Methoxychlor

Gross Pathology

Gross pathology findings are summarized in Table 6 and are presented individually in Appendix 5.

There were treatment related gross pathology findings in the adrenal and thyroid. Ten of 12 ketoconazole-treated females (83%) had enlarged adrenals, while adrenals from all other treatment groups appeared normal. The thyroid appeared enlarged in 83% of PTU-treated females, as compared to 8% of the control females.

Organ Weights

Organ weights and organ-to-body weight ratios are summarized in Tables 7 and 8, respectively, and presented individually in Appendix 6. Organ weights which were outside of physiological range were excluded from group means and analysis by the study director. Exclusions are noted on Appendix 6.

Trends in organ weight changes were similar for both strains of rat when both absolute organ weights and organ to body weights were considered. Figure 6 details which organs were affected as absolute and/or as a percent of body weight in each strain. In agreement with the gross findings, the mean adrenal weight in ketoconazole-treated females, both absolute weight and as a percent of body weight, was approximately twice that of control females. Adrenal weights were decreased in tamoxifen and PTU-treated SD females as compared to control means.

Kidney weights were lower in females treated with ethynyl estradiol (LE), tamoxifen (LE), PTU, pimozide and methoxychlor (LE), but higher in ketoconazole-treated rats. The affects on liver weights were similar. Pituitary weights were only significantly affected by tamoxifen in LE females. As the pituitaries are very small in these juvenile animals, the variability in weights may be an artifact. Most of these effects were not significant as a percentage of body weight and as no histology was performed on these organs, whether these effects are test article related cannot be determined.

Uterine weights were decreased by tamoxifen and PTU as compared to control means. Ovary weight was decreased by PTU in both strains and by ethynyl estradiol, tamoxifen,

ketoconazole and pimozide in LE females.

Figure 6: Organ Weights

Tissue	Ethynyl Estradiol		Tamoxifen		PTU		Ketocon-azole		Pimozide		Methoxy-chlor	
	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%	Wt.	%
Adrenals			↓SD		↓SD		↑	↑				
Kidneys	↓LE		↓LE	↑	↓		↑SD	↑	↓		↓LE	
Liver	↓LE		↓		↓		↑SD	↑	↓LE		↓	
Pituitary			↓LE									
Ovaries	↓LE		↓LE	↓LE	↓		↓LE		↓LE			
Uterus			↓	↓SD	↓				↓LE			
Uterus - dry			↓LE	↓	↓LE							

KEY: ↓ = significantly decreased compared to control mean Wt. = absolute organ weight
↑ = significantly increased compared to control mean % = organ weight as a percentage of body weight
LE = Long Evans only SD = Sprague Dawley only

Histopathology

Summary and individual pathology findings are shown in Figure 7 and presented and discussed in the Pathology Report located in Appendix 6.

The three tissues examined in this study were thyroids, ovaries, and uterus. All test articles affected histopathology. Reproductive organs were affected in both strains by all test articles. PTU was the only treatment which affected thyroid histopathology.

Figure 7: Histopathology Findings

Tissue	Ethynyl Estradiol	Tamoxifen	PTU	Ketocon-azole	Pimozide	Methoxy-chlor
Thyroid			✓			
Ovaries	✓	✓	✓	✓	✓	
Uterus	✓	✓	✓			✓

KEY: ✓= affected histopathology

Serum T4 and TSH

Serum T4 and TSH data are summarized in Table 9 and presented individually in Appendix 8.

The control group mean serum T4 and TSH were similar for both strains. Control levels of T4 were 3.59 and 4.42 μg/DL and TSH were 1.67 and 1.34 ng/mL for SD and LE females, respectively. The largest effect was seen with PTU treatment. PTU-treated females had mean T4 levels which were less than 1% of the control mean. These females had TSH levels which were more than 16 times the control mean. There were no other significant differences among groups in mean serum TSH. There was a small, but significant increase in T4 levels in tamoxifen-treated females.

DISCUSSION

The purpose of this study was to quantify the effects of endocrine disrupting chemicals on pubertal development and thyroid function in the intact juvenile female rat. The compounds used disrupt normal endocrine function in a variety of ways and have differing effects on growth and sexual maturation.

The effect of endogenous estrogens are mimicked by ethynyl estradiol and methoxychlor which act as estrogen receptor agonists. The two estrogenic compounds significantly decreased the age of vaginal opening and first estrus, and disrupted estrous cycling, with many animals in persistent estrus. Histological changes included ovary atrophy, and hypertrophy/hyperplasia of the uterus.

Tamoxifen also acts at the estrogen receptor, but usually functions as an antagonist. Tamoxifen-treatment had mixed effects - body and uterus weights were significantly reduced and females never exhibited estrus, as would be expected of an anti-estrogen, but the age of vaginal opening was decreased. Histology findings included atrophy and interstitial cell hyperplasia in the ovary and atrophy and epithelial cell hypertrophy of the uterus. It is believed that tamoxifen's ability to act as either an estrogen receptor agonist or antagonist may be related to the dosage and the subset of receptors that are expressed in different tissues.

Ketoconazole disrupts endocrine function by inhibiting cytochrome P_{450} enzymes which are necessary for steroidogenesis. Females treated with ketoconazole had lower body weights (LE only), increased adrenal size and weights, age at first estrus, and disrupted estrous cycling. The only histology findings were in the ovaries. Ovaries findings were lack of corpora lutea, atrophy, and interstitial cell hyperplasia.

Pimozide is a D_2 receptor antagonist, which suppresses the action of dopamine, resulting in an increase in prolactin secretion. Pimozide treatment resulted in severe weight loss in the first two days of treatment. Subsequently, the females gained weight, but body weights were always significantly lower than controls. One pimozide-treated SD female never achieved vaginal opening. Ovarian histology revealed lutinized follicles and a general

reduction in size.

PTU acts by inhibiting iodination of thyroid hormones. This inhibition disrupts growth and, indirectly, sexual maturation. The effect of PTU treatment on thyroid function were dramatic. PTU was very effective at inhibiting T4 levels and without the negative feedback TSH levels soared and the thyroid appeared grossly enlarged. As expected, PTU-treated females had the lowest mean body weights. Treatment increased the age of vaginal opening significantly in SD females and one LE never achieved vaginal opening. Age at first estrus was also increased in SD females and one SD and two LE PTU-treated females never exhibited estrus. Thyroid histology was severly affected in all animals and ovaries and uteri were atrophic.

Another goal of this study was to assess whether Sprague Dawley and Long Evans rats respond differently to endocrine disruptors. While the magnitude of the effect varied, both strains of rat responded similarly to treatment.

CONCLUSION

This study design accurately identified endocrine disrupting compounds which were estrogenic, anti-estrogenic, inhibitors of steroid and thyroid hormone synthesis, or a dopamine antagonist and quantified their effect on the juvenile female rat. Although there were some differences between strains, when all endpoints were considered the results were consistent. As evidenced by changes observed in body weights, vaginal opening, age of first estrus, vaginal cytology, organ weights, gross and histopathology and TSH and T4 levels, dosing female juvenile rats of the Sprague Dawley or Long-Evans strain from postnatal day 22 through 42 or 43 is a good model for identifying endocrine disrupting compounds.

Study Director:

Meredith S. Rocca 6-29-00

Meredith S. Rocca, Ph.D./ Date

Project Leader:

Suzanne M Borst 6-29-00

Suzanne M. Borst, B.S. / Date

TABLE 1
SUMMARY OF WEEKLY CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: PERIOD	1	2	3	4	5	6	7
PND 21							
NO. OBSERVED	12	12	12	12	12	12	12
NORMAL	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
PND 28							
NO. OBSERVED	12	12	12	12	12	11	12
NORMAL	12 100%	12 100%	12 100%	12 100%	12 100%	11 100%	12 100%
PND 35							
NO. OBSERVED	12	12	12	12	12	12	12
NORMAL	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
PND 42							
NO. OBSERVED	12	12	12	12	12	12	12
NORMAL	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
TERMINAL KILL	6 50%	6 50%	6 50%	6 50%	6 50%	6 50%	6 50%
PND 43							
NO. OBSERVED	6	6	6	6	6	6	6
TERMINAL KILL	6 100%	6 100%	6 100%	6 100%	6 100%	6 100%	6 100%

KEY:

GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

PND = POSTNATAL DAY

TABLE 2
SUMMARY OF BODY WEIGHTS (GRAMS) - SPRAGUE DAWLEY
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: PERIOD	1	2	3	4	5	6	7
PND21	48.6 ± 0.44 (6)	48.6 ± 0.53 (6)	48.6 ± 0.51 (6)	48.6 ± 0.53 (6)	48.6 ± 0.51 (6)	48.6 ± 0.54 (6)	48.6 ± 0.34 (6)
PND22	47.63 ± 0.84 (6)	49.13 ± 0.84 (6)	48.70 ± 0.75 (6)	47.60 ± 0.87 (6)	49.72 ± 0.55 (6)	48.80 ± 0.88 (6)	48.98 ± 0.47 (6)
PND23	50.55 ± 0.83 (6)	*53.42 ± 0.79 (6)	52.43 ± 0.62 (6)	49.77 ± 1.43 (6)	51.75 ± 0.45 (6)	42.58 ± 0.77 (6)	53.57 ± 0.43 (6)
PND24	57.43 ± 0.79 (6)	58.72 ± 0.88 (6)	56.08 ± 0.80 (6)	**52.42 ± 1.81 (6)	56.68 ± 0.51 (6)	42.63 ± 0.75 (6)	58.38 ± 0.50 (6)
PND25	61.48 ± 1.07 (6)	62.97 ± 0.76 (6)	59.85 ± 0.93 (6)	59.72 ± 1.33 (6)	60.95 ± 0.54 (6)	50.12 ± 0.87 (6)	62.62 ± 0.51 (6)
PND26	65.82 ± 1.15 (6)	67.97 ± 0.93 (6)	65.13 ± 1.03 (6)	64.97 ± 1.26 (6)	65.05 ± 0.41 (6)	55.50 ± 1.13 (6)	66.78 ± 0.58 (6)
PND27	70.18 ± 1.39 (6)	71.75 ± 1.16 (6)	67.83 ± 1.13 (6)	69.92 ± 1.30 (6)	69.62 ± 0.39 (6)	61.13 ± 1.25 (6)	70.95 ± 0.48 (6)
PND28	76.28 ± 1.62 (6)	76.68 ± 0.88 (6)	*71.87 ± 1.14 (6)	74.58 ± 1.35 (6)	74.45 ± 0.43 (6)	65.27 ± 1.47 (6)	76.20 ± 0.83 (6)
PND29	80.52 ± 1.65 (6)	80.58 ± 1.06 (6)	**74.87 ± 1.34 (6)	78.32 ± 1.75 (6)	78.70 ± 0.61 (6)	**64.57 ± 2.15 (6)	*80.35 ± 1.04 (6)
PND30	85.77 ± 2.03 (6)	84.87 ± 1.27 (6)	**77.48 ± 1.28 (6)	83.47 ± 1.47 (6)	82.62 ± 0.43 (6)	**74.38 ± 2.55 (6)	85.17 ± 0.80 (6)
PND31	90.88 ± 2.33 (6)	90.53 ± 0.83 (6)	**81.37 ± 1.24 (6)	87.85 ± 1.30 (6)	88.78 ± 0.47 (6)	**80.85 ± 1.89 (6)	91.20 ± 0.88 (6)
PND32	95.85 ± 2.30 (6)	95.45 ± 1.06 (6)	**85.18 ± 1.43 (6)	*90.58 ± 1.90 (6)	93.30 ± 0.61 (6)	**81.90 ± 2.09 (6)	92.88 ± 1.36 (6)
PND33	100.83 ± 2.48 (6)	99.33 ± 1.22 (6)	**89.45 ± 1.50 (6)	**91.50 ± 1.95 (6)	97.55 ± 0.60 (6)	**83.37 ± 2.29 (6)	97.97 ± 1.42 (6)
PND34	106.77 ± 2.63 (6)	105.63 ± 1.63 (6)	**91.77 ± 1.99 (6)	**96.77 ± 2.36 (6)	104.37 ± 1.03 (6)	**87.85 ± 3.85 (6)	102.77 ± 1.01 (6)
PND35	112.28 ± 2.76 (6)	111.03 ± 1.37 (6)	**96.07 ± 1.99 (6)	**97.67 ± 1.83 (6)	109.53 ± 0.83 (6)	**95.08 ± 2.66 (6)	*105.85 ± 2.68 (6)
PND36	114.63 ± 3.09 (6)	112.43 ± 1.85 (6)	**98.47 ± 2.09 (6)	**97.72 ± 2.70 (6)	112.05 ± 1.10 (6)	**101.52 ± 2.79 (6)	110.42 ± 1.05 (6)
PND37	120.23 ± 3.75 (6)	117.83 ± 2.24 (6)	**101.70 ± 2.05 (6)	**96.18 ± 2.29 (6)	117.27 ± 1.43 (6)	**101.73 ± 2.63 (6)	114.92 ± 1.53 (6)
PND38	125.30 ± 3.49 (6)	121.68 ± 1.99 (6)	**105.82 ± 2.14 (6)	**98.72 ± 2.82 (6)	124.50 ± 1.73 (6)	**109.48 ± 3.16 (6)	120.43 ± 1.39 (6)
PND39	131.75 ± 3.85 (6)	127.32 ± 2.72 (6)	**110.65 ± 2.65 (6)	**97.85 ± 2.34 (6)	127.77 ± 1.75 (6)	**113.73 ± 3.21 (6)	*123.98 ± 1.64 (6)
PND40	135.65 ± 3.59 (6)	131.03 ± 2.56 (6)	**112.55 ± 1.94 (6)	**98.05 ± 2.87 (6)	130.73 ± 0.89 (6)	**119.58 ± 3.78 (6)	129.60 ± 2.07 (6)
PND41	138.45 ± 3.75 (6)	132.87 ± 2.86 (6)	**114.58 ± 2.44 (6)	**97.52 ± 2.83 (6)	132.70 ± 2.43 (6)	**117.12 ± 3.55 (6)	131.53 ± 2.21 (6)
PND42	145.70 ± 3.87 (6)	138.08 ± 3.10 (6)	**120.70 ± 2.41 (6)	**100.72 ± 2.20 (6)	142.82 ± 2.21 (6)	**126.53 ± 3.51 (6)	137.73 ± 2.93 (6)
PND43	150.70 ± 7.36 (3)	137.83 ± 5.42 (3)	124.67 ± 3.95 (3)	96.67 ± 1.47 (3)	146.03 ± 3.17 (3)	132.47 ± 4.91 (3)	142.57 ± 4.34 (3)

KEY:

GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

PND = POSTNATAL DAY

*=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.05
**=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.01

TABLE CONTINUED

TABLE 2 (CONTINUED)
SUMMARY OF BODY WEIGHTS (GRAMS) - LONG EVANS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: PERIOD	1	2	3	4	5	6	7
PND21	44.9 ± 0.81 (6)	45.0 ± 0.78 (6)	45.0 ± 0.89 (6)	44.9 ± 0.92 (6)	44.9 ± 0.84 (6)	44.9 ± 0.83 (6)	44.9 ± 0.85 (6)
PND22	45.88 ± 1.35 (6)	46.40 ± 0.84 (6)	45.92 ± 0.89 (6)	44.57 ± 1.19 (6)	44.05 ± 0.95 (6)	44.82 ± 0.64 (6)	46.13 ± 1.15 (6)
PND23	50.77 ± 1.27 (6)	52.22 ± 0.95 (6)	49.27 ± 1.16 (6)	**45.17 ± 1.62 (6)	*46.83 ± 0.96 (6)	**40.77 ± 0.66 (6)	50.82 ± 1.08 (6)
PND24	55.92 ± 1.15 (6)	57.68 ± 1.37 (6)	54.52 ± 1.00 (6)	53.33 ± 1.64 (6)	53.42 ± 1.40 (6)	**39.80 ± 0.99 (6)	56.58 ± 1.02 (6)
PND25	62.55 ± 1.02 (6)	62.90 ± 0.95 (6)	59.38 ± 1.10 (6)	*58.48 ± 1.18 (6)	*57.77 ± 1.61 (6)	**44.95 ± 1.83 (6)	60.85 ± 0.90 (6)
PND26	68.42 ± 1.13 (6)	66.60 ± 1.30 (6)	*63.62 ± 1.12 (6)	*63.87 ± 1.36 (6)	**61.52 ± 1.82 (6)	**51.12 ± 1.49 (6)	66.35 ± 1.06 (6)
PND27	73.77 ± 1.12 (6)	71.20 ± 1.35 (6)	**68.00 ± 1.19 (6)	**68.48 ± 1.11 (6)	**65.68 ± 1.50 (6)	**55.60 ± 1.67 (6)	71.52 ± 1.16 (6)
PND28	79.97 ± 1.51 (6)	76.85 ± 1.16 (6)	**72.48 ± 1.14 (6)	**74.15 ± 1.18 (6)	**71.97 ± 1.66 (6)	**59.82 ± 1.73 (6)	77.12 ± 1.25 (6)
PND29	85.78 ± 1.77 (6)	81.57 ± 1.47 (6)	**76.20 ± 0.93 (6)	**76.10 ± 1.02 (6)	**76.25 ± 1.98 (6)	**59.55 ± 2.23 (6)	81.50 ± 1.15 (6)
PND30	91.85 ± 1.80 (6)	*86.05 ± 1.33 (6)	**80.13 ± 0.81 (6)	**82.58 ± 1.46 (6)	**81.68 ± 1.90 (6)	**67.40 ± 1.95 (6)	*86.87 ± 1.63 (6)
PND31	100.75 ± 1.88 (6)	*94.45 ± 1.41 (6)	**85.72 ± 0.71 (6)	**82.85 ± 1.28 (6)	**88.03 ± 2.19 (6)	**75.40 ± 2.48 (6)	**94.15 ± 1.32 (6)
PND32	105.88 ± 1.80 (6)	**98.18 ± 1.38 (6)	**89.87 ± 0.88 (6)	**83.65 ± 1.36 (6)	**92.08 ± 2.11 (6)	**77.48 ± 1.81 (6)	**97.37 ± 1.06 (6)
PND33	112.03 ± 2.48 (6)	**102.88 ± 1.56 (6)	**94.33 ± 0.91 (6)	**83.05 ± 0.70 (6)	**97.35 ± 1.92 (6)	**79.53 ± 2.31 (6)	**102.43 ± 1.37 (6)
PND34	120.60 ± 2.08 (6)	**110.23 ± 0.92 (6)	**98.22 ± 1.05 (6)	**85.10 ± 0.90 (6)	**106.50 ± 2.54 (6)	**84.18 ± 2.33 (6)	**108.30 ± 1.30 (6)
PND35	128.12 ± 2.50 (6)	**116.42 ± 0.96 (6)	**103.43 ± 1.25 (6)	**87.87 ± 0.77 (6)	**111.10 ± 2.48 (6)	**89.75 ± 2.43 (6)	**114.90 ± 1.84 (6)
PND36	131.73 ± 2.22 (6)	**118.73 ± 1.21 (6)	**105.28 ± 1.36 (6)	**85.88 ± 1.47 (6)	**115.38 ± 2.13 (6)	**91.23 ± 2.95 (6)	**115.43 ± 1.55 (6)
PND37	137.27 ± 2.11 (6)	**122.10 ± 2.52 (6)	**109.47 ± 1.68 (6)	**87.20 ± 1.35 (6)	**119.85 ± 2.45 (6)	**98.27 ± 2.30 (6)	**121.27 ± 1.99 (6)
PND38	142.75 ± 1.79 (6)	**126.83 ± 2.16 (6)	**113.22 ± 1.97 (6)	**87.55 ± 1.18 (6)	**123.73 ± 2.39 (6)	**102.17 ± 2.69 (6)	**123.77 ± 2.74 (6)
PND39	148.70 ± 2.07 (6)	**133.13 ± 2.07 (6)	**117.20 ± 2.02 (6)	**88.57 ± 1.32 (6)	**128.67 ± 2.90 (6)	**107.02 ± 2.92 (6)	**129.85 ± 2.22 (6)
PND40	153.52 ± 2.05 (6)	**136.28 ± 1.99 (6)	**119.82 ± 2.12 (6)	**89.28 ± 1.19 (6)	**134.00 ± 2.77 (6)	**110.08 ± 2.53 (6)	**130.93 ± 2.75 (6)
PND41	157.18 ± 2.58 (6)	**138.25 ± 2.13 (6)	**122.20 ± 2.17 (6)	**86.95 ± 1.17 (6)	**137.65 ± 2.18 (6)	**112.93 ± 2.71 (6)	**134.40 ± 2.35 (6)
PND42	163.02 ± 2.73 (6)	**142.52 ± 1.84 (6)	**126.63 ± 2.17 (6)	**91.33 ± 1.12 (6)	**147.68 ± 2.00 (6)	**120.05 ± 1.66 (6)	**138.58 ± 2.75 (6)
PND43	166.43 ± 5.01 (3)	144.00 ± 2.75 (3)	133.20 ± 0.78 (3)	87.70 ± 1.01 (3)	151.97 ± 6.48 (3)	128.00 ± 1.29 (3)	144.50 ± 4.25 (3)

KEY:

GROUP:
1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

PND = POSTNATAL DAY

*=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.05
**=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.01

TABLE 3
SUMMARY OF BODY WEIGHT CHANGE FROM PND 21 TO NECROPSY
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
				SPRAGUE DAWLEY			
	98.9 ±10.38(6)	89.9 ±7.36 (6)	75.7 ±5.84 (6)	51.7 ±4.26 (6)	95.4 ±5.80 (6)	80.8 ±8.98 (6)	89.4 ±8.31 (6)
				LONG-EVANS			
	119.8 ±7.28 (6)	99.3 ±5.40 (6)	84.3 ±7.84 (6)	45.6 ±2.19 (6)	105.2 ±7.78 (6)	78.6 ±5.36 (6)	95.6 ±7.35 (6)

KEY: GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR
PND = POSTNATAL DAY

TABLE 4
SUMMARY OF VAGINAL OPENING DATA
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
				SPRAGUE-DAWLEY			
VAG OPENING BW	108.50 ± 2.01 (6)	**67.97 ± 0.93 (6)	**66.40 ± 2.07 (6)	*97.97 ± 2.77 (6)	114.30 ± 4.24 (6)	**88.52 ± 4.59 (6)	**68.33 ± 1.52 (6)
DAY OF VAG OPENING	34.33 ± 0.21 (6)	**26.00 ± 0.00 (6)	**26.33 ± 0.33 (6)	36.67 ± 0.80 (6)	36.17 ± 0.87 (6)	33.83 ± 1.56 (6)	**26.33 ± 0.33 (6)
				LONG-EVANS			
VAG OPENING BW	135.60 ± 1.98 (6)	**65.10 ± 0.95 (6)	**64.18 ± 0.84 (6)	**86.07 ± 0.62^A(6)	**110.57 ± 4.26 (6)	**95.00 ± 4.22 (6)	**67.38 ± 1.82 (6)
DAY OF VAG OPENING	36.50 ± 0.34 (6)	**25.67 ± 0.21 (6)	**26.17 ± 0.17 (6)	35.00 ± 2.03^A(6)	35.17 ± 0.70 (6)	36.67 ± 1.33 (6)	**26.17 ± 0.17 (6)

KEY: GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

BW = BODY WEIGHT (GRAMS)

*=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.05
**=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.01
A = THIS MEAN INCLUDES CENSORED DATA

TABLE 5
SUMMARY OF AGE OF FIRST ESTRUS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
				SPRAGUE-DAWLEY			
AGE (PND)	35.2 ± 0.31(6)	**26.0 ± 0.00 (6)	**43.5 ± 0.22^A(6)	*38.2 ± 1.14^A(6)	**41.7 ± 1.28^A(6)	35.5 ± 1.93^A(6)	**29.0 ± 1.21 (6)
				LONG-EVANS			
AGE (PND)	37.2 ± 1.60(6)	**25.7 ± 0.21 (6)	**43.5 ± 0.22^A(6)	35.6 ± 2.14^A(5)	*41.7 ± 1.33^A(6)	37.8 ± 1.56 (6)	**31.2 ± 1.68 (6)

KEY: GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

PND = POSTNATAL DAY

*=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.05
**=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.01
A = THIS MEAN INCLUDES CENSORED DATA

TABLE 6
SUMMARY OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
LIVER							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
KIDNEYS (PAIRED)							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
UTERUS WITH CERVIX							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
UTERUS-CYST	0	0	0	0	0	0	1 8%
UTERUS-DISTENDED	1 8%	2 17%	0	1 8%	1 8%	0	4 33%
UTERUS-SMALL	0	0	0	0	1 8%	0	0
NO GROSS FINDINGS	11 92%	10 83%	12 100%	11 92%	10 83%	12 100%	7 58%
UTERUS WITH CERVIX(DRY)							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
OVARIES							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
ADRENALS							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
ENLARGED	0	0	0	0	10 83%	0	0
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	2 17%	12 100%	12 100%
PITUITARY							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
ENLARGED	0	0	0	1 8%	0	0	0
NO GROSS FINDINGS	12 100%	12 100%	12 100%	11 92%	12 100%	12 100%	12 100%
THYROID							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
ENLARGED	1 8%	0	0	10 83%	1 8%	0	1 8%
NO GROSS FINDINGS	11 92%	12 100%	12 100%	2 17%	11 92%	12 100%	11 92%

KEY: GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

TABLE CONTINUED

TABLE 6 (CONTINUED)
SUMMARY OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
MESENTERIC LYMPH NODE							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
TRACHEA							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
BRAIN							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
SPINAL CORDS							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
CLITORAL GLAND							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
ESOPHAGUS							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
EYE							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
HARDERIAN GLAND							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
DUODENUM							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
JEJUNUM							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%

KEY: GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

TABLE CONTINUED

TABLE 6 (CONTINUED)
SUMMARY OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
ILEUM							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
CECUM							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
COLON							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
RECTUM							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
LACRIMAL GLAND							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
LARYNX							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
HEART							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
MANDIBULAR LYMPH NODE							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
MAMMARY GLAND							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%

KEY: GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

TABLE CONTINUED

TABLE 6 (CONTINUED)
SUMMARY OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
MESENTERY							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
NOSE							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
MUSCLE							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
SALIVARY GLANDS							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
PANCREAS							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
PERIPHERAL NERVE							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
PHARYNX							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
SKIN							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%
OTHER							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%

KEY: GROUP: 1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

TABLE 7
SUMMARY OF BODY AND ORGAN WEIGHTS AT NECROPSY
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
SPRAGUE-DAWLEY							
BODY (G)	147.5 ± 4.32 (6)	*138.6 ± 3.26 (6)	**121.4 ± 2.44 (6)	**100.2 ± 2.22 (6)	144.0 ± 2.13 (6)	**129.4 ± 4.09 (6)	*138.0 ± 3.11 (6)
ADRENALS (MG)	41.17 ± 3.33 (6)	40.50 ± 2.77 (6)	**31.33 ± 1.65 (6)	**23.00 ± 1.26 (6)	**77.50 ± 3.49 (6)	34.33 ± 1.48 (6)	35.17 ± 2.21 (6)
KIDNEYS (MG)	1238.50 ±39.55 (6)	1177.50 ±52.65 (6)	1129.50 ±34.62 (6)	**751.33 ±17.68 (6)	*1361.50 ±42.38 (6)	**1072.83 ±32.90 (6)	1138.67 ±55.98 (6)
LIVER (G)	6.14 ± 0.23 (6)	6.10 ± 0.43 (6)	*5.29 ± 0.17 (6)	**4.26 ± 0.10 (6)	**7.31 ± 0.22 (6)	5.48 ± 0.29 (6)	*5.37 ± 0.24 (6)
OVARIES (MG)	61.50 ± 6.40 (6)	49.83 ± 6.77 (6)	41.00 ± 4.91 (6)	*34.33 ± 4.20 (6)	86.67 ±35.08 (6)	56.50 ± 2.81 (6)	48.83 ± 8.80 (6)
PITUITARY(MG)	8.02 ± 0.85 (6)	9.22 ± 2.20 (6)	4.90 ± 0.96 (6)	4.20 ± 1.48 (5)	10.32 ± 3.80 (6)	8.13 ± 1.53 (6)	6.05 ± 0.45 (6)
UTERUS-WET (G)	0.26 ± 0.05 (6)	0.26 ± 0.02 (6)	**0.12 ± 0.01 (6)	**0.15 ± 0.02 (6)	0.19 ± 0.02 (6)	0.21 ± 0.01 (6)	0.30 ± 0.03 (6)
UTERUS-DRY(G)	0.22 ± 0.03 (6)	0.24 ± 0.01 (6)	**0.11 ± 0.01 (6)	0.13 ± 0.02 (6)	0.18 ± 0.02 (6)	0.19 ± 0.01 (6)	0.23 ± 0.02 (6)
LONG EVANS							
BODY (G)	164.7 ± 2.68 (6)	**144.2 ± 1.66 (6)	**129.3 ± 2.65 (6)	**90.4 ± 1.39 (6)	**150.1 ± 3.10 (6)	**123.6 ± 2.59 (6)	**140.5 ± 3.14 (6)
ADRENALS (MG)	37.83 ± 2.32 (6)	36.17 ± 3.40 (6)	33.67 ± 3.17 (6)	29.67 ± 3.59 (6)	**69.33 ± 3.54 (6)	36.67 ± 6.76 (6)	37.67 ± 3.21 (6)
KIDNEYS (MG)	1533.67 ±29.58 (6)	**1261.17 ±30.44 (6)	**1259.17 ±49.79 (6)	**786.00 ±16.41 (6)	1483.33 ±34.98 (6)	**1124.33 ±31.67 (6)	**1340.83 ±28.63 (6)
LIVER (G)	7.12 ± 0.17 (6)	**6.11 ± 0.17 (6)	**5.95 ± 0.31 (6)	**3.78 ± 0.18 (6)	7.40 ± 0.38 (6)	**5.52 ± 0.13 (6)	**5.85 ± 0.18 (6)
OVARIES (G)	0.08 ± 0.00 (6)	*0.06 ± 0.01 (6)	**0.04 ± 0.00 (6)	**0.04 ± 0.00 (6)	*0.06 ± 0.01 (6)	**0.05 ± 0.01 (6)	0.08 ± 0.01 (6)
PITUITARY(MG)	6.40 ± 2.41 (5)	6.50 ± 0.67 (6)	*4.17 ± 0.40 (6)	5.33 ± 0.84 (6)	6.00 ± 0.93 (6)	7.17 ± 0.48 (6)	5.83 ± 0.70 (6)
UTERUS-WET (G)	0.29 ± 0.05 (6)	0.36 ± 0.05 (6)	**0.10 ± 0.01 (6)	**0.16 ± 0.04 (6)	0.23 ± 0.04 (6)	*0.16 ± 0.02 (6)	0.28 ± 0.05 (6)
UTERUS-DRY(G)	0.23 ± 0.03 (6)	0.28 ± 0.03 (6)	**0.09 ± 0.00 (6)	**0.12 ± 0.02 (6)	0.19 ± 0.03 (6)	*0.15 ± 0.01 (6)	0.24 ± 0.02 (6)

KEY: GROUP:
1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

*=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.05
**=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.01

TABLE 8
SUMMARY OF ORGAN-TO-BODY WEIGHT RATIOS AT NECROPSY (% OF BODY WEIGHT)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
				SPRAGUE-DAWLEY			
ADRENALS	0.28 ± 0.02 (6)	0.29 ± 0.02 (6)	0.26 ± 0.01 (6)	0.23 ± 0.01 (6)	**0.54 ± 0.02 (6)	0.27 ± 0.02 (6)	0.26 ± 0.01 (6)
KIDNEYS	8.40 ± 0.17 (6)	8.48 ± 0.20 (6)	**9.30 ± 0.15 (6)	7.52 ± 0.26 (6)	**9.45 ± 0.25 (6)	8.29 ± 0.05 (6)	8.24 ± 0.26 (6)
LIVER	41.59 ± 0.52 (6)	43.76 ± 2.16 (6)	43.59 ± 0.99 (6)	42.49 ± 0.22 (6)	**50.77 ± 1.32 (6)	42.24 ± 1.33 (6)	38.82 ± 0.90 (6)
OVARIES	0.42 ± 0.04 (6)	0.36 ± 0.04 (6)	0.34 ± 0.04 (6)	0.35 ± 0.05 (6)	0.60 ± 0.24 (6)	0.44 ± 0.01 (6)	0.35 ± 0.06 (6)
PITUITARY	0.06 ± 0.01 (6)	0.07 ± 0.01 (6)	0.04 ± 0.01 (6)	0.04 ± 0.01 (5)	0.07 ± 0.03 (6)	0.06 ± 0.01 (6)	0.04 ± 0.00 (6)
UTERUS-WET	1.75 ± 0.34 (6)	1.92 ± 0.15 (6)	**0.96 ± 0.04 (6)	1.53 ± 0.24 (6)	1.32 ± 0.10 (6)	1.62 ± 0.08 (6)	2.18 ± 0.28 (6)
UTERUS-DRY	1.49 ± 0.22 (6)	1.74 ± 0.10 (6)	**0.88 ± 0.03 (6)	1.33 ± 0.18 (6)	1.22 ± 0.10 (6)	1.44 ± 0.09 (6)	1.69 ± 0.11 (6)
				LONG EVANS			
ADRENALS	0.23 ± 0.01 (6)	0.25 ± 0.02 (6)	0.26 ± 0.02 (6)	0.33 ± 0.04 (6)	**0.46 ± 0.03 (6)	0.30 ± 0.06 (6)	0.27 ± 0.02 (6)
KIDNEYS	9.32 ± 0.20 (6)	8.75 ± 0.20 (6)	*9.73 ± 0.27 (6)	8.69 ± 0.07 (6)	*9.89 ± 0.10 (6)	9.12 ± 0.30 (6)	9.55 ± 0.11 (6)
LIVER	43.26 ± 1.12 (6)	42.32 ± 0.99 (6)	45.88 ± 1.68 (6)	41.77 ± 1.55 (6)	**49.15 ± 1.57 (6)	44.74 ± 0.97 (6)	41.63 ± 0.81 (6)
OVARIES	0.50 ± 0.03 (6)	0.41 ± 0.07 (6)	**0.28 ± 0.03 (6)	0.50 ± 0.04 (6)	0.42 ± 0.06 (6)	0.42 ± 0.05 (6)	0.55 ± 0.05 (6)
PITUITARY	0.04 ± 0.02 (5)	0.04 ± 0.00 (6)	0.03 ± 0.00 (6)	0.06 ± 0.01 (6)	0.04 ± 0.00 (6)	0.06 ± 0.00 (6)	0.04 ± 0.01 (6)
UTERUS-WET	1.77 ± 0.35 (6)	2.52 ± 0.34 (6)	0.78 ± 0.05 (6)	1.73 ± 0.44 (6)	1.54 ± 0.29 (6)	1.29 ± 0.15 (6)	1.95 ± 0.29 (6)
UTERUS-DRY	1.41 ± 0.22 (6)	*1.95 ± 0.18 (6)	*0.71 ± 0.04 (6)	1.36 ± 0.23 (6)	1.26 ± 0.19 (6)	1.19 ± 0.13 (6)	1.69 ± 0.14 (6)

KEY: GROUP:
1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

*=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.05
**=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.01

TABLE 9
SUMMARY OF SERUM T4 AND TSH LEVELS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
				SPRAGUE-DAWLEY			
T4, TOTAL (UG/DL)	3.59 ± 0.08 (6)	3.84 ± 0.22 (6)	*5.81 ± 0.12 (6)	**0.02 ± 0.02 (6)	3.94 ± 0.49 (6)	3.42 ± 0.24 (6)	4.19 ± 0.33 (6)
TSH (NG/ML)	1.67 ± 0.07 (6)	1.62 ± 0.16 (6)	2.19 ± 0.16 (6)	**26.77 ± 1.73 (6)	1.72 ± 0.16 (6)	1.51 ± 0.23 (6)	1.64 ± 0.19 (6)
				LONG EVANS			
T4, TOTAL (UG/DL)	4.42 ± 0.34 (6)	5.36 ± 0.60 (6)	*5.78 ± 0.27 (6)	**0.01 ± 0.01 (6)	4.48 ± 0.83 (6)	3.34 ± 0.20 (6)	4.35 ± 0.23 (6)
TSH (NG/ML)	1.34 ± 0.11 (6)	1.72 ± 0.27 (6)	1.76 ± 0.20 (6)	**36.59 ± 6.38 (6)	1.26 ± 0.12 (6)	1.56 ± 0.22 (6)	1.30 ± 0.12 (6)

KEY: GROUP:
1 = 2.5 ML/KG/DAY CORN OIL
2 = 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
3 = 10 MG/KG/DAY TAMOXIFEN
4 = 240 MG/KG/DAY PROPYLTHIOURACIL
5 = 100 MG/KG/DAY KETOCONAZOLE
6 = 30 MG/KG/DAY PIMOZIDE
7 = 100 MG/KG/DAY METHOXYCHLOR

*=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.05
**=STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE CONTROL GROUP; P<0.01

APPENDIX 1
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 1-F - 2.5 ML/KG/DAY CORN OIL STRAIN: SPRAGUE DAWLEY

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15146	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15147	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15148	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15149	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15150	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15151	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 1-F - 2.5 ML/KG/DAY CORN OIL STRAIN: LONG EVANS

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15152	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15153	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15154	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15155	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15156	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15157	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 2-F - 0.005 MG/KG/DAY ETHINYL ESTRADIOL STRAIN: SPRAGUE DAWLEY

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15158	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15159	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15160	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15161	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15162	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15163	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 2-F - 0.005 MG/KG/DAY ETHINYL ESTRADIOL STRAIN: LONG EVANS

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15164	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15165	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15166	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15167	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15168	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15169	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 3-F - 10 MG/KG/DAY TAMOXIFEN STRAIN: SPRAGUE DAWLEY

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15170	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15171	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15172	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15173	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15174	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15175	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 3-F - 10 MG/KG/DAY TAMOXIFEN STRAIN: LONG EVANS

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15176	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15177	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15178	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15179	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15180	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15181	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 4-F - 240 MG/KG/DAY PROPYLTHIOURACIL STRAIN: SPRAGUE DAWLEY

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15182	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15183	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15184	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15185	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15186	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15187	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 4-F - 240 MG/KG/DAY PROPYLTHIOURACIL STRAIN: LONG EVANS

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15188	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15189	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15190	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15191	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15192	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15193	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 5-F - 100 MG/KG/DAY KETOCONAZOLE STRAIN: SPRAGUE DAWLEY

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15194	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15195	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15196	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15197	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15198	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15199	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 5-F - 100 MG/KG/DAY KETOCONAZOLE STRAIN: LONG EVANS

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15200	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15201	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15202	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15203	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15204	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15205	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 6-F - 30 MG/KG/DAY PIMOZIDE STRAIN: SPRAGUE DAWLEY

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15206	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15207	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15208	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15209	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15210	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15211	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 6-F - 30 MG/KG/DAY PIMOZIDE STRAIN: LONG EVANS

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15212	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15213	NORMAL			PND 21
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15214	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15215	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15216	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15217	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 7-F - 100 MG/KG/DAY METHOXYCHLOR STRAIN: SPRAGUE DAWLEY

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15218	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15219	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15220	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15221	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15222	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15223	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 1 (CONTINUED)
INDIVIDUAL CLINICAL OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 7-F - 100 MG/KG/DAY METHOXYCHLOR STRAIN: LONG EVANS

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15224	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15225	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15226	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15227	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15228	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15229	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43

PND = POSTNATAL DAY

APPENDIX 2
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 1-F - 2.5 ML/KG/DAY CORN OIL

ANIMAL #	PND 21	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32
R15146	47.9	45.3	48.5	55.7	58.8	63.2	66.8	74.1	76.5	81.5	84.9	89.8
R15147	49.8	48.3	49.2	56.6	60.8	64.2	68.5	73.4	79.8	83.1	87.0	92.9
R15148	48.8	48.9	51.3	59.3	64.6	69.1	73.2	82.0	85.6	91.8	98.1	103.2
R15149	48.3	46.2	50.5	56.5	59.1	63.3	67.6	72.7	76.8	81.0	87.0	91.3
R15150	49.8	50.8	54.2	60.4	64.8	69.4	75.4	80.6	85.3	92.0	97.6	102.1
R15151	47.1	46.3	49.6	56.1	60.8	65.7	69.6	74.9	79.1	85.2	90.7	95.8
R15152	47.8	50.6	55.2	58.9	66.2	71.7	76.7	85.3	91.0	97.6	107.1	111.2
R15153	46.1	49.2	53.7	56.0	63.6	69.3	75.9	82.8	89.3	95.4	104.6	110.1
R15154	42.5	44.0	48.9	51.2	60.7	64.9	70.6	75.4	81.2	86.6	96.6	103.8
R15155	44.5	42.3	47.1	54.5	59.4	65.7	70.3	76.7	80.1	87.0	95.0	99.2
R15156	43.0	43.6	48.9	56.5	61.4	67.9	73.8	79.8	86.2	92.0	100.6	106.4
R15157	45.5	45.6	50.8	58.4	64.0	71.0	75.3	79.8	86.9	92.5	100.6	104.6
MEAN	46.8	46.8	50.7	56.7	62.0	67.1	72.0	78.1	83.2	88.8	95.8	100.9
S.D.	2.47	2.79	2.52	2.43	2.51	2.98	3.49	4.13	4.85	5.49	7.14	7.12
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15146-R15151 ARE SPRAGUE-DAWLEY
ANIMALS R15152-R15157 ARE LONG EVANS

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 1-F - 2.5 ML/KG/DAY CORN OIL - CONTINUED

ANIMAL #	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43
R15146	94.4	99.3	106.5	107.0	111.2	117.2	120.9	124.9	127.0	134.6	
R15147	97.6	103.1	106.3	107.9	113.9	120.3	131.2	134.4	136.5	145.2	
R15148	108.5	113.8	119.6	123.6	130.6	132.4	139.4	144.0	147.3	153.2	
R15149	96.6	102.5	107.0	110.5	112.3	116.6	121.4	125.7	128.9	134.2	136.2
R15150	108.2	115.2	121.0	123.9	131.5	137.1	144.3	144.5	148.5	156.3	160.1
R15151	99.7	106.7	113.3	114.9	121.9	128.2	133.3	140.4	142.5	150.7	155.8
R15152	119.0	126.4	133.0	136.1	139.1	142.0	147.7	157.1	157.8	161.1	
R15153	116.0	126.1	135.3	136.7	144.8	147.4	152.9	157.6	162.2	168.3	
R15154	103.9	117.1	123.7	126.1	133.3	137.3	142.9	148.8	149.8	159.2	
R15155	105.9	113.7	119.8	126.4	132.2	140.2	143.9	146.4	150.7	155.9	160.6
R15156	116.0	121.9	131.9	137.2	141.1	148.6	156.0	158.3	166.0	173.8	176.4
R15157	111.4	118.4	125.0	127.9	133.1	141.0	148.8	152.9	156.6	159.8	162.3
MEAN	106.4	113.7	120.2	123.2	128.8	134.0	140.2	144.6	147.8	154.4	158.6
S.D.	8.23	9.11	10.30	10.92	11.39	11.17	11.42	11.56	12.34	11.96	13.0
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15146-R15151 ARE SPRAGUE-DAWLEY
ANIMALS R15152-R15157 ARE LONG EVANS

A: TERMINAL KILL
PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 2-F - 0.005 MG/KG/DAY ETHINYL ESTRADIOL

ANIMAL #	PND 21	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32
R15158	48.1	49.5	54.6	59.1	63.5	70.3	74.9	79.6	84.6	89.0	93.7	98.7
R15159	48.9	50.9	55.2	59.9	63.2	69.4	72.6	76.6	80.5	85.1	90.3	95.8
R15160	48.3	49.1	54.2	58.0	62.6	66.5	71.5	75.0	79.4	82.0	90.1	93.6
R15161	49.1	49.4	52.8	58.4	63.1	66.7	68.4	76.1	80.3	84.2	89.4	94.9
R15162	50.6	50.7	53.9	61.7	65.6	70.1	74.6	78.8	81.9	87.8	91.9	97.9
R15163	46.7	45.2	49.8	55.2	59.8	64.8	68.5	74.0	76.8	81.1	87.8	91.8
R15164	44.7	46.6	52.1	59.0	63.7	68.4	73.4	79.5	84.6	87.9	95.3	98.2
R15165	46.4	48.9	54.5	59.7	64.3	68.4	73.2	78.4	84.0	88.4	97.2	101.6
R15166	47.5	47.6	54.5	61.9	66.2	70.5	74.6	79.9	85.4	90.2	99.4	102.5
R15167	45.4	47.4	53.3	58.1	62.5	66.7	71.8	75.7	80.3	84.7	91.5	97.1
R15168	43.4	44.5	49.7	54.2	60.0	62.6	66.7	72.9	76.7	82.0	90.6	93.6
R15169	42.3	43.4	49.2	53.2	60.7	63.0	67.5	74.7	78.4	83.1	92.7	96.1
MEAN	46.8	47.8	52.8	58.2	62.9	67.3	71.5	76.8	81.1	85.5	92.5	96.8
S.D.	2.47	2.43	2.14	2.74	2.01	2.73	2.96	2.41	3.04	3.10	3.39	3.20
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15158-R15163 ARE SPRAGUE-DAWLEY
ANIMALS R15164-R15169 ARE LONG EVANS

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 2-F - 0.005 MG/KG/DAY ETHINYL ESTRADIOL - CONTINUED

ANIMAL #	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43
R15158	104.8	112.8	117.0	119.4	127.0	129.4	137.8	140.4	142.0	146.6	
R15159	98.6	107.2	111.9	115.1	120.1	123.6	128.5	131.3	135.1	141.0	
R15160	96.8	103.1	106.9	108.5	115.2	118.9	124.0	128.2	132.1	130.2	
R15161	97.4	104.1	110.2	111.1	117.3	123.3	128.7	134.2	136.9	142.2	145.6
R15162	100.6	105.0	110.6	113.4	116.9	119.6	127.5	130.6	129.6	141.3	140.5
R15163	97.8	101.6	109.6	107.1	110.5	115.3	117.4	121.5	121.5	127.2	127.4
R15164	103.7	110.2	117.5	121.2	123.3	128.8	132.8	138.5	139.4	147.2	
R15165	106.8	111.3	118.2	122.0	127.4	131.4	139.3	142.3	146.5	146.7	
R15166	107.8	113.1	119.6	120.8	129.3	133.7	139.0	139.0	138.2	139.5	
R15167	100.5	107.2	113.9	116.2	112.4	120.0	126.7	129.4	131.1	135.3	138.5
R15168	98.7	108.0	114.2	114.9	121.9	124.0	131.0	131.5	134.6	142.8	146.7
R15169	99.8	111.6	115.1	117.3	118.3	123.1	130.0	137.0	139.7	143.6	146.8
MEAN	101.1	107.9	113.7	115.6	120.0	124.3	130.2	133.7	135.6	140.3	140.9
S.D.	3.76	3.91	3.94	4.91	6.00	5.55	6.41	6.02	6.52	6.39	7.47
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15158-R15163 ARE SPRAGUE-DAWLEY
ANIMALS R15164-R15169 ARE LONG EVANS

A: TERMINAL KILL
PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 3-F - 10 MG/KG/DAY TAMOXIFEN

ANIMAL #	PND 21	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32
R15170	48.5	49.1	52.7	55.4	59.3	64.5	67.1	71.0	73.8	75.8	80.3	82.5
R15171	48.8	49.3	52.0	55.1	59.8	64.6	65.9	70.8	73.9	75.7	79.5	83.9
R15172	50.4	51.2	54.4	58.6	61.5	67.6	68.9	73.9	78.8	80.0	83.4	86.6
R15173	48.0	47.8	52.0	55.2	58.9	64.5	66.9	71.8	73.0	77.1	81.6	83.7
R15174	49.3	49.1	53.5	58.4	63.1	68.3	72.9	75.9	78.9	82.4	86.0	91.7
R15175	46.7	45.7	50.0	53.8	56.5	61.3	65.3	67.8	70.8	73.9	77.4	82.7
R15176	48.0	48.0	49.1	54.5	62.8	66.5	71.1	74.6	78.9	82.2	87.7	92.8
R15177	41.5	42.2	44.0	50.3	55.6	59.8	63.2	67.5	73.5	78.2	83.4	89.3
R15178	46.0	47.7	50.3	54.3	59.5	61.8	67.1	71.8	74.4	78.2	84.1	86.7
R15179	44.0	44.7	48.9	54.1	56.9	62.1	66.9	71.9	75.1	78.7	85.5	88.8
R15180	45.6	45.9	51.8	56.9	61.2	65.8	70.6	75.0	78.8	82.4	86.2	91.6
R15181	44.9	47.0	51.5	57.0	60.3	65.7	69.1	74.1	76.5	81.1	87.4	90.0
MEAN	46.8	47.3	50.9	55.3	59.6	64.4	67.9	72.2	75.5	78.8	83.5	87.5
S.D.	2.53	2.41	2.73	2.26	2.39	2.64	2.72	2.68	2.78	2.87	3.27	3.70
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15170-R15175 ARE SPRAGUE-DAWLEY
ANIMALS R15176-R15181 ARE LONG EVANS

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 3-F - 10 MG/KG/DAY TAMOXIFEN - CONTINUED

ANIMAL #	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43
R15170	86.6	89.7	93.2	94.0	99.5	103.2	103.2	110.1	109.7	115.7	
R15171	87.0	87.7	91.7	94.0	97.4	100.2	106.3	107.4	109.3	116.8	
R15172	92.8	94.3	100.7	100.9	105.3	108.1	113.8	114.8	116.6	121.6	
R15173	88.6	91.1	95.2	97.8	98.8	102.7	108.2	109.5	111.9	118.0	119.9
R15174	95.2	100.3	103.4	107.5	110.3	115.0	121.6	120.7	125.3	131.9	132.5
R15175	86.5	87.5	92.2	96.6	98.9	105.7	110.8	112.8	114.7	120.2	121.6
R15176	96.9	97.8	102.6	105.2	107.9	111.6	115.7	118.8	122.9	125.9	
R15177	94.5	99.1	107.3	109.1	112.6	117.8	121.4	124.4	127.4	132.8	
R15178	90.5	93.3	98.7	99.2	102.0	104.8	108.4	109.9	112.1	117.6	
R15179	93.5	98.7	105.8	105.6	111.4	114.9	119.6	122.2	124.3	129.9	134.3
R15180	95.9	100.2	101.9	107.3	113.0	117.5	121.5	122.5	124.9	124.5	133.6
R15181	94.7	100.2	104.3	105.3	109.9	112.7	116.6	121.1	121.6	129.1	131.7
MEAN	91.9	95.0	99.8	101.9	105.6	109.5	113.9	116.2	118.4	123.7	128.9
S.D.	3.86	5.02	5.46	5.45	5.97	6.16	6.47	6.08	6.70	6.19	6.42
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15170-R15175 ARE SPRAGUE-DAWLEY
ANIMALS R15176-R15181 ARE LONG EVANS

A: TERMINAL KILL
PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 4-F - 240 MG/KG/DAY PROPYLTHIOURACIL

ANIMAL #	PND 21	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32
R15182	50.6	51.0	54.2	58.8	65.7	69.4	73.6	78.4	84.3	88.7	93.8	96.1
R15183	48.8	48.8	52.2	53.4	60.0	65.1	70.9	78.5	80.7	82.9	87.7	90.5
R15184	49.1	46.0	44.6	45.0	57.5	63.0	69.0	71.5	78.2	82.3	88.3	90.5
R15185	47.9	46.0	48.9	52.4	57.1	64.5	68.4	72.6	72.7	79.9	85.1	86.7
R15186	46.7	45.6	47.4	51.7	57.5	60.6	64.9	71.3	74.2	80.1	85.2	84.3
R15187	48.4	48.2	51.3	53.2	60.5	67.2	72.7	75.2	79.8	86.9	87.0	95.4
R15188	46.9	45.7	45.2	52.2	59.1	66.1	70.7	74.8	77.4	86.3	87.0	84.0
R15189	45.6	45.3	45.6	55.5	59.3	63.2	70.5	75.1	74.7	84.3	85.6	88.5
R15190	47.6	48.7	50.7	58.8	63.2	69.1	71.1	78.0	79.7	86.5	83.0	86.6
R15191	44.5	44.3	45.5	55.3	57.7	63.3	67.5	75.3	76.8	80.4	82.7	81.3
R15192	41.9	39.8	38.3	47.5	54.5	59.5	64.4	69.7	72.4	78.1	79.0	80.0
R15193	42.8	43.6	45.7	50.7	57.1	62.0	66.7	72.0	75.6	79.9	79.8	81.5
MEAN	46.7	46.1	47.5	52.9	59.1	64.4	69.2	74.4	77.2	83.0	85.4	87.1
S.D.	2.61	2.90	4.29	4.06	3.01	3.12	2.91	2.97	3.54	3.46	3.99	5.29
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15182-R15187 ARE SPRAGUE-DAWLEY
ANIMALS R15188-R15193 ARE LONG EVANS

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 4-F - 240 MG/KG/DAY PROPYLTHIOURACIL - CONTINUED

ANIMAL #	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43
R15182	100.0	104.2	106.6	104.8	106.0	109.0	108.0	111.6	107.9	109.5	
R15183	89.3	95.0	97.0	89.6	92.1	93.7	94.1	92.9	93.5	98.7	
R15184	91.1	91.3	95.5	102.2	98.1	101.2	99.8	97.3	102.1	103.2	
R15185	88.5	94.8	96.9	93.6	90.9	91.9	94.6	94.3	93.3	98.4	95.2
R15186	86.6	91.6	95.4	92.3	92.8	93.0	92.1	93.4	88.9	93.5	95.2
R15187	93.5	103.7	94.6	103.8	97.2	103.5	98.5	98.8	99.4	101.0	99.6
R15188	81.8	89.0	90.1	87.3	91.5	91.7	93.5	94.9	91.5	94.8	
R15189	85.9	84.2	87.6	91.0	89.2	89.6	90.2	89.1	88.6	93.5	
R15190	83.2	85.9	89.2	87.0	89.0	88.2	89.9	89.4	86.9	91.2	
R15191	84.0	83.9	88.8	85.5	86.6	84.9	87.3	88.2	86.7	90.8	89.0
R15192	81.3	82.6	86.5	80.1	83.1	84.0	85.0	87.1	84.5	90.9	88.4
R15193	82.1	85.0	85.0	84.4	83.8	86.9	85.5	87.0	83.5	86.8	85.7
MEAN	87.3	90.9	92.8	91.8	91.7	93.1	93.2	93.7	92.2	96.0	92.2
S.D.	5.58	7.38	6.08	8.00	6.42	7.72	6.58	6.88	7.49	6.37	5.29
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15182-R15187 ARE SPRAGUE-DAWLEY
ANIMALS R15188-R15193 ARE LONG EVANS

PND = POSTNATAL DAY
A: TERMINAL KILL

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 5-F - 100 MG/KG/DAY KETOCONAZOLE

ANIMAL #	PND 21	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32
R15194	47.9	48.1	51.0	55.7	59.2	63.6	68.5	73.7	79.1	83.0	90.3	94.5
R15195	50.1	49.8	52.1	55.8	60.7	65.2	70.2	74.2	77.1	82.2	87.2	90.6
R15196	46.6	48.4	50.6	55.9	60.2	64.7	69.4	74.8	78.7	83.2	88.2	93.5
R15197	49.6	51.5	53.7	59.0	63.1	66.7	71.2	75.7	81.0	84.2	89.1	94.7
R15198	48.9	51.0	51.9	56.9	61.6	65.2	69.5	75.4	79.3	81.7	89.8	93.8
R15199	48.6	49.5	51.2	56.8	60.9	64.9	68.9	72.9	77.0	81.4	88.1	92.7
R15200	42.9	43.1	45.7	54.0	58.1	62.7	66.2	73.2	78.3	82.7	90.1	92.1
R15201	44.9	44.7	47.7	54.2	59.8	59.9	64.7	72.7	75.1	83.2	89.3	93.1
R15202	47.8	46.2	49.2	55.8	60.8	67.0	69.4	77.2	82.3	84.5	91.9	96.2
R15203	46.8	46.9	49.5	56.8	60.6	64.8	68.6	73.1	78.1	85.0	90.7	96.6
R15204	42.7	40.7	45.3	52.6	57.0	60.5	66.1	70.7	75.9	82.3	88.9	92.2
R15205	44.2	42.7	43.6	47.1	50.3	54.2	59.1	64.9	67.8	72.4	77.3	82.3
MEAN	46.8	46.9	49.3	55.1	59.4	63.3	67.7	73.2	77.5	82.2	88.4	92.7
S.D.	2.54	3.47	3.11	2.99	3.26	3.59	3.28	3.11	3.65	3.26	3.72	3.69
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15194-R15199 ARE SPRAGUE-DAWLEY
ANIMALS R15200-R15205 ARE LONG EVANS

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 5-F - 100 MG/KG/DAY KETOCONAZOLE - CONTINUED

ANIMAL #	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43
R15194	98.0	106.1	111.1	113.4	119.4	125.4	130.8	131.9	138.4	146.1	
R15195	95.3	101.9	108.0	110.5	112.2	119.3	125.6	128.4	127.2	136.3	
R15196	99.8	106.4	112.3	115.9	120.9	129.4	131.6	132.0	135.0	143.6	
R15197	97.4	104.5	109.8	112.1	117.0	120.7	121.4	128.0	124.0	137.5	141.7
R15198	96.9	106.6	109.3	112.5	120.0	129.1	131.9	133.5	138.5	150.8	152.2
R15199	97.9	100.7	106.7	107.9	114.1	123.1	125.3	130.6	133.1	142.6	144.2
R15200	96.4	107.2	114.5	115.5	119.9	123.0	127.1	133.1	138.3	147.6	
R15201	100.0	108.6	113.6	119.1	122.4	125.2	132.1	137.7	140.4	151.0	
R15202	99.6	109.7	113.1	115.3	120.5	120.0	126.1	129.5	133.7	145.8	
R15203	101.0	110.3	114.3	119.3	125.2	130.0	137.6	143.1	144.5	153.9	159.8
R15204	98.8	109.2	112.3	117.8	122.9	129.5	132.2	136.8	139.6	148.3	157.0
R15205	88.3	94.0	98.8	105.3	108.2	114.7	116.9	123.8	129.4	139.5	139.1
MEAN	97.5	105.4	110.3	113.7	118.6	124.1	128.2	132.4	135.2	145.3	149.0
S.D.	3.32	4.66	4.40	4.33	4.88	4.88	5.61	5.09	5.98	5.54	8.55
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15194-R15199 ARE SPRAGUE-DAWLEY
ANIMALS R15200-R15205 ARE LONG EVANS

PND = POSTNATAL DAY
A: TERMINAL KILL

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 6-F - 30 MG/KG/DAY PIMOZIDE

ANIMAL #	PND 21	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32
R15206	47.9	48.0	41.2	41.5	49.4	54.3	60.5	65.3	62.0	74.9	79.2	81.2
R15207	46.4	45.3	39.9	38.4	47.0	51.6	55.5	59.3	55.9	62.8	72.9	73.6
R15208	50.1	48.0	42.1	44.9	53.4	60.1	63.8	69.2	70.7	81.2	85.0	88.1
R15209	49.6	51.0	45.0	42.1	49.2	56.1	61.8	64.0	64.8	73.7	80.9	81.5
R15210	48.8	49.7	43.4	40.6	50.9	55.0	63.8	68.8	69.0	77.8	85.7	86.5
R15211	48.7	50.8	43.9	41.8	50.8	55.9	61.4	65.0	65.0	75.9	81.4	80.5
R15212	44.6	43.4	39.6	36.6	41.8	49.3	53.8	60.8	58.6	71.0	78.6	79.4
R15213	41.8	43.7	40.3	41.6	49.1	53.0	57.6	59.8	63.1	70.0	78.0	81.7
R15214	47.6	47.6	43.8	41.8	51.6	57.2	62.3	67.0	68.4	73.7	84.6	82.5
R15215	46.1	44.4	39.9	36.9	40.1	47.6	50.6	53.9	53.0	62.0	69.2	71.5
R15216	45.8	45.7	41.3	41.7	44.5	51.6	56.2	58.5	55.9	63.9	72.7	75.9
R15217	43.7	44.1	39.7	40.2	42.6	48.0	53.1	58.9	58.3	63.8	69.3	73.9
MEAN	46.8	46.8	41.7	40.7	47.5	53.3	58.4	62.5	62.1	70.9	78.1	79.7
S.D.	2.51	2.75	1.92	2.36	4.30	3.84	4.50	4.71	5.76	6.43	5.88	5.11
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15206-R15211 ARE SPRAGUE-DAWLEY
ANIMALS R15212-R15217 ARE LONG EVANS

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 6-F - 30 MG/KG/DAY PIMOZIDE - CONTINUED

ANIMAL #	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43
R15206	79.6	92.0	92.9	98.1	104.9	111.7	115.3	120.8	119.5	128.1	
R15207	74.4	71.0	85.6	90.8	90.3	97.1	101.5	107.3	108.9	113.2	
R15208	87.6	96.7	101.1	108.8	106.5	117.7	123.3	131.8	130.6	137.5	
R15209	86.9	92.3	96.8	101.1	101.0	109.9	117.6	121.6	112.0	127.6	133.0
R15210	89.0	92.3	103.0	108.8	108.0	116.3	117.2	125.7	122.7	132.2	140.7
R15211	82.7	82.8	91.1	101.5	99.7	104.2	107.5	110.3	109.0	120.6	123.7
R15212	79.9	83.5	92.6	89.6	100.5	102.4	109.0	109.5	112.7	114.8	
R15213	80.5	88.4	91.3	89.4	96.5	102.1	103.9	108.5	104.4	116.5	
R15214	89.3	91.9	99.2	105.1	108.6	114.7	120.5	122.0	124.7	126.0	
R15215	72.0	75.1	82.1	83.6	93.2	96.3	104.0	108.0	111.6	122.7	127.6
R15216	78.5	83.4	86.5	89.9	96.4	99.8	100.5	103.6	110.4	120.1	130.4
R15217	77.0	82.8	86.8	89.8	94.4	97.7	104.2	108.9	113.8	120.2	126.0
MEAN	81.5	86.0	92.4	96.4	100.0	105.8	110.4	114.8	115.0	123.3	130.2
S.D.	5.74	7.67	6.57	8.58	6.05	7.85	7.98	9.01	7.69	7.25	6.08
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15206-R15211 ARE SPRAGUE-DAWLEY
ANIMALS R15212-R15217 ARE LONG EVANS

PND = POSTNATAL DAY
A: TERMINAL KILL

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 7-F - 100 MG/KG/DAY METHOXYCHLOR

ANIMAL #	PND 21	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32
R15218	49.2	49.9	55.1	60.2	64.6	69.2	72.5	79.0	84.9	88.4	94.0	96.5
R15219	49.8	50.7	54.4	59.5	63.5	67.0	71.6	75.0	79.9	83.4	89.1	88.6
R15220	48.9	48.8	52.7	57.6	62.7	67.0	70.9	78.0	81.6	86.5	92.2	95.4
R15221	48.2	48.7	53.0	57.9	61.4	64.9	69.8	73.5	78.3	83.4	88.2	89.4
R15222	47.8	47.4	52.4	57.0	61.6	66.1	71.5	75.4	79.2	84.8	91.6	92.3
R15223	47.7	48.4	53.8	58.1	61.9	66.5	69.4	76.3	78.2	84.5	92.1	95.1
R15224	46.2	47.6	53.6	58.5	63.0	69.1	75.3	79.3	85.3	91.1	98.1	97.8
R15225	45.7	46.2	49.8	56.0	59.6	66.4	69.7	76.3	80.2	83.9	93.4	98.5
R15226	44.8	44.2	50.1	54.7	59.8	64.6	70.4	76.0	81.3	88.4	94.1	97.2
R15227	47.5	50.2	46.5	59.0	61.9	67.2	73.2	78.9	81.5	88.3	93.7	98.0
R15228	43.9	46.6	51.5	58.5	63.2	68.6	73.0	80.3	83.6	89.2	96.8	100.2
R15229	41.5	42.0	53.4	52.8	57.6	62.2	67.5	71.9	77.1	80.3	88.8	92.5
MEAN	46.8	47.6	52.2	57.5	61.7	66.6	71.2	76.7	80.9	86.0	92.7	95.1
S.D.	2.44	2.54	2.40	2.10	1.94	2.01	2.09	2.53	2.64	3.12	3.04	3.68
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15218-R15223 ARE SPRAGUE-DAWLEY
ANIMALS R15224-R15229 ARE LONG EVANS

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 2 (CONTINUED)
INDIVIDUAL BODY WEIGHTS (GRAMS)
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 7-F - 100 MG/KG/DAY METHOXYCHLOR - CONTINUED

ANIMAL #	PND 33	PND 34	PND 35	PND 36	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43
R15218	99.8	105.0	112.1	114.6	116.5	122.8	125.6	129.5	134.1	139.0	
R15219	91.3	99.4	105.4	110.1	110.9	117.8	119.7	123.1	124.0	129.1	
R15220	97.9	103.6	94.1	109.5	112.1	119.4	120.8	129.1	127.7	132.1	
R15221	97.9	99.9	103.9	106.9	112.7	115.7	120.9	125.2	129.2	134.3	133.9
R15222	100.3	104.0	108.4	111.7	121.0	122.1	128.6	135.5	136.5	147.3	146.4
R15223	100.6	104.7	111.2	109.7	116.3	124.8	128.3	135.2	137.7	144.6	147.4
R15224	104.1	107.9	118.8	119.2	124.7	126.2	132.4	135.2	138.2	140.3	
R15225	104.0	109.4	112.2	113.7	114.3	117.3	126.9	121.4	128.5	128.7	
R15226	103.9	109.3	118.9	116.3	121.6	129.9	134.4	133.1	139.4	140.6	
R15227	103.1	110.6	117.3	119.6	126.7	130.8	135.0	138.7	139.3	148.0	150.2
R15228	103.9	110.5	114.9	114.3	123.8	124.1	129.8	133.2	134.8	140.7	147.1
R15229	95.6	102.1	107.3	109.5	116.5	114.3	120.6	124.0	126.2	133.2	136.2
MEAN	100.2	105.5	110.4	112.9	118.1	122.1	126.9	130.3	133.0	138.2	143.5
S.D.	4.01	3.97	7.16	4.05	5.31	5.36	5.49	5.73	5.53	6.66	6.74
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15218-R15223 ARE SPRAGUE-DAWLEY
ANIMALS R15224-R15229 ARE LONG EVANS

PND = POSTNATAL DAY
A: TERMINAL KILL

APPENDIX 3
INDIVIDUAL AGE AND WEIGHT AT VAGINAL OPENING - SPRAGUE-DAWLEY
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	WEIGHT AT VAGINAL OPENING (GRAMS)	AGE AT VAGINAL OPENING (DAYS)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL	
R15146	106.5	35
R15147	106.3	35
R15148	113.8	34
R15149	102.5	34
R15150	115.2	34
R15151	106.7	34
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15158	70.3	26
R15159	69.4	26
R15160	66.5	26
R15161	66.7	26
R15162	70.1	26
R15163	64.8	26
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN	
R15170	64.5	26
R15171	64.6	26
R15172	67.6	26
R15173	64.5	26
R15174	75.9	28
R15175	61.3	26
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15182	106.6	35
R15183	89.6	36
R15184	101.2	38
R15185	94.8	34
R15186	92.1	39
R15187	103.5	38
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15194	98.0	33
R15195	119.3	38
R15196	120.9	37
R15197	104.5	34
R15198	120.0	37
R15199	123.1	38
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE	
R15206	81.2	32
R15207	108.9	41
R15208	88.1	32
R15209	92.3	34
R15210	77.8	30
R15211	82.8	34
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15218	69.2	26
R15219	67.0	26
R15220	67.0	26
R15221	64.9	26
R15222	75.4	28
R15223	66.5	26

APPENDIX CONTINUED

APPENDIX 3 (CONTINUED)
INDIVIDUAL AGE AND WEIGHT AT VAGINAL OPENING - LONG EVANS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	WEIGHT AT VAGINAL OPENING (GRAMS)	AGE AT VAGINAL OPENING (DAYS)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL	
R15152	133.0	35
R15153	144.8	37
R15154	133.3	37
R15155	132.2	37
R15156	137.2	36
R15157	133.1	37
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15164	63.7	25
R15165	68.4	26
R15166	66.2	25
R15167	66.7	26
R15168	62.6	26
R15169	63.0	26
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN	
R15176	66.5	26
R15177	63.2	27
R15178	61.8	26
R15179	62.1	26
R15180	65.8	26
R15181	65.7	26
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15188	84.0	32
R15189	85.9	33
R15190	86.5	30
R15191	86.6	37
R15192	NV	NV
R15193	85.0	34
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15200	119.9	37
R15201	113.6	35
R15202	113.1	35
R15203	119.3	36
R15204	92.2	32
R15205	105.3	36
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE	
R15212	79.4	32
R15213	89.4	36
R15214	91.9	34
R15215	108.0	40
R15216	103.6	40
R15217	97.7	38
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15224	75.3	27
R15225	66.4	26
R15226	64.6	26
R15227	67.2	26
R15228	68.6	26
R15229	62.2	26

NV = NO VAGINAL OPENING WAS OBSERVED

APPENDIX 4

INDIVIDUAL DAILY VAGINAL OPENING DATA

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (SPRAGUE DAWLEY)

	POSTNATAL DAY																					
ANIMAL ID	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
GROUP: 1 - 2.5 ML/KG/DAY CORN OIL																						
R15146	N	N	N	N	N	N	N	N	N	N	N	N	T	Y								
R15147	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15148	N	N	N	N	N	N	N	N	N	N	P	P	Y									
R15149	N	N	N	N	N	N	N	N	N	N	N	N	Y									
R15150	N	N	N	N	N	N	N	N	N	N	N	T	Y									
R15151	N	N	N	N	N	N	N	N	N	N	N	N	Y									
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																						
R15158	N	N	N	N	Y																	
R15159	N	N	N	N	Y																	
R15160	N	N	N	N	Y																	
R15161	N	N	N	N	Y																	
R15162	N	N	N	N	Y																	
R15163	N	N	N	N	Y																	
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																						
R15170	N	N	N	N	Y																	
R15171	N	N	N	N	Y																	
R15172	N	N	N	N	Y																	
R15173	N	N	N	N	Y																	
R15174	N	N	N	N	N	N	Y															
R15175	N	N	N	N	Y																	
GROUP: 4 - 240MG/KG/DAY PROPYLTHIOURACIL																						
R15182	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15183	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y							
R15184	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y					
R15185	N	N	N	N	N	N	N	N	N	N	N	N	Y									
R15186	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y				
R15187	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y					

Y = Yes, vagina opened
N = No, vagina not opened
P = Pinhole opening
T = Thread (a piece of tissue) presented

APPENDIX 4 (CONTINUED)
INDIVIDUAL DAILY VAGINAL OPENING DATA
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (SPRAGUE DAWLEY)

	POSTNATAL DAY																				
ANIMAL ID	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE																						
R15194	N	N	N	N	N	N	N	N	N	N	N	Y										
R15195	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y					
R15196	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
R15197	N	N	N	N	N	N	N	N	N	N	N	N	Y									
R15198	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
R15199	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y					
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																						
R15206	N	N	N	N	N	N	N	N	N	N	Y											
R15207	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y		
R15208	N	N	N	N	N	N	N	N	N	N	Y											
R15209	N	N	N	N	N	N	N	N	N	N	N	N	Y									
R15210	N	N	N	N	N	N	N	N	Y													
R15211	N	N	N	N	N	N	N	N	N	N	N	N	Y									
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																						
R15218	N	N	N	N	Y																	
R15219	N	N	N	N	Y																	
R15220	N	N	N	N	Y																	
R15221	N	N	N	P	Y																	
R15222	N	N	N	P	P	P	Y															
R15223	N	N	N	P	Y																	

Y = Yes, vagina opened
P = Pinhole opening
N = No, vagina not opened
T = Thread (a piece of tissue) presented

APPENDIX 4 (CONTINUED)
INDIVIDUAL DAILY VAGINAL OPENING DATA
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

	POSTNATAL DAY																					
ANIMAL ID	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
GROUP: 1 - 2.5 ML/KG/DAY CORN OIL																						
R15152	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15153	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
R15154	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	Y						
R15155	N	N	N	N	N	N	N	N	N	N	N	N	N	N	T	Y						
R15156	N	N	N	N	N	N	N	N	N	N	N	N	N	T	Y							
R15157	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																						
R15164	N	N	N	Y																		
R15165	N	N	N	N	Y																	
R15166	N	N	N	Y																		
R15167	N	N	N	N	Y																	
R15168	N	N	N	N	Y																	
R15169	N	N	N	N	Y																	
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																						
R15176	N	N	N	N	Y																	
R15177	N	N	N	N	N	Y																
R15178	N	N	N	N	Y																	
R15179	N	N	N	N	Y																	
R15180	N	N	N	N	Y																	
R15181	N	N	N	N	Y																	
GROUP: 4 - 240MG/KG/DAY PROPYLTHIOURACIL																						
R15188	N	N	N	N	N	N	N	N	N	N	Y											
R15189	N	N	N	N	N	N	N	N	N	N	P	Y										
R15190	N	N	N	N	N	N	N	N	Y													
R15191	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
R15192	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
R15193	N	N	N	N	N	N	N	N	N	N	N	N	Y									

Y = Yes, vagina opened
P = Pinhole opening
N = No, vagina not opened
T = Thread (a piece of tissue) presented

APPENDIX 4 (CONTINUED)
INDIVIDUAL DAILY VAGINAL OPENING DATA
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

	POSTNATAL DAY																					
ANIMAL ID	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE																						
R15200	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
R15201	N	N	N	N	N	N	N	N	N	N	N	N	P	Y								
R15202	N	N	N	N	N	N	N	N	N	N	N	N	P	Y								
R15203	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y							
R15204	N	N	N	N	N	N	N	N	N	N	Y											
R15205	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y							
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																						
R15212	N	N	N	N	N	N	N	N	N	N	Y											
R15213	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y							
R15214	N	N	N	N	N	N	N	N	N	N	N	N	Y									
R15215	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y			
R15216	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	T	Y			
R15217	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y					
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																						
R15224	N	N	N	N	T	Y																
R15225	N	N	N	N	Y																	
R15226	N	N	N	N	Y																	
R15227	N	N	N	N	Y																	
R15228	N	N	N	N	Y																	
R15229	N	N	N	N	Y																	

Y = Yes, vagina opened
P = Pinhole opening
N = No, vagina not opened
T = Thread (a piece of tissue) presented

APPENDIX 5

INDIVIDUAL VAGINAL CYTOLOGY ANALYSES - SPRAGUE-DAWLEY

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	AGE AT FIRST ESTRUS (DAYS)	POSTNATAL DAY 25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
GROUP: 1 - 2.5 ML/KG/DAY CORN OIL																				
R15146	36											P	E	M	D	D	D	D	D	
R15147	35											E	M	D	D	D	P	E	E	
R15148	36										D	P	E	M	D	D	P	E	E	
R15149	35										P	E	E	M	D	D	P	E	M	D
R15150	34										E	E	E	M	D	D	P	E	E	M
R15151	35										P	E	M	D	D	D	D	D	D	D
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																				
R15158	26		E	E	E	E	E	M	D	D	P	E	M	D	P	E	E	D	P	
R15159	26		E	E	E	E	E	M	D	P	E	E	E	E	D	E	E	D	P	
R15160	26		E	E	E	E	E	E	M	D	P	E	E	M	D	D	P	E	E	
R15161	26		E	E	E	E	E	M	D	D	P	E	M	D	D	D	P	E	D	P
R15162	26		E	E	E	E	E	M	D	D	P	E	D	P	E	E	E	M	D	D
R15163	26		E	E	E	E	E	M	D	D	P	E	E	D	P	E	E	E	E	E
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																				
R15170	NE		M	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15171	NE		M	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15172	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15173	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15174	NE				D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15175	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL																				
R15182	NE											D	D	D	D	D	D	D	D	
R15183	36												E	M	D	D	D	D	D	
R15184	38														E	M	D	D	D	
R15185	35										P	E	M	D	D	D	D	D	D	D
R15186	39															E	M	D	D	D
R15187	38														E	M	D	D	D	D
GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE																				
R15194	36									D	D	D	E	M	D	D	D	D	D	
R15195	NE														D	D	D	D	D	
R15196	NE													D	D	D	D	D	P	
R15197	NE										D	D	D	D	D	D	D	D	D	D
R15198	NE													D	D	D	D	D	D	D
R15199	40														D	D	E	M	D	D
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																				
R15206	33								D	E	E	M	D	E	E	M	D	D	D	
R15207	NE																	M	D	
R15208	33								P	E	M	D	D	D	E	E	M	D	D	
R15209	35										P	E	E	E	E	M	D	D	D	D
R15210	30						E	E	E	E	E	E	M	D	D	D	D	D	D	D
R15211	39										D	D	D	D	P	E	M	D	D	D
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																				
R15218	27		D	E	E	E	M	D	D	P	E	E	M	D	D	D	D	D	E	
R15219	32		D	D	D	D	D	D	E	M	D	D	D	E	M	D	D	D	P	
R15220	26		E	E	E	E	E	M	D	D	P	E	M	D	E	D	E	M	D	
R15221	26		E	M	D	D	D	D	D	D	D	P	E	E	M	D	P	E	M	D
R15222	32				D	D	D	P	E	M	D	D	D	D	D	D	D	D	D	D
R15223	31		D	D	D	D	P	E	M	D	D	D	D	D	D	D	D	D	D	D

NE = NO ESTRUS OBSERVED P = PROESTRUS E = ESTRUS M = METESTRUS D = DIESTRUS

APPENDIX CONTINUED

APPENDIX 5 (CONTINUED)
INDIVIDUAL VAGINAL CYTOLOGY ANALYSES - LONG EVANS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	AGE AT FIRST ESTRUS (DAYS)	POSTNATAL DAY 25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
GROUP: 1 - 2.5 ML/KG/DAY CORN OIL																				
R15152	35											E	M	D	D	D	D	P	E	
R15153	37													E	M	D	D	D	D	
R15154	39													M	D	E	M	D	D	
R15155	38													P	E	M	D	D	D	D
R15156	36												E	M	D	D	P	E	E	M
R15157	38													P	E	M	D	D	D	P
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																				
R15164	25	E	E	E	E	E	E	M	D	D	P	E	E	M	D	D	D	P	E	
R15165	26		E	E	E	E	E	M	D	D	P	E	E	E	E	E	E	D	P	
R15166	25	E	E	E	E	E	E	M	D	D	E	E	E	D	P	E	E	E	E	
R15167	26		E	E	E	E	E	M	D	D	P	E	E	M	D	E	M	D	P	E
R15168	26		E	E	D	E	E	M	D	D	D	P	E	E	M	D	D	P	E	E
R15169	26		E	E	E	E	E	M	D	D	E	M	D	D	D	E	D	P	E	E
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																				
R15176	NE		M	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15177	NE			M	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15178	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15179	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15180	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15181	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL																				
R15188	34								D	D	E	E	E	M	D	D	D	D	P	
R15189	34									D	E	M	D	D	D	D	E	M	D	
R15190	32						D	P	E	M	D	D	D	D	D	D	D	D	D	
R15191	NE													D	D	D	D	D	D	D
R15192	NV																			
R15193	34										E	M	D	D	D	D	D	D	D	D
GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE																				
R15200	38													D	E	M	D	D	D	
R15201	37											D	D	E	M	D	D	D	D	
R15202	NE											D	D	D	D	D	D	D	D	
R15203	NE												D	D	D	D	D	D	D	D
R15204	NE								D	D	D	D	D	D	D	D	D	D	D	D
R15205	NE												D	D	D	D	D	D	D	D
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																				
R15212	32								E	M	D	D	D	D	E	M	D	D	D	
R15213	40												D	D	D	P	E	M	D	
R15214	34										E	M	D	P	E	E	M	D	D	
R15215	41																P	E	M	D
R15216	40																E	E	M	D
R15217	40														D	P	E	M	D	D
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																				
R15224	34			D	D	D	D	D	D	D	E	E	M	D	D	D	E	E	E	
R15225	27		P	E	E	E	M	D	D	D	D	E	E	M	D	D	D	P	E	
R15226	30		D	D	D	P	E	E	M	D	D	D	D	D	D	D	D	D	D	
R15227	34		D	D	D	D	D	D	D	P	E	E	M	D	D	P	E	E	M	D
R15228	36		D	D	D	D	D	D	D	D	D	P	E	E	M	D	D	D	P	D
R15229	26		E	M	D	D	D	D	D	D	P	E	E	E	M	D	D	P	E	E

NE = NO ESTRUS OBSERVED P = PROESTRUS E = ESTRUS M = METESTRUS D = DIESTRUS
NV = NO VAGINAL OPENING WAS OBSERVED

APPENDIX 6
INDIVIDUAL NECROPSY OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 1-F - 2.5 ML/KG/DAY CORN OIL

ANIMAL ID	FATE	DAY	LOCATION	OBSERVATION
R15146	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15147	TERMINAL KILL	42	THYROID	ENLARGED,SLIGHT
R15148	TERMINAL KILL	42	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,SEVERE
R15149	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15150	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15151	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15152	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15153	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15154	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15155	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15156	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15157	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15146-R15151 ARE SPRAGUE-DAWLEY
ANIMALS R15152-R15157 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 6 (CONTINUED)
INDIVIDUAL NECROPSY OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 2-F - 0.005 MG/KG/DAY ETHINYL ESTRADIOL

ANIMAL ID	FATE	DAY	LOCATION	OBSERVATION
R15158	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15159	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15160	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15161	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15162	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15163	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15164	TERMINAL KILL	42	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,SEVERE
R15165	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15166	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15167	TERMINAL KILL	43	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,SEVERE
R15168	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15169	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15158-R15163 ARE SPRAGUE-DAWLEY
ANIMALS R15164-R15169 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 6 (CONTINUED)
INDIVIDUAL NECROPSY OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 3-F - 10 MG/KG/DAY TAMOXIFEN

ANIMAL ID	FATE	DAY	LOCATION	OBSERVATION
R15170	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15171	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15172	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15173	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15174	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15175	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15176	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15177	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15178	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15179	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15180	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15181	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15170-R15175 ARE SPRAGUE-DAWLEY
ANIMALS R15176-R15181 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 6 (CONTINUED)
INDIVIDUAL NECROPSY OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 4-F - 240 MG/KG/DAY PROPYLTHIOURACIL

ANIMAL ID	FATE	DAY	LOCATION	OBSERVATION
R15182	TERMINAL KILL	42	THYROID	ENLARGED,SEVERE
R15183	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15184	TERMINAL KILL	42	PITUITARY	ENLARGED,SEVERE
R15185	TERMINAL KILL	43	THYROID	ENLARGED,SEVERE
R15186	TERMINAL KILL	43	THYROID	ENLARGED,SEVERE
R15187	TERMINAL KILL	43	THYROID	ENLARGED,SEVERE
R15188	TERMINAL KILL	42	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,MODERATE
			THYROID	ENLARGED,SEVERE
R15189	TERMINAL KILL	42	THYROID	ENLARGED,SEVERE
R15190	TERMINAL KILL	42	THYROID	ENLARGED,SEVERE
R15191	TERMINAL KILL	43	THYROID	ENLARGED,MODERATE
R15192	TERMINAL KILL	43	THYROID	ENLARGED,SEVERE
R15193	TERMINAL KILL	43	THYROID	ENLARGED,SEVERE

KEY: ANIMALS R15182-R15187 ARE SPRAGUE-DAWLEY
ANIMALS R15188-R15193 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 6 (CONTINUED)
INDIVIDUAL NECROPSY OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 5-F - 100 MG/KG/DAY KETOCONAZOLE

ANIMAL ID	FATE	DAY	LOCATION	OBSERVATION
R15194	TERMINAL KILL	42	ADRENALS	ENLARGED,3X3X3MM
R15195	TERMINAL KILL	42	ADRENALS	ENLARGED,4X4X4MM
R15196	TERMINAL KILL	42	ADRENALS	ENLARGED,4X4X4MM
R15197	TERMINAL KILL	43	ADRENALS	ENLARGED,4X4X4MM
R15198	TERMINAL KILL	43	ADRENALS	ENLARGED,MODERATE
R15199	TERMINAL KILL	43	ADRENALS	ENLARGED,SLIGHT
R15200	TERMINAL KILL	42	ADRENALS	ENLARGED,3X3X3MM
R15201	TERMINAL KILL	42	ADRENALS	ENLARGED,3X3X3MM
R15202	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15203	TERMINAL KILL	43	ADRENALS	ENLARGED,MODERATE
R15204	TERMINAL KILL	43	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,MODERATE
R15205	TERMINAL KILL	43	UTERUS WITH CERVIX	UTERUS-SMALL,BILATERAL
			ADRENALS	ENLARGED,MODERATE
			THYROID	ENLARGED,SLIGHT

KEY: ANIMALS R15194-R15199 ARE SPRAGUE-DAWLEY
ANIMALS R15200-R15205 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 6 (CONTINUED)
INDIVIDUAL NECROPSY OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 6-F - 30 MG/KG/DAY PIMOZIDE

ANIMAL ID	FATE	DAY	LOCATION	OBSERVATION
R15206	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15207	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15208	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15209	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15210	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15211	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15212	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15213	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15214	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15215	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15216	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15217	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15206-R15211 ARE SPRAGUE-DAWLEY
ANIMALS R15212-R15217 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 6 (CONTINUED)
INDIVIDUAL NECROPSY OBSERVATIONS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 7-F - 100 MG/KG/DAY METHOXYCHLOR

ANIMAL ID	FATE	DAY	LOCATION	OBSERVATION
R15218	TERMINAL KILL	42	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,MODERATE
			THYROID	ENLARGED,MODERATE
R15219	TERMINAL KILL	42	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,MODERATE
R15220	TERMINAL KILL	42	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,MODERATE
R15221	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15222	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15223	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15224	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15225	TERMINAL KILL	42	UTERUS WITH CERVIX	UTERUS-CYST,RIGHT HORN,ONE,CLEAR,1X1X1MM
R15226	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15227	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15228	TERMINAL KILL	43	UTERUS WITH CERVIX	UTERUS-DISTENDED,BILATERAL,MODERATE
R15229	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15218-R15223 ARE SPRAGUE-DAWLEY
ANIMALS R15224-R15229 ARE LONG EVANS

APPENDIX 7

INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE RATS

GROUP: 1-F - 2.5 ML/KG/DAY CORN OIL

ANIMAL ID:	R15146	R15147	R15148	R15149	R15150	R15151	R15152	R15153	R15154
BODY WEIGHT (G)	134.6	145.2	153.2	136.2	160.1	155.8	161.1	168.3	159.2
LIVER (G)	5.257	6.112	6.421	5.748	6.669	6.638	7.164	6.392	7.040
% BODY WEIGHT	3.906	4.209	4.191	4.220	4.166	4.261	4.447	3.798	4.422
KIDNEYS (PAIRED) (G)	1.148	1.205	1.171	1.186	1.344	1.377	1.488	1.484	1.460
% BODY WEIGHT	0.853	0.830	0.764	0.871	0.839	0.884	0.924	0.882	0.917
UTERUS WITH CERVIX (G)	0.161	0.288	0.504	0.173	0.261	0.171	0.322	0.152	0.151
% BODY WEIGHT	0.120	0.198	0.329	0.127	0.163	0.110	0.200	0.090	0.095
UTERUS WITH CERVIX(DRY) (G)	0.157	0.268	0.362	0.147	0.234	0.156	0.291	0.137	0.134
% BODY WEIGHT	0.117	0.185	0.236	0.108	0.146	0.100	0.181	0.081	0.084
OVARIES (G)	0.067	0.045	0.044	0.061	0.086	0.066	0.066	0.078	0.089
% BODY WEIGHT	0.050	0.031	0.029	0.045	0.054	0.042	0.041	0.046	0.056
ADRENALS (G)	0.038	0.036	0.036	0.034	0.050	0.053	0.033	0.034	0.042
% BODY WEIGHT	0.028	0.025	0.023	0.025	0.031	0.034	0.020	0.020	0.026
PITUITARY (G)	0.005	0.008	0.007	0.012	0.009	0.008	0.009	0.004	0.005
% BODY WEIGHT	0.004	0.006	0.005	0.009	0.006	0.005	0.006	0.002	0.003

KEY: ANIMALS R15146-R15151 ARE SPRAGUE-DAWLEY
ANIMALS R15152-R15154 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)

INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 1-F - 2.5 ML/KG/DAY CORN OIL - CONTINUED

ANIMAL ID:	R15155	R15156	R15157
BODY WEIGHT (G)	160.6	176.4	162.3
LIVER (G)	7.377	7.607	7.115
% BODY WEIGHT	4.593	4.312	4.384
KIDNEYS (PAIRED) (G)	1.534	1.586	1.650
% BODY WEIGHT	0.955	0.899	1.017
UTERUS WITH CERVIX (G)	0.474	0.231	0.404
% BODY WEIGHT	0.295	0.131	0.249
UTERUS WITH CERVIX(DRY) (G)	0.292	0.208	0.327
% BODY WEIGHT	0.182	0.118	0.201
OVARIES (G)	0.078	0.085	0.100
% BODY WEIGHT	0.049	0.048	0.062
ADRENALS (G)	0.040	0.046	0.032
% BODY WEIGHT	0.025	0.026	0.020
PITUITARY (G)	0.005	0.009	1.220[a]
% BODY WEIGHT	0.003	0.005	0.752[a]

KEY: ANIMALS R15155-R15157 ARE LONG EVANS

APPENDIX CONTINUED

A WEIGHT OUT OF RANGE - EXCLUDED FROM SUMMARY DATA

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 2-F - 0.005 MG/KG/DAY ETHINYL ESTRADIOL

ANIMAL ID:	R15158	R15159	R15160	R15161	R15162	R15163	R15164	R15165	R15167
BODY WEIGHT (G)	146.6	141.0	130.2	145.6	140.5	127.4	147.2	146.7	139.5
LIVER (G)	6.969	6.604	4.924	7.124	6.308	4.653	6.414	5.786	6.139
% BODY WEIGHT	4.754	4.684	3.782	4.893	4.490	3.652	4.357	3.944	4.401
KIDNEYS (PAIRED) (G)	1.356	1.146	1.070	1.286	1.193	1.014	1.209	1.214	1.188
% BODY WEIGHT	0.925	0.813	0.822	0.883	0.849	0.796	0.821	0.828	0.852
UTERUS WITH CERVIX (G)	0.248	0.212	0.325	0.275	0.250	0.276	0.510	0.233	0.275
% BODY WEIGHT	0.169	0.150	0.250	0.189	0.178	0.217	0.346	0.159	0.197
UTERUS WITH CERVIX(DRY) (G)	0.235	0.199	0.264	0.259	0.226	0.253	0.362	0.221	0.216
% BODY WEIGHT	0.160	0.141	0.203	0.178	0.161	0.199	0.246	0.151	0.155
OVARIES (G)	0.079	0.051	0.037	0.057	0.036	0.039	0.077	0.045	0.023
% BODY WEIGHT	0.054	0.036	0.028	0.039	0.026	0.031	0.052	0.031	0.016
ADRENALS (G)	0.049	0.046	0.042	0.037	0.039	0.030	0.043	0.026	0.031
% BODY WEIGHT	0.033	0.033	0.032	0.025	0.028	0.024	0.029	0.018	0.022
PITUITARY (G)	0.020	0.010	0.005	0.008	0.005	0.008	0.009	0.008	0.005
% BODY WEIGHT	0.014	0.007	0.004	0.005	0.004	0.006	0.006	0.005	0.004

KEY: ANIMALS R15158-R15163 ARE SPRAGUE-DAWLEY
ANIMALS R15164-R15166 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 2-F - 0.005 MG/KG/DAY ETHINYL ESTRADIOL - CONTINUED

ANIMAL ID:	R15167	R15168	R15169
BODY WEIGHT (G)	138.5	146.7	146.8
LIVER (G)	5.500	6.129	6.668
% BODY WEIGHT	3.971	4.178	4.542
KIDNEYS (PAIRED) (G)	1.259	1.315	1.382
% BODY WEIGHT	0.909	0.896	0.941
UTERUS WITH CERVIX (G)	0.434	0.260	0.469
% BODY WEIGHT	0.313	0.177	0.319
UTERUS WITH CERVIX(DRY) (G)	0.333	0.239	0.314
% BODY WEIGHT	0.240	0.163	0.214
OVARIES (G)	0.076	0.081	0.050
% BODY WEIGHT	0.055	0.055	0.034
ADRENALS (G)	0.030	0.040	0.047
% BODY WEIGHT	0.022	0.027	0.032
PITUITARY (G)	0.006	0.006	0.005
% BODY WEIGHT	0.004	0.004	0.003

KEY: ANIMALS R15167-R15169 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 3-F - 10 MG/KG/DAY TAMOXIFEN

ANIMAL ID:	R15170	R15171	R15172	R15173	R15174	R15175	R15176	R15177	R15178
BODY WEIGHT (G)	115.7	116.8	121.6	119.9	132.5	121.6	125.9	132.8	117.6
LIVER (G)	5.300	4.951	4.773	5.279	5.994	5.453	5.331	6.352	5.044
% BODY WEIGHT	4.581	4.239	3.925	4.403	4.524	4.484	4.234	4.783	4.289
KIDNEYS (PAIRED) (G)	1.101	1.104	1.074	1.092	1.301	1.105	1.180	1.212	1.132
% BODY WEIGHT	0.952	0.945	0.883	0.911	0.982	0.909	0.937	0.913	0.963
UTERUS WITH CERVIX (G)	0.100	0.113	0.112	0.112	0.151	0.113	0.081	0.109	0.109
% BODY WEIGHT	0.086	0.097	0.092	0.093	0.114	0.093	0.064	0.082	0.093
UTERUS WITH CERVIX(DRY) (G)	0.096	0.100	0.102	0.102	0.132	0.108	0.076	0.099	0.099
% BODY WEIGHT	0.083	0.086	0.084	0.085	0.100	0.089	0.060	0.075	0.084
OVARIES (G)	0.019	0.048	0.044	0.038	0.054	0.043	0.041	0.027	0.029
% BODY WEIGHT	0.016	0.041	0.036	0.032	0.041	0.035	0.033	0.020	0.025
ADRENALS (G)	0.029	0.029	0.038	0.034	0.027	0.031	0.035	0.025	0.024
% BODY WEIGHT	0.025	0.025	0.031	0.028	0.020	0.025	0.028	0.019	0.020
PITUITARY (G)	0.004	0.004	0.003	0.004	0.005	0.010	0.006	0.003	0.004
% BODY WEIGHT	0.003	0.003	0.002	0.003	0.004	0.008	0.005	0.002	0.003

KEY: ANIMALS R15170-R15175 ARE SPRAGUE-DAWLEY
ANIMALS R15176-R15178 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 3-F - 10 MG/KG/DAY TAMOXIFEN - CONTINUED

ANIMAL ID:	R15179	R15180	R15181
BODY WEIGHT (G)	134.3	133.6	131.7
LIVER (G)	7.160	6.007	5.784
% BODY WEIGHT	5.331	4.496	4.392
KIDNEYS (PAIRED) (G)	1.473	1.319	1.239
% BODY WEIGHT	1.097	0.987	0.941
UTERUS WITH CERVIX (G)	0.089	0.112	0.102
% BODY WEIGHT	0.066	0.084	0.077
UTERUS WITH CERVIX(DRY) (G)	0.079	0.102	0.092
BODY WEIGHT	0.059	0.076	0.070
OVARIES (G)	0.030	0.051	0.038
% BODY WEIGHT	0.022	0.038	0.029
ADRENALS (G)	0.038	0.044	0.036
% BODY WEIGHT	0.028	0.033	0.027
PITUITARY (G)	0.004	0.004	0.004
% BODY WEIGHT	0.003	0.003	0.003

KEY: ANIMALS R15179-R15181 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 4-F - 240 MG/KG/DAY PROPYLTHIOURACIL

ANIMAL ID:	R15182	R15183	R15184	R15185	R15186	R15187	R15188	R15189	R15190
BODY WEIGHT (G)	109.5	98.7	103.2	95.2	95.2	99.6	94.8	93.5	91.2
LIVER (G)	4.666	4.285	4.387	4.039	3.984	4.194	4.616	3.780	3.465
% BODY WEIGHT	4.261	4.341	4.251	4.243	4.185	4.211	4.869	4.043	3.799
KIDNEYS (PAIRED) (G)	0.758	0.704	0.758	0.829	0.735	0.724	0.833	0.826	0.785
% BODY WEIGHT	0.692	0.713	0.734	0.871	0.772	0.727	0.879	0.883	0.861
UTERUS WITH CERVIX (G)	0.174	0.109	0.140	0.251	0.100	0.140	0.373	0.149	0.108
% BODY WEIGHT	0.159	0.110	0.136	0.264	0.105	0.141	0.393	0.159	0.118
UTERUS WITH CERVIX(DRY) (G)	0.164	0.096	0.128	0.201	0.088	0.122	0.235	0.138	0.094
% BODY WEIGHT	0.150	0.097	0.124	0.211	0.092	0.122	0.248	0.148	0.103
OVARIES (G)	0.031	0.046	0.038	0.040	0.035	0.016	0.054	0.050	0.042
% BODY WEIGHT	0.028	0.047	0.037	0.042	0.037	0.016	0.057	0.053	0.046
ADRENALS (G)	0.019	0.026	0.026	0.025	0.020	0.022	0.045	0.027	0.021
% BODY WEIGHT	0.017	0.026	0.025	0.026	0.021	0.022	0.047	0.029	0.023
PITUITARY (G)	0.006	0.004	0.004	0.002	0.005	0.001[a]	0.009	0.005	0.006
% BODY WEIGHT	0.005	0.004	0.004	0.002	0.005	0.001[a]	0.009	0.005	0.007

KEY: ANIMALS R15182-R15187 ARE SPRAGUE-DAWLEY
ANIMALS R15188-R15190 ARE LONG EVANS

APPENDIX CONTINUED

A WEIGHT OUT OF RANGE - EXCLUDED FROM SUMMARY DATA

APPENDIX 7 (CONTINUED)

INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 4-F - 240 MG/KG/DAY PROPYLTHIOURACIL - CONTINUED

ANIMAL ID:	R15191	R15192	R15193
BODY WEIGHT (G)	89.0	88.4	85.7
LIVER (G)	3.496	3.795	3.541
% BODY WEIGHT	3.928	4.293	4.132
KIDNEYS (PAIRED) (G)	0.758	0.787	0.727
% BODY WEIGHT	0.852	0.890	0.848
UTERUS WITH CERVIX (G)	0.104	0.105	0.114
% BODY WEIGHT	0.117	0.119	0.133
UTERUS WITH CERVIX(DRY) (G)	0.089	0.093	0.097
% BODY WEIGHT	0.100	0.105	0.113
OVARIES (G)	0.033	0.039	0.051
% BODY WEIGHT	0.037	0.044	0.060
ADRENALS (G)	0.030	0.033	0.022
% BODY WEIGHT	0.034	0.037	0.026
PITUITARY (G)	0.005	0.004	0.003
% BODY WEIGHT	0.006	0.005	0.004

KEY: ANIMALS R15191-R15193 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)

INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 5-F - 100 MG/KG/DAY KETOCONAZOLE

ANIMAL ID:	R15194	R15195	R15196	R15197	R15198	R15199	R15200	R15201	R15202
BODY WEIGHT (G)	146.1	136.3	143.6	141.7	152.2	144.2	147.6	151.0	145.8
LIVER (G)	7.464	6.371	7.233	7.992	7.430	7.382	7.365	7.469	6.500
% BODY WEIGHT	5.109	4.674	5.037	5.640	4.882	5.119	4.990	4.946	4.458
KIDNEYS (PAIRED) (G)	1.476	1.216	1.304	1.470	1.398	1.305	1.440	1.568	1.442
% BODY WEIGHT	1.010	0.892	0.908	1.037	0.919	0.905	0.976	1.038	0.989
UTERUS WITH CERVIX (G)	0.253	0.159	0.145	0.177	0.222	0.191	0.139	0.358	0.263
% BODY WEIGHT	0.173	0.117	0.101	0.125	0.146	0.132	0.094	0.237	0.180
UTERUS WITH CERVIX(DRY) (G)	0.239	0.146	0.136	0.157	0.199	0.177	0.131	0.273	0.234
% BODY WEIGHT	0.164	0.107	0.095	0.111	0.131	0.123	0.089	0.181	0.160
OVARIES (G)	0.095	0.028	0.039	0.033	0.071	0.254	0.046	0.058	0.097
% BODY WEIGHT	0.065	0.021	0.027	0.023	0.047	0.176	0.031	0.038	0.067
ADRENALS (G)	0.091	0.068	0.071	0.084	0.076	0.075	0.058	0.069	0.076
% BODY WEIGHT	0.062	0.050	0.049	0.059	0.050	0.052	0.039	0.046	0.052
PITUITARY (G)	0.029[a]	0.005	0.005	0.007	0.006	0.010	0.004	0.006	0.007
% BODY WEIGHT	0.020[a]	0.004	0.003	0.005	0.004	0.007	0.003	0.004	0.005

KEY: ANIMALS R15194-R15199 ARE SPRAGUE-DAWLEY
ANIMALS R15200-R15202 ARE LONG EVANS

A WEIGHT OUT OF RANGE - EXCLUDED FROM SUMMARY DATA

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 5-F - 100 MG/KG/DAY KETOCONAZOLE - CONTINUED

ANIMAL ID:	R15203	R15204	R15205
BODY WEIGHT (G)	159.8	157.0	139.1
LIVER (G)	8.457	8.380	6.212
% BODY WEIGHT	5.292	5.338	4.466
KIDNEYS (PAIRED) (G)	1.564	1.533	1.353
% BODY WEIGHT	0.979	0.976	0.973
UTERUS WITH CERVIX (G)	0.199	0.349	0.092
% BODY WEIGHT	0.125	0.222	0.066
UTERUS WITH CERVIX(DRY) (G)	0.164	0.251	0.086
% BODY WEIGHT	0.103	0.160	0.062
OVARIES (G)	0.071	0.064	0.041
% BODY WEIGHT	0.044	0.041	0.029
ADRENALS (G)	0.079	0.060	0.074
% BODY WEIGHT	0.049	0.038	0.053
PITUITARY (G)	0.010	0.004	0.005
% BODY WEIGHT	0.006	0.003	0.004

KEY: ANIMALS R15203-R15205 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)

INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 6-F - 30 MG/KG/DAY PIMOZIDE ANIMAL ID:	R15206	R15207	R15208	R15209	R15210	R15211	R15212	R15213	R15214
BODY WEIGHT (G)	128.1	113.2	137.5	133.0	140.7	123.7	114.8	116.5	126.0
LIVER (G)	5.642	4.801	6.192	5.566	6.204	4.458	5.097	5.602	5.279
% BODY WEIGHT	4.404	4.241	4.503	4.185	4.409	3.604	4.440	4.809	4.190
KIDNEYS (PAIRED) (G)	1.052	0.936	1.150	1.111	1.144	1.044	1.157	1.021	1.158
% BODY WEIGHT	0.821	0.827	0.836	0.835	0.813	0.844	1.008	0.876	0.919
UTERUS WITH CERVIX (G)	0.225	0.179	0.203	0.184	0.216	0.241	0.224	0.131	0.186
% BODY WEIGHT	0.176	0.158	0.148	0.138	0.154	0.195	0.195	0.112	0.148
UTERUS WITH CERVIX(DRY) (G)	0.216	0.154	0.186	0.156	0.194	0.210	0.204	0.121	0.170
% BODY WEIGHT	0.169	0.136	0.135	0.117	0.138	0.170	0.178	0.104	0.135
OVARIES (G)	0.057	0.044	0.060	0.054	0.063	0.061	0.060	0.056	0.050
% BODY WEIGHT	0.044	0.039	0.044	0.041	0.045	0.049	0.052	0.048	0.040
ADRENALS (G)	0.035	0.034	0.030	0.031	0.036	0.040	0.067	0.029	0.021
% BODY WEIGHT	0.027	0.030	0.022	0.023	0.026	0.032	0.058	0.025	0.017
PITUITARY (G)	0.007	0.004	0.007	0.006	0.010	0.015	0.006	0.007	0.009
% BODY WEIGHT	0.005	0.004	0.005	0.005	0.007	0.012	0.005	0.006	0.007

KEY: ANIMALS R15206-R15211 ARE SPRAGUE-DAWLEY
ANIMALS R15212-R15214 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)

INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 6-F - 30 MG/KG/DAY PIMOZIDE - CONTINUED

ANIMAL ID:	R15215	R15216	R15217
BODY WEIGHT (G)	127.6	130.4	126.0
LIVER (G)	6.013	5.690	5.454
% BODY WEIGHT	4.712	4.363	4.329
KIDNEYS (PAIRED) (G)	1.192	1.031	1.187
% BODY WEIGHT	0.934	0.791	0.942
UTERUS WITH CERVIX (G)	0.152	0.127	0.131
% BODY WEIGHT	0.119	0.097	0.104
UTERUS WITH CERVIX(DRY) (G)	0.139	0.113	0.130
% BODY WEIGHT	0.109	0.087	0.103
OVARIES (G)	0.053	0.027	0.062
% BODY WEIGHT	0.042	0.021	0.049
ADRENALS (G)	0.035	0.026	0.042
% BODY WEIGHT	0.027	0.020	0.033
PITUITARY (G)	0.007	0.008	0.006
% BODY WEIGHT	0.005	0.006	0.005

KEY: ANIMALS R15215-R15217 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 7-F - 100 MG/KG/DAY METHOXYCHLOR

ANIMAL ID:	R15218	R15219	R15220	R15221	R15222	R15223	R15224	R15225	R15226
BODY WEIGHT (G)	139.0	129.1	132.1	133.9	146.4	147.4	140.3	128.7	140.6
LIVER (G)	5.648	4.627	4.874	5.093	6.006	5.968	5.952	5.395	6.268
% BODY WEIGHT	4.063	3.584	3.690	3.804	4.102	4.049	4.242	4.192	4.458
KIDNEYS (PAIRED) (G)	1.141	0.995	1.081	1.058	1.389	1.168	1.334	1.242	1.307
% BODY WEIGHT	0.821	0.771	0.818	0.790	0.949	0.792	0.951	0.965	0.930
UTERUS WITH CERVIX (G)	0.361	0.417	0.300	0.171	0.275	0.267	0.252	0.171	0.283
% BODY WEIGHT	0.260	0.323	0.227	0.128	0.188	0.181	0.180	0.133	0.201
UTERUS WITH CERVIX(DRY) (G)	0.254	0.246	0.237	0.156	0.250	0.258	0.245	0.160	0.274
% BODY WEIGHT	0.183	0.191	0.179	0.117	0.171	0.175	0.175	0.124	0.195
OVARIES (G)	0.027	0.031	0.055	0.035	0.063	0.082	0.084	0.057	0.059
% BODY WEIGHT	0.019	0.024	0.042	0.026	0.043	0.056	0.060	0.044	0.042
ADRENALS (G)	0.038	0.026	0.039	0.033	0.034	0.041	0.044	0.034	0.028
% BODY WEIGHT	0.027	0.020	0.030	0.025	0.023	0.028	0.031	0.026	0.020
PITUITARY (G)	0.005	0.006	0.006	0.006	0.007	0.008	0.008	0.005	0.006
% BODY WEIGHT	0.004	0.005	0.005	0.004	0.005	0.005	0.006	0.004	0.004

KEY: ANIMALS R15218-R15223 ARE SPRAGUE-DAWLEY
ANIMALS R15224-R15226 ARE LONG EVANS

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS (GRAMS) AND ORGAN-TO-BODY-WEIGHT RATIOS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP: 7-F - 100 MG/KG/DAY METHOXYCHLOR - CONTINUED

ANIMAL ID:	R15227	R15228	R15229
BODY WEIGHT (G)	150.2	147.1	136.2
LIVER (G)	6.251	5.982	5.251
% BODY WEIGHT	4.162	4.067	3.855
KIDNEYS (PAIRED) (G)	1.381	1.450	1.331
% BODY WEIGHT	0.919	0.986	0.977
UTERUS WITH CERVIX (G)	0.217	0.488	0.244
% BODY WEIGHT	0.144	0.332	0.179
UTERUS WITH CERVIX(DRY) (G)	0.205	0.321	0.226
% BODY WEIGHT	0.136	0.218	0.166
OVARIES (G)	0.077	0.093	0.095
% BODY WEIGHT	0.051	0.063	0.070
ADRENALS (G)	0.041	0.048	0.031
% BODY WEIGHT	0.027	0.033	0.023
PITUITARY (G)	0.006	0.007	0.003
% BODY WEIGHT	0.004	0.005	0.002

KEY: ANIMALS R15227-R15229 ARE LONG EVANS

APPENDIX 8
PATHOLOGY REPORT
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

Pathology Associates International
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An Employee-Owned Company

PATHOLOGY REPORT
FOR

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

PREPARED FOR
THERIMMUNE RESEARCH CORPORATION

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PATHOLOGY REPORT

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

TherImmune Research Corporation 1143-101

INTRODUCTION

The purpose of this protocol was to quantify the effects of environmental compounds on pubertal development and thyroid function in the intact juvenile female rat. This report prepared by Pathology Associates International (PAI) for TherImmune Research Corporation, 15 Firstfield Road, Gaithersburg, MD 20878, presents the results of the evaluation of pathology endpoints. The portion of this study performed by PAI was conducted in accordance with the Environmental Protection Agency (EPA) FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

EXPERIMENTAL DESIGN AND METHODS

The procedures described below were performed on two strains of juvenile rats concurrently to compare inter-strain variability. Forty-two female Sprague-Dawley rats and forty-two female Long-Evans rats were randomly distributed into seven groups as depicted in Text Table 1.

Text Table 1. Group Designation and Dosage Levels

Group	Treatment	Dosage (per kg/day)	# of females per strain
1	Corn Oil	2.5 ml	6
2	Ethynyl estradiol	0.005 mg	6
3	Tamoxifen	10 mg	6
4	Propylthiouracil (PTU)	240 mg	6
5	Ketoconazole	100 mg	6
6	Pimozide	30 mg	6
7	Methoxychlor	100 mg	6

Juvenile rats, approximately 22 days old, were dosed by oral gavage at a volume of 2.5 ml/kg body weight. The animals were dosed daily, between 0700 and 0900 hours, for at least 21 days.

Animals surviving to the scheduled terminal sacrifice time point (between 1300 and 1700 hours on post-natal day [PND] 42 or 43) were killed by decapitation and necropsied in accordance with the study protocol. The thyroid, ovaries and uterus were placed in Bouin's fixative for approximately 24 hours, after which they were rinsed and stored in 70% ethanol. These selected tissues were embedded in paraffin, sectioned at

approximately 5 microns, stained with hematoxylin and eosin (H&E) and examined microscopically by the undersigned pathologist.

RESULTS

Gross Pathology

All rats survived to the scheduled terminal sacrifice. Text Table 2 shows the number of rats with specific gross lesions by group and strain of rat.

Text Table 2. Number of Rats with Specific Gross Lesions by Group and Strain

	Groups													
	1		2		3		4		5		6		7	
Organ/Lesion	6SD	6LE	6SD	6LE	6SD	6LE	6SD	6LE	6SD	6LE	6SD	6LE	6SD	6LE
Uterus/Distended	1	0	0	2	0	0	0	1	0	1	0	0	3	1
Uterus/Small	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Uterus/Cyst	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Thyroid/Enlarged	1	0	0	0	0	0	4	6	0	1	0	0	1	0
Adrenals/Enlarged	0	0	0	0	0	0	0	0	6	4	0	0	0	0
Pituitary/Enlarged	0	0	0	0	0	0	1	0	0	0	0	0	0	0

6SD = Six Sprague-Dawley rats; 6LE = Six Long-Evans rats

All gross lesions in the thyroid and adrenals were considered to be test article related.

Histopathology

Microscopic findings for all groups are summarized by strain on the Project Summary Table (Section II) in which the numbers of animals per group and lesions per group are indicated. Microscopic findings are presented by treatment group with all diagnoses for individual animals in the Tabulated Animal Data Tables (Section III). Microscopic lesions are correlated to gross findings, when applicable, in the Correlation of Gross and Microscopic Findings (Section IV). Comments for individual animals, where appropriate, are in the Comments Report (Section V). The codes used as entries in these tables are explained in the Reports Code Table, Appendix 1, and abbreviations are explained in the Abbreviations List, Appendix 2.

Corn Oil Controls

With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. These cysts were characterized by variable sized follicles lined with keritinized squamous epithelium. Some cysts were completely filled with epithelium but most had a central lumen. No test article related lesions were identified in the ovary or uteri. All findings in all tissues were considered to be spontaneous changes of no significance to the animal.

Ethynyl Estradiol (0.005 mg/kg/day)

Sprague-Dawley - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related changes in the ovary were atrophy (1/6), follicular cyst (1/6), luteinized follicles (2/6), and absence of corpora lutea (2/6). In general normal follicular formation and maturation was present in most ovaries. Most also contained corpora lutea. It is difficult to determine whether an ovary is atrophic in a short term study since the development of follicles to the tertiary stage is autonomous and corpora lutea may last for a prolonged period. Some ovaries were called atrophic based on the absence of corpora lutea and the presence of inactive interstitial glands. These glands were characterized by periglandular cells with nuclei that were hyperchromatic and oval to elongate with a reduced amount of cytoplasm. The ovarian changes are most likely related to deranged pituitary hormone secretion as a result of the test article. Test article related changes in the uterus consisted of epithelial (3/6) and myometrial hypertrophy/hyperplasia (6/6). Most uteri were enlarged as a result of increased thickness of the endometrial and myometrial layers. The endometrial epithelium was for the most part hyperplastic and characterized by increased surface folding and tall columnar cells. Uterine glands were not necessarily increased but some were dilated. The uterine changes were considered a direct effect of the test article. Some animals exhibited uterine morphology appropriate to metestrus despite drug treatment and were therefore considered normal.

Long-Evans - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related changes in the ovary were atrophy (2/6), luteinized follicles (1/6), and absence of corpora lutea (2/6). Histologic changes are as described above. Test article related changes in the uterus consisted of epithelial (4/6) and myometrial hypertrophy/hyperplasia (4/6).

Tamoxifen (10 mg/kg/day)

Sprague-Dawley - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related changes in the ovary were atrophy (6/6) and interstitial cell hyperplasia (1/6). It is difficult to determine whether an ovary is atrophic in a short term study since the development of follicles to the tertiary stage is autonomous and corpora lutea may last for a prolonged period. All ovaries in this group were small and most lacked corpora lutea (6/6). The interstitial cell hyperplasia was felt to be relative due to the lack of corpora lutea. Test article related changes in the uterus consisted of atrophy (6/6), squamous metaplasia (3/6), and epithelial hypertrophy (6/6). The uteri were small. The endometrial epithelium was not hyperplastic but was hypertrophic and consisted of tall columnar cells indicative of the influence of estrogen. Occasional squamous metaplasia was present.

Long-Evans - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related changes in the ovary were atrophy (6/6) and interstitial cell

hyperplasia (5/6). It is difficult to determine whether an ovary is atrophic in a short term study since the development of follicles to the tertiary stage is autonomous and corpora lutea may last for a prolonged period. All ovaries in this group were small and lacked corpora lutea. In addition, ovaries were called atrophic based on the presence of inactive interstitial glands. Test article related changes in the uterus consisted of atrophy (6/6) and epithelial hypertrophy (6/6).

Propylthiouracil (240 mg/kg/day)

Sprague-Dawley - Test article related findings in the thyroid were hyperplasia/ hypertrophy of the follicular cells (6/6) and colloid depletion (6/6). Both changes were diffuse throughout the thyroid glands. All follicles were uniformly large and lined by large cuboidal to low columnar follicular cells with abundant eosinophilic cytoplasm. Most follicles were devoid of colloid. In general, the ovaries and uteri were normal. An occasional ovary was considered atrophic as described previously. Some ovaries described as atrophic lacked corpora lutea but appeared to have interstitial cell hyperplasia. This may have been a relative increase in an otherwise active ovary. Interstitial cell hyperplasia was present in one ovary but its relationship to the test article is uncertain. Test article related findings in the uteri consisted of atrophy (3/6). Atrophic changes in the ovary and uterus are most likely related to hypothyroidism.

Long-Evans - Test article related findings in the thyroid were hyperplasia/hypertrophy of the follicular cells (6/6) and colloid depletion (6/6). Both changes were diffuse throughout the thyroid glands. Test article related findings in the ovaries (2/6) and uteri (4/6) consisted of atrophy. This change is most likely related to hypothyroidism.

Ketoconazole (100 mg/kg/day)

Sprague-Dawley - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related lesions in the ovaries consisted of interstitial cell hyperplasia (6/6), absence of corpora lutea (3/6), atrophy (3/6), and a follicular cyst. The ovaries were within normal limits for size. Many of the ovaries lacked corpora lutea and most likely would have been reduced in size. An expanded population of interstitial cells most likely resulted in the maintenance of ovarian size, however. The interstitial cell hypertrophy/hyperplasia represented a diffuse change throughout all the ovaries examined and was characterized by cords of plump, polygonal, cells with well-defined borders. The cytoplasm was clear to lightly eosinophilic and the nuclei round and heterochromatic with inconspicuous nucleoli. Interstitial cell hyperplasia was considered a direct effect of the test article. The lack of corpora lutea were considered an indirect effects of the test article as a result of deranged pituitary hormone secretion. In general, the uteri were inactive exhibiting morphology consistent with diestrus. Lesions were not identified in the uterus.

Long-Evans - The thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related findings in the ovary consisted of interstitial cell hyperplasia (6/6), depletion of corpora lutea (5/6), and follicular cysts (1/6). In general, the uteri were inactive exhibiting morphology consistent with diestrus.

In addition, some uteri were atrophied characterized by minimal gland development and cuboidal epithelium. Occasional uteri, exhibited tissue changes consistent with proestrus.

Pimozide (30 mg/kg/day)

Sprague-Dawley - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related changes in the ovary consisted of luteal cysts (2/6), luteinized follicles (4/6) and depleted corpora lutea (1/6). The majority of ovaries examined from both groups of rats were reduced in size. They were not considered atrophic, however, since follicular formation appeared normal. In addition, cells surrounding interstitial glands were polygonal with moderate amounts of eosinophilic granular cytoplasm characteristic of actively cycling ovaries. Most ovaries contained one to several corpora lutea. In both groups there appeared to be a tendency toward abnormal follicular maturation characterized by increased follicular atresia, luteal cysts, and partial lutinization of follicles. The ovarian changes are thought to be related to the test article most likely as a result of deranged pituitary hormone secretion. Test article related changes in the uterus consisted of atrophy (1/6), and epithelial hypertrophy/hyperplasia (1/6) and supportive endometritis (1/6). In general uteri appeared inactive and exhibited morphology consistent with diestrus.

Long-Evans - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related changes in the ovary consisted of follicular cysts (2/6), and luteinized follicles (3/6). Test article related changes were not evident in the uterus.

Group 7: Methoxychlor (100 mg/kg/day)

Sprague-Dawley - With the exception of occasional ultimobranchial cysts the thyroid glands were within normal limits. There were no test article related findings in the thyroid glands. Test article related findings in the ovary consisted of an absence of corpora lutea (2/6). This change may be related to ovarian atrophy secondary to deranged pituitary hormone secretion. Test article related findings in the uterus consisted of epithelial hypertrophy/hyperplasia (5/6) and myometrial hypertrophy/hyperplasia (1/6). Uteri consistently appeared as though they were in proestrus. In these uteri the myometrium was increased in thickness and the epithelium was tall columnar and hyperplastic. Only a few of the uteri appeared to be in an inactive state. The epithelial hypertrophy/hyperplasia is considered direct effects of the test article.

Long-Evans - There were no test article related findings in the thyroid glands. There were no test article related findings in the ovary. Test article related findings in the uterus consisted of epithelial hypertrophy/hyperplasia (4/6) and papilloma (1/6). The uterine changes are considered direct effects of the test article.

CONCLUSION

Eighty-four juvenile female rats (42 Sprague-Dawley rats and 42 Long-Evans rats) were randomly assigned to seven groups and gavaged daily for at least 21 days. Text Table 1 summarizes the group designations and dosage levels. Following at least 21 days of dosing, all animals were killed by decapitation. Protocol specified tissues were collected at necropsy and preserved. Thyroid, uterus, and ovaries from all animals were processed through paraffin and rendered to H&E stained ~5-micron sections, which were evaluated microscopically for pathological changes.

Under the conditions of this study, ethynyl estradiol, tamoxifen, ketoconazole, pimozide, and methoxychlor caused morphologic changes in the female reproductive organs (ovary and uterus) consistent with their respective reported actions. The Sprague-Dawley strain appeared to be more sensitive to changes associated with ethynyl estradiol and exhibited more consistent changes with this test article. In addition, changes in the uterus were more consistent with ethynyl estradiol. Changes associated with pimozide and methoxychlor were not consistent.

Propylthiouracil caused morphologic changes in the thyroid glands consistent with its reported action. Secondary changes in the ovary and uterus were considered to be a result of hypothyroidism.

Study Pathologist:

David N. Peters, DVM, PhD — Date: 20-JUNE00

Appendix 1:
Reports Code Table

PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

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 INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

Abbreviations List

# EX	Number Examined
1143101	1143-101
TK	Terminal Kill

II. Project Summary Tables

PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

PROJECT SUMMARY

STUDY ID : 1143-101
STUDY NUMBER: 1143101
FATE: TK Sprague-Dawley
SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)	5 (5)
NUMBER OF ANIMALS:		6	6	6	6	6
		#	#	#	#	#
THYROID	# EX	6	5	6	6	6
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0	0	6	0
ULTIMOBRANCHIAL CYST(S)		0	1	1	0	1
CYSTIC DILATION, FOLLICLE		0	0	0	0	0
COLLOID DEPLETION		0	0	0	6	0
OVARIES	# EX	6	6	6	6	6
CYST, PAROVARIAN		1	0	1	0	0
CYST, FOLLICULAR		0	1	0	0	1
ATROPHY		0	1	6	1	3
INTERSTITIAL CELL HYPERPLASIA		0	0	1	1	6
LUTEAL CYST		0	0	0	0	0
LUTEINIZED FOLLICLES		0	2	0	0	0
CORPORA LUTEA ABSENT		0	2	6	1	3
INACTIVE INTERSTITIAL GLANDS		0	1	5	0	0
UTERUS	# EX	6	6	6	6	6
SQUAMOUS METAPLASIA		0	1	3	0	0
EPITHELIAL HYPERTROPHY/HYPERPLASIA		0	3	0	0	0
SUPPURATIVE ENDOMETRITIS		0	0	0	0	0
MYOMETRIAL HYPERTROPHY/HYPERPLASIA		0	6	0	0	0
ATROPHY		0	0	6	3	0
EPITHELIAL HYPERTROPHY		0	0	6	0	0

(1) - Corn Oil (2.5ml/kg/d)
(2) - Ethynyl estradiol (0.005mg/kg/d)
(3) - Tamoxifen (10mg/kg/d)
(4) - Propylthiouracil (PTU) (240mg/kg/d)
(5) - Ketoconazole (100mg/kg/d)

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

PROJECT SUMMARY

STUDY ID : 1143-101 STUDY NUMBER: 1143101
FATE: TK Sprague-Dawley SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		6 (1)	7 (2)
NUMBER OF ANIMALS:		6	6
		#	#
THYROID	# EX	6	6
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0
ULTIMOBRANCHIAL CYST(S)		1	0
CYSTIC DILATION, FOLLICLE		0	1
COLLOID DEPLETION		0	0
OVARIES	# EX	6	6
CYST, PAROVARIAN		0	0
CYST, FOLLICULAR		0	0
ATROPHY		0	0
INTERSTITIAL CELL HYPERPLASIA		0	0
LUTEAL CYST		2	0
LUTEINIZED FOLLICLES		4	0
CORPORA LUTEA ABSENT		1	2
INACTIVE INTERSTITIAL GLANDS		0	0
UTERUS	# EX	6	6
SQUAMOUS METAPLASIA		0	0
EPITHELIAL HYPERTROPHY/HYPERPLASIA		1	5
SUPPURATIVE ENDOMETRITIS		1	0
MYOMETRIAL HYPERTROPHY/HYPERPLASIA		0	1
ATROPHY		1	0
EPITHELIAL HYPERTROPHY		0	0

(1) - Pimozide (30mg/kg/d) (2) - Methoxychlor (100mg/kg/d)

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

PROJECT SUMMARY

STUDY ID : 1143-101
FATE: TK Long-Evans

STUDY NUMBER: 1143101

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)	5 (5)
NUMBER OF ANIMALS:		6	6	6	6	6
		#	#	#	#	#
THYROID	# EX	5	6	6	6	6
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0	0	6	1
ULTIMOBRANCHIAL CYST(S)		1	1	2	0	0
COLLOID DEPLETION		0	0	0	6	0
OVARIES	# EX	6	6	6	6	6
CYST, FOLLICULAR		0	0	0	0	1
ATROPHY		0	2	6	2	5
INTERSTITIAL CELL HYPERPLASIA		0	0	5	2	6
LUTEAL CYST		0	0	0	1	0
LUTEINIZED FOLLICLES		0	1	0	0	0
CORPORA LUTEA ABSENT		0	2	6	2	5
INACTIVE INTERSTITIAL GLANDS		0	3	1	0	0
UTERUS	# EX	6	6	6	6	6
EPITHELIAL HYPERTROPHY/HYPERPLASIA		0	4	0	0	0
PAPILLOMA		0	0	0	0	0
MYOMETRIAL HYPERTROPHY/HYPERPLASIA		0	4	0	0	0
ATROPHY		0	0	6	4	2
EPITHELIAL HYPERTROPHY		0	0	6	0	0

(1) - Corn Oil (2.5ml/kg/d)
(2) - Ethynyl estradiol (0.005mg/kg/d)
(3) - Tamoxifen (10mg/kg/d)
(4) - Propylthiouracil (PTU) (240mg/kg/d)
(5) - Ketoconazole (100mg/kg/d)

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

PROJECT SUMMARY

STUDY ID : 1143-101 STUDY NUMBER: 1143101
FATE: TK Long-Evans SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		6 (1)	7 (2)
NUMBER OF ANIMALS:		6	6
		#	#
THYROID	# EX	5	6
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0
ULTIMOBRANCHIAL CYST(S)		1	0
COLLOID DEPLETION		0	0
OVARIES	# EX	6	6
CYST, FOLLICULAR		2	0
ATROPHY		1	0
INTERSTITIAL CELL HYPERPLASIA		0	1
LUTEAL CYST		0	0
LUTEINIZED FOLLICLES		3	0
CORPORA LUTEA ABSENT		0	0
INACTIVE INTERSTITIAL GLANDS		0	0
UTERUS	# EX	6	6
EPITHELIAL HYPERTROPHY/HYPERPLASIA		0	4
PAPILLOMA		0	1
MYOMETRIAL HYPERTROPHY/HYPERPLASIA		0	0
ATROPHY		0	0
EPITHELIAL HYPERTROPHY		0	0

(1) - Pimozide (30mg/kg/d) (2) - Methoxychlor (100mg/kg/d)

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III. Tabulated Animal Data

PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143101
GROUP: 1: Corn Oil (2.5ml/kg/d)
SEX: FEMALE

ANIMAL ID:	R15146	R15147	R15148	R15149	R15150	R15151
THYROID	N	N	N	N	N	N
OVARIES	N	N	N	-	N	N
CYST, PAROVARIAN	-	-	-	P	-	-
UTERUS	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions

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ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101 STUDY NUMBER: 1143101
FATE: TK Sprague-Dawley GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15158	R15159	R15160	R15161	R15162	R15163
THYROID	U	N	N	-	N	N
ULTIMOBRANCHIAL CYST(S)	-	-	-	(P)	-	-
OVARIES	-	-	-	N	-	-
CYST, FOLLICULAR	(P)	-	-	-	-	-
ATROPHY	-	-	P	-	-	-
LUTEINIZED FOLLICLES	-	<P>	-	-	(P)	-
CORPORA LUTEA ABSENT	-	-	P	-	-	P
INACTIVE INTERSTITIAL GLANDS	-	-	P	-	-	-
UTERUS	-	-	-	-	-	-
SQUAMOUS METAPLASIA	-	(P)	-	-	-	-
EPITHELIAL HYPERTROPHY/HYPERPLASIA	-	-	[P]	[P]	-	[P]
MYOMETRIAL HYPERTROPHY/HYPERPLASIA	P	P	P	[P]	[P]	[P]

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THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101 STUDY NUMBER: 1143101
FATE: TK Sprague-Dawley GROUP: 3: Tamoxifen (10mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15170	R15171	R15172	R15173	R15174	R15175
THYROID	N	N	-	N	N	N
ULTIMOBRANCHIAL CYST(S)	-	-	<P>	-	-	-
OVARIES	-	-	-	-	-	-
CYST, PAROVARIAN	-	P	-	-	-	-
ATROPHY	P	P	P	P	P	P
INTERSTITIAL CELL HYPERPLASIA	-	-	-	-	-	P
CORPORA LUTEA ABSENT	P	P	P	P	P	P
INACTIVE INTERSTITIAL GLANDS	P	P	P	P	P	-
UTERUS	-	-	-	-	-	-
SQUAMOUS METAPLASIA	P	-	-	-	P	P
ATROPHY	P	P	P	P	P	P
EPITHELIAL HYPERTROPHY	P	P	P	P	P	P

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101 STUDY NUMBER: 1143101
FATE: TK Sprague-Dawley GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15182	R15183	R15184	R15185	R15186	R15187
THYROID	-	-	-	-	-	-
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY	[4]	[4]	[4]	[4]	[4]	[4]
COLLOID DEPLETION	[4]	[4]	[4]	[4]	[4]	[4]
OVARIES	N	N	N	N	N	-
ATROPHY	-	-	-	-	-	P
INTERSTITIAL CELL HYPERPLASIA	-	-	-	-	-	P
CORPORA LUTEA ABSENT	-	-	-	-	-	P
UTERUS	N	N	-	N	-	-
ATROPHY	-	-	P	-	P	P
Non-Protocol Tissues:						
PITUITARY	-	-	*	-	-	-

See Reports Code Table for Symbol Definitions

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ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143101
GROUP: 5: Ketoconazole (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15194	R15195	R15196	R15197	R15198	R15199
THYROID	-	N	N	N	N	N
ULTIMOBRANCHIAL CYST(S)	(P)	-	-	-	-	-
OVARIES	-	-	-	-	-	-
CYST, FOLLICULAR	-	-	(P)	-	-	-
ATROPHY	-	P	P	P	-	-
INTERSTITIAL CELL HYPERPLASIA	P	P	P	P	[P]	P
CORPORA LUTEA ABSENT	-	P	P	P	-	-
UTERUS	N	N	N	N	N	N
Non-Protocol Tissues:						
ADRENALS	*	*	*	*	*	*

See Reports Code Table for Symbol Definitions

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143101
GROUP: 6: Pimozide (30mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15206	R15207	R15208	R15209	R15210	R15211
THYROID	-	N	N	N	N	N
ULTIMOBRANCHIAL CYST(S)	(P)	-	-	-	-	-
OVARIES	-	-	-	-	N	-
LUTEAL CYST	-	-	(P)	-	-	P
LUTEINIZED FOLLICLES	<P>	-	<P>	<P>	-	<P>
CORPORA LUTEA ABSENT	-	P	-	-	-	-
UTERUS	N	-	N	N	-	N
EPITHELIAL HYPERTROPHY/HYPERPLASIA	-	P	-	-	-	-
SUPPURATIVE ENDOMETRITIS	-	-	-	-	1	-
ATROPHY	-	P	-	-	-	-

See Reports Code Table for Symbol Definitions

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143101
GROUP: 7: Methoxychlor (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15218	R15219	R15220	R15221	R15222	R15223
THYROID	-	N	N	N	N	N
CYSTIC DILATION, FOLLICLE	P	-	-	-	-	-
OVARIES	-	N	-	N	N	N
CORPORA LUTEA ABSENT	P	-	P	-	-	-
UTERUS	-	-	-	N	-	-
EPITHELIAL HYPERTROPHY/HYPERPLASIA	[3]	[3]	[3]	-	[1]	[3]
MYOMETRIAL HYPERTROPHY/HYPERPLASIA	-	[2]	-	-	-	-

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Long-Evans

STUDY NUMBER: 1143101
GROUP: 1: Corn Oil (2.5ml/kg/d)
SEX: FEMALE

ANIMAL ID:	R15152	R15153	R15154	R15155	R15156	R15157
THYROID	N	U	N	N	-	N
ULTIMOBRANCHIAL CYST(S)	-	-	-	-	(P)	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N

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PATHOLOGY ASSOCIATES INTERNATIONAL
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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Long-Evans

STUDY NUMBER: 1143101
GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15164	R15165	R15166	R15167	R15168	R15169
THYROID	-	N	N	N	N	N
ULTIMOBRANCHIAL CYST(S)	P	-	-	-	-	-
OVARIES	N	-	-	N	N	-
ATROPHY	-	-	P	-	-	P
LUTEINIZED FOLLICLES	-	(P)	-	-	-	-
CORPORA LUTEA ABSENT	-	-	P	-	-	P
INACTIVE INTERSTITIAL GLANDS	-	P	P	-	-	P
UTERUS	-	-	N	-	N	-
EPITHELIAL HYPERTROPHY/HYPERPLASIA	[P]	[P]	-	[P]	-	[P]
MYOMETRIAL HYPERTROPHY/HYPERPLASIA	[P]	[P]	-	[P]	-	[P]

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ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Long-Evans

STUDY NUMBER: 1143101
GROUP: 3: Tamoxifen (10mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15176	R15177	R15178	R15179	R15180	R15181
THYROID	N	N	-	-	N	N
ULTIMOBRANCHIAL CYST(S)	-	-	P	P	-	-
OVARIES	-	-	-	-	-	-
ATROPHY	P	P	P	P	P	P
INTERSTITIAL CELL HYPERPLASIA	P	P	-	P	P	P
CORPORA LUTEA ABSENT	P	P	P	P	P	P
INACTIVE INTERSTITIAL GLANDS	-	-	P	-	-	-
UTERUS	-	-	-	-	-	-
ATROPHY	P	P	P	P	P	P
EPITHELIAL HYPERTROPHY	P	P	P	P	P	P

See Reports Code Table for Symbol Definitions

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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Long-Evans

STUDY NUMBER: 1143101
GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15188	R15189	R15190	R15191	R15192	R15193
THYROID	-	-	-	-	-	-
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY	[4]	[4]	[4]	[3]	[4]	[4]
COLLOID DEPLETION	[4]	[4]	[4]	[3]	[4]	[4]
OVARIES	N	N	-	-	-	N
ATROPHY	-	-	-	P	P	-
INTERSTITIAL CELL HYPERPLASIA	-	-	-	P	P	-
LUTEAL CYST	-	-	P	-	-	-
CORPORA LUTEA ABSENT	-	-	-	P	P	-
UTERUS	N	N	-	-	-	-
ATROPHY	-	-	P	P	P	P

See Reports Code Table for Symbol Definitions

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101 STUDY NUMBER: 1143101
FATE: TK Long-Evans GROUP: 5: Ketoconazole (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15200	R15201	R15202	R15203	R15204	R15205
THYROID	N	-	N	N	N	N
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY	-	(1)	-	-	-	-
OVARIES	-	-	-	-	-	-
CYST, FOLLICULAR	(P)	-	-	-	-	-
ATROPHY	P	-	P	P	P	P
INTERSTITIAL CELL HYPERPLASIA	[P]	P	[P]	[P]	[P]	[P]
CORPORA LUTEA ABSENT	P	-	P	P	P	P
UTERUS	N	N	N	-	N	-
ATROPHY	-	-	-	P	-	P
Non-Protocol Tissues:						
ADRENALS	*	*	-	*	-	*

See Reports Code Table for Symbol Definitions

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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Long-Evans

STUDY NUMBER: 1143101
GROUP: 6: Pimozide (30mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15212	R15213	R15214	R15215	R15216	R15217
THYROID	N	N	U	N	N	-
ULTIMOBRANCHIAL CYST(S)	-	-	-	-	-	P
OVARIES	-	N	-	-	-	-
CYST, FOLLICULAR	-	-	-	-	P	<P>
ATROPHY	-	-	-	-	P	-
LUTEINIZED FOLLICLES	P	-	(P)	P	-	-
UTERUS	N	N	N	N	N	N

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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

TABULATED ANIMAL DATA

STUDY ID : 1143-101
FATE: TK Long-Evans

STUDY NUMBER: 1143101
GROUP: 7: Methoxychlor (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15224	R15225	R15226	R15227	R15228	R15229
THYROID	N	N	N	N	N	N
OVARIES	-	N	N	N	N	N
INTERSTITIAL CELL HYPERPLASIA	P	-	-	-	-	-
UTERUS	N	-	-	N	-	-
EPITHELIAL HYPERTROPHY/HYPERPLASIA	-	[1]	[2]	-	[3]	[3]
PAPILLOMA	-	-	P	-	-	-

See Reports Code Table for Symbol Definitions

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IV. Correlation of Gross and Microscopic Findings

PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 1: Corn Oil (2.5ml/kg/d)

Animal ID: R15147 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, SLIGHT

Related Histopathology:
THYROID - No Corollary change detected

Animal ID: R15148 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
UTERUS WITH CERVIX - DISTENDED, BILATERAL, SEVERE

Related Histopathology:
UTERUS WITH CERVIX - No Corollary change detected

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101
STUDY NUMBER: 1143101
SEX: FEMALE
GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)

Animal ID: R15164
Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
UTERUS WITH CERVIX - UTERUS, DISTENDED, BILATERAL, SEVERE

Related Histopathology:
UTERUS - No Corollary change detected

Animal ID: R15167
Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
UTERUS WITH CERVIX - UTERUS, DISTENDED, BILATERAL, SEVERE

Related Histopathology:
UTERUS - No Corollary change detected

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 3: Tamoxifen (10mg/kg/d)

No Gross Observations for any animal in this group

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PATHOLOGY ASSOCIATES INTERNATIONAL
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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101 — STUDY NUMBER: 1143101
SEX: FEMALE — GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)

Animal ID: R15182 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, SEVERE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15184 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
PITUITARY - ENLARGED, SEVERE

Related Histopathology:
PITUITARY - Histopathology Not Required

Animal ID: R15185 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, SEVERE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15186 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, SEVERE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15187 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, SEVERE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)

Animal ID: R15188 Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
UTERUS - DISTENDED, BILATERAL, MODERATE	UTERUS - No Corollary change detected
THYROID - ENLARGED, SEVERE	THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15189 Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGED, SEVERE	THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15190 Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGED, SEVERE	THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15191 Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGED, MODERATE	THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15192 Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGED, SEVERE	THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101
SEX: FEMALE

STUDY NUMBER: 1143101
GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)

Animal ID: R15193
Animal Fate: TK Long-Evans

Pathologist: DNP

Reference to Necropsy Record:
THYROID - ENLARGED, SEVERE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15194 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
ADRENALS - ENLARGED, 3X3X3MM

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15195 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
ADRENALS - ENLARGED, 4X4X4MM

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15196 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
ADRENALS - ENLARGED, 4X4X4MM

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15197 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
ADRENALS - ENLARGED, 4X4X4MM

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15198 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
ADRENALS - ENLARGED, MODERATE

Related Histopathology:
ADRENALS - Histopathology Not Required

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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101
STUDY NUMBER: 1143101
SEX: FEMALE
GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15199
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
ADRENALS - ENLARGED, SLIGHT

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15200
Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
ADRENALS - ENLARGED, 3X3X3MM

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15201
Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
ADRENALS - ENLARGED, 3X3X3MM

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15203
Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
ADRENALS - ENLARGED, MODERATE

Related Histopathology:
ADRENALS - Histopathology Not Required

Animal ID: R15204
Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
UTERUS WITH CERVIX - UTERUS, DISTENDED, BILATERAL, MODERATE

Related Histopathology:
UTERUS - No Corollary change detected

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GRCUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15205 Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - UTERUS, SMALL, BILATERAL	UTERUS - Atrophy
ADRENALS - ENLARGED, MODERATE	ADRENALS - Histopathology Not Required
THYROID - ENLARGED, SLIGHT	THYROID - No Corollary Change Detected

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101
SEX: FEMALE

STUDY NUMBER: 1143101
GROUP: 6: Pimozide (30mg/kg/d)

No Gross Observations for any animal in this group

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-101 — STUDY NUMBER: 1143101
SEX: FEMALE — GROUP: 7: Methoxychlor (100mg/kg/d)

Animal ID: R15218 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - UTERUS, DISTENDED, BILATERAL, MODERATE	UTERUS - No Corollary change detected
THYROID - ENLARGED, MODERATE	THYROID - No Corollary change detected

Animal ID: R15219 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - UTERUS, DISTENDED, BILATERAL, MODERATE	UTERUS - No Corollary change detected

Animal ID: R15220 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - UTERUS, DISTENDED, BILATERAL, MODERATE	UTERUS - NO Corollary change detected

Animal ID: R15225 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - UTERUS, CYST, RIGHT HORN, ONE, CLEAR, 1X1X1MM	UTERUS - No Corollary change detected

Animal ID: R15228 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - UTERUS, DISTENDED, BILATERAL, MODERATE	UTERUS - No Corollary change detected

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V. Comment Report

PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 1: Corn Oil (2.5ml/kg/d)

No Comments for any animal in this group

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101
SEX: FEMALE

STUDY NUMBER: 1143101
GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)

No Comments for any animal in this group

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION
IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 3: Tamoxifen (10mg/kg/d)

Animal ID: R15173 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: MYOSITI, CHRONIC, FOCAL
OVARIES - ONLY ONE OVARY AVAILABLE FOR EXAMINATION

Animal ID: R15174 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
OVARIES - OVIDUCT: LARGE VACUOLES ARE PRESENT IN SEVERAL OVIDUCT CELLS.

Animal ID: R15178 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: MYOSITIS, CHRONIC, FOCAL

Animal ID: R15180 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
OVARIES - OVIDUCT: THERE ARE NUMEROUS VACUOLES PRESENT IN THE CYTOPLASM OF OVIDUCTAL EPITHELIUM.

PATHOLOGY ASSOCIATES INTERNATIONAL
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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)

Animal ID: R15190 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: FOCAL EPITHELIAL HYPERPLASIA IS PRESENT IN THE OVIDUCT.

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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15194 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: THERE IS FOCAL CYSTIC DILATION OF THE OVIDUCT. THERE IS MULTIFOCAL EPITHELIAL HYPERPLASIA OF THE OVIDUCT.

Animal ID: R15195 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - THERE IS A FOCAL AREA OF EPITHELIAL NEROSIS AND HYPERPLASIA IN THE TRACHEA.

Animal ID: R15196 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: THERE IS FOCAL HYPERPLASIA OF THE OVIDUCT EPITHELIUM.

Animal ID: R15199 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: THERE IS A FOCAL AREA OF EPTHELIAL HYPERPLAIA IN THE OVIDUCT.

Animal ID: R15202 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: THERE IS FOCAL EPITHELIAL HYPERPLASIA IN THE OVIDUCT.

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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15204 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
OVARIES - OVARY: THERE IS A FOCAL, PERIVASCULAR

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PATHOLOGY ASSOCIATES INTERNATIONAL
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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 6: Pimozide (30mg/kg/d)

Animal ID: R15208 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
OVARIES - OVIDUCT: THERE IS CYSTIC DILATION OF THE OVIDUCT.

Animal ID: R15209 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: THERE IS FOCAL EPITHELIAL HYPERPLASIA IN THE OVIDUCT.

Animal ID: R15210 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: THERE IS A FOCUS OF EPITHELIAL HYPERPLASIA IN THE OVIDUT.

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PATHOLOGY ASSOCIATES INTERNATIONAL
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IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-101

COMMENT REPORT

STUDY ID: 1143-101 STUDY NUMBER: 1143101
SEX: FEMALE GROUP: 7: Methoxychlor (100mg/kg/d)

Animal ID: R15221 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Animal Comments:
THERE IS MILD, DIFFUSE NECROTIZING AND SUPPURATIVE TRACHEITIS.

Animal ID: R15224 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
OVARIES - OVARY AND OVIDUCT: THERE IS CYSTIC DILATION OF THE OVIDUCT.

Animal ID: R15225 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: MYOSITIS, FOCAL, CHRONIC.

Animal ID: R15229 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
OVARIES - OVIDUCT: THERE IS CYSTIC DILATION OF THE OVIDUCT.

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VI. Quality Assurance Statement

Pathology Associates International
A Company of Science Applications International Corporation
An Employee-Owned Company

Pathology Report

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

TherImmune Research Corporation Study Number: 1143-101

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. Environmental Protection Agency (EPA-FIFRA). 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
02/09/00	Vaginal Cytology Staining	02/09/00
04/06,07/00	Individual Animal Data	04/07/00
04/06,07/00	Draft Pathology Report	04/07/00
06/15/00	Final Pathology Report	06/15/00

Karen E. Butler
Quality Assurance Officer

6/15/00
Date

15 Worman's Mill Court, Suite I • Frederick, Maryland 21701 • (301) 663-1644 • (301) 663-8994 FAX

APPENDIX 9

INDIVIDUAL SERUM T4 AND TSH LEVELS - SPRAGUE DAWLEY

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	T4, TOTAL (UG/DL)	TSH (NG/ML)
	GROUP 1 - 2.5 ML/KG/DAY CORN OIL	
R15146	4.44	1.66
R15147	3.31	1.48
R15148	3.42	1.80
R15149	3.75	2.32
R15150	3.33	1.07
R15151	3.27	1.70
	GROUP 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15158	4.73	1.42
R15159	3.98	2.30
R15160	3.31	1.19
R15161	3.62	1.71
R15162	4.06	1.65
R15163	3.35	1.43
	GROUP 3 - 10 MG/KG/DAY TAMOXIFEN	
R15170	5.89	1.78
R15171	6.10	2.06
R15172	5.28	2.58
R15173	5.99	1.83
R15174	5.66	2.72
R15175	5.93	2.15
	GROUP 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15182	0.00	27.40
R15183	0.01	25.69
R15184	0.00	23.40
R15185	0.10	21.42
R15186	0.00	33.12
R15187	0.00	29.60
	GROUP 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15194	5.46	1.80
R15195	3.03	2.21
R15196	2.63	1.99
R15197	5.34	1.57
R15198	3.82	1.62
R15199	3.37	1.11
	GROUP 6 - 30 MG/KG/DAY PIMOZIDE	
R15206	3.16	2.53
R15207	3.80	1.07
R15208	2.91	1.50
R15209	4.39	1.23
R15210	3.46	1.72
R15211	2.81	1.02
	GROUP 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15218	5.36	1.73
R15219	3.25	1.72
R15220	3.41	0.78
R15221	4.65	1.60
R15222	3.85	2.02
R15223	4.59	2.00

APPENDIX CONTINUED

APPENDIX 9 (CONTINUED)

INDIVIDUAL SERUM T4 AND TSH LEVELS - LONG EVANS

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	T4, TOTAL (UG/DL)	TSH (NG/ML)
	GROUP 1 - 2.5 ML/KG/DAY CORN OIL	
R15152	5.57	1.30
R15153	4.92	1.26
R15154	3.72	1.01
R15155	4.90	1.59
R15156	3.34	1.19
R15157	4.09	1.70
	GROUP 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15164	7.93	1.96
R15165	5.24	1.28
R15166	3.39	1.04
R15167	5.09	1.85
R15168	4.84	2.85
R15169	5.64	1.33
	GROUP 3 - 10 MG/KG/DAY TAMOXIFEN	
R15176	6.13	1.83
R15177	5.62	1.53
R15178	5.04	1.20
R15179	6.91	2.60
R15180	5.50	1.53
R15181	5.45	1.88
	GROUP 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15188	0.00	42.44
R15189	0.00	29.27
R15190	0.00	24.98
R15191	0.04	27.23
R15192	0.00	65.96
R15193	0.00	29.66
	GROUP 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15200	3.81	1.18
R15201	3.72	1.66
R15202	2.79	0.96
R15203	8.51	1.19
R15204	4.39	1.57
R15205	3.67	0.98
	GROUP 6 - 30 MG/KG/DAY PIMOZIDE	
R15212	4.28	1.70
R15213	3.41	1.39
R15214	3.19	1.63
R15215	3.18	0.85
R15216	2.85	2.50
R15217	3.12	1.31
	GROUP 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15224	4.80	1.28
R15225	4.28	1.09
R15226	4.89	1.16
R15227	3.42	1.26
R15228	4.67	1.89
R15229	4.03	1.11

APPENDIX 10
INDIVIDUAL DAY OF DEATH - SPRAGUE DAWLEY
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	DAY OF DEATH (PND)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL
R15146	42
R15147	42
R15148	42
R15149	43
R15150	43
R15151	43
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
R15158	42
R15159	42
R15160	42
R15161	43
R15162	43
R15163	43
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN
R15170	42
R15171	42
R15172	42
R15173	43
R15174	43
R15175	43
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL
R15182	42
R15183	42
R15184	42
R15185	43
R15186	43
R15187	43
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE
R15194	42
R15195	42
R15196	42
R15197	43
R15198	43
R15199	43
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE
R15206	42
R15207	42
R15208	42
R15209	43
R15210	43
R15211	43
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR
R15218	42
R15219	42
R15220	42
R15221	43
R15222	43
R15223	43

PND = POSTNATAL DAY

APPENDIX CONTINUED

APPENDIX 10 (CONTINUED)
INDIVIDUAL DAY OF DEATH - LONG EVANS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

ANIMAL ID	DAY OF DEATH (PND)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL
R15152	42
R15153	42
R15154	42
R15155	43
R15156	43
R15157	43
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
R15164	42
R15165	42
R15166	42
R15167	43
R15168	43
R15169	43
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN
R15176	42
R15177	42
R15178	42
R15179	43
R15180	43
R15181	43
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL
R15188	42
R15189	42
R15190	42
R15191	43
R15192	43
R15193	43
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE
R15200	42
R15201	42
R15202	42
R15203	43
R15204	43
R15205	43
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE
R15212	42
R15213	42
R15214	42
R15215	43
R15216	43
R15217	43
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR
R15224	42
R15225	42
R15226	42
R15227	43
R15228	43
R15229	43

PND = POSTNATAL DAY

APPENDIX 11
STATISTICAL ANALYSIS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

Experiment R114301 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Table 1
Test of Homogeneity of Variance Over All Groups
Brown-Forsyth Version of Levene's Test

Dependent	ProbF	Transformation
TERM	0.2796	
LIVER	0.1842	
KIDNEY	0.4405	
UTERUS	0.0829	Logarithm
UTERUSD	0.0318	No transformation successful
OVARY	0.3396	1/ovary
ADREN	0.5593	
PIT	0.5705	
T4	0.3799	Logarithm(t4+.01)
TSH	0.8637	Logarithm
VAGOP	0.0890	Logarithm(vagop-20)
ESTRUS	0.0022	No transformation successful

Experiment R114301 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Table 2
Analysis of Covariance: Test for PND22 x Dose Interaction

Dependent	ProbF
TERM	0.0815
LIVER	0.2003
KIDNEY	0.2543
UTERUS	0.3903
OVARY	0.7613
ADREN	0.9048
PIT	0.8986
T4	0.9514
TSH	0.7285
VAGOP	0.2564

Experiment R114301 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Table 3
Results of MANCOVA for All Endpoints

Hypothesis	ProbF
Control_vs_Dose2	<.0001
Control_vs_Dose3	<.0001
Control_vs_Dose4	<.0001
Control_vs_Dose5	<.0001
Control_vs_Dose6	0.0101
Control_vs_Dose7	<.0001

Experiment R1143-101 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Table 1
Test of Homogeneity of Variance Over All Groups
Brown-Forsyth Version of Levene's Test

Dependent	ProbF
TERM	0.2796
LIVER	0.1842
KIDNEY	0.4405
UTERUS	0.0829
OVARY	0.3396
ADREN	0.5593
PIT	0.5705
T4	0.3799
TSH	0.8637
VAGOP	0.0890

Experiment R1143-101 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Table 2
Analysis of Covariance: Test for PND22 x Dose Interaction

Dependent	ProbF
TERM	0.0815
LIVER	0.2003
KIDNEY	0.2543
UTERUS	0.3903
OVARY	0.7613
ADREN	0.9048
PIT	0.8986
T4	0.9514
TSH	0.7285
VAGOP	0.2564

Experiment R1143-101 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Table 3
Results of MANCOVA for All Endpoints

Hypothesis	ProbF
Control_vs_Dose2	<.0001
Control_vs_Dose3	<.0001
Control_vs_Dose4	<.0001
Control_vs_Dose5	<.0001
Control_vs_Dose6	0.0101
Control_vs_Dose7	<.0001

Experiment R1143-101 - Sprague Dawley Juvenile Female Rats
Ratio Data
Table 4
Ratios: Test of Homogeneity of Variance Over All Groups
Ratios: Brown-Forsyth Version of Levene's Test

Dependent	ProbF
LIVER	0.1060
KIDNEY	0.7433
UTERUS	0.1448
UTERUSD	0.1518
OVARY	0.1076
ADREN	0.9501
PIT	0.6086

Experiment R1143-101 - Sprague Dawley Juvenile Female Rats
Ratio Data
Table 5
Analysis of Covariance: Test for Weaning Body Weight x Dose Inter

Dependent	ProbF
LIVER	0.2786
KIDNEY	0.5661
UTERUS	0.1386
UTERUSD	0.4185
OVARY	0.9907
ADREN	0.8255
PIT	0.9106

Experiment R1143-101 - Sprague Dawley Juvenile Female Rats
Ratio Data
Table 6
Ratios: Results of MANCOVA

Hypothesis	ProbF
Control_vs_Dose2	0.5226
Control_vs_Dose3	0.0109
Control_vs_Dose4	0.1223
Control_vs_Dose5	<.0001
Control_vs_Dose6	0.9884
Control_vs_Dose7	0.7565

Experiment R1143-101 - Long Evans Juvenile Female Rats
Body and Organ Weights
Table 1
Test of Homogeneity of Variance Over All Groups
Brown-Forsyth Version of Levene's Test

Dependent	ProbF
TERM	0.8489
LIVER	0.1843
KIDNEY	0.6934
UTERUS	0.2708
UTERUSD	0.2887
OVARY	0.1296
ADREN	0.6254
PIT	0.6417
t4	0.3716
TSH	0.7294
VAGOP	0.2347
ESTRUS	0.2295

Experiment R1143-101 - Long Evans Juvenile Female Rats
Body and Organ Weights
Table 2
Analysis of Covariance: Test for PND22 x Dose Interaction

Dependent	ProbF
TERM	0.4762
LIVER	0.4740
KIDNEY	0.5254
UTERUS	0.6918
UTERUSD	0.6612
OVARY	0.0597
ADREN	0.1953
PIT	0.6530
t4	0.5911
TSH	0.7055
VAGOP	0.0867
ESTRUS	0.6257

Experiment R1143-101 - Long Evans Juvenile Female Rats
Body and Organ Weights
Table 3
Results of MANCOVA for All Endpoints

Hypothesis	ProbF
Control_vs_Dose2	<.0001
Control_vs_Dose3	<.0001
Control_vs_Dose4	<.0001
Control_vs_Dose5	<.0001
Control_vs_Dose6	<.0001
Control_vs_Dose7	<.0001

Experiment R1143-101 - Long Evans Juvenile Female Rats
Ratio Data
Table 4
Ratios: Test of Homogeneity of Variance Over All Groups
Ratios: Brown-Forsyth Version of Levene's Test

Dependent	ProbF
LIVER	0.8172
KIDNEY	0.3588
UTERUS	0.2267
UTERUSD	0.1765
OVARY	0.3070
ADREN	0.3498
PIT	0.6997

Experiment R1143-101 - Long Evans Juvenile Female Rats
Ratio Data
Table 5
Analysis of Covariance: Test for Weaning Body Weight x Dose Inter

Dependent	ProbF
LIVER	0.6319
KIDNEY	0.8844
UTERUS	0.6417
UTERUSD	0.6129
OVARY	0.0672
ADREN	0.4402
PIT	0.4705

Experiment R1143-101 - Long Evans Juvenile Female Rats
Ratio Data
Table 6
Ratios: Results of MANCOVA

Hypothesis	ProbF
Control_vs_Dose2	0.0174
Control_vs_Dose3	0.0217
Control_vs_Dose4	0.0627
Control_vs_Dose5	0.0013
Control_vs_Dose6	0.0774
Control_vs_Dose7	0.3170

Experiment R114301 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Results of Pairwise Analysis of Covariance for All Endpoints
Least Squares Means Adjusted for Covariate Weaning Body Weight

Endpoint	dose1	dose2	star2	dose3	star3	dose4	star4	dose5	star5	dose6	star6	dose7	star7
ADREN	0.041	0.041		0.031	**	0.023	**	0.078	**	0.034		0.035	
ESTRUS $	35.167	26.000	**	43.500	**	38.167	*	41.667	**	35.500		29.000	**
KIDNEY	1.238	1.177		1.129		0.751	**	1.361	*	1.073	**	1.139	
LIVER	6.140	6.096		5.290	*	4.261	**	7.311	**	5.479		5.370	*
OVARY	0.058	0.046		0.037		0.031	*	0.050		0.056		0.042	
PIT	0.008	0.009		0.005		0.004		0.010		0.008		0.006	
T4	3.566	3.813		5.804	*	0.007	**	3.798		3.378		4.118	
TERM	147.493	138.526	*	121.326	**	100.276	**	143.993		129.409	**	137.993	*
TSH	1.629	1.582		2.159		26.491	**	1.678		1.436		1.570	
UTERUS	0.238	0.262		0.116	**	0.145	**	0.188		0.207		0.288	
UTERUSD $	0.221	0.239		0.107	**	0.133		0.175		0.186		0.234	
VAGOP	34.330	26.002	**	26.297	**	36.559		36.047		33.445		26.294	**

$ Endpoints excluded from MANCOVA. Unadjusted means reported with results of Wilcoxon Rank Sum test.

TABLE (1143-101)
ADJUSTED MEANS FROM PAIRWISE ANALYSIS OF COVARIANCE (ORGAN-TO-BODY WEIGHT RATIOS)
PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (SPRAGUE-DAWLEY)

	DOSE GROUP						
ORGAN	CORN OIL	ETHYNYL ESTRADIOL	TAMOXIFEN	PROPYLTHIOURACIL	KETOCONAZOLE	PIMOZIDE	METHOXYCHLOR
ADRENALS	0.028	0.029	0.026	0.023	**0.054	0.027	0.025
KIDNEYS	0.840	0.848	**0.930	0.751	**0.945	0.829	0.824
LIVER	4.159	4.376	4.359	4.249	**5.077	4.225	3.882
OVARIES	0.042	0.036	0.034	0.034	0.060	0.044	0.035
PITUITARY	0.005	0.007	0.004	0.004	0.007	0.006	0.004
UTERUS	0.174	0.192	**0.096	0.153	0.132	0.161	0.218
UTERUS-DRY	0.149	0.174	**0.088	0.133	0.122	0.144	0.169

TABLE (1143-101)
ADJUSTED MEANS FROM ANALYSIS OF COVARIANCE
PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

	DOSE GROUP						
	CORN OIL	ETHYNYL ESTRADIOL	TAMOXIFEN	PROPYLTHIOURACIL	KETOCONAZOLE	PIMOZIDE	METHOXYCHLOR
ADRENALS	0.038	0.036	0.034	0.030	**0.069	0.037	0.038
ESTRUS	37.165	**25.668	**43.505	35.664	*41.664	37.834	**31.167
KIDNEYS	1.534	**1.261	**1.259	**0.786	1.483	**1.124	**1.341
LIVER	7.115	**6.107	**5.950	**3.780	7.395	**5.523	**5.850
OVARIES	0.083	*0.059	**0.036	**0.044	*0.063	**0.051	0.078
PITUITARY	0.006	0.006	*0.004	0.005	0.006	0.007	0.006
T4, TOTAL	4.424	5.354	*5.773	**0.008	4.483	3.338	4.348
TERMINAL	164.654	**144.230	**129.306	**90.440	**150.056	**123.549	**140.516
TSH	1.320	1.624	1.715	**34.346	1.227	1.485	1.274
UTERUS	0.263	0.346	**0.100	**0.140	0.209	*0.155	0.261
UTERUS-DRY	0.218	0.275	**0.090	**0.116	0.176	*0.143	0.233
VAGINAL OP.	36.462	**25.589	**26.147	34.117	35.021	36.291	**26.140

TABLE (1143-101)
ADJUSTED MEANS FROM PAIRWISE ANALYSIS OF COVARIANCE (ORGAN-TO-BODY WEIGHT RATIOS)
PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

	DOSE GROUP						
ORGAN	CORN OIL	ETHYNYL ESTRADIOL	TAMOXIFEN	PROPYLTHIOURACIL	KETOCONAZOLE	PIMOZIDE	METHOXYCHLOR
ADRENALS	0.024	0.025	0.026	0.033	**0.046	0.030	0.027
KIDNEYS	0.915	0.875	*0.973	0.869	*0.988	0.912	0.955
LIVER	4.310	4.234	4.591	4.176	**4.914	4.475	4.163
OVARIES	0.048	0.041	**0.028	0.050	0.042	0.042	0.055
PITUITARY	0.004	0.004	0.003	0.006	0.004	0.006	0.004
UTERUS	0.162	0.252	0.078	0.173	0.154	0.129	0.195
UTERUS-DRY	0.129	*0.195	*0.071	0.136	0.126	0.119	0.169

APPENDIX 12
PROTOCOL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

THERIMMUNE
Research Corporation

STUDY PROTOCOL

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

APPROVED:

TherImmune Research Corporation:

M S. Rocca 12.14.99
Meredith S. Rocca, Ph.D. Date
Study Director

EPA:

H 12/5/99
Kenneth H. Elstein Date
Project Officer

REVIEWED:

12-07-99
Carole L. Brown Date
Quality Assurance Auditor

EPA Requisition No. AC5001 - TherImmune No. 1143-101
EPA Reference No. QT-RT-99-002276

PROTOCOL

I. Study Title

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

II. Purpose

The purpose of this protocol is to quantify the effects of environmental compounds on pubertal development and thyroid function in the intact juvenile female rat. The larger goal is to use this study and its replicate (1143-103) to: 1) provide preliminary validation of the protocol for future EPA studies and 2) assess intra-laboratory and inter-strain variation.

III. Study Location

TherImmune Research Corporation (TherImmune)
15 Firstfield Road
Gaithersburg, Maryland 20878
Phone: 301-330-3737
Fax: 301-330-3738

IV. Sponsor and Address

Environmental Protection Agency
Kenneth H. Elstein, Project Officer
Phone: 919-541-3581 Fax: 919-541-1499
Julio E. Lopez, Contracting/Ordering Officer
Phone: 919-541-4474 Fax: 919-541-4273
RTP: MD-71 NHEERL
Research Triangle Park, NC 27711

V. TherImmune Staff

A. Principal Investigator	Gary W. Wolfe, Ph.D., D.A.B.T.
B. Study Director	Meredith S. Rocca, Ph.D.
C. Pathologist	John M. Pletcher, D.V.M., M.P.H., D.A.C.V.P., D.A.C.V.P.M.
D. Quality Assurance Director	James Carignan, B.S.
E. Veterinarian	Edward T. Greenstein, D.V.M., A.C.L.A.M.

VI. Regulatory Compliance

This study will be conducted in accordance with the EPA FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

VII. **Quality Assurance**

The protocol, in-life phases, and the final report will be audited by Quality Assurance in accordance with TherImmune Standard Operating Procedures. Data will be examined for completeness, consistency, and proper documentation.

VIII. **Proposed Study Timetable**

Initiation of Dosing:	January 7, 2000
Last Terminal Sacrifice:	January 27, 2000
Progress Report:	December 31, 1999
Draft Report	February 25, 2000
Final Report:	April 28, 2000

IX. **Test Articles**

A. **Identification**

Vehicle:	Corn oil
Test Article 1:	Ethynyl estradiol
Test Article 2:	Tamoxifen
Test Article 3:	Propylthiouracil (PTU)
Test Article 4:	Ketoconazole
Test Article 5:	Pimozide
Test Article 6:	Methoxychlor

B. **Purity**

Purity will be provided by the supplier.

C. **Characteristics**

Information on the methods of synthesis and stability, as well as data on composition or other characteristics which define the test articles, is on file with the manufacturer.

D. **Reserve Samples**

1. A sample of each reagent as provided by the vendor in the following quantities:
   100 mg each of tamoxifen, propylthiouracil, and ketoconazole
   1 g each of ethynyl estradiol, pimozide and methoxychlor

2. 1 ml of the initial stock solution made from the reagent (if applicable).

3. 1 ml of the first and last dosing solutions administered to the animals.

Samples shall be stored according to the manufacturer's recommendations to minimize degradation. Samples shall be stored for at least six months after the final report is issued, or sent to the Sponsor on request.

X. **Husbandry**

A. **Housing**

Animals will be housed in polycarbonate boxes with Sani-Chip Hardwood laboratory bedding as follows:

Pregnant/lactating females: 1/cage
Juvenile females 3/cage, if possible

B. **Food**

Teklad 7012 Certified Rodent Diet will be provided *ad libitum*. Fresh food will be provided weekly.
Feed is analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. Specified nutrients analyses are on file at TherImmune.

C. **Water**

Tap water will be provided *ad libitum* via an automatic watering system or water bottles. The water is routinely analyzed for contaminants and specific microbes. The results of these analyses are on file at TherImmune.

D. **Contaminants**

The Study Director and/or Sponsor have considered possible interfering substances potentially present in animal feed and water, including the test material itself or possible structurally related materials as well as the items listed in (B) and (C) above. None of these contaminants are reasonably expected to be present in animal feed or water at levels sufficient to interfere with this study.

E. **Environment**

The targeted temperature range is 20- 24° C with a relative humidity of 40-50%. Temperature and humidity are monitored continuously. A 14-hour light/10-hour dark cycle (lights on at 0500 h, off at 1900 h), will be maintained. Ten or greater air changes/hour will be maintained.

F. **Acclimation**

Pregnant females will be acclimated to the facility for approximately 7 days prior to expected parturition. Animals will be observed for general health and suitability for testing during this period. Animals that are diseased or unsuitable for testing will be removed from the study.

XI. **Experimental Design - Production of Juvenile Animals**

This section describes procedures for producing the juvenile animals which will be used as study animals on protocols 1143-100 and 1143-101. Briefly, one set of timed pregnant females will arrive at TherImmune on Gestation Day (GD) 12 and will be used for both protocols. The females will be allowed to deliver and rear pups. At weaning, the female pups will be used on this protocol and the male pups on protocol 1143-100.

A. **Animals**

1. **Strain/Source**

Hsd: Sprague Dawley®SD® Rats
Harlan Sprague Dawley, Inc., Indianapolis, IN

Long-Evans Hooded Rats
Harlan Sprague Dawley, Inc., Indianapolis, IN

2. **Number/Sex**

20 timed pregnant Sprague-Dawley females
20 timed pregnant Long-Evans females

3. **Identification**

Females will be identified by individual ear tag and cage label.

4. **Justification**

Rats will be used because of the extensive historical data base.

B. **Observation of Animals**

1. **Clinical Observations**

Clinical observations for mortality and morbundity will be performed twice daily by cage-side observation.

2. **Litter Observations**

a. Parturition

Pregnant females will be observed at least twice daily for signs of parturition.

b. Body Weights

Pups will be weighed on post-natal day PND 1 and weekly thereafter. (The objective is to identify runted pups and unthrifty litters; pups will not be individually identified.)

c. Culling

On PND 3 or 4, litters will be culled to 8 to 10 pups (approximately equal numbers of male and females pups, when possible). Culled pups will be euthanized with sodium pentobarbital overdose.

d. Weaning

Pups will be weaned on PND 21.

3. **Terminal Sacrifice/Necropsy - Dams and Untreated Pups**

a. Unscheduled Sacrifices and Deaths

Moribund dams will be anesthetized via carbon dioxide inhalation and discarded without necropsy.

Moribund pups will be sacrificed with sodium pentobarbital overdose or carbon dioxide inhalation, and discarded without necropsy.

Animals found dead will be discarded without necropsy.

b. Scheduled Sacrifices

After total litter loss or litter weaning on PND 21, dams will be anesthetized via carbon dioxide inhalation and discarded without necropsy.

Culled pups will be euthanized with sodium pentobarbital overdose, and discarded without necropsy.

C. **Selection of Study Animals**

On PND 21, female pups will be weighed to the nearest 0.1 g, weight ranked and assigned to groups using computer-generated random numbers. At the time of randomization, the weight variation of each female used should not exceed 8 grams above or below the mean weight, and the mean body weights for each group will not be statistically different. Unthrifty or runted pups will not be selected.

Procedures for selected females are described in Section XII.

Unselected female pups may be returned to the animal colony or sacrificed by carbon dioxide inhalation and discarded without necropsy. Male pups will be used on Protocol 1143-100.

XII. **Experimental Design - Treatment and Assessment of Juvenile Animals**
The procedures described below will be performed on both strains of rats concurrently to compare inter-strain variability.

A. **Animals**

1. **Number/Sex**
42 Sprague-Dawley females
42 Long-Evans females

2. **Identification**
Individual ear tag and cage label.

B. **Group Designation and Dosage Levels**

Group	Treatment	Dosage (per kg/day)	# of females per strain
1	Corn Oil	2.5 ml	6
2	Ethynyl estradiol	0.005 mg	6
3	Tamoxifen	10 mg	6
4	Propylthiouracil (PTU)	240 mg	6
5	Ketoconazole	100 mg	6
6	Pimozide	30 mg	6
7	Methoxychlor	100 mg	6

C. **Dosing Procedures**

1. **Method of Administration**
Oral gavage, using an 18-gauge gavage needle (1" long, with a 2.25 mm ball) and a 1 cc glass tuberculin syringe for each treatment.

2. **Frequency**
Daily, between 0700 and 0900 h, PND 22 through 42 or 43

3. **Volume**
   2.5 ml/kg body weight, adjusted on a daily basis.

4. **Formulations**
   Test articles will be suspended in corn oil.

5. **Absorption**
   Toxic or pathologic effects will serve as evidence of absorption.

D. **Observation of Animals**

1. **Clinical Observations**
   Clinical observations for mortality and morbundity will be performed twice daily by cage-side observation.

2. **Physical Examinations**
   Detailed clinical observations will be performed weekly.

3. **Body Weights**
   Rats will be weighed daily. Body weight on the day of complete vaginal opening will also be noted.

4. **Food Consumption**
   Not required.

5. **Water Consumption**
   Not required.

6. **Vaginal Opening**
   Females will be examined daily for vaginal opening beginning on PND 22. The appearance of a small "pinhole", a vaginal thread and complete vaginal opening will be recorded on the days observed. The day of complete vaginal opening will be used for analysis.

7. **Vaginal Cytology**
   Following vaginal opening, daily vaginal smears will be taken, stained and examined for stage of estrous.

E. **Termination**

1. **Unscheduled Sacrifices and Deaths**
Necropsies will be conducted on all moribund animals and on all animals not surviving to termination. Moribund animals will be weighed and killed by decapitation. Trunk blood and tissues will be collected as described below. Animals will be necropsied as close as possible to the time of death.

2. **Terminal Sacrifice**
Between 1300 and 1700 h on PND 42 or 43, all surviving animals will be killed by decapitation. Decapitation shall occur in a room separate from the housing area and within 15 seconds of removing the animal from its cage.

F. **Postmortem Procedures**

1. **Serum Collection and Analysis**
Trunk blood (supplemented by cardiac puncture, if necessary) will be collected immediately after decapitation. Serum will be separated by centrifugation.
A minimum of 500 µl/animal will be aliquoted into 1.7 ml siliconized microcentrifuge tubes, stored at -20° C, and shipped by express carrier to:

Dr. Ralph Cooper
US EPA/NHEERL/RTD, MD-72
2525 NC Highway 54
Durham, NC 27713.

A minimum of 550 µl/animal will be aliquoted into 1 ml microcentrifuge tubes, stored at -20° C, and shipped to Ani Lytics (Gaithersburg, MD) for T4 and TSH analysis. Low, medium and high internal RIA standards will be used for each assay.

2. **Gross Necropsy**
All animals will be subjected to a full gross necropsy, which includes examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents.

3. **Organ Weights**
Connective tissue and fat shall be carefully removed from the following tissues using small surgical scissors. The following organs will be weighed immediately after dissection to avoid drying of the trimmed tissues.

(1) ovaries

(2) uterus with cervix
(3) thyroid
(4) liver
(5) kidney
(6) pituitary
(7) adrenals

The uterus will then be placed on a paper towel, slit to allow the fluid contents to leak out, gently blotted dry and reweighed.

4. **Tissue Preservation**
The thyroid, ovaries and uterus will be placed in Bouin's fixative for approximately 24 hours, after which they shall be rinsed and stored in 70% ethanol.

5. **Histopathology**
The preserved thyroid, ovaries and uterus from all animals will be embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically by a pathologist at Pathology Associates International.

XII. Final Report

At termination of the study, a final report which includes the following information (as appropriate) will be prepared and submitted:

A. **Abstract**

B. **Experimental Design and Methods**

C. **Results**
1. mortality
2. clinical observations
3. body weights
4. age and weight at vaginal opening
5. estrous cycling
6. gross pathology
7. organ weights and organ/body ratios
8. histopathology
9. serum T4 and TSH

D. **Statistical Analyses**

Data shall be analyzed using multivariate analysis of covariance (MANCOVA), using body weight at weaning as a covariate. If the treatment x body weight at interaction is not significant, then the intercepts shall be tested for difference among treatments using a two-tailed test. If serum hormone levels, or any other data, display heterogeneity of variance, then appropriate data transformations (i.e. log transformation) shall be employed.

E. **Statistical Evaluation** (as deemed appropriate)

1. age and weight at vaginal opening
2. age at first estrus
3. body weight
4. organ weights and organ/body weight ratios
5. serum T4 and TSH

F. **Tables** (including mean, standard error, and sample size)

1. mean age and weight at vaginal opening
2. mean age at first estrus
3. mean daily body weight
4. mean body weight change from PND 21 to necropsy
5. summary of clinical signs for each test group to include a list of each findings and number of animals affected
6. mean serum T4 and TSH
7. mean organ weights and organ to body weight ratios
8. summary incidence of gross pathology findings
9. summary incidence of histopathology findings

G. **Appendices**

1. day of death for each animal
2. individual age and weight at vaginal opening
3. individual vaginal cytology
4. individual body weights
5. individual clinical signs for each animal to include the week of observation of each sign, a description of each sign and its subsequent course
6. individual serum T4 and TSH
7. individual organ weights and organ to body weight ratios
8. individual gross pathology findings
9. individual histopathology findings

XIII. **Record Retention**

All study records, study protocols, final reports, protocol and report revisions, and any written letters, memorandums or communications concerning the conduct of the study

shall be retained at the TherImmune Archive for at least one year from study completion. Documentation of any transfer of study records, specimens, and reports will be maintained by TherImmune for a period of one year.

XIV. **Amendments**

Amendments to this protocol will be approved by the EPA Project Officer, justified, dated, and signed by the Study Director. Amendments will include a statement noting the impact, if any, on the study.

XV. **Deviations**

Deviations from the GLP Regulations, Protocol, and Standard Operating Procedures will be immediately reported to the TherImmune Study Director. The Study Director will note in the study records any deviation, the effect of the deviation on the study, any corrective action taken, and will inform the EPA Project Officer.

PROTOCOL AMENDMENT

TherImmune No.: 1143-101	
AMENDMENT NUMBER: 1	
STUDY TITLE: Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats	
DISTRIBUTION:	
STUDY DIRECTORS/Wolfe and Rocca **OPERATIONS DIRECTOR/Morgan** **FACILITY MANAGER/Blackford** **TECHNICAL SUPERVISOR/Hatcher** **VETERINARIAN/Greenstein** **QUALITY ASSURANCE/Carignan** **SPONSOR/Elstein** **HEALTH AND SAFETY OFFICER/Blackford** **ANALYTICAL CHEM/NA** **SALES-MARKETING/Zemo**	**STUDY NOTEBOOK/Musselman (2)** **CENTRAL FILE/Wolfe** **DOSE PREPARATION/Nyakiti** **IACUC CHAIR/Rocca** **PROJECT LEADER /Borst/Pepperl** **NECROPSY/Hackett** **PAI/Delaney (3)** **HEAD TECH/Musselman** **CONTRACTS/Allen**
ORIGINAL FILED IN QA	
SPONSOR AUTHORIZATION: 1/24/00 e-mail from Kenneth Elstein	

1. Subject: Organ Weights (XII, F, 3)

The thyroids will not be weighed. The thyroids with parathyroids, trachea and esophagus attached will be submitted for histology.

Justification: Thyroids weights were deemed unnecessary as hormone profiles and histology will provide more meaningful data on thyroid effects.

2. Subject: Serum Collection (XII, F, 1)

All serum samples will be aliquoted into 1.7 ml siliconized microcentrifuge tubes and stored at ~-80°C.

Justification: One type of tube is being used for both serum samples for consistency. Samples are being stored at a lower temperature for better preservation.

Approval:

M S. Rocca 1 26 00

Meredith S. Rocca, Ph.D. Date