Final Report - 103

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

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Study Completion Date:
June 29, 2000

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TherImmune Study Number:
1143-103

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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:

Meredith S. Rocca 6.29.00

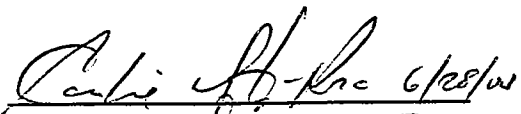
Meredith S. Rocca, Ph.D./Date

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. Brown
Phase Inspection 01/14/00	01/31/00	C. Brown
Final Report and Data Audit 05/01-03,08-12/00	05/12/00	C. Matos-Rosa
Post-Audit 06/22,23,26/00	06/26/00	C. Matos-Rosa

Quality Assurance Unit:



C. Matos-Rosa /Date

STUDY IDENTIFICATION

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

TherImmune Study Number:	1143-103
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 14, 2000
Experimental Termination:	February 4, 2000

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

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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. Females 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	↓	↓	↓	✓		↓SD
Tamoxifen	↓	↓	↑	✓	↑SD	↑
Propylthiouracil	↓	↑SD	↑SD	✓	↑	↓
Ketoconazole	↓	↑SD	↑SD	✓		↓
Pimozide	↓	↑		✓	↓LE	↓
Methoxychlor	↓	↓	↓ SD	✓	↓LE	↓SD

KEY: ↓ = significantly decreased compared to control mean LE = Long Evans only
↑ = significantly increased compared to control mean SD = Sprague Dawley only
✓ = affected histopathology 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 14, 2000. The last terminal necropsies were performed on February 4, 2000.

TEST AND CONTROL ARTICLES

The control article, corn oil, and 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. 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 of ethynyl estradiol; and a 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-four timed-pregnant female Hsd: Sprague Dawley® SD® Rats and twenty timed-pregnant female Long-Evans Hooded Rats, were received on December 14, 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*. 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 (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. 1143-102).

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. The weight variation of selected females did not exceed 4 and 5 grams above or below the mean body weight for Sprague Dawley (SD) rats and Long-Evans (LE) rats respectively, and 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 1143-102. This protocol was conducted as a "blind study" - with the technicians performing the study activities having 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 first use. 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, 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 and continuing through the day of necropsy, 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.

The groups for which the null hypothesis could not be rejected by MANCOVA for organ to body weight ratios were PTU (LE only), 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 protocol, report, protocol and report revisions, written communications, 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 9. 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

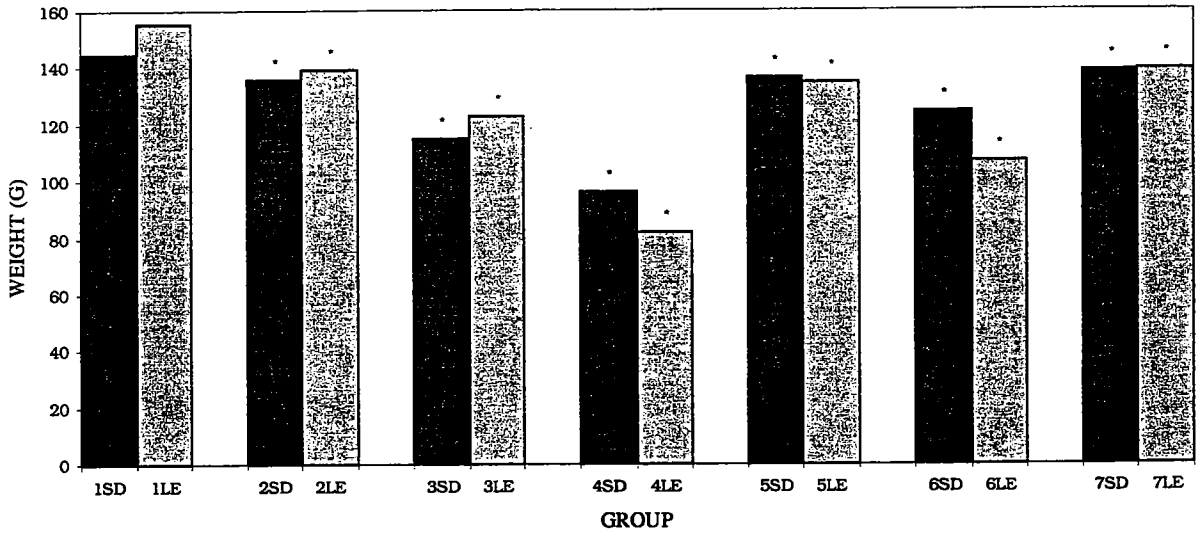
Mean daily body weights are shown in Figure 2, summarized in Table 2 and presented individually in Appendix 2. Mean body weight changes from PND 21 to necropsy are summarized in Table 3.

The mean body weight of females treated with corn oil (control), ethynyl estradiol, tamoxifen, and methoxychlor increased daily, although the mean body weight of tamoxifen-treated females was significantly lower than controls from PND 27 or 28 until termination. The PND 42 mean body weight of tamoxifen-treated females was only 79% of their control groups.

In contrast, females treated with PTU, ketoconazole (SD only), and pimozide lost weight during a portion of the treatment period. The most severe weight loss was in pimozide-treated females whose mean body weight decreased 14.1% for SD and 12.3% for LE from PND 22 to 24. Body weight in the pimozide group then steadily increased (except on PND 29 and 33 for LE), but remained significantly lower than controls, being only 85.8 and 68.9% of control mean body weights on PND 42 for SD and LE females, respectively. Ketoconazole-treated SD females lost 2.38 g on PND 29 and gained steadily thereafter, but had lower mean body weights for the remainder of the study with PND 42 mean body weight of 94.2% of controls. Ketoconazole-

treated LE females gained weight daily, but had significantly lower mean body weights from PND 23 to 42, which was 86.7% of controls. PTU treatment had the greatest effect on PND 42 mean body weight. Mean body weight for PTU-treated females was significantly lower than controls (PND 23-28, 32-42 for SD and PND 23-42 for LE), being only 66.6 and 52.7% of control mean body weight on PND 42 for SD and LE females, respectively.

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
5 = 100 mg/kg/day Ketoconazole
6 = 30 mg/kg/day Pimozide
7 = 100 mg/kg/day Methoxychlor

* = significantly different than control

Age and Weight at Vaginal Opening

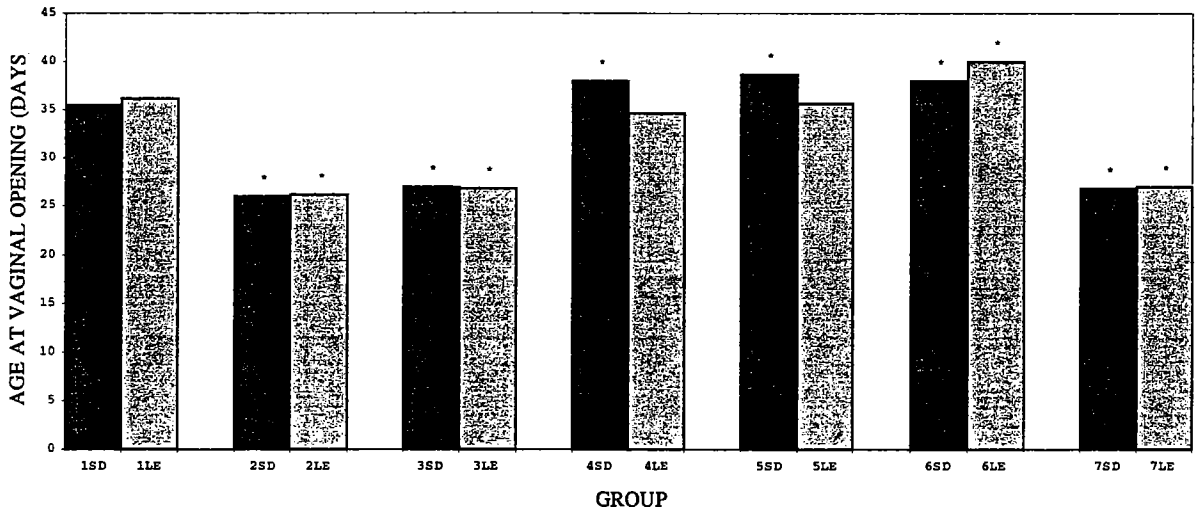
Vaginal opening data are shown in Figure 3, summarized in Table 4 and presented individually in Appendix 3.

The mean age of vaginal opening was similar for both SD and LE females. Control

females opened on PND 36. The mean age of vaginal opening was significantly decreased in females treated with ethynyl estradiol (PND 26), tamoxifen (PND 27), and methoxychlor (PND 27). In contrast, pimozide treatment increased the mean age of vaginal opening to PND 38 and 40 in SD and LE females, respectively. The means for pimozide-treated females are actually artificially low as one female of each strain never achieved vaginal opening and therefore the day after necropsy was used as the day of vaginal opening for statistical purposes for these females. In SD females only, the mean age of vaginal opening was also increased by PTU (PND 38) and ketoconazole (PND 39) treatment.

The mean body weight of females at vaginal opening was significantly lower than controls for all groups except ketoconazole-treated SD females.

Figure 3: Mean Age at Vaginal Opening



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5 = 100 mg/kg/day Ketoconazole
6 = 30 mg/kg/day Pimozide
7 = 100 mg/kg/day Methoxychlor

* = significantly different than control

Vaginal Cytology

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

The mean age at first estrus was similar for control SD and LE females; PND 36.5 and 38.2, respectively. 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. In SD females only, methoxychlor treatment also significantly reduced the mean age at first estrus to PND 28.7.

The mean age at first estrus was significantly increased by tamoxifen (PND 43.5), PTU (SD only, PND 41.3) and ketoconazole (SD only, PND 41.8). The means for tamoxifen, PTU, and ketoconazole-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%). Only 50% of SD females and 66.6% of LE females treated with PTU or ketoconazole exhibited estrus. Although there was no significant difference in mean age at first estrus between controls and pimozide treated females, one female of each strain never achieved vaginal opening and two additional LE females never exhibited estrus in the pimozide treated group. Therefore, only 83.3% of SD and 50% of LE females treated with pimozide ever exhibited estrus.

Figure 4: Mean Age at First Estrus

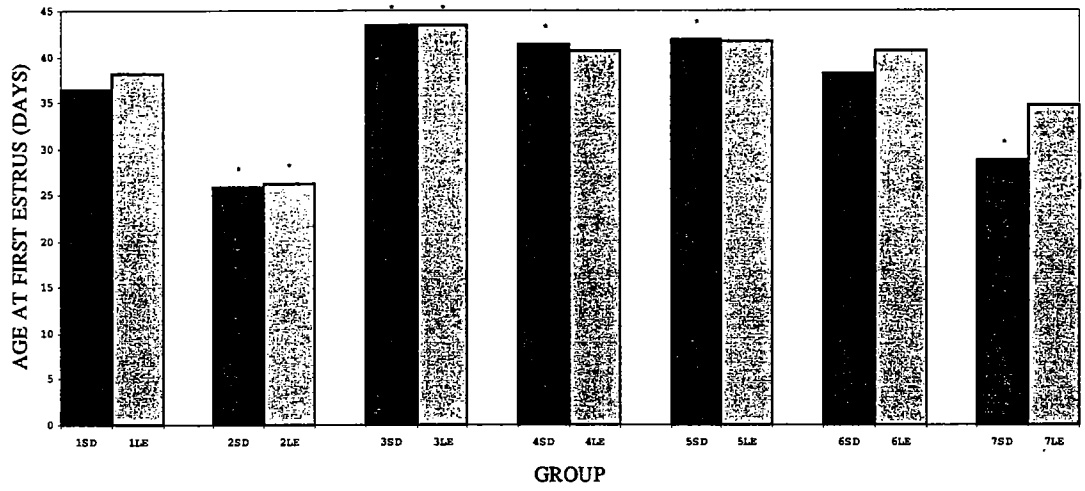
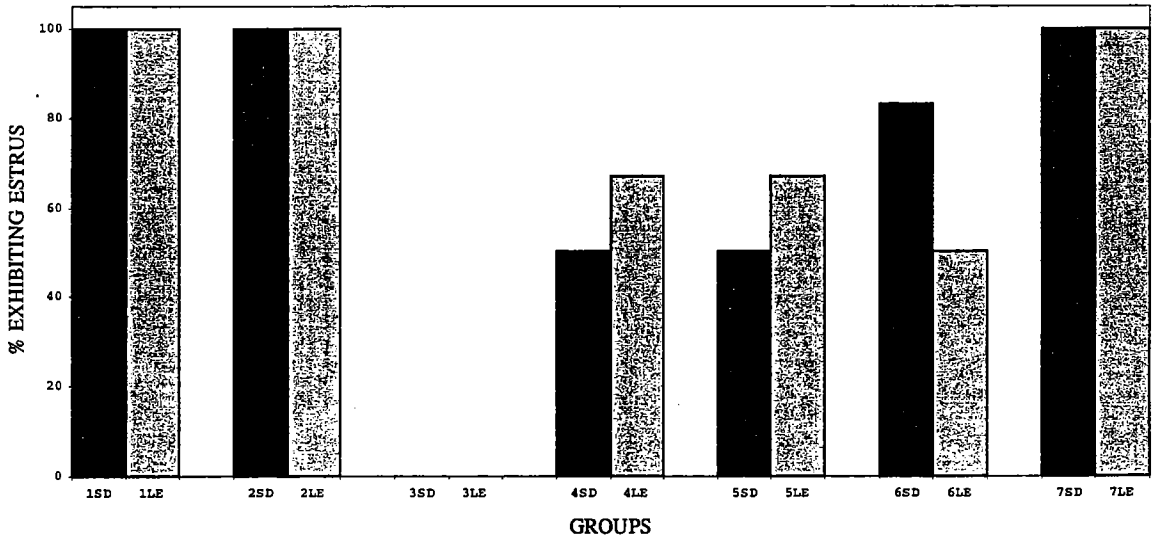


Figure 5: Percent of Females Exhibiting Estrus



KEY: PND = Postnatal Day

GROUP: 1 = 2.5 mL/kg/day Corn Oil
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4 = 240 mg/kg/day Propylthiouracil
5 = 100 mg/kg/day Ketoconazole
6 = 30 mg/kg/day Pimozide
7 = 100 mg/kg/day Methoxychlor

* = significantly different than control

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 ovary, adrenal and thyroid. Two tamoxifen-treated LE females had ovarian cysts, and 11 of 12 ketoconazole-treated females had enlarged adrenals, while the ovaries and adrenals from all other treatment groups appeared normal. The thyroid appeared enlarged in 50% of PTU-treated females, as compared to 17% 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 weight ratios 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, were twice the weight of control adrenals. Adrenal weights were decreased in tamoxifen (SD), PTU, and pimozide-treated females. Compared to controls, kidney weights were decreased in ethynyl estradiol (SD), PTU, pimozide, and methoxychlor-treated females. Liver weights were increased by ketoconazole and decreased by pimozide. As no histology was performed on these organs and body weight varied greatly among groups, whether changes in organ weights are test article related effects cannot be determined.

Ovary weight was decreased in both strains by ethynyl estradiol, tamoxifen, and pimozide. Results for the uterus were the same for both wet and dry weights. Uterine weights were decreased by tamoxifen, PTU(SD), ketoconazole, pimozide and methoxychlor (SD) as compared to control means.

Figure 6: Organ Weights

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

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	✓	✓		✓	✓	✓SD
Uterus	✓	✓				✓SD

KEY: ✓= affected histopathology SD = Sprague Dawley only

Serum T4 and TSH

Serum T4 and TSH levels 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 4.25 and 4.23 μg/DL and TSH were 1.53 and 1.77 ng/mL for SD and LE females, respectively. There were small, but significant decreases in T4 levels in ethynyl estradiol (SD only), ketoconazole, pimozide, and methoxychlor (SD only) treated females, while tamoxifen treatment resulted in a small increase. The largest effect was seen with PTU treatment. PTU-treated females had mean T4 levels which were less than 3% of the control mean. These females had TSH levels which were more than 13 times the control mean. TSH levels were also increased in tamoxifen-treated SD females, but decreased in pimozide and methoxychlor-treated LE 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, ovary, 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 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, increased adrenal size and weights, increased age of vaginal opening and age at first estrus, and disrupted estrous cycling. Ovary 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. The age of vaginal opening was increased and two of twelve pimozide-treated females never achieved vaginal opening. Ovaries were small, cystic and corpora

lutea were deleted.

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 and first estrus (SD only), and decreased the number of females ever exhibiting estrus. Although the absolute weight of most organs was reduced, as a percent of body weight, only the uterus was significantly lower than controls. All thyroids had hyperplasia/hypertrophy of the follicular cells.

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:

Stefanie Pepperl 6-29-00

Stefanie Pepperl, 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
PND21							
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	12	12
NORMAL	12 100%	12 100%	12 100%	12 100%	12 100%	12 100%	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%

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

GROUP: PERIOD	1	2	3	4	5	6	7
PND21	47.1 ±0.92 (6)	47.1 ±0.85 (6)	47.1 ±0.84 (6)	47.1 ±0.81 (6)	47.0 ±0.83 (6)	47.1 ±0.95 (6)	47.0 ±0.79 (6)
PND22	47.82±1.13 (6)	48.25±0.87 (6)	48.58±1.02 (6)	46.57±0.77 (6)	47.53±0.99 (6)	47.47±0.81 (6)	47.82±0.51 (6)
PND23	50.82±0.76 (6)	51.90±0.83 (6)	50.73±1.13 (6)	*47.62±1.74 (6)	49.50±0.92 (6)	**41.23±0.95 (6)	50.30±0.60 (6)
PND24	56.40±0.89 (6)	57.08±0.63 (6)	54.75±1.18 (6)	**50.93±1.89 (6)	54.43±1.26 (6)	**40.77±0.83 (6)	55.95±0.65 (6)
PND25	60.37±0.94 (6)	61.40±0.52 (6)	59.08±1.31 (6)	*56.30±1.93 (6)	60.15±1.29 (6)	**47.98±0.71 (6)	60.75±0.44 (6)
PND26	65.45±0.88 (6)	65.83±0.38 (6)	63.12±1.21 (6)	**61.02±1.70 (6)	63.32±1.16 (6)	**52.33±0.95 (6)	64.62±0.67 (6)
PND27	70.67±0.87 (6)	70.50±0.71 (6)	**65.83±1.27 (6)	*66.68±2.06 (6)	67.38±1.19 (6)	**56.77±0.79 (6)	69.32±0.44 (6)
PND28	74.22±0.84 (6)	74.92±0.69 (6)	*69.45±1.47 (6)	*69.88±2.46 (6)	71.30±1.35 (6)	**61.42±1.02 (6)	73.20±0.65 (6)
PND29	78.12±1.18 (6)	78.95±0.81 (6)	*72.87±1.42 (6)	74.63±1.74 (6)	**68.82±3.23 (6)	**63.67±1.77 (6)	78.50±0.55 (6)
PND30	83.23±1.26 (6)	83.13±0.71 (6)	*77.03±1.27 (6)	81.25±1.95 (6)	**76.87±2.86 (6)	**65.33±2.45 (6)	82.38±0.65 (6)
PND31	89.75±1.32 (6)	88.07±0.65 (6)	**81.73±1.37 (6)	85.28±2.11 (6)	**80.57±2.43 (6)	**71.48±2.29 (6)	87.60±0.97 (6)
PND32	95.22±1.23 (6)	94.45±0.94 (6)	**86.15±1.47 (6)	**87.38±1.93 (6)	**87.47±2.65 (6)	**77.03±1.94 (6)	92.63±0.78 (6)
PND33	100.68±1.53 (6)	97.08±0.83 (6)	**88.07±1.35 (6)	**86.25±2.23 (6)	**92.50±2.84 (6)	**80.08±2.40 (6)	96.15±0.92 (6)
PND34	106.17±1.48 (6)	103.38±1.00 (6)	**92.00±1.51 (6)	**87.83±2.22 (6)	**97.10±2.78 (6)	**84.48±2.44 (6)	101.65±0.99 (6)
PND35	111.87±1.36 (6)	106.95±1.15 (6)	**94.70±1.88 (6)	**90.30±2.43 (6)	**103.75±3.01 (6)	**87.67±2.54 (6)	107.17±1.53 (6)
PND36	117.28±1.38 (6)	*111.35±1.21 (6)	**98.12±1.51 (6)	**90.15±1.99 (6)	**107.75±2.71 (6)	**89.85±2.81 (6)	*110.47±1.24 (6)
PND37	123.08±1.48 (6)	116.83±1.78 (6)	**101.17±1.93 (6)	**92.50±2.08 (6)	**114.05±2.87 (6)	**98.03±3.41 (6)	117.70±1.64 (6)
PND38	127.18±1.76 (6)	120.30±1.92 (6)	**104.72±1.98 (6)	**93.40±2.23 (6)	120.17±3.56 (6)	**105.28±3.70 (6)	121.93±2.04 (6)
PND39	132.48±1.62 (6)	*124.50±1.80 (6)	**108.47±1.97 (6)	**94.17±2.23 (6)	*123.95±2.93 (6)	**107.58±3.31 (6)	128.43±2.23 (6)
PND40	134.45±1.80 (6)	*126.05±2.39 (6)	**110.03±1.93 (6)	**93.35±2.56 (6)	*126.47±2.95 (6)	**109.97±3.67 (6)	130.58±2.12 (6)
PND41	140.50±1.91 (6)	**129.88±1.83 (6)	**111.48±2.04 (6)	**93.78±2.53 (6)	*131.13±2.61 (6)	**118.62±4.27 (6)	134.70±2.71 (6)
PND42	144.70±2.21 (6)	*135.55±2.00 (6)	**115.03±2.87 (6)	**96.35±2.29 (6)	*136.32±2.38 (6)	**124.17±3.07 (6)	138.60±2.25 (6)
PND43	149.90±2.76 (3)	142.93±2.21 (3)	119.60±3.70 (3)	96.50±3.20 (3)	143.50±3.03 (3)	129.10±6.70 (3)	141.93±6.07 (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 CONTROL GROUP; P < 0.05.
** = STATISTICALLY SIGNIFICANT DIFFERENCE FROM CONTROL GROUP; P < 0.01.

TABLE CONTINUED

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

GROUP: PERIOD	1	2	3	4	5	6	7
PND21	42.6 ±0.97 (6)	42.7 ±0.90 (6)	42.6 ±1.06 (6)	42.7 ±1.05 (6)	42.7 ±0.95 (6)	42.6 ±1.06 (6)	42.6 ±0.91 (6)
PND22	43.43±0.60 (6)	43.88±1.38 (6)	42.12±0.63 (6)	41.33±1.24 (6)	43.18±1.31 (6)	42.77±0.72 (6)	42.78±0.49 (6)
PND23	48.18±0.52 (6)	48.52±1.55 (6)	46.85±1.10 (6)	**41.73±1.53 (6)	**43.42±1.84 (6)	**38.72±0.59 (6)	46.43±0.71 (6)
PND24	54.72±0.79 (6)	52.98±1.62 (6)	51.40±1.07 (6)	**47.27±1.47 (6)	**47.50±3.07 (6)	**37.52±0.57 (6)	50.65±0.81 (6)
PND25	59.17±0.55 (6)	58.73±1.98 (6)	56.28±1.34 (6)	*52.85±1.26 (6)	*52.12±3.93 (6)	**44.28±1.08 (6)	57.17±0.80 (6)
PND26	64.87±0.75 (6)	63.78±2.33 (6)	61.32±1.33 (6)	*59.52±1.83 (6)	**57.17±3.20 (6)	**48.05±0.82 (6)	61.93±0.99 (6)
PND27	70.30±0.57 (6)	68.68±2.20 (6)	65.08±1.51 (6)	*64.50±2.15 (6)	**61.13±3.59 (6)	**52.15±1.03 (6)	66.88±1.01 (6)
PND28	75.78±0.81 (6)	73.30±2.69 (6)	*69.00±1.48 (6)	*68.00±2.43 (6)	**65.58±3.60 (6)	**56.22±0.92 (6)	72.82±1.51 (6)
PND29	80.45±0.67 (6)	79.00±2.78 (6)	*72.83±1.24 (6)	**71.60±2.26 (6)	**69.72±2.93 (6)	**55.82±1.85 (6)	77.40±1.33 (6)
PND30	86.40±0.93 (6)	83.55±2.97 (6)	**77.48±1.45 (6)	**74.73±2.56 (6)	**73.87±3.32 (6)	**57.85±1.51 (6)	82.62±0.97 (6)
PND31	93.35±1.00 (6)	88.85±3.21 (6)	**82.32±1.70 (6)	**77.45±2.87 (6)	**79.60±3.15 (6)	**65.38±1.33 (6)	87.62±1.86 (6)
PND32	99.78±1.51 (6)	94.93±3.48 (6)	**88.48±1.85 (6)	**77.33±3.13 (6)	**85.80±3.15 (6)	**69.80±1.28 (6)	94.37±1.91 (6)
PND33	105.23±1.60 (6)	98.70±3.81 (6)	**91.37±1.66 (6)	**74.03±2.26 (6)	**89.58±2.75 (6)	**69.08±1.27 (6)	99.75±1.52 (6)
PND34	111.13±1.95 (6)	104.30±3.90 (6)	**95.10±1.80 (6)	**75.83±2.36 (6)	**93.62±2.81 (6)	**74.73±1.52 (6)	104.73±1.64 (6)
PND35	116.85±2.14 (6)	110.03±4.41 (6)	**100.48±1.52 (6)	**77.78±2.97 (6)	**100.32±3.11 (6)	**80.90±1.78 (6)	110.73±1.97 (6)
PND36	122.70±2.90 (6)	113.97±4.47 (6)	**101.42±1.38 (6)	**78.35±3.23 (6)	*104.50±3.55 (6)	**83.15±2.28 (6)	115.10±2.56 (6)
PND37	129.78±2.72 (6)	**118.73±3.98 (6)	**108.30±1.65 (6)	**79.68±2.82 (6)	*110.13±3.02 (6)	**88.27±2.42 (6)	**118.83±2.48 (6)
PND38	135.48±2.71 (6)	**122.58±4.45 (6)	**110.28±1.41 (6)	**80.92±3.05 (6)	*117.58±2.65 (6)	**92.02±2.43 (6)	**123.82±2.55 (6)
PND39	139.22±2.92 (6)	**126.38±4.65 (6)	**113.53±1.62 (6)	**81.08±2.87 (6)	*120.62±3.11 (6)	**93.10±2.81 (6)	**126.15±2.94 (6)
PND40	143.88±3.34 (6)	**131.03±4.13 (6)	**117.43±1.42 (6)	**80.90±2.63 (6)	*125.00±2.80 (6)	**98.47±3.15 (6)	**130.05±2.14 (6)
PND41	150.20±3.65 (6)	**134.57±4.53 (6)	**119.77±1.66 (6)	**80.62±2.67 (6)	*128.30±3.09 (6)	**103.93±2.87 (6)	**134.18±1.98 (6)
PND42	155.43±3.23 (6)	**138.82±5.00 (6)	**122.47±1.92 (6)	**81.88±2.71 (6)	*134.82±3.23 (6)	**107.07±3.81 (6)	**139.18±2.67 (6)
PND43	165.20±4.94 (3)	139.10±5.37 (3)	123.43±1.82 (3)	81.37±2.01 (3)	133.90±2.27 (3)	105.80±5.41 (3)	139.53±1.07 (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 CONTROL GROUP; P < 0.05.
** = STATISTICALLY SIGNIFICANT DIFFERENCE FROM 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			
	99.4 ±5.50 (6)	90.9 ±7.29 (6)	68.7 ±7.27 (6)	50.0 ±4.63 (6)	91.9 ±5.93 (6)	79.3 ±8.75 (6)	92.5 ±6.85 (6)
				LONG-EVANS			
	116.1 ±9.50 (6)	99.2 ±9.16 (6)	82.0 ±3.49 (6)	39.5 ±5.57 (6)	94.6 ±4.21 (6)	66.5 ±8.14 (6)	98.1 ±5.26 (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			
AGE (PND)	35.50±0.67 (6)	**26.00±0.26 (6)	**27.00±0.26 (6)	*38.00±1.15 (6)	**38.67±0.80 (6)	*38.00±1.24^A (6)	**26.83±0.17 (6)
WEIGHT (GRAMS)	114.72±2.49 (6)	**65.85±1.41 (6)	**66.15±0.87 (6)	**92.20±1.80 (6)	120.92±5.21 (6)	**100.98±4.41^A (6)	**68.50±0.88 (6)
				LONG-EVANS			
AGE (PND)	36.17±1.19 (6)	**26.17±0.17 (6)	**26.83±0.17 (6)	34.67±0.56 (6)	35.67±0.84 (6)	**40.00±1.21^A (6)	**27.00±0.79 (6)
WEIGHT (GRAMS)	125.37±8.27 (6)	**64.65±2.08 (6)	**64.35±1.77 (6)	**77.10±2.88 (6)	**104.42±4.29 (6)	**97.32±2.89^A (6)	**66.88±1.01 (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 CONTROL GROUP; $P < 0.05$.
** = STATISTICALLY SIGNIFICANT DIFFERENCE FROM 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)	36.5 ±1.02 (6)	**26.0 ±0.26 (6)	**43.5 ±0.22[A] (6)	**41.3 ±1.05[A] (6)	**41.8 ±0.95[A] (6)	38.2±1.59[A] (5)	**28.7±1.02 (6)
				LONG-EVANS			
AGE (PND)	38.2±1.39 (5)	**26.2±0.17 (6)	**43.5±0.22[A] (6)	40.7±1.69[A] (6)	41.6±1.36[A] (5)	40.6±1.40[A] (5)	34.7±2.17 (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

PND = POSTNATAL DAY
* = STATISTICALLY SIGNIFICANT DIFFERENCE FROM CONTROL GROUP; P < 0.05.
** = STATISTICALLY SIGNIFICANT DIFFERENCE FROM CONTROL GROUP; P < 0.01.
A = THIS MEAN INCLUDES CENSORED DATA

TABLE 6
INCIDENCE 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	
ACCESSORY LOBE	0		0		1	8%	0		1	8%	0		0	
RAISED AREA	1	8%	0		0		0		0		1	8%	0	
NO GROSS FINDINGS	11	92%	12	100%	11	92%	12	100%	11	92%	11	92%	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	
CYST	0		1	8%	0		0		0		0		0	
DISTENDED	3	25%	0		0		0		1	8%	1	8%	1	8%
NO GROSS FINDINGS	9	75%	11	92%	12	100%	12	100%	11	92%	11	92%	11	92%
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	
CYST	0		0		2	17%	0		0		0		0	
NO GROSS FINDINGS	12	100%	12	100%	10	83%	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)
INCIDENCE OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
ADRENALS							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
ENLARGED	0	0	0	0	11 92%	0	0
PALE	0	0	0	0	1 8%	0	0
NO GROSS FINDINGS	12 100%	12 100%	12 100%	12 100%	1 8%	12 100%	12 100%
PITUITARY							
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%
THYROID							
NO. OF OBSERVATIONS	12	12	12	12	12	12	12
ENLARGED	2 17%	0	1 8%	6 50%	1 8%	0	0
NO GROSS FINDINGS	10 83%	12 100%	11 92%	6 50%	11 92%	12 100%	12 100%
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 CORD							
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)
INCIDENCE OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
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%
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%
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%
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%

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)
INCIDENCE OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
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%
SCIATIC 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%
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%

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)
INCIDENCE OF GROSS PATHOLOGY FINDINGS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

GROUP:	1	2	3	4	5	6	7
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%
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%
SALIVARY 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%
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% 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)	146.5 ± 2.18 (6)	*138.0 ± 2.74 (6)	**115.9 ± 2.62 (6)	**97.1 ± 2.40 (6)	*138.9 ± 2.66 (6)	**126.4 ± 3.55 (6)	*139.5 ± 2.98 (6)
ADRENALS(G)	0.046 ± 0.004 (5)	0.039 ± 0.002 (6)	**0.032 ± 0.003 (6)	**0.024 ± 0.001 (6)	**0.091 ± 0.005 (6)	**0.030 ± 0.003 (6)	0.039 ± 0.002 (6)
KIDNEYS(G)	1.226 ± 0.034 (6)	**1.082 ± 0.046 (6)	**1.033 ± 0.035 (6)	**0.751 ± 0.021 (6)	1.274 ± 0.048 (6)	**1.028 ± 0.034 (6)	*1.121 ± 0.016 (6)
LIVER(G)	5.982 ± 0.138 (6)	6.145 ± 0.326 (6)	**5.085 ± 0.163 (6)	**4.428 ± 0.171 (6)	**7.552 ± 0.218 (6)	*5.140 ± 0.219 (6)	5.836 ± 0.184 (6)
OVARIES(G)	0.069 ± 0.004 (5)	*0.051 ± 0.003 (6)	**0.033 ± 0.005 (6)	**0.040 ± 0.007 (6)	0.057 ± 0.003 (6)	*0.051 ± 0.007 (6)	0.056 ± 0.005 (6)
PITUITARY(G)	0.007 ± 0.001 (6)	0.007 ± 0.002 (5)	0.003 ± 0.000 (6)	0.006 ± 0.000 (6)	0.007 ± 0.002 (5)	0.006 ± 0.002 (5)	0.007 ± 0.001 (6)
UTERUS-WET(G)	0.326 ± 0.066 (6)	0.289 ± 0.023 (6)	**0.104 ± 0.009 (6)	**0.124 ± 0.009 (6)	**0.216 ± 0.032 (6)	**0.196 ± 0.036 (6)	*0.224 ± 0.020 (6)
UTERUS-DRY(G)	0.266 ± 0.031 (6)	0.270 ± 0.018 (6)	**0.097 ± 0.009 (6)	**0.116 ± 0.009 (6)	**0.186 ± 0.022 (6)	**0.162 ± 0.019 (6)	**0.205 ± 0.018 (6)
LONG-EVANS							
BODY(G)	158.6 ± 4.24 (6)	**141.9 ± 4.51 (6)	**124.6 ± 1.20 (6)	**82.2 ± 2.81 (6)	**137.2 ± 2.60 (6)	**109.1 ± 3.71 (6)	**140.7 ± 2.40 (6)
ADRENALS(G)	0.037 ± 0.003 (6)	0.046 ± 0.005 (6)	0.035 ± 0.003 (6)	**0.023 ± 0.002 (6)	**0.074 ± 0.009 (6)	*0.025 ± 0.001 (6)	0.046 ± 0.006 (6)
KIDNEYS(G)	1.369 ± 0.054 (6)	1.288 ± 0.087 (6)	**1.196 ± 0.022 (6)	**0.665 ± 0.025 (6)	1.356 ± 0.029 (6)	**0.934 ± 0.039 (6)	*1.257 ± 0.029 (6)
LIVER(G)	6.804 ± 0.390 (6)	6.338 ± 0.364 (6)	**5.631 ± 0.203 (6)	**3.599 ± 0.174 (6)	7.426 ± 0.230 (6)	**4.329 ± 0.259 (6)	**5.634 ± 0.257 (6)
OVARIES(G)	0.081 ± 0.009 (6)	*0.058 ± 0.013 (6)	**0.052 ± 0.007 (6)	**0.041 ± 0.003 (6)	0.067 ± 0.006 (6)	**0.045 ± 0.003 (6)	0.075 ± 0.006 (6)
PITUITARY(G)	0.008 ± 0.001 (6)	0.006 ± 0.002 (5)	0.004 ± 0.001 (5)	0.005 ± 0.001 (6)	0.007 ± 0.001 (6)	0.005 ± 0.001 (6)	0.005 ± 0.000 (6)
UTERUS-WET(G)	0.260 ± 0.040 (6)	0.271 ± 0.009 (6)	**0.103 ± 0.004 (6)	0.155 ± 0.023 (6)	*0.181 ± 0.034 (6)	*0.158 ± 0.024 (6)	0.237 ± 0.032 (6)
UTERUS-DRY(G)	0.233 ± 0.026 (6)	0.254 ± 0.004 (6)	**0.095 ± 0.004 (6)	**0.134 ± 0.013 (6)	*0.170 ± 0.032 (6)	**0.147 ± 0.022 (6)	0.212 ± 0.021 (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 CONTROL GROUP; $P < 0.05$.
** = STATISTICALLY SIGNIFICANT DIFFERENCE FROM CONTROL GROUP; $P < 0.01$.

TABLE 8

SUMMARY OF ORGAN-TO-BODY WEIGHT RATIOS AT NECROPSY

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

GROUP:	1	2	3	4	5	6	7
				SPRAGUE DAWLEY			
ADRENALS	0.031 ± 0.003 (5)	0.029 ± 0.001 (6)	0.028 ± 0.003 (6)	*0.025 ± 0.001 (6)	**0.065 ± 0.003 (6)	0.024 ± 0.002 (6)	0.029 ± 0.002 (6)
KIDNEYS	0.836 ± 0.014 (6)	*0.783 ± 0.020 (6)	*0.891 ± 0.019 (6)	*0.774 ± 0.017 (6)	**0.917 ± 0.023 (6)	0.813 ± 0.010 (6)	0.804 ± 0.011 (6)
LIVER	4.084 ± 0. 69 (6)	*4.440 ± 0.156 (6)	*4.385 ± 0.070 (6)	**4.557 ± 0.095 (6)	**5.437 ± 0.105 (6)	4.066 ± 0.129 (6)	4.183 ± 0.092 (6)
OVARIES	0.046 ± 0.002 (5)	0. 37 ± 0.003 (6)	**0.028 ± 0.005 (6)	0.041 ± 0.007 (6)	0.041 ± 0.003 (6)	0.040 ± 0.005 (6)	0.041 ± 0.004 (6)
PITUITARY	0.005 ± 0.000 (6)	0.005 ± 0.001 (56)	0.003 ± 0.000 (6)	0.007 ± 0.001 (6)	0.005 ± 0.001 (5)	0.005 ± 0.002 (5)	0.005 ± 0.001 (6)
UTERUS-WET	0.222 ± 0.045 (6)	0.209 ± 0.014 (6)	**0.091 ± 0.010 (6)	**0.127 ± 0.008 (6)	*0.155 ± 0.022 (6)	0.157 ± 0.033 (6)	0.160 ± 0.013 (6)
UTERUS-DRY	0.181 ± 0.020 (6)	0.195 ± 0.011 (6)	**0.084 ± 0.010 (6)	**0.119 ± 0.008 (6)	*0.133 ± 0.015 (6)	0.130 ± 0.017 (6)	0.147 ± 0.011 (6)
				LONG-EVANS			
ADRENALS	0.024 ± 0.002 (6)	*0.032 ± 0.003 (6)	0.028 ± 0.003 (6)	0.028 ± 0.002 (6)	**0.054 ± 0.006 (6)	0.023 ± 0.002 (6)	0.032 ± 0.004 (6)
KIDNEYS	0.864 ± 0.028 (6)	0.903 ± 0.032 (6)	**0.960 ± 0.015 (6)	0.809 ± 0.020 (6)	**0.989 ± 0.019 (6)	0.857 ± 0.023 (6)	0.893 ± 0.011 (6)
LIVER	4.273 ± 0.146 (6)	4.451 ± 0.119 (6)	4.515 ± 0.121 (6)	4.377 ± 0.141 (6)	**5.417 ± 0.160 (6)	3.953 ± 0.110 (6)	3.996 ± 0.127 (6)
OVARIES	0.051 ± 0.005 (6)	0.040 ± 0.008 (6)	0.042 ± 0.005 (6)	0.050 ± 0.004 (6)	0.049 ± 0.004 (6)	0.042 ± 0.004 (6)	0.054 ± 0.004 (6)
PITUITARY	0.005 ± 0.001 (6)	0.005 ± 0.001 (5)	0.003 ± 0.001 (5)	0.007 ± 0.001 (6)	0.005 ± 0.000 (6)	0.004 ± 0.000 (6)	0.004 ± 0.000 (6)
UTERUS-WET	0.166 ± 0.028 (6)	0.192 ± 0.009 (6)	*0.083 ± 0.003 (6)	0.192 ± 0.033 (6)	0.131 ± 0.023 (6)	0.144 ± 0.020 (6)	0.169 ± 0.024 (6)
UTERUS-DRY	0.149 ± 0.019 (6)	0.180 ± 0.009 (6)	**0.076 ± 0.003 (6)	0.164 ± 0.017 (6)	0.123 ± 0.022 (6)	0.134 ± 0.018 (6)	0.151 ± 0.016 (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 CONTROL GROUP; $P < 0.05$.

** = STATISTICALLY SIGNIFICANT DIFFERENCE FROM 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 MG/DL	4.25±0.22 (6)	*3.00±0.17 (6)	**5.03±0.21 (6)	**0.02±0.01 (6)	**3.10±0.15 (6)	**3.13±0.25 (6)	**3.34±0.20 (6)
TSH NG/ML	1.53±0.21 (6)	1.65±0.13 (6)	*2.35±0.23 (6)	**26.26±1.28 (6)	1.56±0.08 (6)	1.33±0.15 (6)	1.30±0.13 (6)
				LONG-EVANS			
T4, TOTAL MG/DL	4.23±0.16 (6)	4.56±0.36 (6)	**5.42±0.16 (6)	**0.09±0.08 (6)	*3.30±0.27 (6)	*3.47±0.38 (6)	4.18±0.21 (6)
TSH NG/ML	1.77±0.30 (6)	1.67±0.24 (6)	1.70±0.26 (6)	**23.23±2.79 (6)	1.75±0.25 (6)	**0.89±0.07 (6)	*1.08±0.11 (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 CONTROL GROUP; $P < 0.05$
** = STATISTICALLY SIGNIFICANT DIFFERENCE FROM 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
PND 21- 43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15314	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15315	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15316	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15317	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15318	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15319	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15320	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15321	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15322	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15323	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15324	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15325	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15326	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15327	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15328	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15329	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15330	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15331	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15332	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15333	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15334	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15335	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15336	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15337	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15338	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15339	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15340	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15341	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15342	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15343	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15344	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15345	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15346	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15347	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15348	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15349	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15350	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15351	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15352	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15353	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15354	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15355	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15356	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15357	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15358	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15359	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15360	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15361	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15362	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15363	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15364	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15365	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15366	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15367	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15368	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15369	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15370	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15371	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15372	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15373	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15374	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15375	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15376	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15377	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15378	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15379	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15380	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15401	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15402	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15403	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15404	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15405	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15406	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15407	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15408	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15409	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15410	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15411	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
PND 21-43

ANIMAL #	OBSERVATIONS	SEVERITY	LOCATION	TIME OCCURRED
R15412	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15413	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15414	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 42
R15415	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15416	NORMAL			PND 21
	NORMAL			PND 28
	NORMAL			PND 35
	NORMAL			PND 42
	TERMINAL KILL			PND 43
R15417	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
R15314	48.5	48.2	52.1	57.8	61.4	66.9	71.1	74.9	76.2	81.3	87.5	92.2
R15315	43.5	43.5	48.1	53.1	56.3	62.0	66.9	70.5	74.4	79.4	85.5	91.2
R15316	46.6	45.8	50.7	55.9	60.4	65.6	70.6	74.5	78.0	82.6	90.3	96.8
R15317	46.1	48.6	49.4	55.0	59.4	64.1	70.2	73.5	77.2	82.2	88.4	95.0
R15318	50.1	51.2	53.3	59.2	62.6	68.1	73.2	76.1	82.2	87.1	93.2	97.1
R15319	47.7	49.6	51.3	57.4	62.1	66.0	72.0	75.8	80.7	86.8	93.6	99.0
R15320	40.6	42.2	46.8	51.1	57.3	61.7	68.4	73.5	79.0	85.1	92.1	98.5
R15321	43.1	45.4	50.1	56.6	59.9	64.9	70.4	76.4	79.7	86.6	94.5	99.0
R15322	42.0	43.7	48.8	55.4	59.0	64.7	70.3	73.4	79.1	82.8	88.9	93.4
R15323	44.4	43.9	48.5	56.0	60.9	66.9	72.0	76.7	80.6	86.8	94.3	103.7
R15324	39.5	41.3	46.7	54.4	58.0	64.5	69.1	76.2	80.9	87.6	95.3	102.1
R15325	45.9	44.1	48.2	54.8	59.9	66.5	71.6	78.5	83.4	89.5	95.0	102.0
MEAN	44.8	45.6	49.5	55.6	59.8	65.2	70.5	75.0	79.3	84.8	91.6	97.5
S.D.	3.23	3.11	2.06	2.16	1.90	1.94	1.73	2.09	2.55	3.07	3.32	4.01
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15314 - R15319 ARE SPRAGUE DAWLEY
ANIMALS R15320 - R15325 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
R15314	95.8	103.2	108.3	112.5	118.1	121.9	128.2	130.6	134.8	138.9	
R15315	97.2	102.7	109.7	117.1	121.4	124.2	131.2	134.2	140.4	145.4	
R15316	102.9	108.5	115.0	121.6	126.2	131.9	135.7	138.9	144.2	144.8	
R15317	99.4	103.1	108.8	114.6	120.8	126.2	128.7	129.8	137.1	142.0	145.5
R15318	104.1	108.2	113.6	117.8	124.2	126.1	132.8	132.6	139.0	142.4	149.2
R15319	104.7	111.3	115.8	120.1	127.8	132.8	138.3	140.6	147.5	154.7	155.0
R15320	103.1	109.0	112.6	115.9	124.0	130.0	131.4	136.0	143.3	148.5	
R15321	106.6	113.6	118.9	125.4	134.2	142.1	143.8	148.3	155.4	161.4	
R15322	98.7	102.6	108.4	112.9	120.2	127.6	130.8	132.3	138.3	146.3	
R15323	106.9	112.5	118.7	123.1	129.8	132.0	138.1	145.2	147.1	151.9	156.9
R15324	105.9	112.9	120.3	127.1	132.3	137.4	142.4	147.3	154.4	157.8	164.7
R15325	110.2	116.2	122.2	131.8	138.2	143.8	148.8	154.2	162.7	166.7	174.0
MEAN	103.0	108.7	114.4	120.0	126.4	131.3	135.9	139.2	145.4	150.1	157.6
S.D.	4.36	4.81	4.93	6.01	6.20	6.88	6.54	7.98	8.48	8.55	10.43
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15314 - R15319 ARE SPRAGUE DAWLEY
ANIMALS R15320 - R15325 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

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
R15326	45.5	47.9	51.8	56.5	61.5	65.5	72.6	76.5	81.9	85.1	89.6	97.1
R15327	44.1	44.3	48.0	54.4	59.5	64.3	68.6	72.6	76.5	81.8	86.1	95.0
R15328	49.5	50.1	52.9	58.7	61.5	66.9	71.5	75.4	78.2	82.4	86.5	91.9
R15329	49.0	49.9	53.9	58.1	63.5	66.5	72.0	77.0	79.1	84.3	88.9	96.4
R15330	47.9	49.2	52.1	56.9	61.3	65.6	69.0	73.8	77.5	80.7	87.5	91.5
R15331	46.8	48.1	52.7	57.9	61.1	66.2	69.3	74.2	80.5	84.5	89.8	94.8
R15332	45.2	49.7	54.8	59.5	66.9	73.4	77.6	84.2	90.4	96.0	102.6	110.2
R15333	40.4	42.3	48.1	50.7	55.0	59.7	64.6	68.0	72.7	76.7	82.1	88.8
R15334	43.6	45.4	50.2	55.4	61.3	66.7	71.5	76.6	81.7	85.9	91.0	97.2
R15335	44.6	43.6	47.9	52.0	59.0	64.7	69.9	75.0	81.2	85.8	91.1	96.5
R15336	39.8	39.9	43.4	48.1	53.7	58.4	63.6	67.5	73.4	78.1	82.7	87.9
R15337	42.5	42.4	46.7	52.2	56.5	59.8	64.9	68.5	74.6	78.8	83.6	89.0
MEAN	44.9	46.1	50.2	55.0	60.1	64.8	69.6	74.1	79.0	83.3	88.5	94.7
S.D.	3.10	3.53	3.41	3.58	3.66	4.05	3.94	4.67	4.79	5.05	5.43	5.96
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15326 - R15331 ARE SPRAGUE DAWLEY
ANIMALS R15332 - R15337 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
R15326	99.8	106.7	109.9	114.7	121.1	122.1	128.0	125.5	131.7	135.9	
R15327	97.3	102.9	107.2	110.1	115.7	119.3	123.0	126.8	127.8	135.9	
R15328	93.7	99.2	102.2	106.2	109.4	111.5	116.6	115.5	122.0	127.3	
R15329	98.0	104.6	105.8	113.4	115.9	123.7	126.0	130.9	131.0	134.6	142.4
R15330	96.3	103.6	107.0	111.5	117.7	120.7	124.7	125.6	131.9	137.1	139.4
R15331	97.4	103.3	109.6	112.2	121.2	124.5	128.7	132.0	134.9	142.5	147.0
R15332	115.3	122.4	130.4	135.2	137.4	143.8	147.8	150.8	155.4	160.7	
R15333	93.1	98.6	103.7	108.2	114.1	118.1	122.2	129.1	129.7	135.0	
R15334	100.9	104.2	110.4	112.4	119.5	121.5	123.9	126.0	130.5	138.3	
R15335	100.9	105.7	111.4	114.9	118.4	122.6	129.1	131.6	138.5	142.3	148.5
R15336	91.3	98.1	103.2	107.8	111.5	115.4	115.8	123.3	125.7	125.0	129.9
R15337	90.7	96.8	101.1	105.3	111.5	114.1	119.5	125.4	127.6	131.6	138.9
MEAN	97.9	103.8	108.5	112.7	117.8	121.4	125.4	128.5	132.2	137.2	141.0
S.D.	6.49	6.67	7.70	7.78	7.27	8.10	8.29	8.30	8.44	9.05	6.70
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15326 - R15331 ARE SPRAGUE DAWLEY
ANIMALS R15332 - R15337 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

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
R15338	48.8	48.1	49.7	52.7	57.1	61.1	64.2	65.4	69.3	73.9	78.8	83.7
R15339	48.2	51.3	52.5	57.7	61.4	65.7	68.0	71.8	75.4	79.1	84.1	88.2
R15340	49.3	50.1	53.5	57.6	62.9	65.9	69.6	72.4	76.1	78.3	83.5	90.0
R15341	46.0	49.7	50.7	54.2	57.7	62.5	65.0	68.7	72.6	78.4	83.3	84.2
R15342	43.8	44.1	45.8	50.4	54.4	58.5	60.9	65.1	68.1	72.4	76.3	81.2
R15343	46.7	48.2	52.2	55.9	61.0	65.0	67.3	73.3	75.7	80.1	84.4	89.6
R15344	46.4	46.4	51.7	54.8	61.9	66.7	71.2	74.4	76.9	82.7	88.2	94.9
R15345	44.4	43.8	47.3	52.3	58.0	63.2	67.7	71.8	75.5	80.2	85.8	91.7
R15346	41.8	41.2	44.3	47.9	53.7	59.6	64.0	66.7	71.2	75.4	81.4	89.1
R15347	39.3	40.6	44.9	49.5	54.0	58.2	61.5	66.9	70.4	74.3	79.3	84.5
R15348	43.0	44.3	47.4	53.6	56.6	61.7	64.0	69.5	73.8	78.4	82.4	88.1
R15349	40.5	41.5	45.5	50.3	53.5	58.5	62.1	64.7	69.2	73.9	76.8	82.6
MEAN	44.9	45.8	48.8	53.1	57.7	62.2	65.5	69.2	72.9	77.3	82.0	87.3
S.D.	3.27	3.71	3.30	3.16	3.43	3.11	3.29	3.45	3.12	3.19	3.63	4.09
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15338 - R15343 ARE SPRAGUE DAWLEY
ANIMALS R15344 - R15349 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
R15338	85.8	88.7	88.8	94.3	97.2	98.9	102.7	104.9	105.2	107.0	
R15339	89.8	92.7	95.7	99.1	103.1	105.8	109.2	109.6	111.6	115.0	
R15340	90.7	95.6	98.0	99.4	102.3	104.9	108.3	113.1	111.4	114.4	
R15341	86.8	92.1	95.3	99.6	99.4	106.2	109.2	110.7	112.9	116.6	119.4
R15342	83.3	86.8	89.8	93.2	96.0	100.1	104.7	104.9	107.9	109.8	113.3
R15343	92.0	96.1	100.6	103.1	109.0	112.4	116.7	117.0	119.9	127.4	126.1
R15344	96.8	100.9	105.1	104.9	111.1	114.0	116.2	120.4	120.4	123.0	
R15345	95.0	98.6	103.1	104.3	111.2	113.5	119.3	121.6	125.4	128.6	
R15346	92.3	94.3	100.6	102.9	107.7	109.8	113.7	119.2	121.9	125.5	
R15347	87.3	91.4	96.8	98.1	104.0	109.5	110.7	115.9	118.8	122.0	125.4
R15348	89.9	96.3	101.9	101.7	112.7	110.5	113.3	114.7	119.0	121.1	125.1
R15349	86.9	89.1	95.4	96.6	103.1	104.4	108.0	112.8	113.1	114.6	119.8
MEAN	89.7	93.6	97.6	99.8	104.7	107.5	111.0	113.7	115.6	118.8	121.5
S.D.	3.93	4.21	5.01	3.79	5.61	4.96	4.98	5.53	6.13	6.91	4.98
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15338 - R15343 ARE SPRAGUE DAWLEY
ANIMALS R15344 - R15349 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

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
R15350	49.1	49.7	54.1	56.5	62.4	66.5	73.0	76.3	79.4	86.6	91.9	91.9
R15351	43.7	44.0	43.3	48.0	51.3	59.0	62.8	65.1	68.5	76.0	78.8	81.1
R15352	46.2	46.9	49.3	54.3	58.3	64.2	70.6	73.6	76.4	84.6	87.8	88.7
R15353	47.0	47.1	42.8	43.6	50.3	55.1	60.3	60.5	70.5	75.3	80.1	82.2
R15354	47.8	45.8	46.8	50.7	56.1	58.8	63.9	69.9	75.3	80.5	84.2	88.3
R15355	48.8	45.9	49.4	52.5	59.4	62.5	69.5	73.9	77.7	84.5	88.9	92.1
R15356	40.9	37.6	40.3	45.4	49.7	55.8	58.8	62.6	65.1	67.8	67.5	67.1
R15357	42.9	43.3	46.3	51.8	56.7	65.1	69.5	77.3	78.9	84.0	87.1	87.3
R15358	46.8	44.0	40.5	48.5	53.6	62.7	69.6	72.0	75.7	79.6	82.1	84.8
R15359	44.0	42.9	42.7	45.5	53.0	60.6	64.7	66.0	71.8	70.0	75.4	75.4
R15360	39.4	37.3	35.8	42.1	48.8	52.9	57.5	61.6	65.3	70.9	72.7	71.8
R15361	42.1	42.9	44.8	50.3	55.3	60.0	66.9	68.5	72.8	76.1	79.9	77.6
MEAN	44.9	44.0	44.7	49.1	54.6	60.3	65.6	68.9	73.1	78.0	81.4	82.4
S.D.	3.18	3.64	4.90	4.39	4.21	4.19	5.04	5.80	4.97	6.31	7.16	8.03
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15350 - R15355 ARE SPRAGUE DAWLEY
ANIMALS R15356 - R15361 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
R15350	91.1	92.9	97.3	94.9	97.3	97.8	100.4	99.1	100.3	102.3	
R15351	79.3	81.2	80.9	83.7	84.8	85.4	85.5	84.8	86.5	89.1	
R15352	88.7	90.7	91.4	93.3	96.6	99.5	98.8	98.8	98.3	101.4	
R15353	79.8	80.7	86.2	84.8	88.2	88.9	90.9	86.4	85.9	90.1	90.9
R15354	86.9	89.8	91.1	90.1	92.1	92.7	93.7	94.2	94.3	96.6	96.6
R15355	91.7	91.7	94.9	94.1	96.0	96.1	95.7	96.8	97.4	98.6	102.0
R15356	65.6	66.1	66.5	67.5	70.4	70.3	72.4	71.5	72.0	71.2	
R15357	80.6	81.2	82.8	84.0	86.1	87.6	86.9	87.4	87.7	88.4	
R15358	78.0	80.7	86.1	89.5	87.7	89.9	90.9	88.0	88.8	89.3	
R15359	74.5	75.8	77.7	77.3	76.6	78.0	77.7	79.0	78.4	81.0	80.1
R15360	69.6	72.5	72.3	72.3	74.4	76.0	75.9	76.6	76.5	79.0	78.7
R15361	75.9	78.7	81.3	79.5	82.9	83.7	82.7	82.9	80.3	82.4	85.3
MEAN	80.1	81.8	84.0	84.3	86.1	87.2	87.6	87.1	87.2	89.1	88.9
S.D.	8.26	8.24	9.10	8.79	8.85	9.02	9.09	8.89	9.17	9.57	9.27
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15350 - R15355 ARE SPRAGUE DAWLEY
ANIMALS R15356 - R15361 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

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
R15362	49.4	49.8	51.5	57.1	63.6	66.6	71.1	74.1	66.0	73.7	78.5	87.4
R15363	47.5	48.1	49.7	54.2	60.2	62.5	67.1	71.2	63.8	73.1	77.6	81.0
R15364	45.3	45.4	46.5	49.7	55.6	59.3	63.4	66.4	58.4	68.1	72.8	80.9
R15365	46.5	48.4	51.4	56.8	60.9	64.3	68.1	71.8	74.5	82.0	83.3	92.2
R15366	44.3	43.8	46.9	51.9	57.4	61.2	64.8	68.9	69.6	76.6	80.8	85.8
R15367	49.0	49.7	51.0	56.9	63.2	66.0	69.8	75.4	80.6	87.7	90.4	97.5
R15368	44.5	46.4	47.5	52.9	58.0	62.4	66.8	71.9	74.3	77.0	82.6	90.6
R15369	39.8	39.7	36.3	33.5	33.7	42.2	45.0	49.0	56.7	60.3	67.4	94.4
R15370	46.1	47.9	49.0	54.6	60.7	63.8	69.1	72.0	76.5	83.6	88.4	94.4
R15371	42.2	41.5	43.2	50.1	55.9	60.5	65.4	70.3	72.9	79.2	85.8	91.1
R15372	40.8	41.7	42.6	46.7	51.2	56.6	57.9	62.9	66.7	70.4	74.9	80.2
R15373	42.5	41.9	41.9	47.2	53.2	57.5	62.6	67.4	71.2	72.7	78.5	84.5
MEAN	44.8	45.4	46.5	51.0	56.1	60.2	64.3	68.4	69.3	75.4	80.1	86.6
S.D.	3.08	3.54	4.65	6.57	8.02	6.47	7.05	7.02	7.22	7.40	6.59	6.85
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15362 - R15367 ARE SPRAGUE DAWLEY
ANIMALS R15368 - R15373 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
R15362	92.7	98.2	105.2	107.4	113.6	117.6	123.5	123.7	130.6	137.3	
R15363	88.1	93.7	99.4	102.7	108.3	113.9	121.2	123.6	124.6	135.2	
R15364	84.3	87.1	94.3	99.4	105.7	108.9	112.7	116.9	123.3	130.1	
R15365	94.4	100.1	105.4	110.7	117.4	125.6	127.5	129.5	138.3	136.5	143.6
R15366	90.8	96.0	102.0	107.8	113.7	121.6	124.3	126.6	132.0	132.0	138.2
R15367	104.7	107.5	116.2	118.5	125.6	133.4	134.5	138.5	138.0	146.8	148.7
R15368	95.9	98.1	106.4	111.6	120.0	124.7	128.4	131.7	132.1	141.9	
R15369	79.7	85.3	93.0	96.6	104.5	110.3	116.5	122.4	127.0	132.6	
R15370	95.9	103.0	111.8	117.0	117.7	125.3	128.9	133.2	140.4	147.0	
R15371	92.9	96.0	100.8	105.0	107.2	118.1	120.3	124.0	127.6	128.5	134.8
R15372	83.4	86.3	92.7	94.3	101.3	110.8	108.6	114.3	117.7	127.0	129.6
R15373	89.7	93.0	97.2	102.5	110.1	116.3	121.0	124.4	125.0	131.9	137.3
MEAN	91.0	95.4	102.0	106.1	112.1	118.9	122.3	125.7	129.7	135.6	138.7
S.D.	6.70	6.77	7.37	7.56	7.18	7.45	7.28	6.76	6.84	6.67	6.70
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15362 - R15367 ARE SPRAGUE DAWLEY
ANIMALS R15368 - R15373 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

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
R15374	47.3	48.3	41.2	43.8	49.7	51.6	56.3	62.8	63.0	68.6	71.2	77.4
R15375	45.2	45.8	38.8	40.6	47.2	53.8	55.6	61.0	63.7	63.1	71.7	77.4
R15376	43.5	44.9	38.3	39.7	47.2	53.1	55.2	60.7	61.4	59.2	65.7	76.4
R15377	48.9	49.6	43.9	40.2	46.4	47.9	55.1	57.1	57.1	58.7	65.5	68.8
R15378	49.6	49.6	43.5	42.3	50.6	54.0	59.5	64.3	68.2	73.8	80.4	83.5
R15379	48.1	46.6	41.7	38.0	46.8	53.6	58.9	62.6	68.6	68.6	74.4	78.7
R15380	46.0	44.7	41.0	39.0	45.6	49.5	55.3	59.0	61.6	60.0	69.6	74.2
R15401	45.2	43.6	39.4	35.7	41.3	48.2	51.1	57.3	59.3	62.1	67.3	71.6
R15402	42.6	43.2	39.0	37.0	42.6	48.8	54.0	56.6	54.8	57.7	63.4	69.9
R15403	40.7	42.6	38.4	39.3	48.5	50.0	52.2	56.0	51.8	53.2	62.4	66.0
R15404	39.2	39.5	36.9	37.4	42.5	44.4	48.0	52.2	49.8	53.7	61.8	66.4
R15405	42.0	43.0	37.6	36.7	45.2	47.4	52.3	56.2	57.6	60.4	67.8	70.7
MEAN	44.9	45.1	40.0	39.1	46.1	50.2	54.5	58.8	59.7	61.6	68.4	73.4
S.D.	3.32	3.04	2.26	2.37	2.88	3.05	3.23	3.53	5.88	6.15	5.41	5.39
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15374 - R15379 ARE SPRAGUE DAWLEY
ANIMAL R15380 AND ANIMALS R15401 - R15405 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
R15374	80.7	83.8	87.0	88.3	97.0	105.3	106.4	104.5	110.9	120.7	
R15375	78.4	82.5	85.0	87.4	97.1	98.9	104.7	104.8	108.6	120.0	
R15376	79.7	84.1	90.3	91.4	99.6	109.3	112.7	113.8	124.8	130.1	
R15377	71.3	75.7	78.2	78.5	84.8	90.9	93.4	98.1	108.4	113.0	115.7
R15378	89.7	94.1	97.1	97.8	111.0	115.6	115.8	122.2	131.3	132.9	135.7
R15379	80.7	86.7	88.4	95.7	98.7	111.7	112.5	116.4	127.7	128.3	135.9
R15380	73.7	81.1	88.4	91.2	97.1	102.2	103.6	108.5	117.7	123.0	
R15401	70.9	73.8	78.3	84.2	89.6	91.2	95.0	101.7	103.9	108.1	
R15402	69.4	73.7	79.1	82.3	89.0	91.5	85.7	94.5	100.0	105.9	
R15403	64.5	70.1	75.7	74.2	78.9	84.5	86.0	87.0	98.7	94.4	95.2
R15404	68.4	73.2	82.0	81.4	85.8	88.6	91.4	95.3	100.2	102.8	113.0
R15405	67.6	76.5	81.9	85.6	89.2	94.1	96.9	103.8	103.1	108.2	109.2
MEAN	74.6	79.6	84.3	86.5	93.2	98.7	100.3	104.2	111.3	115.6	117.5
S.D.	7.29	6.97	6.23	6.93	8.58	10.07	10.42	9.98	11.44	12.05	15.87
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15374 - R15379 ARE SPRAGUE DAWLEY
ANIMAL R15380 AND ANIMALS R15401 - R15405 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

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
R15406	45.3	47.2	49.4	54.8	60.1	63.2	68.7	71.9	77.3	82.5	86.2	92.8
R15407	44.3	46.1	48.6	54.2	59.3	62.6	67.5	70.8	76.6	80.3	84.9	90.7
R15408	46.8	48.7	51.0	55.7	61.4	64.4	69.5	73.7	78.6	81.5	88.7	92.1
R15409	48.5	47.2	50.7	56.4	61.0	64.7	69.6	74.8	78.7	81.6	85.5	91.0
R15410	47.9	48.1	49.4	55.8	60.3	65.7	70.2	73.3	80.0	83.7	89.6	93.2
R15411	49.3	49.6	52.7	58.8	62.4	67.1	70.4	74.7	79.8	84.7	90.7	96.0
R15412	42.2	42.5	46.8	50.7	55.2	61.6	66.5	73.3	77.9	83.2	87.9	93.6
R15413	42.5	42.5	46.9	50.5	57.4	61.9	67.1	73.7	77.7	82.0	86.5	93.6
R15414	45.5	45.0	49.4	54.3	60.3	66.2	71.0	78.8	82.8	86.7	95.6	102.0
R15415	45.0	43.1	46.1	50.7	58.0	62.3	67.0	72.7	76.7	81.6	86.4	94.6
R15416	40.2	41.6	44.8	48.9	57.1	60.8	66.5	67.6	76.6	82.7	87.8	95.1
R15417	40.4	42.0	44.6	48.8	55.0	58.8	63.2	70.8	72.7	79.5	81.5	87.3
MEAN	44.8	45.3	48.4	53.3	59.0	63.3	68.1	73.0	78.0	82.5	87.6	93.5
S.D.	3.03	2.88	2.54	3.26	2.40	2.42	2.22	2.71	2.44	1.93	3.47	3.53
N	12	12	12	12	12	12	12	12	12	12	12	12

KEY: ANIMALS R15406 - R15411 ARE SPRAGUE DAWLEY
ANIMALS R15412 - R15417 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
R15406	97.8	99.8	105.0	109.9	117.9	120.4	125.9	128.0	132.1	136.4	
R15407	94.6	101.5	106.5	109.0	116.6	122.2	126.6	130.8	134.4	139.3	
R15408	95.1	100.9	105.2	109.2	116.6	119.6	127.5	127.2	133.3	135.3	
R15409	93.2	98.7	103.3	106.6	111.6	114.6	121.4	124.7	125.1	131.7	130.8
R15410	96.9	104.2	109.4	113.2	119.7	126.1	132.0	133.7	138.3	141.3	143.3
R15411	99.3	104.8	113.6	114.9	123.8	128.7	137.2	139.1	145.0	147.6	151.7
R15412	97.7	103.7	107.9	112.5	118.7	120.6	121.8	128.4	132.2	136.2	
R15413	99.1	102.3	110.6	112.1	114.0	122.7	123.7	128.5	132.5	137.4	
R15414	104.8	111.2	118.7	126.3	130.5	136.2	140.6	140.5	144.0	152.1	
R15415	99.8	105.4	110.4	115.3	117.9	123.0	123.3	129.4	133.0	133.5	137.8
R15416	102.8	106.4	112.5	116.6	117.8	121.5	125.5	127.4	132.2	137.5	141.5
R15417	94.3	99.4	104.3	107.8	114.1	118.9	122.0	126.1	131.2	138.4	139.3
MEAN	98.0	103.2	109.0	112.8	118.3	122.9	127.3	130.3	134.4	138.9	140.7
S.D.	3.48	3.54	4.52	5.29	4.94	5.48	6.21	4.98	5.54	5.78	6.88
N	12	12	12	12	12	12	12	12	12	12	6

KEY: ANIMALS R15406 - R15411 ARE SPRAGUE DAWLEY
ANIMALS R15412 - R15417 ARE LONG-EVANS
PND = POSTNATAL DAY

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

ANIMAL ID	WEIGHT AT VAGINAL OPENING (GRAMS)	AGE AT VAGINAL OPENING (DAYS)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL	
R15314	118.1	37
R15315	121.4	37
R15316	108.5	34
R15317	120.8	37
R15318	108.2	34
R15319	111.3	34
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15326	65.5	26
R15327	64.3	26
R15328	61.5	25
R15329	72.0	27
R15330	65.6	26
R15331	66.2	26
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN	
R15338	64.2	27
R15339	68.0	27
R15340	69.6	27
R15341	65.0	27
R15342	65.1	28
R15343	65.0	26
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15350	97.3	35
R15351	85.5	39
R15352	91.4	35
R15353	90.1	42
R15354	92.1	37
R15355	96.8	40
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15362	107.4	36
R15363	113.9	38
R15364	108.9	38
R15365	136.5	42
R15366	124.3	39
R15367	134.5	39
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE	
R15374	106.4	39
R15375	NV	NV
R15376	90.3	35
R15377	93.4	39
R15378	97.1	35
R15379	98.7	37
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15406	68.7	27
R15407	67.5	27
R15408	69.5	27
R15409	64.7	26
R15410	70.2	27
R15411	70.4	27

NV = NO VAGINAL OPENING WAS OBSERVED

APPENDIX CONTINUED

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

ANIMAL ID	WEIGHT AT VAGINAL OPENING (GRAMS)	AGE AT VAGINAL OPENING (DAYS)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL	
R15320	109.0	34
R15321	118.9	35
R15322	127.6	38
R15323	106.9	33
R15324	127.1	36
R15325	162.7	41
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15332	73.4	26
R15333	59.7	26
R15334	66.7	26
R15335	64.7	26
R15336	63.6	27
R15337	59.8	26
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN	
R15344	71.2	27
R15345	67.7	27
R15346	59.6	26
R15347	61.5	27
R15348	64.0	27
R15349	62.1	27
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15356	66.5	35
R15357	82.8	35
R15358	86.1	35
R15359	77.3	36
R15360	72.3	35
R15361	77.6	32
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15368	90.6	32
R15369	93.0	35
R15370	117.0	36
R15371	105.0	36
R15372	110.8	38
R15373	110.1	37
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE	
R15380	97.1	37
R15401	NV	NV
R15402	91.5	38
R15403	95.2	43
R15404	102.8	42
R15405	89.2	37
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15412	66.5	27
R15413	67.1	27
R15414	71.0	27
R15415	67.0	27
R15416	66.5	27
R15417	63.2	27

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																						
R15314	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P	Y						
R15315	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P	Y						
R15316	N	N	N	N	N	N	N	N	N	N	N	N	Y									
R15317	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P	Y						
R15318	N	N	N	N	N	N	N	N	N	N	N	N	Y									
R15319	N	N	N	N	N	N	N	N	N	N	N	N	Y									
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																						
R15326	N	N	N	N	Y																	
R15327	N	N	N	N	Y																	
R15328	N	N	N	Y																		
R15329	N	N	N	N	P	Y																
R15330	N	N	N	P	Y																	
R15331	N	N	N	P	Y																	
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																						
R15338	N	N	N	N	P	Y																
R15339	N	N	N	N	N	Y																
R15340	N	N	N	N	N	Y																
R15341	N	N	N	N	N	Y																
R15342	N	N	N	N	P	P	Y															
R15343	N	N	N	N	Y																	
GROUP: 4 - 240MG/KG/DAY PROPYLTHIOURACIL																						
R15350	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15351	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y				
R15352	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15353	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y	
R15354	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
R15355	N	N	N	N	N	N	N	N	N	N	N	N	P	T	T	T	T	T	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 (SPRAGUE DAWLEY)

ANIMAL ID	POSTNATAL DAY 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																						
R15362	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y							
R15363	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y					
R15364	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y					
R15365	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	Y	
R15366	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y				
R15367	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y				
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																						
R15374	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y				
R15375	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
R15376	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15377	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y				
R15378	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15379	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																						
R15406	N	N	N	N	N	Y																
R15407	N	N	N	N	N	Y																
R15408	N	N	N	N	N	Y																
R15409	N	N	N	P	Y																	
R15410	N	N	N	N	N	Y																
R15411	N	N	N	P	P	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 (LONG-EVANS)

ANIMAL ID	POSTNATAL DAY 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																						
R15320	N	N	N	N	N	N	N	N	N	N	P	T	Y									
R15321	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15322	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y					
R15323	N	N	N	N	N	N	N	N	N	N	P	Y										
R15324	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y							
R15325	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	T	T	Y		
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																						
R15332	N	N	N	N	Y																	
R15333	N	N	N	N	Y																	
R15334	N	N	N	N	Y																	
R15335	N	N	N	N	Y																	
R15336	N	N	N	N	N	Y																
R15337	N	N	N	N	Y																	
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																						
R15344	N	N	N	N	N	Y																
R15345	N	N	N	N	N	Y																
R15346	N	N	N	N	Y																	
R15347	N	N	N	N	N	Y																
R15348	N	N	N	N	N	Y																
R15349	N	N	N	N	N	Y																
GROUP: 4 - 240MG/KG/DAY PROPYLTHIOURACIL																						
R15356	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15357	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15358	N	N	N	N	N	N	N	N	N	N	N	N	P	Y								
R15359	N	N	N	N	N	N	N	N	N	N	N	N	N	T	Y							
R15360	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15361	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																						
R15368	N	N	N	N	N	N	N	N	N	N	Y											
R15369	N	N	N	N	N	N	N	N	N	N	N	N	N	Y								
R15370	N	N	N	N	N	N	N	N	N	N	N	N	N	P	Y							
R15371	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y							
R15372	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y					
R15373	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																						
R15380	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
R15401	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
R15402	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y					
R15403	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
R15404	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	P	Y	
R15405	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y						
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																						
R15412	N	N	N	N	N	Y																
R15413	N	N	N	N	N	Y																
R15414	N	N	N	N	N	Y																
R15415	N	N	N	N	N	Y																
R15516	N	N	N	N	N	Y																
R15417	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 5
INDIVIDUAL VAGINAL CYTOLOGY ANALYSES
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (SPRAGUE DAWLEY)

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																				
R15314	37													E	M	D	D	D	D	
R15315	37													E	M	D	D	D	E	
R15316	35										P	E	M	D	D	P	E	M	D	
R15317	41													M	D	D	P	E	E	M
R15318	35										P	E	M	D	D	P	E	M	D	D
R15319	34										E	M	D	D	P	E	M	D	D	P
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																				
R15326	26		E	E	E	E	E	D	P	E	E	E	E	M	D	D	D	D	D	
R15327	26		E	E	E	E	E	E	E	E	E	E	D	D	P	E	E	E	E	
R15328	25	E	E	E	E	E	M	D	D	P	E	E	E	E	E	E	*	E	E	
R15329	27			E	E	M	D	P	E	E	M	D	E	E	E	E	E	E	E	E
R15330	26		E	E	E	E	E	M	D	E	E	E	E	M	D	D	D	P	E	M
R15331	26		E	E	E	E	E	E	E	E	E	E	E	E	E	E	M	D	P	E
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																				
R15338	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15339	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15340	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15341	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15342	NE				D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15343	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
GROUP: 4 - 240MG/KG/DAY PROPYLTHIOURACIL																				
R15350	NE											D	D	D	D	D	D	D	D	
R15351	40															P	E	M	D	
R15352	NE											M	D	D	D	D	D	D	D	
R15353	NE																		D	D
R15354	37													E	M	D	D	D	D	D
R15355	41																P	E	M	D
GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE																				
R15362	41												D	D	D	D	P	E	E	
R15363	NE														M	D	D	D	D	
R15364	38														E	M	D	D	P	
R15365	NE																		D	D
R15366	NE															D	D	D	D	D
R15367	41															D	P	E	M	D
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																				
R15374	42															D	D	P	E	
R15375	NV																			
R15376	35											E	E	M	D	D	D	D	D	
R15377	42															D	D	D	E	E
R15378	35											E	M	D	D	D	D	D	D	D
R15379	37													E	E	M	D	D	D	D
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																				
R15406	27			E	M	D	D	D	P	E	D	E	M	E	E	E	M	D	D	
R15407	28			P	E	M	D	D	D	D	D	D	D	P	E	E	E	E	M	
R15408	28			P	E	E	E	D	D	D	D	D	D	P	E	M	D	D	D	
R15409	26		E	M	D	D	D	D	D	E	M	D	D	D	D	E	D	P	E	M
R15410	33			D	D	D	D	D	D	E	D	E	E	M	D	D	D	D	D	D
R15411	30			M	D	P	E	M	D	E	E	E	M	D	D	D	D	P	E	E

NE = NO ESTRUS OBSERVED
NV = NO VAGINAL CYTOLOGY EXAMINATIONS WERE PERFORMED FOR THIS ANIMAL BECAUSE VAGINA NEVER OPENED.
* = NO SAMPLE WAS TAKEN.
P = PROESTRUS E = ESTRUS M = METESTRUS D = DIESTRUS

APPENDIX CONTINUED

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

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																				
R15320	38										M	D	D	P	E	E	M	D	P	
R15321	35											E	M	D	D	P	E	M	D	
R15322	39														P	E	M	D	D	
R15323	UD									*	M	D	P	E	E	M	D	D	P	E
R15324	36												E	M	D	D	P	E	M	D
R15325	43																	D	P	E
GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL																				
R15332	26		E	E	E	E	M	D	E	M	D	P	E	E	D	E	D	P	E	
R15333	26		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	
R15334	26		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	
R15335	26		E	E	M	D	D	D	D	D	E	E	M	D	D	D	D	D	E	E
R15336	27			E	E	E	E	E	M	D	D	E	E	E	E	E	E	E	E	E
R15337	26		E	E	E	M	D	D	D	D	D	E	E	E	E	E	E	E	E	E
GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN																				
R15344	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15345	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15346	NE		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	
R15347	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15348	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
R15349	NE			D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
GROUP: 4 - 240 MG/KG/DAY PROPOTHIOURACIL																				
R15356	39											M	D	D	P	E	M	D	D	
R15357	NE											M	D	D	D	D	D	D	D	
R15358	42											M	D	D	D	D	D	D	E	
R15359	NE												M	D	D	D	D	D	D	D
R15360	43											M	D	D	D	D	D	D	D	E
R15361	33								P	E	M	D	D	D	D	D	D	D	D	D
GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE																				
R15368	UD								D	*	D	D	D	P	E	E	M	D	D	
R15369	NE											D	D	D	D	D	D	D	D	
R15370	37												D	E	D	D	D	D	P	
R15371	40												D	D	D	D	E	M	D	D
R15372	43														D	D	D	D	E	D
R15373	NE													D	D	D	D	D	D	D
GROUP: 6 - 30 MG/KG/DAY PIMOZIDE																				
R15380	38													P	E	E	M	D	D	
R15401	NV																			
R15402	39														P	E	M	D	D	
R15403	NE																			P
R15404	NE																		D	D
R15405	38													P	E	M	D	D	P	E
GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR																				
R15412	39			D	D	D	D	D	D	D	D	D	D	D	D	E	E	M	D	
R15413	39			D	D	D	D	D	D	D	D	D	D	D	D	E	E	M	D	
R15414	38			D	P	E	E	M	D	D	D	D	D	D	E	E	M	D	D	
R15415	27			E	M	D	D	D	D	D	D	E	E	M	D	D	D	D	E	E
R15416	29			D	P	E	E	E	E	E	M	D	D	D	P	E	E	E	M	D
R15417	36			D	D	D	D	D	D	D	D	P	E	E	M	D	D	P	E	E

NE = NO ESTRUS OBSERVED
NV = NO VAGINAL CYTOLOGY EXAMINATIONS WERE PERFORMED FOR THIS ANIMAL BECAUSE VAGINA NEVER OPENED.
* = NO SAMPLE WAS TAKEN.
UD = FIRST DAY OF ESTRUS COULD NOT BE CONCLUSIVELY DETERMINED FOR THIS ANIMAL BECAUSE NO SAMPLE WAS TAKEN ON PND 33.
P = PROESTRUS E = ESTRUS M = METESTRUS D = DIESTRUS

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
R15314	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15315	TERMINAL KILL	42	UTERUS WITH CERVIX	DISTENDED
R15316	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15317	TERMINAL KILL	43	LIVER	RAISED AREA,MEDIAN LOBE,ONE,TAN,5X4X2MM
R15318	TERMINAL KILL	43	THYROID	ENLARGED,SLIGHTLY
R15319	TERMINAL KILL	43	UTERUS WITH CERVIX	DISTENDED,BILATERAL,MODERATE
R15320	TERMINAL KILL	42	UTERUS WITH CERVIX	DISTENDED,BILATERAL
R15321	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15342	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15343	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15324	TERMINAL KILL	43	THYROID	ENLARGED,MODERATE
R15325	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15314 - R15319 ARE SPRAGUE DAWLEY
ANIMALS R15320 - R15325 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
R15326	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15327	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15328	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15329	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15330	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15331	TERMINAL KILL	43	UTERUS WITH CERVIX	CYST,RIGHT HORN,ONE,CLEAR,1X1X1MM
R15332	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15333	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15334	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15335	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15336	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15337	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15326 - R15331 ARE SPRAGUE DAWLEY
ANIMALS R15332 - R15337 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
R15338	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15339	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15340	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15341	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15342	TERMINAL KILL	43	LIVER	ACCESSORY LOBE,MEDIAN LOBE,AT CLEFT, 15X4X2MM
			THYROID	ENLARGED,SLIGHT
R15343	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15344	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15345	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15346	TERMINAL KILL	42	OVARIES	CYST,RIGHT,ONE,CLEAR,6X3X4MM
R15347	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15348	TERMINAL KILL	43	OVARIES	CYST,RIGHT,ONE,CLEAR,4X3X3MM
R15349	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15338 - R15343 ARE SPRAGUE DAWLEY
ANIMALS R15344 - R15349 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
R15350	TERMINAL KILL	42	THYROID	ENLARGED, MODERATE
R15351	TERMINAL KILL	42	THYROID	ENLARGED, MODERATE
R15352	TERMINAL KILL	42	THYROID	ENLARGED
R15353	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15354	TERMINAL KILL	43	THYROID	ENLARGED,SEVERE
R15355	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15356	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15357	TERMINAL KILL	42	THYROID	ENLARGED
R15358	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15359	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15360	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15361	TERMINAL KILL	43	THYROID	ENLARGED,SEVERE

KEY: ANIMALS R15350 - R15355 ARE SPRAGUE DAWLEY
ANIMALS R15356 - R15361 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
R15362	TERMINAL KILL	42	ADRENALS	ENLARGED, BILATERAL,5X5X4MM; PALE, BILATERAL
R15363	TERMINAL KILL	42	ADRENALS	ENLARGED,BILATERAL, 5X5X4 MM
			THYROID	ENLARGED, SLIGHT
R15364	TERMINAL KILL	42	UTERUS WITH CERVIX	DISTENDED, MODERATE
			ADRENALS	ENLARGED, SEVERE
R15365	TERMINAL KILL	43	ADRENALS	ENLARGED,BILATERAL,3X3X3MM
R15366	TERMINAL KILL	43	ADRENALS	ENLARGED,BILATERAL,4X3X3MM
R15367	TERMINAL KILL	43	ADRENALS	ENLARGED,BILATERAL,5X5X3MM
R15368	TERMINAL KILL	42	ADRENALS	ENLARGED,BILATERAL, SEVERE
R15369	TERMINAL KILL	42	ADRENALS	ENLARGED,BILATERAL, SEVERE
R15370	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15371	TERMINAL KILL	43	ADRENALS	ENLARGED,BILATERAL,5X5X3MM
R15372	TERMINAL KILL	43	LIVER	ACCESSORY LOBE,MEDIAN LOBE AT CLEFT,7X3X2MM
			ADRENALS	ENLARGED,BILATERAL, 4X3X3MM
R15373	TERMINAL KILL	43	ADRENALS	ENLARGED,BILATERAL,4X3X3MM

KEY: ANIMALS R15362 - R15367 ARE SPRAGUE DAWLEY
ANIMALS R15368 - R15373 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
R15374	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15375	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15376	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15377	TERMINAL KILL	43	LIVER	RAISED AREA,LEFT LATERAL,ONE,TAN,6X4X3MM
			UTERUS WITH CERVIX	DISTENDED,SEVERE
R15378	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15379	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15380	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15401	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15402	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15403	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15404	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15405	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15374 - R15379 ARE SPRAGUE DAWLEY
ANIMAL R15380 AND ANIMALS R15401 - R15405 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
R15406	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15407	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15408	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15409	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15410	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15411	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15412	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15413	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15414	TERMINAL KILL	42		<NO ORGANS WITH GROSS FINDINGS>
R15415	TERMINAL KILL	43	UTERUS WITH CERVIX	DISTENDED,MODERATE
R15416	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>
R15417	TERMINAL KILL	43		<NO ORGANS WITH GROSS FINDINGS>

KEY: ANIMALS R15406 - R15411 ARE SPRAGUE DAWLEY
ANIMALS R15412 - R15417 ARE LONG-EVANS

APPENDIX 7
INDIVIDUAL ORGAN WEIGHTS 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 STRAIN: SPRAGUE DAWLEY

ANIMAL ID:	R15314	R15315	R15316	R15317	R15318	R15319
BODY WEIGHT(G)	138.9	145.4	144.8	145.5	149.2	155.0
LIVER(G)	5.878	6.115	5.505	5.757	6.176	6.460
% BODY WEIGHT	4.232	4.206	3.802	3.957	4.139	4.168
KIDNEYS (PAIRED)(G)	1.153	1.184	1.264	1.149	1.239	1.366
% BODY WEIGHT	0.830	0.814	0.873	0.790	0.830	0.881
UTERUS WITH CERVIX(G)	0.185	0.620	0.261	0.198	0.298	0.395
% BODY WEIGHT	0.133	0.426	0.180	0.136	0.200	0.255
UTERUS WITH CERVIX(DRY)(G)	0.169	0.359	0.252	0.190	0.289	0.334
% BODY WEIGHT	0.122	0.247	0.174	0.131	0.194	0.215
OVARIES(G)	0.236[a]	0.072	0.068	0.055	0.073	0.075
% BODY WEIGHT	0.170[a]	0.050	0.047	0.038	0.049	0.048
ADRENALS(G)	0.267[a]	0.052	0.037	0.037	0.056	0.046
% BODY WEIGHT	0.192[a]	0.036	0.026	0.025	0.038	0.030
PITUITARY(G)	0.0051	0.0075	0.0074	0.0069	0.0049	0.0083
% BODY WEIGHT	0.0037	0.0052	0.0051	0.0047	0.0033	0.0054

APPENDIX CONTINUED

a Weight out of range - excluded from summary data.

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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 — STRAIN: LONG-EVANS

ANIMAL ID:	R15323	R15324	R15325	R15320	R15321	R15322
BODY WEIGHT(G)	156.9	164.7	174.0	148.5	161.4	146.3
LIVER(G)	7.202	6.868	8.142	5.711	7.208	5.690
% BODY WEIGHT	4.590	4.170	4.679	3.846	4.466	3.889
KIDNEYS (PAIRED)(G)	1.426	1.385	1.379	1.204	1.573	1.246
% BODY WEIGHT	0.909	0.841	0.793	0.811	0.975	0.852
UTERUS WITH CERVIX(G)	0.285	0.171	0.231	0.443	0.209	0.223
% BODY WEIGHT	0.182	0.104	0.133	0.298	0.129	0.152
UTERUS WITH CERVIX(DRY)(G)	0.275	0.159	0.216	0.336	0.199	0.213
% BODY WEIGHT	0.175	0.097	0.124	0.226	0.123	0.146
OVARIES (G)	0.102	0.076	0.097	0.048	0.097	0.064
% BODY WEIGHT	0.065	0.046	0.056	0.032	0.060	0.044
ADRENALS (G)	0.036	0.029	0.036	0.027	0.045	0.048
% BODY WEIGHT	0.023	0.018	0.021	0.018	0.028	0.033
PITUITARY (G)	0.0086	0.0133	0.0082	0.0033	0.0068	0.0059
% BODY WEIGHT	0.0055	0.0081	0.0047	0.0022	0.0042	0.0040

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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 STRAIN: SPRAGUE-DAWLEY

ANIMAL ID:	R15326	R15327	R15328	R15329	R15330	R15331
BODY WEIGHT(G)	135.9	135.9	127.3	142.4	139.4	147.0
LIVER(G)	6.008	5.539	4.964	6.593	6.642	7.125
% BODY WEIGHT	4.421	4.076	3.899	4.630	4.765	4.847
KIDNEYS (PAIRED)(G)	1.063	1.067	0.912	1.211	1.036	1.204
% BODY	0.782	0.785	0.716	0.850	0.743	0.819
UTERUS WITH CERVIX(G)	0.327	0.270	0.239	0.270	0.245	0.383
% BODY WEIGHT	0.241	0.199	0.188	0.190	0.176	0.261
UTERUS WITH CERVIX(DRY)(G)	0.302	0.261	0.238	0.245	0.230	0.344
% BODY WEIGHT	0.222	0.192	0.187	0.172	0.165	0.234
OVARIES(G)	0.060	0.054	0.059	0.039	0.045	0.051
% BODY WEIGHT	0.044	0.040	0.046	0.027	0.032	0.035
ADRENALS(G)	0.043	0.037	0.030	0.041	0.039	0.046
% BODY WEIGHT	0.032	0.027	0.024	0.029	0.028	0.031
PITUITARY(G)	0.0053	0.0064	0.0079	0.0390[a]	0.0043	0.0094
% BODY WEIGHT	0.0039	0.0047	0.0062	0.0274[a]	0.0031	0.0064

APPENDIX CONTINUED

a Weight out of range - excluded from summary data.

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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 STRAIN: LONG-EVANS

ANIMAL ID:	R15335	R15336	R15337	R15332	R15333	R15334
BODY WEIGHT(G)	148.5	129.9	138.9	160.7	135.0	138.3
LIVER(G)	6.709	5.375	6.474	7.860	5.757	5.854
% BODY WEIGHT	4.518	4.138	4.661	4.891	4.264	4.233
KIDNEYS (PAIRED)(G)	1.338	1.137	1.218	1.697	1.130	1.207
% BODY WEIGHT	0.901	0.875	0.877	1.056	0.837	0.873
UTERUS WITH CERVIX(G)	0.239	0.257	0.287	0.285	0.298	0.260
% BODY	0.161	0.198	0.207	0.177	0.221	0.188
UTERUS WITH CERVIX(DRY)(G)	0.222	0.243	0.255	0.270	0.288	0.248
% BODY WEIGHT	0.149	0.187	0.184	0.168	0.213	0.179
OVARIES(G)	0.045	0.051	0.061	0.119	0.027	0.044
% BODY WEIGHT	0.030	0.039	0.044	0.074	0.020	0.032
ADRENALS(G)	0.061	0.038	0.053	0.056	0.028	0.038
% BODY WEIGHT	0.041	0.029	0.038	0.035	0.021	0.027
PITUITARY(G)	0.0040	0.0069	0.0050	0.0086	0.0075	0.0197[a]
% BODY WEIGHT	0.0027	0.0053	0.0036	0.0054	0.0056	0.0142[a]

APPENDIX CONTINUED

a Weight out of range - excluded from summary data.

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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					STRAIN: SPRAGUE DAWLEY	
ANIMAL ID:	R15338	R15339	R15340	R15341	R15342	R15343
BODY WEIGHT(G)	107.0	115.0	114.4	119.4	113.3	126.1
LIVER(G)	4.477	5.221	4.785	5.456	5.059	5.511
% BODY WEIGHT	4.184	4.540	4.183	4.570	4.465	4.370
KIDNEYS (PAIRED)(G)	0.971	1.038	1.063	1.010	0.935	1.182
% BODY WEIGHT	0.907	0.903	0.929	0.846	0.825	0.937
UTERUS WITH CERVIX(G)	0.140	0.105	0.122	0.086	0.082	0.091
% BODY WEIGHT	0.131	0.091	0.107	0.072	0.072	0.072
UTERUS WITH CERVIX(DRY)(G)	0.130	0.102	0.116	0.079	0.072	0.082
% BODY WEIGHT	0.121	0.089	0.101	0.066	0.064	0.065
OVARIES(G)	0.037	0.045	0.044	0.035	0.016	0.019
% BODY WEIGHT	0.035	0.039	0.038	0.029	0.014	0.015
ADRENALS(G)	0.030	0.045	0.031	0.032	0.025	0.028
% BODY WEIGHT	0.028	0.039	0.027	0.027	0.022	0.022
PITUITARY(G)	0.0027	0.0023	0.0030	0.0050	0.0031	0.0037
% BODY WEIGHT	0.0025	0.0020	0.0026	0.0042	0.0027	0.0029

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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					STRAIN: LONG-EVANS	
ANIMAL ID:	R15347	R15348	R15349	R15344	R15345	R15346
BODY WEIGHT(G)	125.4	125.1	119.8	123.0	128.6	125.5
LIVER(G)	5.796	5.772	4.843	5.351	6.327	5.697
% BODY WEIGHT	4.622	4.614	4.043	4.350	4.920	4.539
KIDNEYS (PAIRED)(G)	1.207	1.189	1.126	1.251	1.259	1.143
% BODY WEIGHT	0.963	0.950	0.940	1.017	0.979	0.911
UTERUS WITH CERVIX(G)	0.114	0.109	0.098	0.107	0.102	0.086
% BODY WEIGHT	0.091	0.087	0.082	0.087	0.079	0.069
UTERUS WITH CERVIX(DRY)(G)	0.106	0.099	0.090	0.096	0.099	0.078
% BODY WEIGHT	0.085	0.079	0.075	0.078	0.077	0.062
OVARIES(G)	0.061	0.073	0.050	0.054	0.054	0.022
% BODY WEIGHT	0.049	0.058	0.042	0.044	0.042	0.018
ADRENALS(G)	0.029	0.039	0.035	0.033	0.048	0.024
% BODY WEIGHT	0.023	0.031	0.029	0.027	0.037	0.019
PITUITARY(G)	0.0043	0.0040	0.0035	0.0036	0.0062	0.0170[a]
% BODY WEIGHT	0.0034	0.0032	0.0029	0.0029	0.0048	0.0135[a]

APPENDIX CONTINUED

a Weight out of range - excluded from summary data.

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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 STRAIN: SPRAGUE DAWLEY

ANIMAL ID:	R15350	R15351	R15352	R15353	R15354	R15355
BODY WEIGHT(G)	102.3	89.1	101.4	90.9	96.6	102.0
LIVER(G)	4.633	3.837	4.488	4.000	4.716	4.896
% BODY WEIGHT	4.529	4.306	4.426	4.400	4.882	4.800
KIDNEYS (PAIRED)(G)	0.819	0.665	0.730	0.750	0.781	0.761
% BODY WEIGHT	0.801	0.746	0.720	0.825	0.808	0.746
UTERUS WITH CERVIX(G)	0.124	0.120	0.142	0.084	0.125	0.148
% BODY WEIGHT	0.121	0.135	0.140	0.092	0.129	0.145
UTERUS WITH CERVIX(DRY)(G)	0.117	0.114	0.137	0.077	0.113	0.137
% BODY WEIGHT	0.114	0.128	0.135	0.085	0.117	0.134
OVARIES(G)	0.032	0.031	0.043	0.017	0.055	0.063
% BODY WEIGHT	0.031	0.035	0.042	0.019	0.057	0.062
ADRENALS(G)	0.021	0.022	0.024	0.025	0.026	0.026
% BODY WEIGHT	0.021	0.025	0.024	0.028	0.027	0.025
PITUITARY(G)	0.0069	0.0075	0.0055	0.0052	0.0046	0.0066
% BODY WEIGHT	0.0067	0.0084	0.0054	0.0057	0.0048	0.0065

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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 STRAIN: LONG-EVANS

ANIMAL ID:	R15359	R15360	R15361	R15356	R15357	R15358
BODY WEIGHT(G)	80.1	78.7	85.3	71.2	88.4	89.3
LIVER(G)	3.961	3.485	3.577	2.873	3.613	4.083
% BODY WEIGHT	4.945	4.428	4.193	4.035	4.087	4.572
KIDNEYS (PAIRED)(G)	0.690	0.593	0.644	0.603	0.704	0.753
% BODY WEIGHT	0.861	0.753	0.755	0.847	0.796	0.843
UTERUS WITH CERVIX(G)	0.086	0.240	0.138	0.196	0.107	0.162
% BODY WEIGHT	0.107	0.305	0.162	0.275	0.121	0.181
UTERUS WITH CERVIX(DRY)(G)	0.078	0.169	0.128	0.142	0.134	0.154
% BODY WEIGHT	0.097	0.215	0.150	0.199	0.152	0.172
OVARIES(G)	0.039	0.046	0.040	0.042	0.029	0.048
% BODY WEIGHT	0.049	0.058	0.047	0.059	0.033	0.054
ADRENALS(G)	0.021	0.020	0.025	0.021	0.032	0.020
% BODY WEIGHT	0.026	0.025	0.029	0.029	0.036	0.022
PITUITARY(G)	0.0073	0.0047	0.0029	0.0040	0.0045	0.0068
% BODY WEIGHT	0.0091	0.0060	0.0034	0.0056	0.0051	0.0076

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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 STRAIN: SPRAGUE DAWLEY

ANIMAL ID:	R15362	R15363	R15364	R15365	R15366	R15367
BODY WEIGHT(G)	137.3	135.2	130.1	143.6	138.2	148.7
LIVER(G)	7.149	7.742	6.668	7.749	7.943	8.059
% BODY WEIGHT	5.207	5.726	5.125	5.396	5.747	5.420
KIDNEYS (PAIRED)(G)	1.256	1.106	1.208	1.292	1.325	1.457
% BODY WEIGHT	0.915	0.818	0.929	0.900	0.959	0.980
UTERUS WITH CERVIX(G)	0.214	0.140	0.280	0.204	0.130	0.328
% BODY WEIGHT	0.156	0.104	0.215	0.142	0.094	0.221
UTERUS WITH CERVIX(DRY)(G)	0.202	0.131	0.217	0.184	0.118	0.262
% BODY WEIGHT	0.147	0.097	0.167	0.128	0.085	0.176
OVARIES(G)	0.072	0.049	0.057	0.052	0.054	0.058
% BODY WEIGHT	0.052	0.036	0.044	0.036	0.039	0.039
ADRENALS(G)	0.089	0.083	0.084	0.081	0.091	0.117
% BODY	0.065	0.061	0.065	0.056	0.066	0.079
PITUITARY(G)	0.0081	0.0057	0.0013[a]	0.0071	0.0095	0.0054
% BODY WEIGHT	0.0059	0.0042	0.0010[a]	0.0049	0.0069	0.0036

APPENDIX CONTINUED

a Weight out of range - excluded from summary data.

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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					STRAIN: LONG-EVANS	
ANIMAL ID:	R15371	R15372	R15373	R15368	R15369	R15370
BODY WEIGHT(G)	134.8	129.6	137.3	141.9	132.6	147.0
LIVER(G)	7.406	6.692	7.213	8.349	7.726	7.172
% BODY WEIGHT	5.494	5.164	5.253	5.884	5.827	4.879
KIDNEYS (PAIRED)(G)	1.404	1.329	1.253	1.358	1.331	1.461
% BODY WEIGHT	1.042	1.025	0.913	0.957	1.004	0.994
UTERUS WITH CERVIX(G)	0.136	0.200	0.076	0.269	0.123	0.284
% BODY WEIGHT	0.101	0.154	0.055	0.190	0.093	0.193
UTERUS WITH CERVIX(DRY)(G)	0.125	0.186	0.069	0.255	0.121	0.263
% BODY WEIGHT	0.093	0.144	0.050	0.180	0.091	0.179
OVARIES(G)	0.052	0.063	0.051	0.068	0.078	0.090
% BODY WEIGHT	0.039	0.049	0.037	0.048	0.059	0.061
ADRENALS(G)	0.081	0.048	0.054	0.096	0.097	0.067
% BODY WEIGHT	0.060	0.037	0.039	0.068	0.073	0.046
PITUITARY(G)	0.0082	0.0062	0.0065	0.0042	0.0081	0.0076
% BODY WEIGHT	0.0061	0.0048	0.0047	0.0030	0.0061	0.0052

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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					STRAIN: SPRAGUE DAWLEY	
ANIMAL ID:	R15374	R15375	R15376	R15377	R15378	R15379
BODY WEIGHT(G)	120.7	120.0	130.1	115.7	135.7	135.9
LIVER(G)	5.170	5.295	5.457	4.069	5.429	5.417
% BODY WEIGHT	4.283	4.413	4.194	3.517	4.001	3.986
KIDNEYS (PAIRED)(G)	0.981	0.932	1.104	0.949	1.092	1.107
% BODY WEIGHT	0.813	0.777	0.849	0.820	0.805	0.815
UTERUS WITH CERVIX(G)	0.202	0.104	0.174	0.365	0.151	0.177
% BODY WEIGHT	0.167	0.087	0.134	0.315	0.111	0.130
UTERUS WITH CERVIX(DRY)(G)	0.195	0.093	0.172	0.223	0.133	0.158
% BODY WEIGHT	0.162	0.078	0.132	0.193	0.098	0.116
OVARIES(G)	0.055	0.042	0.047	0.022	0.068	0.070
% BODY WEIGHT	0.046	0.035	0.036	0.019	0.050	0.052
ADRENALS(G)	0.031	0.028	0.024	0.027	0.043	0.028
% BODY WEIGHT	0.026	0.023	0.018	0.023	0.032	0.021
PITUITARY(G)	0.0055	0.0042	0.0063	0.0103	0.0009[a]	0.0058
% BODY WEIGHT	0.0046	0.0035	0.0048	0.0089	0.0007[a]	0.0043

APPENDIX CONTINUED

a Weight out of range - excluded from summary data.

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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					STRAIN: LONG-EVANS	
ANIMAL ID:	R15403	R15404	R15405	R15380	R15401	R15402
BODY WEIGHT(G)	95.2	113.0	109.2	123.0	108.1	105.9
LIVER(G)	3.491	4.804	4.338	5.277	3.983	4.081
% BODY WEIGHT	3.667	4.251	3.973	4.290	3.685	3.854
KIDNEYS (PAIRED)(G)	0.881	0.888	0.883	1.125	0.945	0.884
% BODY WEIGHT	0.925	0.786	0.809	0.915	0.874	0.835
UTERUS WITH CERVIX(G)	0.160	0.141	0.180	0.251	0.067	0.147
% BODY WEIGHT	0.168	0.125	0.165	0.204	0.062	0.139
UTERUS WITH CERVIX(DRY)(G)	0.150	0.131	0.168	0.228	0.064	0.142
% BODY WEIGHT	0.158	0.116	0.154	0.185	0.059	0.134
OVARIES(G)	0.052	0.047	0.055	0.031	0.044	0.041
% BODY WEIGHT	0.055	0.042	0.050	0.025	0.041	0.039
ADRENALS(G)	0.027	0.025	0.026	0.021	0.024	0.027
% BODY WEIGHT	0.028	0.022	0.024	0.017	0.022	0.025
PITUITARY(G)	0.0049	0.0020	0.0056	0.0064	0.0036	0.0051
% BODY WEIGHT	0.0051	0.0018	0.0051	0.0052	0.0033	0.0048

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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 — STRAIN: SPRAGUE-DAWLEY

ANIMAL ID:	R15406	R15407	R15408	R15409	R15410	R15411
BODY WEIGHT(G)	136.4	139.3	135.3	130.8	143.3	151.7
LIVER(G)	6.201	5.810	5.603	5.059	6.152	6.188
% BODY WEIGHT	4.546	4.171	4.141	3.868	4.293	4.079
KIDNEYS (PAIRED)(G)	1.127	1.166	1.088	1.067	1.111	1.165
% BODY WEIGHT	0.826	0.837	0.804	0.816	0.775	0.768
UTERUS WITH CERVIX(G)	0.246	0.194	0.262	0.176	0.174	0.292
% BODY WEIGHT	0.180	0.139	0.194	0.135	0.121	0.192
UTERUS WITH CERVIX(DRY)(G)	0.234	0.182	0.215	0.161	0.165	0.272
% BODY WEIGHT	0.172	0.131	0.159	0.123	0.115	0.179
OVARIES(G)	0.065	0.065	0.071	0.046	0.054	0.037
% BODY WEIGHT	0.048	0.047	0.052	0.035	0.038	0.024
ADRENALS(G)	0.045	0.041	0.044	0.035	0.038	0.033
% BODY	0.033	0.029	0.033	0.027	0.027	0.022
PITUITARY(G)	0.0054	0.0109	0.0066	0.0092	0.0058	0.0042
% BODY WEIGHT	0.0040	0.0078	0.0049	0.0070	0.0040	0.0028

APPENDIX CONTINUED

APPENDIX 7 (CONTINUED)
INDIVIDUAL ORGAN WEIGHTS 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					STRAIN: LONG-EVANS	
ANIMAL ID:	R15415	R15416	R15417	R15412	R15413	R15414
BODY WEIGHT(G)	137.8	141.5	139.3	136.2	137.4	152.1
LIVER(G)	5.115	5.438	6.135	5.165	5.279	6.669
% BODY WEIGHT	3.712	3.843	4.404	3.792	3.842	4.385
KIDNEYS (PAIRED)(G)	1.214	1.290	1.282	1.230	1.164	1.364
% BODY WEIGHT	0.881	0.912	0.920	0.903	0.847	0.897
UTERUS WITH CERVIX(G)	0.393	0.227	0.228	0.196	0.180	0.200
% BODY WEIGHT	0.285	0.160	0.164	0.144	0.131	0.131
UTERUS WITH CERVIX(DRY)(G)	0.312	0.208	0.212	0.186	0.174	0.180
% BODY WEIGHT	0.226	0.147	0.152	0.137	0.127	0.118
OVARIES(G)	0.064	0.066	0.095	0.062	0.076	0.089
% BODY WEIGHT	0.046	0.047	0.068	0.046	0.055	0.059
ADRENALS(G)	0.042	0.035	0.067	0.031	0.043	0.057
% BODY WEIGHT	0.030	0.025	0.048	0.023	0.031	0.037
PITUITARY(G)	0.0035	0.0052	0.0054	0.0053	0.0050	0.0065
% BODY WEIGHT	0.0025	0.0037	0.0039	0.0039	0.0036	0.0043

APPENDIX 8
PATHOLOGY REPORT
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (SPRAGUE DAWLEY)

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

PATHOLOGY REPORT
FOR

ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS

THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

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 1143-103

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/Dilated	1	1	0	0	0	0	0	0	1	0	0	0	0	0
Uterus/Distended	1	0	0	0	0	0	0	0	0	0	1	0	0	1
Uterus/Cyst	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Ovary/Cyst	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Thyroid/Enlarged	0	1	0	0	1	0	4	2	0	0	0	0	0	0
Adrenals/Enlarged	0	0	0	0	0	0	0	0	6	5	0	0	0	0
Adrenals/Pale	0	0	0	0	0	0	0	0	1	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 – Generally, the thyroid glands were within normal limits. In one animal there was a locally extensive area of intra-glandular hemorrhage accompanied by compression of surrounding follicles. There were no test article related findings in the thyroid glands. Test article related changes in the ovary were atrophy (3/6). 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 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 (5/6) and myometrial hypertrophy/hyperplasia (5/6) and squamous metaplasia (1/6). 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 in number 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 (3/6) and luteal cyst (1/6). Histologic changes are as described above. Test article related changes in the uterus consisted of epithelial (2/6) and myometrial hypertrophy/hyperplasia (2/6).

Tamoxifen (10 mg/kg/day)

Sprague-Dawley - There were no test article related findings in the thyroid glands. Test article related changes in the ovary were atrophy (6/6). 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), squamous metaplasia (3/6), and epithelial hyperplasia/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.

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). 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 hyperplasia/hypertrophy (6/6).

Propylthiouracil (240 mg/kg/day)

Sprague-Dawley - Test article related findings in the thyroid were hyperplasia/ hypertrophy of the follicular cells (5/5) and colloid depletion (5/5). 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. A follicular cyst was present in one ovary but its relationship to the test article is uncertain. Test article related findings in the uteri consisted of atrophy (1/6). This change is 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 and uteri consisted of atrophy (1/6). 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 findings in the ovary consisted of interstitial cell hyperplasia (6/6), depletion of corpora lutea (6/6), and follicular cysts (4/6). The ovaries were within normal limits for size. Most 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 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. Follicular cysts were common and composed of a large, central, fluid filled cavity lined by a thin layer of often degenerate granulosa cells. External to the granulosa cells was a thick layer of prominent theca interna cells similar in appearance to the interstitial cells. Interstitial cell hyperplasia was considered a direct effect of the test article. The follicular cysts and the lack of corpora lutea were considered 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. Occasional uteri exhibited tissue changes consistent with proestrus or estrus. Uterine changes were considered a secondary effect of the test article as a result of deranged steroid hormone synthesis.

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 findings in the ovary consisted of interstitial cell hyperplasia (6/6), depletion of corpora lutea (4/6), and follicular cysts (4/6). The presence of corpora lutea in some ovaries indicates incomplete dosing or absence of a drug effect in some

animals. Alternatively, these structures may have been the result of earlier cycles prior to the drug effect. In general, the uteri were inactive exhibiting morphology consistent with diestrus.

Pimozide (30 mg/kg/day)

Sprague-Dawley - There were no test article related findings in the thyroid glands. Test article related changes in the ovary consisted of follicular cysts (2/6), luteal cysts (1/6), polyovular follicles (1/6), and depleted corpora lutea (2/6). The majority of ovaries examined appeared small. 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. There appeared to be an increased tendency toward abnormal follicular maturation characterized by polyovular follicles, increased follicular atresia, luteal cysts, partial lutinization of follicles and a reduced number of late tertiary or Graafian follicles. The ovarian changes are thought to be directly related to the test article most likely as a result of deranged pituitary hormone secretion. Test article related changes in the uteri consisted of atrophy (1/6), and epithelial hyperplasia/hypertrophy (2/6). The majority of uteri were inactive but of normal size. One animal had uterine changes consistent with metesterus. The epithelium was hyperplastic, however, characterized by increased epithelial folding.

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 (3/6), and depleted corpora lutea (6/6). Test article related changes in the uteri consisted of atrophy (4/6) and epithelial hyperplasia/hypertrophy (2/6). The majority of uteri were inactive but two exhibited changes consistent with metestrus.

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 follicular cysts (3/6). This change is presumable related to premature follicular atresia as a result of reduced pituitary hormone influence. Test article related findings in the uterus consisted of epithelial hyperplasia/hypertrophy (2/6), and endometrial gland dilation (1/6). The uterine changes are considered direct effects of the test article.

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. There were no test article related findings in the ovary. Test article related findings in the uterus consisted of epithelial hyperplasia/hypertrophy (1/6), and endometrial gland dilation (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 pimozide.

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 JUNE 00

Appendix 1:
Reports Code Table

PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

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: 1143-103

Abbreviations List

# EX	Number Examined
1143103	1143-103
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: 1143-103

PROJECT SUMMARY

STUDY ID : 1143-103
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143103

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	6	6	5	6
MINERALIZATION, FOLLIULAR		1	0	0	0	0
ULTIMOBRANCHIAL CYST		1	0	0	0	1
HEMORRHAGE AND EDEMA, ORGANIZING		0	1	0	0	0
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0	0	5	0
COLLOID DEPLETION		0	0	0	5	0
OVARIES	# EX	6	6	6	6	6
CYST, FOLLICULAR		1	0	0	1	4
ATROPHY		0	3	6	0	0
CYST, LUTEUAL		0	0	0	0	0
INTERSTITIAL CELL HYPERPLASIA		0	0	0	0	6
CORPORA LUTEA, ABSENT		0	0	6	0	6
POLYOVULAR FOLLICLES		0	0	0	0	0
REDUCED TERTIARY FOLLICLES		0	0	0	0	0
UTERUS	# EX	6	6	6	6	6
ENDOMETRIAL HYPERPLASIA		0	5	0	0	0
HYPERTROPHY, MYOMETRIUM		0	5	0	0	0
SQUAMOUS METAPLASIA		0	1	3	0	0
CYST, PERIUTERINE		0	1	0	0	0
ATROPHY		0	0	6	1	0
EPITHELIAL HYPERPLASIA/HYPERTROPHY		0	0	6	0	0
CYSTIC DILATION, ENDOMETRIAL GLAND(S)		0	0	0	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: 1143-103

PROJECT SUMMARY

STUDY ID : 1143-103 STUDY NUMBER: 1143103
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
MINERALIZATION, FOLLIULAR		0	0
ULTIMOBRANCHIAL CYST		0	1
HEMORRHAGE AND EDEMA, ORGANIZING		0	0
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0
COLLOID DEPLETION		0	0
OVARIES	# EX	6	6
CYST, FOLLICULAR		2	3
ATROPHY		0	0
CYST, LUTEUAL		1	0
INTERSTITIAL CELL HYPERPLASIA		0	0
CORPORA LUTEA, ABSENT		2	0
POLYOVULAR FOLLICLES		1	0
REDUCED TERTIARY FOLLICLES		4	0
UTERUS	# EX	6	6
ENDOMETRIAL HYPERPLASIA		0	0
HYPERTROPHY, MYOMETRIUM		0	0
SQUAMOUS METAPLASIA		0	0
CYST, PERIUTERINE		0	0
ATROPHY		1	0
EPITHELIAL HYPERPLASIA/HYPERTROPHY		2	2
CYSTIC DILATION, ENDOMETRIAL GLAND(S)		0	1

(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: 1143-103

PROJECT SUMMARY

STUDY ID : 1143-103 STUDY NUMBER: 1143103
FATE: TK Long-Evans 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	6	6	6	6
DEGENERATION, FOLLICULAR		1	0	0	0	0
ULTIMOBRANCHIAL CYST		1	1	1	0	2
INFILTRATING LYMPHOCYTES		1	0	0	0	0
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0	0	6	0
COLLOID DEPLETION		0	0	0	6	0
OVARIES	# EX	6	6	6	6	6
CYST, FOLLICULAR		0	0	0	0	4
ATROPHY		0	3	6	1	0
CYST, LUTEUAL		0	1	0	0	0
CYSTIC DILATION, BURSA		0	0	1	0	0
INTERSTITIAL CELL HYPERPLASIA		0	0	0	0	6
CORPORA LUTEA, ABSENT		0	0	6	0	4
REDUCED TERTIARY FOLLICLES		0	0	0	0	0
UTERUS	# EX	6	6	6	6	6
ENDOMETRIAL HYPERPLASIA		0	2	0	0	0
HYPERTROPHY, MYOMETRIUM		0	2	0	0	0
ATROPHY		0	0	6	1	0
EPITHELIAL HYPERPLASIA/HYPERTROPHY		0	0	6	0	0
CYSTIC DILATION, ENDOMETRIAL GLAND(S)		0	2	0	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: 1143-103

PROJECT SUMMARY

STUDY ID : 1143-103 STUDY NUMBER: 1143103
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	6	6
DEGENERATION, FOLLICULAR		0	0
ULTIMOBRANCHIAL CYST		1	3
INFILTRATING LYMPHOCYTES		0	0
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY		0	0
COLLOID DEPLETION		0	0
OVARIES	# EX	6	6
CYST, FOLLICULAR		3	0
ATROPHY		0	0
CYST, LUTEUAL		0	0
CYSTIC DILATION, BURSA		0	0
INTERSTITIAL CELL HYPERPLASIA		0	0
CORPORA LUTEA, ABSENT		6	0
REDUCED TERTIARY FOLLICLES		2	0
UTERUS	# EX	6	6
ENDOMETRIAL HYPERPLASIA		0	0
HYPERTROPHY, MYOMETRIUM		0	0
ATROPHY		4	0
EPITHELIAL HYPERPLASIA/HYPERTROPHY		2	1
CYSTIC DILATION, ENDOMETRIAL GLAND(S)		0	1

(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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143103
GROUP: 1: Corn Oil (2.5ml/kg/d)
SEX: FEMALE

ANIMAL ID:	R15314	R15315	R15316	R15317	R15318	R15319
THYROID	N	N	-	N	N	N
MINERALIZATION, FOLLIULAR	-	-	<1>	-	-	-
ULTIMOBRANCHIAL CYST	-	-	<P>	-	-	-
OVARIES	N	N	N	N	-	N
CYST, FOLLICULAR	-	-	-	-	<1>	-
UTERUS	N	N	N	N	N	N
Non-Protocol Tissues:						
LIVER	-	-	-	*	-	-

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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143103
GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15326	R15327	R15328	R15329	R15330	R15331
THYROID	N	N	N	-	N	N
HEMORRHAGE AND EDEMA, ORGANIZING	-	-	-	3	-	-
OVARIES	N	-	-	-	N	N
ATROPHY	-	P	P	P	-	-
UTERUS	-	-	-	-	-	-
ENDOMETRIAL HYPERPLASIA	[P]	[P]	[P]	-	[P]	[P]
HYPERTROPHY, MYOMETRIUM	[P]	[P]	[P]	-	[P]	[P]
SQUAMOUS METAPLASIA	-	-	-	(1)	-	-
CYST, PERIUTERINE	-	-	-	-	-	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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143103
GROUP: 3: Tamoxifen (10mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15338	R15339	R15340	R15341	R15342	R15343
THYROID	N	N	N	N	N	N
OVARIES	-	-	-	-	-	-
ATROPHY	P	P	P	P	P	P
CORPORA LUTEA, ABSENT	P	P	P	P	P	P
UTERUS	-	-	-	-	-	-
SQUAMOUS METAPLASIA	-	-	<2>	-	(2)	(2)
ATROPHY	P	P	P	P	P	P
EPITHELIAL HYPERPLASIA/HYPERTROPHY	P	P	P	P	P	P
Non-Protocol Tissues:						
LIVER	-	-	-	-	*	-

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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103 STUDY NUMBER: 1143103
FATE: TK Sprague-Dawley GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15350	R15351	R15352	R15353	R15354	R15355
THYROID	-	-	-	-	-	U
FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY	[4]	[4]	[4]	[4]	[4]	-
COLLOID DEPLETION	[4]	[4]	[4]	[4]	[4]	-
OVARIES	N	N	N	-	N	N
CYST, FOLLICULAR	-	-	-	P	-	-
UTERUS	N	N	N	-	N	N
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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143103
GROUP: 5: Ketoconazole (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15362	R15363	R15364	R15365	R15366	R15367
THYROID	N	N	-	N	N	N
ULTIMOBRANCHIAL CYST	-	-	(1)	-	-	-
OVARIES	-	-	-	-	-	-
CYST, FOLLICULAR	<P>	(P)	P	-	-	P
INTERSTITIAL CELL HYPERPLASIA	[2]	[3]	[2]	[2]	[3]	3
CORPORA LUTEA, ABSENT	P	P	P	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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143103
GROUP: 6: Pimozide (30mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15374	R15375	R15376	R15377	R15378	R15379
THYROID	N	N	N	N	N	N
OVARIES	-	-	N	-	-	-
CYST, FOLLICULAR	<P>	-	-	-	<P>	-
CYST, LUTEUAL	-	-	-	-	P	-
CORPORA LUTEA, ABSENT	-	P	-	P	-	-
POLYOVULAR FOLLICLES	-	-	-	P	-	-
REDUCED TERTIARY FOLLICLES	P	-	-	P	P	P
UTERUS	-	-	N	-	N	N
ATROPHY	-	P	-	-	-	-
EPITHELIAL HYPERPLASIA/HYPERTROPHY	[3]	-	-	[3]	-	-
Non-Protocol Tissues:						
LIVER	-	-	-	*	-	-

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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Sprague-Dawley

STUDY NUMBER: 1143103
GROUP: 7: Methoxychlor (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15406	R15407	R15408	R15409	R15410	R15411
THYROID	-	N	N	N	N	N
ULTIMOBRANCHIAL CYST	<P>	-	-	-	-	-
OVARIES	N	N	-	-	-	N
CYST, FOLLICULAR	-	-	(P)	<P>	(P)	-
UTERUS	N	N	-	N	N	-
EPITHELIAL HYPERPLASIA/HYPERTROPHY	-	-	[2]	-	-	[3]
CYSTIC DILATION, ENDOMETRIAL GLAND(S)	-	-	-	-	-	(P)

See Reports Code Table for Symbol Definitions

LABCAT HP4.33

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Long-Evans

STUDY NUMBER: 1143103
GROUP: 1: Corn Oil (2.5ml/kg/d)
SEX: FEMALE

ANIMAL ID:	R15320	R15321	R15322	R15323	R15324	R15325
THYROID	N	-	N	N	-	N
DEGENERATION, FOLLICULAR	-	-	-	-	(1)	-
ULTIMOBRANCHIAL CYST	-	-	-	-	<2>	-
INFILTRATING LYMPHOCYTES	-	(1)	-	-	-	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N

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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103 STUDY NUMBER: 1143103
FATE: TK Long-Evans GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15332	R15333	R15334	R15335	R15336	R15337
THYROID	-	N	N	N	N	N
ULTIMOBRANCHIAL CYST	(1)	-	-	-	-	-
OVARIES	N	-	-	-	-	N
ATROPHY	-	-	P	P	P	-
CYST, LUTEUAL	-	P	-	-	-	-
UTERUS	-	-	-	N	N	N
ENDOMETRIAL HYPERPLASIA	[P]	[P]	-	-	-	-
HYPERTROPHY, MYOMETRIUM	[P]	[P]	-	-	-	-
CYSTIC DILATION, ENDOMETRIAL GLAND(S)	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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Long-Evans

STUDY NUMBER: 1143103
GROUP: 3: Tamoxifen (10mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15344	R15345	R15346	R15347	R15348	R15349
THYROID	N	N	N	-	N	N
ULTIMOBRANCHIAL CYST	-	-	-	(1)	-	-
OVARIES	-	-	-	-	-	-
ATROPHY	P	P	P	P	P	P
CYSTIC DILATION, BURSA	-	-	-	-	P	-
CORPORA LUTEA, ABSENT	P	P	P	P	P	P
UTERUS	-	-	-	-	-	-
ATROPHY	P	P	P	P	P	P
EPITHELIAL HYPERPLASIA/HYPERTROPHY	P	P	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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103 STUDY NUMBER: 1143103
FATE: TK Long-Evans GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15356	R15357	R15358	R15359	R15360	R15361
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	-	-	-	-
UTERUS	N	N	N	-	N	N
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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Long-Evans

STUDY NUMBER: 1143103
GROUP: 5: Ketoconazole (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15368	R15369	R15370	R15371	R15372	R15373
THYROID	-	N	-	N	N	N
ULTIMOBRANCHIAL CYST	(1)	-	(1)	-	-	-
OVARIES	-	-	-	-	-	-
CYST, FOLLICULAR	P	P	-	P	<P>	-
INTERSTITIAL CELL HYPERPLASIA	[3]	[2]	[2]	[4]	[3]	[3]
CORPORA LUTEA, ABSENT	P	P	-	P	-	P
UTERUS	N	N	N	N	N	N
Non-Protocol Tissues: ADRENALS	*	*	-	*	*	*
Non-Protocol Tissues: LIVER	-	-	-	-	*	-

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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103 STUDY NUMBER: 1143103
FATE: TK Long-Evans GROUP: 6: Pimozide (30mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15380	R15401	R15402	R15403	R15404	R15405
THYROID	N	N	N	N	-	N
ULTIMOBRANCHIAL CYST	-	-	-	-	(P)	-
OVARIES	-	-	-	-	-	-
CYST, FOLLICULAR	<P>	-	<P>	-	-	(P)
CORPORA LUTEA, ABSENT	P	P	P	P	P	P
REDUCED TERTIARY FOLLICLES	-	-	P	-	-	P
UTERUS	N	-	-	-	-	N
ATROPHY	-	P	P	P	P	-
EPITHELIAL HYPERPLASIA/HYPERTROPHY	-	-	-	[3]	[3]	-

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: 1143-103

TABULATED ANIMAL DATA

STUDY ID : 1143-103
FATE: TK Long-Evans

STUDY NUMBER: 1143103
GROUP: 7: Methoxychlor (100mg/kg/d)
SEX: FEMALE

ANIMAL ID:	R15412	R15413	R15414	R15415	R15416	R15417
THYROID	-	N	N	-	N	-
ULTIMOBRANCHIAL CYST	(P)	-	-	<P>	-	(P)
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	-	N	N
EPITHELIAL HYPERPLASIA/HYPERTROPHY	-	-	-	[3]	-	-
CYSTIC DILATION, ENDOMETRIAL GLAND(S)	-	-	-	(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: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103
STUDY NUMBER: 1143103
SEX: FEMALE
GROUP: 1: Corn Oil (2.5ml/kg/d)

Animal ID: R15315
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - DILATED	UTERUS - No Corollary change detected

Animal ID: R15317
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
LIVER - RAISED AREA, MEDIAN LOBE, ONE, TAN, 5X4X2MM	LIVER - Histopathology Not Required

Animal ID: R15318
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
THYROID - ENLARGED, SLIGHTLY	THYROID - No Corollary change detected

Animal ID: R15319
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

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

Animal ID: R15320
Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - DILATED	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: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 1: Corn Oil (2.5ml/kg/d)

Animal ID: R15324 Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
THYROID - ENLARGED, MODERATE

Related Histopathology:
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: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103
STUDY NUMBER: 1143103
SEX: FEMALE
GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)

Animal ID: R15331
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
UTERUS WITH CERVIX - CYST, RIGH HORN, ONE, CLEAR, 1X1X1MM

Related Histopathology:
UTERUS - CYST, PERIUTERINE

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103 — STUDY NUMBER: 1143103
SEX: FEMALE — GROUP: 3: Tamoxifen (10mg/kg/d)

Animal ID: R15342 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
LIVER - ACCESSORY LOBE, MEDIAN LOBE, AT CLEFT, 15X4X2MM	LIVER - Histopathology Not Required
THYROID - ENLARGED, SLIGHT	THYROID - No Corollary change detected

Animal ID: R15346 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
OVARIES - RIGHT OVARIE: CYST, ONE, CLEAR, 6X3X4MM	OVARIES - No Corollary change detected

Animal ID: R15348 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
OVARIES - CYST, RIGHT, ONE, CLEAR, 4X3X3MM	OVARIES - CYSTIC DILATION, BURSA

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103 — STUDY NUMBER: 1143103
SEX: FEMALE — GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)

Animal ID: R15350 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, MODERATE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPREPLASIA/HYPERTROPHY

Animal ID: R15351 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, MODERATE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15352 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15354 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:
THYROID - ENLARGED, SEVERE

Related Histopathology:
THYROID - FOLLICULAR CELL HYPERPLASIA/HYPERTROPHY

Animal ID: R15357 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:
THYROID - ENLARGED

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: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 4: Propylthiouracil (PTU) (240mg/kg/d)

Animal ID: R15361 Pathologist: DNP
Animal Fate: TK Long-Evans

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: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103
STUDY NUMBER: 1143103
SEX: FEMALE
GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15362
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGED, 5X5X4MM, BILATERAL, PALE	ADRENALS - Histopathology Not Required
	ADRENALS - Histopathology Not Required

Animal ID: R15363
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGEDL, 5X5X4MM, BILATERAL	ADRENALS - Histopathology Not Required

Animal ID: R15364
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
UTERUS WITH CERVIX - DILATED, MODERATE	UTERUS - No Corollary change detected
ADRENALS - ENLARGED, SEVERE	ADRENALS - Histopathology not required

Animal ID: R15365
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGED, BILATERAL, 3X3X3MM	ADRENALS - Histopathology Not Required

Animal ID: R15366
Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGED, BILATERAL, 4X3X3MM	ADRENALS - Histopathology Not Required

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ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103 — STUDY NUMBER: 1143103
SEX: FEMALE — GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15367 — Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGED, BILATERAL, 5X5X3MM	ADRENALS - Histopathology Not Required

Animal ID: R15368 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGED, SEVERE	ADRENALS - Histopathology Not Required

Animal ID: R15369 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGEDL, SEVERE	ADRENALS - Histopathology Not Required

Animal ID: R15371 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
ADRENALS - ENLARGED, BILATERAL, 5X5X3MM	ADRENALS - Histopathology Not Required

Animal ID: R15372 — Pathologist: DNP
Animal Fate: TK Long-Evans

Reference to Necropsy Record:	Related Histopathology:
LIVER - ACCESSORY LOBE, MEDIAN LOBE AT CLEFT, 7X3X2MM	LIVER - Histopathology Not Required
ADRENALS - ENLARGED, 4X3X3MM	ADRENALS - Histopathology Not Required

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103
SEX: FEMALE

STUDY NUMBER: 1143103
GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15373
Animal Fate: TK Long-Evans

Pathologist: DNP

Reference to Necropsy Record:
ADRENALS - ENLARGED, BILATERAL, 4X3X3MM

Related Histopathology:
ADRENALS - Histopathology Not Required

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 6: Pimozide (30mg/kg/d)

Animal ID: R15377 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

Reference to Necropsy Record:	Related Histopathology:
LIVER - RAISED AREA, LEFT LATERAL, ONE, TAN, 6X4X3MM	LIVER - Histopathology Not Required
UTERUS WITH CERVIX - DISTENDED, BILATERAL, SEVERE	UTERUS - No Corollary change detected

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THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

CORRELATION OF GROSS & MICRO

STUDY ID: 1143-103
STUDY NUMBER: 1143103
SEX: FEMALE
GROUP: 7: Methoxychlor (100mg/kg/d)

Animal ID: R15415
Pathologist: DNP
Animal Fate: TK Long-Evans

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

Related Histopathology:
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: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 1: Corn Oil (2.5ml/kg/d)

Animal ID: R15315 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
UTERUS - NORMAL PROESTRUS.

Animal ID: R15317 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - ESOPHAGUS: THERE IS A FOCUS OF CHRONIC ACTIVE INFLAMMATION IN THE PERI-ESOPHAGEAL MUSCULATURE ACCOMPANIED BY LOSS OF MYOFIBERS AND PROLIFERATION OF BLOOD VESSELS AND FIBROBLASTS.

Animal ID: R15318 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - ESOPHAGUS: MYOSITIS, MODERATE, FOCAL, CHRONIC

Animal ID: R15319 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
UTERUS - NORMAL PROESTRUS.

Animal ID: R15320 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
THYROID - PERIESOPHAGEAL MUSCE: FIBROSIS, FOCAL. PERIEOPHAGEAL CONNECTIVE TISSUE: HEMORRHAGE, LOCALLY EXTENSIVE.
UTERUS - NORMAL PROESTRUS.

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 1: Corn Oil (2.5ml/kg/d)

Animal ID: R15321 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
THYROID - CERVICAL CONNECTIVE TISSUE: CELLULITIS, SUPPURATIVE, LOCALLY EXTENSIVE. PERI-ESOPHAGEAL MUSCULATURE: FIBROSIS, FOCAL.

Animal ID: R15323 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
UTERUS - OVIDUCT: CYSTIC DILATION.

Animal ID: R15324 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
THYROID - ESOPHAGUS: MICROABSCESS, FOCAL.

Animal ID: R15325 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
OVARIES - OVIDUCT: DILATION, CYSTIC.

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 2: Ethynyl estradiol (0.005mg/kg/d)

Animal ID: R15329 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - ESOPHAGUS: THERE IS A FOCUS OF CHRONIC ACTIVE MYOSITIS IN THE PERIESOPHAGEAL MUSCLE. EXTENDING LATERALLY AND BETWEEN FASCIAL PLANES IS A LOCALLY EXTENSIVE AREA OF PYOGRANULOMATOUS CELLULITIS.

Animal ID: R15333 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
THYROID - ESOPHAGUS: FIBROSIS, FOCAL

Animal ID: R15336 Pathologist: DNP
Animal Fate: TK Long-Evans

TISSUE COMMENTS:
THYROID - ESOPHAGUS: THERE IS FOCAL FIBROSIS IN THE PERIESOPHAGEAL MUSCLE.

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ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 3: Tamoxifen (10mg/kg/d)

Animal ID: R15342 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: FOCAL FIBROSIS.

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 4: Propylthiouracil (PTU) (240mg/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: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 5: Ketoconazole (100mg/kg/d)

Animal ID: R15365 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: FOCAL FIBROSIS.

Animal ID: R15366 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: FOCAL FIBROSIS.

Animal ID: R15367 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: FOCAL FIBROSIS.

Animal ID: R15372 Pathologist: DNP
Animal Fate: TK Long-Evans

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

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 6: Pimozide (30mg/kg/d)

Animal ID: R15375 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

TISSUE COMMENTS:
THYROID - PERI-ESOPHAGEAL MUSCLE: THERE IS A FOCUS OF FIBROSIS ACCOMPANIED BY REGENERATING SKELETAL MUSCLE FIBERS.

Animal ID: R15377 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

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

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PATHOLOGY ASSOCIATES INTERNATIONAL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS
THERIMMUNE RESEARCH CORPORATION STUDY NUMBER: 1143-103

COMMENT REPORT

STUDY ID: 1143-103 STUDY NUMBER: 1143103
SEX: FEMALE GROUP: 7: Methoxychlor (100mg/kg/d)

Animal ID: R15409 Pathologist: DNP
Animal Fate: TK Sprague-Dawley

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

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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: 1143-103

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
02/17/00	Tissue Trimming	02/17/00
04/05-07/00	Individual Animal Data	04/07/00
04/05-07/00	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
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (SPRAGUE DAWLEY)

ANIMAL ID	T4, TOTAL (UG/DL)	TSH (NG/ML)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL	
R15314	4.84	1.03
R15315	4.78	1.62
R15316	4.54	0.92
R15317	3.78	2.31
R15318	3.54	1.47
R15319	4.01	1.84
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15326	2.94	1.63
R15327	3.36	1.28
R15328	3.35	1.99
R15329	3.32	1.94
R15330	3.62	1.24
R15331	4.18	1.83
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN	
R15338	5.02	3.14
R15339	5.57	1.99
R15340	5.25	1.63
R15341	5.16	1.95
R15342	4.03	2.68
R15343	5.15	2.68
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15350	0.02	28.50
R15351	0.05	30.89
R15352	0.02	22.58
R15353	0.00	25.03
R15354	0.00	26.92
R15355	0.00	23.63
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15362	3.15	1.33
R15363	3.39	1.72
R15364	3.38	1.48
R15365	2.92	1.53
R15366	2.45	1.44
R15367	3.29	1.87
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE	
R15374	4.28	1.80
R15375	2.77	1.05
R15376	3.32	1.36
R15377	2.69	0.93
R15378	2.91	1.13
R15379	2.80	1.70
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15406	3.30	1.12
R15407	3.63	1.38
R15408	2.73	1.83
R15409	4.06	0.87
R15410	3.38	1.38
R15411	2.93	1.20

APPENDIX CONTINUED

APPENDIX 9 (CONTINUED)
INDIVIDUAL SERUM T4 AND TSH LEVELS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

ANIMAL ID	T4, TOTAL (UG/DL)	TSH (NG/ML)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL	
R15320	4.19	2.94
R15321	4.38	1.57
R15322	4.34	1.16
R15323	3.51	2.30
R15324	4.31	1.00
R15325	4.64	1.64
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL	
R15332	5.97	1.77
R15333	4.32	1.83
R15334	3.68	1.06
R15335	5.00	2.66
R15336	4.74	1.62
R15337	3.62	1.09
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN	
R15344	5.45	1.13
R15345	5.79	2.09
R15346	4.73	1.31
R15347	5.56	1.07
R15348	5.71	2.64
R15349	5.28	1.97
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL	
R15356	0.48	30.05
R15357	0.05	24.61
R15358	0.02	26.18
R15359	0.00	26.06
R15360	0.00	22.14
R15361	0.00	10.32
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE	
R15368	4.38	2.32
R15369	2.38	1.39
R15370	3.35	1.16
R15371	3.26	1.37
R15372	3.43	2.69
R15373	3.02	1.58
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE	
R15380	5.21	0.96
R15401	2.81	1.05
R15402	2.86	1.00
R15403	2.77	0.66
R15404	3.45	0.71
R15405	3.69	0.96
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR	
R15412	4.85	1.12
R15413	4.02	1.08
R15414	3.77	0.82
R15415	4.79	1.03
R15416	4.06	0.85
R15417	3.61	1.57

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

ANIMAL ID	DAY OF DEATH (PND)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL
R15314	42
R15315	42
R15316	42
R15317	43
R15318	43
R15319	43
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
R15326	42
R15327	42
R15328	42
R15329	43
R15330	43
R15331	43
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN
R15338	42
R15339	42
R15340	42
R15341	43
R15342	43
R15343	43
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL
R15350	42
R15351	42
R15352	42
R15353	43
R15354	43
R15355	43
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE
R15362	42
R15363	42
R15364	42
R15365	43
R15366	43
R15367	43
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE
R15374	42
R15375	42
R15376	42
R15377	43
R15378	43
R15379	43
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR
R15406	42
R15407	42
R15408	42
R15409	43
R15410	43
R15411	43

PND = POSTNATAL DAY

APPENDIX CONTINUED

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

ANIMAL ID	DAY OF DEATH (PND)
	GROUP: 1 - 2.5 ML/KG/DAY CORN OIL
R15320	42
R15321	42
R15322	42
R15323	43
R15324	43
R15325	43
	GROUP: 2 - 0.005 MG/KG/DAY ETHYNYL ESTRADIOL
R15332	42
R15333	42
R15334	42
R15335	43
R15336	43
R15337	43
	GROUP: 3 - 10 MG/KG/DAY TAMOXIFEN
R15344	42
R15345	42
R15346	42
R15347	43
R15348	43
R15349	43
	GROUP: 4 - 240 MG/KG/DAY PROPYLTHIOURACIL
R15356	42
R15357	42
R15358	42
R15359	43
R15360	43
R15361	43
	GROUP: 5 - 100 MG/KG/DAY KETOCONAZOLE
R15368	42
R15369	42
R15370	42
R15371	43
R15372	43
R15373	43
	GROUP: 6 - 30 MG/KG/DAY PIMOZIDE
R15380	42
R15401	42
R15402	42
R15403	43
R15404	43
R15405	43
	GROUP: 7 - 100 MG/KG/DAY METHOXYCHLOR
R15412	42
R15413	42
R15414	42
R15415	43
R15416	43
R15417	43

PND = POSTNATAL DAY

APPENDIX 11
STATISTICAL ANALYSIS
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

Experiment R1143-103 - 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	0.7230
liver	0.5604
kidney	0.4719
uterus	0.1180
uterusd	0.0772
pit	0.3618
t4	0.3741
tsh	0.1757
vagop	0.0820
estrus	0.0757
ovary	0.2329
adren	0.5378

Experiment R1143-103 - Sprague Dawley Juvenile Female Rats
Body and Organ Weights
Table 2
Analysis of Covariance: Test for PND22 x Dose Interaction

Dependent	ProbF
term	0.8236
liver	0.7392
kidney	0.9266
uterus	0.1285
uterusd	0.5583
pit	0.3612
t4	0.3564
tsh	0.8422
vagop	0.9989
estrus	0.8821
ovary	0.2051
adren	0.4091

Experiment R1143-103 - 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	0.0007
Control_vs_Dose7	<.0001

Experiment R1143-103 - 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	0.4368
kidney	0.5902
uterus	0.2984
uterusd	0.2300
pit	0.3792
ovary	0.1090
adren	0.7096

Experiment R1143-103 - Sprague Dawley Juvenile Female Rats
Ratio Data
Table 5
Analysis of Covariance: Test for Weaning Body Weight x Dose Inter

Dependent	ProbF
liver	0.4207
kidney	0.3938
uterus	0.1415
uterusd	0.5086
pit	0.3328
ovary	0.2443
adren	0.1235

Experiment R1143-103 - Sprague Dawley Juvenile Female Rats
Ratio Data
Table 6
Ratios: Results of MANCOVA

Hypothesis	ProbF
Control_vs_Dose2	0.0043
Control_vs_Dose3	<.0001
Control_vs_Dose4	0.0083
Control_vs_Dose5	<.0001
Control_vs_Dose6	0.0599
Control_vs_Dose7	0.5656

Experiment R1143-103 - 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	0.4405
liver	0.5746
kidney	0.5848
uterus	0.2793
uterusd	0.0768
ovary	0.4025
adren	0.2330
pit	0.5518
t4	0.1744
tsh	0.7372
vagop	0.2103
estrus	0.1084

Experiment R1143-103 - Long Evans Juvenile Female Rats
Body and Organ Weights
Table 2
Analysis of Covariance: Test for PND22 x Dose Interaction

Dependent	ProbF
term	0.4120
liver	0.4004
kidney	0.1766
uterus	0.4272
uterusd	0.4953
ovary	0.1984
adren	0.6128
pit	0.9881
t4	0.7819
tsh	0.6124
vagop	0.6813
estrus	0.4712

Experiment R1143-103 - 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 R1143-103 - 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	0.8961
kidney	0.7716
uterus	0.1795
uterusd	0.1161
ovary	0.8876
adren	0.5858
pit	0.7059

Experiment R1143-103 - Long Evans Juvenile Female Rats
Ratio Data
Table 5
Analysis of Covariance: Test for Weaning Body Weight x Dose Inter

Dependent	ProbF
liver	0.4001
kidney	0.3902
uterus	0.2621
uterusd	0.4399
ovary	0.3102
adren	0.7079
pit	0.9841

Experiment R1143-103 - Long Evans Juvenile Female Rats
Ratio Data
Table 6
Ratios: Results of MANCOVA

Hypothesis	ProbF
Control_vs_Dose2	0.0182
Control_vs_Dose3	0.0031
Control_vs_Dose4	0.2168
Control_vs_Dose5	<.0001
Control_vs_Dose6	0.7227
Control_vs_Dose7	0.2627

TABLE (1143-103)
ADJUSTED MEANS FROM ANALYSIS OF COVARIANCE
PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (SPRAGUE-DAWLEY)

	DOSE GROUP						
	CORN OIL	ETHYNYL ESTRADIOL	TAMOXIFEN	PROPYLTHIOURACIL	KETOCONAZOLE	PIMOZIDE	METHOXYCHLOR
ADRENALS	0.046	0.039	**0.032	**0.024	**0.091	**0.030	0.039
ESTRUS	36.399	**26.000	**43.500	**41.334	**41.833	38.200	**28.667
KIDNEYS	1.242	**1.082	**1.033	**0.751	1.274	**1.027	*1.121
LIVER	6.003	6.145	**5.085	**4.428	**7.552	*5.140	5.835
OVARIES	0.069	*0.051	**0.033	**0.040	0.057	*0.051	0.056
PITUITARY	0.007	0.012	0.003	0.006	0.006	0.005	0.007
T4, TOTAL	4.131	*3.461	**5.030	**0.015	**3.097	**3.128	**3.338
TERMINAL	148.143	*137.926	**115.809	**97.015	*138.881	**126.315	*139.486
TSH	1.560	1.624	*2.287	**26.115	1.551	1.290	1.264
UTERUS	0.354	0.289	**0.104	**0.124	**0.216	**0.196	*0.224
UTERUS-DRY	0.285	0.270	**0.097	**0.116	**0.186	**0.162	**0.205
VAGINAL OP.	35.071	**25.980	**26.986	*37.831	**38.569	*37.811	**26.819

TABLE (1143-103)
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						
	CORN OIL	ETHYNYL ESTRADIOL	TAMOXIFEN	PROPYLTHIOURACIL	KETOCONAZOLE	PIMOZIDE	METHOXYCHLOR
ADRENALS	0.030	0.028	0.027	*0.025	**0.065	0.023	0.028
KIDNEYS	0.838	*0.783	*0.891	*0.774	**0.917	0.812	0.804
LIVER	4.049	*4.442	*4.387	**4.558	**5.436	4.067	4.182
OVARIES	0.046	0.037	**0.028	0.041	0.041	0.040	0.041
PITUITARY	0.005	0.008	0.003	0.007	0.004	0.004	0.005
UTERUS	0.239	0.209	**0.091	**0.127	*0.155	0.157	0.160
UTERUS-DRY	0.193	0.195	**0.084	**0.119	**0.133	0.130	0.146

TABLE (1143-103)
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	0.036	0.044	0.034	**0.023	**0.071	*0.025	0.044
ESTRUS	38.194	**26.174	**43.492	40.674	41.603	40.598	34.667
KIDNEYS	1.370	1.286	**1.197	**0.663	1.356	**0.935	*1.257
LIVER	6.810	6.331	**5.639	**3.592	7.424	**4.331	**5.633
OVARIES	0.081	*0.058	**0.052	**0.041	0.067	**0.045	0.075
PITUITARY	0.008	0.009	0.007	0.005	0.007	0.005	0.005
T4, TOTAL	4.232	4.551	**5.425	**0.088	*3.302	*3.466	4.183
TERMINAL	158.724	**141.784	**124.689	**82.067	**137.164	**109.094	**140.712
TSH	1.650	1.590	1.607	**22.064	1.671	**0.876	*1.053
UTERUS	0.260	0.271	**0.103	0.155	*0.181	*0.158	0.237
UTERUS-DRY	0.233	0.254	**0.095	**0.134	*0.170	**0.147	0.212
VAGINAL OP.	35.888	**26.141	**26.803	35.587	35.496	**39.767	**27.000

TABLE (1143-103)
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						
	CORN OIL	ETHYNYL ESTRADIOL	TAMOXIFEN	PROPYLTHIOURACIL	KETOCONAZOLE	PIMOZIDE	METHOXYCHLOR
ADRENALS	0.023	*0.031	0.027	0.028	**0.052	0.023	0.031
KIDNEYS	0.864	0.903	**0.960	0.809	**0.989	0.857	0.893
LIVER	4.275	4.449	4.517	4.375	**5.416	3.954	3.996
OVARIES	0.051	0.040	0.042	0.050	0.049	0.042	0.053
PITUITARY	0.005	0.006	0.005	0.006	0.005	0.004	0.004
UTERUS	0.166	0.192	*0.082	0.192	0.131	0.144	0.169
UTERUS-DRY	0.149	0.180	**0.076	0.164	0.123	0.134	0.151

APPENDIX 12
PROTOCOL
ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN JUVENILE FEMALE RATS (LONG-EVANS)

TherImmune No. 1143-103

THERIMMUNE
Research Corporation

STUDY PROTOCOL

Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats

APPROVED:

TherImmune Research Corporation:

Meredith S. Rocca 12.14.99

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

EPA:

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. 1143-103**
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 (1143-101) 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 14, 2000
Last Terminal Sacrifice:	February 4, 2000
Progress Report:	December 31, 1999
Draft Report	March 3, 2000
Final Report:	May 5, 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 1143-102 and 1143-103. 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 1143-102.

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 1143-102.

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.: 1143-103	
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:

Meredith S. Rocca 1.26.00

Meredith S. Rocca, Ph.D. Date